Amended Environmental Assessment for Production of AquAdvantage® Salmon at the Bay Fortune and Rollo Bay Facilities on Prince Edward Island, Canada

In support of the
New Animal Drug Application related to AquAdvantage Salmon,
which are triploid, hemizygous, all-female Atlantic salmon (*Salmo salar*)
bearing a single copy of the α-form of the *opAFP-GHc2* recombinant DNA construct
at the α-locus in the EO-1α lineage

September 2024
Prepared by
Center for Veterinary Medicine
United States Food and Drug Administration
Department of Health and Human Services

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A NOTE TO THE READER

This document has been optimized for electronic viewing; the Table of Contents and all individual section headings are hyperlinked for your convenience.

The information and analyses in this amended EA reflect comments and input received from the National Marine Fisheries Service (NMFS) and the Fish and Wildlife Service (FWS) during an Endangered Species Act (ESA) technical assistance review initiated in June 2022 with initial discussions beginning in March 2021. NMFS provided concurrence, on behalf of itself and FWS, on FDA's ESA determination of "may affect, but is not likely to adversely affect" a listed species or their critical habitat in a Letter of Concurrence dated April 24, 2024 (see Appendix I).

This amended EA also takes into account oral comments received from the public during a public meeting held on December 15, 2022, and written comments sent to a public docket and received during a public comment period from November 16, 2022, to January 17, 2023.

LIST OF ACRONYMS AND CONVENTIONS EMPLOYED

~	Approximately
AAFB	AquAdvantage Female Broodstock
AANB	AquAdvantage Neomale Broodstock
AAS	AquAdvantage Salmon
ABRAC	Agricultural Biotechnology Research Advisory Committee
ABT	AquaBounty Technologies, Inc. (the sponsor)
AFP	Antifreeze protein
AGD	Amoebic gill disease
AR	Advanced Rearing (in the Rollo Bay facility)
bp	Base-pair
CEPA	Canadian Environmental Protection Agency
CEQ	Council on Environmental Quality
CFIA	Canadian Food Inspection Agency
CFR	US Code of Federal Regulations
COSEWIC	Committee on the Status of Wildlife in Canada
CSAS	Canadian Science Advisory Secretariat
CVM	Center for Veterinary Medicine
DFO	(Department of) Fisheries and Oceans [Canada]
DNA	Deoxyribonucleic acid
DO	Dissolved oxygen (concentration)
DPS	Distinct Population Segment
DUs	Designatable Units
EA	Environmental assessment
EIS	Environmental impact statement
ECCC	Environment and Climate Change Canada
EO-1α	The integrated form of the AquAdvantage rDNA construct
EPA	US Environmental Protection Agency
ER	Early Rearing (in the Rollo Bay facility)
ERA	Early Rearing Area (in the Bay Fortune facility)
ESA	Endangered Species Act
FDA	US Food and Drug Administration
FD&C Act	Federal Food, Drug, and Cosmetic Act
fn	footnote
FOI	Freedom of Information
FONSI	Finding of No Significant Impact
FWS	US Fish and Wildlife Service, Department of Interior
GE	Genetically engineered
GxE	Genotype-by-environment
GH	Growth hormone
GOA	Grow-Out Area (in the Bay Fortune facility)
HC	Health Canada
ICES	International Council for the Exploration of the Sea
IEF	Isoelectric Focusing
IGA	Intentional genomic alteration
ISA/ISAV	Infectious salmon anemia / infectious salmon anemia virus
mRNA	Messenger ribonucleic acid
MT	17α-methyltestosterone
NAAHP	Canadian National Aquatic Animal Health Program
NADA	New Animal Drug Application
NAH	N-acetyl-histidine
NASCO	North Atlantic Salmon Conservation Organization

NEPA	National Environmental Policy Act
NKA	Na^+, K^+-ATPase
NM	Nautical miles
NMFS	National Marine Fisheries Service, NOAA
NOAA	National Oceanic and Atmospheric Administration
NSN	New Substance Notification
NRC	National Research Council
OECD	Organization of Economic Cooperation and Development
OIE	World Organization for Animal Health
PEI	Prince Edward Island, Canada
ppt	Parts per thousand
PRV	Piscine orthoreovirus
PVC	Polyvinyl chloride
qPCR	Quantitative real time polymerase chain reaction
RAS	Recirculating Aquaculture System
rDNA	Recombinant deoxyribonucleic acid
RFE	Regulatory Fish Encyclopedia
SARA	Canadian Species at Risk Act
SHRU	Salmon Habitat Recovery Units
SMR	Standard metabolic rates
SNAc	Significant New Activity
SOPs	Standard Operating Procedures
SS	Stainless steel
SW	Sea winter
US	United States
USC	United States Code
USDA	US Department of Agriculture
UV	Ultraviolet
VMAC	Veterinary Medicine Advisory Committee
YOY	Year of young

TECHNICAL TERMS

Allele	Any alternative form of a gene that can occupy a particular chromosomal locus (see *heterozygous* or *homozygous*).
AquaBounty Technologies Salmon (ABT salmon)	Any GE Atlantic salmon from the E0-1ɑ lineage irrespective of ploidy, zygosity, or gender (i.e., the set of Atlantic salmon that includes diploid GE salmon that may be used as broodstock, as well as AquAdvantage Salmon).
AquAdvantage Salmon	The triploid, hemizygous, all-female Atlantic salmon from the E0-1ɑ lineage GE Atlantic salmon subject to this application. They are a subset of ABT salmon.
Biological containment (bioconfinement)	Use of biological methods, such as induced sterilization (e.g., triploidy), to prevent gene flow and reproduction in the environment
Chromosome	A physical structure consisting of DNA and supporting proteins called chromatin that carries hereditary information.
°C-day [min]	Compound unit of "time" (°C x days [min]) for relative determination of growth rate that accounts for the effect of water temperature.
Conspecific	An organism (plant or animal) of the same species. Herein, the term typically refers to wild or native Atlantic salmon, as well as salmon that may have been intentionally introduced or stocked into the environment.
Construct (gene or DNA construct)	A synthetic gene comprising regulatory & coding sequences constructed in vitro and usually incorporated into the genome of an organism with the intended purpose of modifying its phenotype. Often used interchangeably with "transgene."
Diploid	A cell, tissue, or organism having two complete sets of chromosomes, one from each parent.
EO-1ɑ	Functional, stably integrated form of *opAFP-GHc2* in the AquAdvantage Salmon genome.
Egg	A mature haploid female germ cell extruded from the ovary at ovulation.
Expression (gene)	The process by which the information encoded in a gene is used in the synthesis of a functional gene product (e.g., cell structures or proteins).
Flow cytometry	A technique for identifying and sorting cells and their components (e.g., DNA) by staining with a fluorescent dye and detecting the fluorescence usually by laser beam illumination. In this EA, flow cytometry is used to confirm ploidy.

Gamete(s)	Haploid reproductive cells produced in sexually mature organisms. A mature reproductive cell capable of fusing with the cell of similar origin but of opposite sex to form a zygote from which a new organism can develop. Gametes normally have haploid chromosomal content. In animals, including fish, gametes are sperm and oocytes (eggs).
GE Animal	Those animals modified by rDNA techniques, including the entire lineage of animals that contain the modification. The term GE animal can refer to both animals with heritable rDNA constructs and animals with non-heritable rDNA constructs (e.g., those modifications intended to be used as gene therapy).
Genome	The entire set of genetic instructions found in a cell.
Genotype	An organism’s collection of genes. The term also can refer to the two alleles inherited for a particular gene. The genotype is expressed when the information encoded in the genes’ DNA is used to make protein and RNA molecules.
GH Transgenic Atlantic salmon	Atlantic salmon containing a growth hormone gene that was exogenously introduced via genetic engineering that may be closely related to AquAdvantage Salmon.
Haploid	A cell, tissue, or organism having a single set of chromosomes (as opposed to diploid or triploid). Haploid cells are generally found in gametes (sex cells) of higher organisms.
Hemizygous	An individual having only one copy (or allele) of a given pair of genes instead of the usual two.
Homozygous	The genetic status in which an individual inherits the same alleles for a particular gene from both parents.
Heterozygous	Having inherited different forms of a particular allele from each parent. A heterozygous genotype stands in contrast to a homozygous genotype, where an individual inherits identical forms of a particular gene (see *allele*) from each parent.
Milt	The sperm-containing secretion of the testes of male fish. Milt is analogous to semen in mammals.
Neomale	A genetically female fish converted to a phenotypic male by hormone treatment.
opAFP-GHc2	The AquAdvantage rDNA construct containing Chinook salmon growth hormone (GH) gene and gene product, ocean pout and Chinook salmon–derived regulatory sequences, and a short synthetic linker.
Phenotype	An organism’s actual observed properties, such as morphology, development, or behavior, which derive predominantly from its genotype.

Plasmid	A circular, self-replicating, non-chromosomal DNA molecule found in many bacteria, although many artificial ones have been made. Often used as vectors for genetic engineering.
Ploidy	The number of complete sets of chromosomes contained within each cell of an organism (see *haploid*, *diploid*, and *triploid*).
Polymerase chain reaction	A standard technique to amplify copies of a DNA sequence often used to confirm genotype.
Promoter	A regulatory sequence of DNA needed to turn a gene on or off. The process of transcription (production of RNA from DNA) is initiated at the promoter. Usually found near the beginning of a gene, the promoter has a binding site for the enzyme used to make a messenger RNA (mRNA) molecule.
Recombinant DNA (rDNA construct)	A technology that uses enzymes to cut and paste together DNA sequences of interest that are linked together. The recombined DNA sequences, or rDNA construct, can be placed into vehicles called vectors (see plasmid) that ferry the DNA into a suitable host cell where it can be copied or expressed.
Redd	A nest created by female Atlantic salmon to deposit their eggs
Regulatory sequence	Non-protein coding DNA sequence of a gene controlling its expression.
Salmonid	A ray-finned finfish of the family Salmonidae, a taxonomic group that includes salmon, trout, chars, freshwater whitefish and graylings. The family includes fish of the following genera, among others: *Salmo, Salvelinus, and Onchorhynchus.*
Sea Winter (SW)	Number of winters spent at sea (e.g., 1SW, 2SW).
Smolt	A freshwater juvenile Atlantic salmon that has undergone the physiological changes necessary to be able to survive in salt water.
Somatic (cell)	Any cell of the body except sperm and egg cells. Most somatic cells of higher organisms are diploid, meaning that they contain two sets of chromosomes, one inherited from each parent.
Transgene	A synthetic gene comprising regulatory and coding sequences constructed in vitro and usually incorporated into the genome of a different species/organism with the intended purpose of modifying its phenotype. Often used interchangeably with “rDNA construct.”
Triploid	Having three complete sets of chromosomes per cell (see *haploid* and *diploid).*

Vector	A small DNA molecule (plasmid, virus, bacteriophage, artificial or cut DNA molecule) used to deliver DNA into a cell; and must be capable of being replicated and contain cloning sites for the introduction of foreign DNA.

*The various sources used for these definitions include: Wiley's *Dictionary of Microbiology and Molecular Biology*, Revised 2nd Ed., John Wiley and Sons, New York, 1994; *Animal Cloning: A Risk Assessment*, U.S. Food and Drug Administration (Center for Veterinary Medicine), 2008, final version and available to download at https://www.fda.gov/media/75280/download (accessed December 8, 2023); National Human Genome Research Institute, *Talking Glossary of Genetic Terms*, accessed at www.genome.gov/Glossary (accessed December 8, 2023).

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1. INTRODUCTION

On November 19, 2015, the United States (US) Food and Drug Administration (FDA) approved new animal drug application (NADA) 141-454 concerning AquAdvantage Salmon (AAS).[1] AAS are triploid, hemizygous, all-female Atlantic salmon (*Salmo salar*) bearing a single copy of the ɑ-form of the *opAFP-GHc2* recombinant DNA (rDNA) construct at the ɑ-locus in the EO-1ɑ lineage. AAS is designed to exhibit a rapid-growth phenotype that allows it to reach smolt size (100 g) faster than farm-raised Atlantic salmon without the *opAFP-GHc2* construct. The November 19, 2015, NADA approval allowed for the AAS to be produced at a facility on Prince Edward Island (PEI), Canada, and grown at a facility in Panama,[2] and allowed for sale of food harvested from AAS in the US.

As a part of the NADA review and approval process under the Federal Food, Drug, and Cosmetic Act (FD&C Act), and consistent with the mandates in the National Environmental Policy Act of 1969 (NEPA), 42 U.S.C. § 4321, et seq. and FDA's environmental impact considerations regulations (21 CFR part 25), FDA's Center for Veterinary Medicine (CVM) prepared an environmental assessment (EA) dated November 12, 2015, for the original approval of AAS.[3] Based on the EA and the specific conditions that were established in the NADA, FDA determined the action would not individually or cumulatively have a significant effect on the quality of the human environment in the US. Therefore, FDA prepared a finding of no significant impact (FONSI).[4] Based on the findings in the EA, the FDA also made a "no effect" determination under the Endangered Species Act (ESA), 16 USC § 1531, et seq., concluding that AAS, when produced and reared under the conditions in the application, and as described in the EA, would not affect US populations of threatened or endangered Atlantic salmon or their critical habitat.

Subsequently, several organizations filed suit in the US District Court, Northern District of California, challenging, among other things, FDA's evaluations under NEPA and the ESA for the 2015 NADA approval. While the Court upheld FDA's jurisdiction to regulate this product under the FD&C Act and left the NADA approval in place, on November 5, 2020, it found that "*FDA did not…meaningfully analyze what might happen to normal salmon in the event the engineered salmon* did *survive and establish themselves in the wild. Even if this scenario was unlikely, the FDA was still required to assess the consequences of it coming to pass*."[5] The Court ordered FDA

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[1] The NADA is for approval of a single copy of the ɑ-form of the *opAFP-GHc2* recombinant DNA construct at the ɑ-locus in the EO-1ɑ line of triploid, all-female Atlantic salmon under the conditions of use specified in the application. For ease of reference, this document generally refers to the application as being for approval of the AquAdvantage Salmon (AAS).

[2] The AquaBounty Technologies, Inc. (ABT) facility in Panama was closed in 2019 and is no longer approved under NADA 141-454.

[3] https://animaldrugsatfda.fda.gov/adafda/app/search/public/document/downloadEA/2243 (accessed December 8, 2023)

[4] https://animaldrugsatfda.fda.gov/adafda/app/search/public/document/downloadFonsi/2223 (accessed December 8, 2023)

[5] *Inst. for Fisheries Res. v. United States Food and Drug Adm'n*, 499 F. Supp. 3d 657, 660 (N.D. Cal. 2020) (emphasis added).

"*to go back and complete the analysis*." In addition, it ordered FDA to reconsider its "no effect" determination under the ESA together with a revised NEPA evaluation.

Following the original approval in November 2015, FDA approved two supplements under NADA 141-454 allowing for grow-out of AAS at a land-based, freshwater aquaculture facility near Albany, Indiana (approval date April 25, 2018), and the production of AAS eyed-eggs[6] in a Hatchery Unit at a second, new land-based aquaculture facility on PEI (approval date November 5, 2019). EAs were also prepared for these supplemental approvals, which resulted in FONSIs. These approvals and EAs were not challenged in the lawsuit.

This amended EA has been prepared to provide additional information and analyses to the 2015 EA for the original approval of NADA 141-454 concerning AAS, pursuant to the Court's November 5, 2020, order in order to address the outstanding issues outlined in that decision. Because this amended EA relates to an action taken in 2015, we prepared this EA based on the requirements in the NEPA implementing regulations in place in 2015. This amended EA discusses the potential impacts on the US environment, specifically the environment of Maine, if it is assumed that Atlantic salmon containing an intentional genomic alteration[7] (IGA), the *opAFP-GHc2* construct (referred to herein as the rDNA construct), were to escape the facilities in PEI, Canada, survive, and establish a persistent population in the US environment. This assessment evaluates the potential environmental impacts associated with all life stages (eyed-eggs to sexually mature adults), genotypes and ploidies of the Atlantic salmon containing the rDNA construct, including the triploid AAS (hemizygous for the rDNA construct) and the diploid AAS and AquAdvantage broodstock (hemizygous or homozygous for the rDNA construct), which will be collectively referred to herein as AquaBounty Technologies (ABT) salmon.[8] In addition, all fish housed at the currently approved facilities on PEI (Bay Fortune and Rollo Bay facilities), as well as planned future expansions and changes at the facilities on PEI, are considered. Finally, this assessment characterizes the risk of environmental impacts occurring in the US, including potential impacts on endangered Atlantic salmon of the Gulf of Maine Distinct Population Segment (DPS), based on the assumed presence of ABT salmon in the US environment.

2. BACKGROUND

The preparation of the EA for the 2015 original approval of AAS and subsequent approvals was the culmination of many individual steps. The major milestones are described below, and an outline of the significant steps taken by FDA during the preparation of all EAs for AAS are provided in detail in Appendix A.

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[6] Eyed-eggs are a life stage where black spot(s) of the eyes are visible through the membrane of the fertilized egg.

[7] In the 2015 EA, AAS were described as "genetically engineered (GE)" or "transgenic", but herein consistent with current FDA practice, the fish are considered to contain an "intentional genomic alteration (IGA)." The terms GE or transgenic may be used herein when referring to genetically altered fish that are not ABT salmon.

[8] AquaBounty Technologies (ABT) salmon will be used collectively herein when referring to triploid and diploid AAS and all AquAdvantage broodstock (see Section 6.3 for definitions of the types of Atlantic salmon with the rDNA construct held at ABT's facilities).

2.1. 2015 Original NADA Approval Action

The rDNA construct as integrated in the genome of AAS – the approved new animal drug - was originally approved by FDA on November 19, 2015, under NADA 141-454. At that time, the approval only permitted the commercial production of eyed-eggs for AAS at the sponsor's facility located near Bay Fortune on PEI (known as the Bay Fortune facility), and the grow-out of AAS at the sponsor's facility in Panama. The approval also included other aspects of the manufacturing process, such as the production and use of AquAdvantage broodstock (all female or neomale, diploid, hemizygous or homozygous for the rDNA construct) to produce AAS eggs.

For the purposes of an NADA approval, an rDNA construct contained in an animal is "defined" in terms of its identity, the claim made for it (i.e., its effectiveness), and any limitations and/or conditions placed on the resulting animals containing the rDNA construct and their use. The following is the product definition for the rDNA construct in AAS, which is also specified in FDA's approval letter:[9]

> Product Identity: A single copy of the α-form of the *opAFP-GHc2* recombinant DNA construct at the α-locus in the EO-1α lineage of triploid, hemizygous, all-female Atlantic salmon (*Salmo salar*) known as AquAdvantage Salmon
>
> Claim: Significantly more AquAdvantage Salmon grow to at least 100 g within 2,700 °C-days than their comparators
>
> Limitations: AquAdvantage Salmon are produced as eyed-eggs and grown-out only in physically contained freshwater culture facilities specified in an FDA-approved application.

The following warnings are required to be prominent on product labeling accompanying all life stages[10] of AAS up to the time of harvest:

- Rear only in a physically contained freshwater culture facility as specified in an FDA-approved application;
- These fish must not be reared in conventional cages or net-pens; and
- Dispose of morbid or dead fish in a manner consistent with local regulations.

The product label must also contain a statement that eggs and fry[11] are not for resale. The current label is provided in Appendix B.

In addition, as part of the Durability Plan to which the sponsor has committed under NADA 141-454, ploidy testing is conducted on a subset of all batches of fertilized AAS eggs distributed to AquaBounty controlled facilities. Per the Durability Plan, if, based on testing, triploidization in these eggs does not exceed 95% (based on the

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[9] 2015 Approval Letter and Appendix: https://www.fda.gov/animal-veterinary/animals-intentional-genomic-alterations/aquadvantage-salmon-approval-letter-and-appendix (accessed December 8, 2023) 2019 Approval Letter and Appendix: https://www.fda.gov/media/112639/download; https://www.fda.gov/media/112646/download (accessed December 8, 2023)

[10] Labeling is placed on all tanks and shipping containers containing AAS.

[11] Fry are included because a portion of the eyed-eggs may hatch during transport from Canada to an approved grow-out facility.

statistical 95% lower confidence limit as described in the 2015 Freedom of Information (FOI) Summary[12]), the batch of eggs is to be destroyed.[13]

Any production or use outside the scope of the approval would be unapproved and, therefore, would render the product unsafe under section 512(a) of the FD&C Act and adulterated under section 501(a)(5) of the FD&C Act. Any changes and/or additions to the conditions of production and use for AAS would require notification of FDA. FDA would consider production in a new facility to be a major change that would require a supplemental NADA approval prior to implementation. Any supplemental approval would constitute a new agency action triggering additional environmental analysis under NEPA (see 21 CFR 25.20(m)) to address the potential individual and cumulative impacts of any proposed changes and/or additions.

2.2. 2018 and 2019 Supplemental NADA Approval Actions

Since the 2015 original NADA approval, FDA has approved the production of AAS eggs or grow-out of AAS at two additional facilities under supplemental NADAs:

- Indiana facility: On April 25, 2018, FDA approved a supplemental NADA to allow grow-out of AAS at a land-based, freshwater aquaculture facility near Albany, Indiana, US. Eyed-eggs can be shipped from a facility specified in an FDA-approved application to the Indiana facility, where they are reared to market size and harvested for processing for food use (e.g., preparation of eviscerated whole fish, fish fillets, steaks, etc.).
- Rollo Bay facility: On November 5, 2019, FDA approved a second supplemental NADA to allow production of AAS in a Hatchery Unit at a second land-based, freshwater aquaculture facility located near Rollo Bay, PEI, Canada. Eyed-eggs can be shipped from the Rollo Bay facility to the grow-out facility in Indiana.

EAs (dated April 20, 2018, and October 29, 2019, respectively) were prepared by ABT, under FDA's direction and oversight, for each of these actions, and issued by FDA following the approval of the supplemental NADAs (21 CFR 25.40(b)).[14] For each action, FDA carefully considered the potential environmental impacts of the proposed agency actions and determined that the actions to approve the supplemental NADAs would not individually or cumulatively have a significant impact on the quality of the human environment in the US. Therefore, FDA prepared FONSIs for each action.[15]

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[12] https://animaldrugsatfda.fda.gov/adafda/app/search/public/document/downloadFoi/2541 (accessed December 8, 2023)

[13] The method used to test the ploidy of eggs is destructive and it is therefore impossible to test every egg before use. FDA approved a sampling method (2015 FOI Summary, see fn 12) that tests between 200 and 900 eggs per batch to determine the ploidy of AAS egg batches.

[14] EA for Indiana facility: https://animaldrugsatfda.fda.gov/adafda/app/search/public/document/downloadEA/862; EA for Rollo Bay facility: https://animaldrugsatfda.fda.gov/adafda/app/search/public/document/downloadEA/2264 (accessed December 8, 2023)

[15] FONSI for Indiana facility: https://animaldrugsatfda.fda.gov/adafda/app/search/public/document/downloadFonsi/802; FONSI for Rollo Bay facility: https://animaldrugsatfda.fda.gov/adafda/app/search/public/document/downloadFonsi/2244 (accessed December 8, 2023)

FDA's approvals of the supplemental NADAs were for the specific set of conditions established in the approvals, and as reflected in FDA's approval letters[9] and as described in the EAs. These include appropriate controls on the production and grow-out of the AAS, such as appropriate physical and biological containment measures. The approved conditions authorized under NADA 141-454, at the time this amended EA was prepared, limit breeding and egg production to two locations on PEI and rearing (grow-out) of AAS to one location in Indiana. In addition, the conditions do not include raising AAS in ocean net pens.

As described above, FDA's approval of supplements under NADA 141-454 included the specific set of conditions of approval. All other conditions of approval, described in the approval letter for the original NADA dated November 19, 2015, remain in effect. Any production or use outside the scope of the approval and supplemental approvals would be unapproved and would result in the article, in this case the construct in AAS, being considered an unsafe new animal drug and, therefore, adulterated within the meaning of section 501(a)(5) of the FD&C Act. The sponsor must continue to notify FDA about proposed changes in any conditions established in an approved application and obtain FDA approval of a supplemental application for any change where necessary (21 CFR 514.8). Major and moderate changes, including any additional production facilities, would require the filing, review and approval of additional supplemental NADAs. Approval of any additional supplemental applications would constitute major agency actions and trigger additional environmental analyses under NEPA, unless otherwise excluded.

2.3. Risk Assessment of AAS Production by Canadian Government

AquaBounty Canada Inc. submitted a New Substance Notification (NSN) under the *New Substances Notification Regulations (Organisms)* of the *Canadian Environmental Protection Act* (CEPA)[16] to Canadian authorities, specifically Health Canada (HC) and Environment and Climate Change Canada (ECCC),[17] for the manufacture and grow-out of AAS at the Bay Fortune and Rollo Bay facilities in PEI, Canada in 2013 and 2018, respectively. As part of ECCC's review of these notifications, HC and ECCC conducted environmental and indirect human health risk assessments. Joint Assessment Reports (DFO, 2013; DFO, 2019; McGowan and Leggatt, 2020; McGowan *et al.*, 2021) were issued for both NSN submissions that include a summary of ECCC's analysis of the potential hazards, likelihoods of exposure, associated uncertainties, and conclusions on risk. In these reports, the Canadian regulatory authorities determined that with the containment measures in place at the

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[16] CEPA, 1999 (S.C. 1999, c. 33), administered by ECCC and HC, is the authority under which the Canadian government ensures that all new substances are assessed for their potential risk to the environment and indirectly to human health through environmental exposure.

[17] Prior to 2015, ECCC was titled Environment Canada.

facilities, EO-1ɑ salmon[18] were "not toxic"[19] under the CEPA due to the low potential for exposure of AAS and AquAdvantage broodstock to the Canadian environment. The Joint Assessment Reports for both the Bay Fortune and Rollo Bay facilities are available online[20] and the information contained in those reports was considered when documenting the potential hazards and harms for this amended EA (see Section 9.3, below).

2.4. 2020 US District Court, Northern District of California, Opinion

Following FDA's 2015 approval of NADA 141-454 concerning AAS, a number of organizations and others filed suit in the US District Court, Northern District of California, challenging, among other things, the NEPA and ESA determinations. In its November 5, 2020, decision, the Court found:[5]

> "*The FDA did not…meaningfully analyze what might happen to normal salmon in the event the engineered salmon did survive and establish themselves in the wild. Even if this scenario was unlikely, the FDA was still required to assess the consequences of it coming to pass. This is especially true because the FDA knew that the company's salmon operations would likely grow, with additional facilities being used for farming*."

Because, in the Court's view, the risk of engineered salmon escaping grew as the company's operations expanded, it concluded that the agency should have conducted a more complete assessment of the risks posed by the company's genetic engineering project, including an assessment of the consequences for "normal" salmon if the engineered salmon established themselves in the wild.

The Court ordered that "*on remand the FDA must*":

1. "*…complete the final step of its own risk analysis by addressing the consequences that would result from the engineered salmon successfully establishing a persistent population outside of captivity*", and
2. "*…reconsider its 'no effect' determination under the ESA together with its revised NEPA evaluation*."

3. PURPOSE OF AND NEED FOR AMENDED EA

This amended EA addresses the Court's considerations relating to FDA's NEPA and ESA evaluations for the 2015 NADA approval of AAS under NADA 141-454, as set

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[18] Per the report, EO-1ɑ salmon is an Atlantic Salmon (*Salmo salar*) containing a single insert of the *opAFP-GHc2* transgene at the EO-1ɑ locus. EO-1ɑ salmon includes AquAdvantage Salmon, which is a triploid (≥95%), all-female subset of the EO-1ɑ lineage.

[19] Under CEPA, a substance or product of biotechnology is toxic if it is entering or may enter the environment in a quantity or concentration or under conditions that: 1) have or may have an immediate or long-term harmful effect on which life depends; or its biological diversity; 2) constitute or may constitute a danger to the environment on which life depends; or 3) constitute or may constitute a danger in Canada to human life or health. See Section 2.5.1 of the 2015 EA for additional information and discussion.

[20] The Joint Assessment Reports and related materials are available through this website: https://www.canada.ca/en/environment-climate-change/services/managing-pollution/evaluating-new-substances/voluntary-public-engagement-initiative.html (accessed December 14, 2023)

forth in the November 5, 2020, opinion by the US District Court, Northern District of California.

In this amended EA, FDA revises and expands Risk-related Question 4 from the 2015 EA[21] and provides revised responses. FDA addresses the Court's considerations by identifying and evaluating the potential harms (herein, the term "harms" is synonymous with "consequences", see additional explanation under Section 4.2 below) to the US environment, specifically the environment of Maine, that could result in the highly unlikely event of escape and establishment of Atlantic salmon containing the rDNA construct (*opAFP-GHc2* construct), including both triploid and diploid AAS and diploid AquAdvantage broodstock (collectively referred to herein as ABT salmon). In addition, the potential for exposure via transmission of pathogens[22] and/or parasites[23] from ABT salmon and the ABT production facilities on PEI is also identified, and the potential for harm, i.e., disease, is evaluated. Finally, FDA also creates a new risk-related question (Question 5) that re-evaluates the risk of these potential harms occurring in the US, including potential effects on endangered Atlantic salmon of the Gulf of Maine DPS, based on the potential for exposure in the US environment, including from establishment and/or presence of ABT salmon in the US environment, as well as from pathogen/parasite transmission to wild US Atlantic salmon. These evaluations account for expansions of ABT's production of ABT salmon on PEI that have occurred since the 2015 NADA approval, as well as planned future expansions and changes to existing facilities on PEI.[24]

This amended EA focuses only on the approved production of AAS at facilities in PEI, Canada. ABT did not renew its lease of the Panama facility and, as of November 5, 2019, that facility is no longer part of FDA's approval of NADA 141-454. Therefore, grow-out of AAS at the Panama facility is not further addressed herein. In addition, an EA and FONSI were prepared for grow-out at the ABT Indiana facility,[14,15] which found that there is no valid exposure pathway for AAS raised at the Indiana facility to affect endangered Atlantic salmon in the US. However, because the eggs sent to the Indiana facility are produced at the PEI facilities, the increased risk of the egg production on PEI for grow-out in the Indiana facility is indirectly evaluated in this EA.

In addition, this amended EA focuses on potential impacts to the US environment. NEPA does not require analysis of effects on the environment in foreign sovereign

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[21] Question 4 in the 2015 EA stated: "What are the likely consequences to, or effects on, the environment of the United States should AquAdvantage Salmon escape the conditions of confinement?" Question 4 was updated in the 2019 EA to also include the evaluation of potential impacts from AquAdvantage broodstock, not just AAS.

[22] Herein, the term "pathogen" will encompass a bacterium, virus, or other microorganism. According to Merriam-Webster Dictionary, a pathogen is defined as a specific causative agent, such as a bacterium or virus, of disease. https://www.merriam-webster.com/dictionary/pathogen (accessed December 8, 2023). Pathogens result in disease that can ultimately harm endangered Atlantic salmon (e.g., mortality).

[23] Parasites can function as pathogens when they are the direct cause of disease but can also act as vectors for disease by spreading other pathogens.

[24] Environmental considerations relating to the grow-out of AAS at the Albany, Indiana facility were considered in connection with approval of that supplemental NADA, were not addressed in the 2020 Court Opinion, and are not included in this evaluation because it was not subject of the 2020 Court Opinion.

countries, such as Canada.[25] In this EA, we have considered the potential for survival, dispersal, reproduction, establishment, and pathogen/parasite transmission in Canada in the context that these events are involved in the pathways that could potentially result in exposure and effects in the US environment. FDA approval of the NADA is independent of any evaluation of effects/impacts, or regulation of ABT salmon, by regulatory authorities in Canada.[26] Regardless of the approval in the US, continued operation of these facilities would be allowed in Canada.

4. PROBLEM FORMULATION AND RISK-RELATED QUESTIONS

4.1. Goal of Amended EA

The goal of this amended EA is to address the Court's considerations described in Section 2.4 above. As directed by the Court, FDA has expanded its assessment beyond that in the 2015 EA in order to conduct an exhaustive analysis of the likelihood of harms that could occur from the assumed presence of ABT salmon in the natural environment. Herein, FDA outlines the pathways necessary for ABT salmon to escape confinement from the PEI facilities and migrate to and establish a persistent population in the US. FDA also evaluates the potential pathways for pathogen/parasite transmission from ABT salmon and the ABT facilities on PEI to wild fish populations, including endangered US Atlantic salmon. Then, FDA identifies and evaluates the potential harms (consequences) to the US environment[27] and the endangered Atlantic salmon population if these highly unlikely scenarios were to occur. Finally, FDA revisits whether there is a potential for significant impacts on the US environment under NEPA, and whether the action would result in effects on threatened and endangered Atlantic salmon and their critical habitat in the US under the ESA. Ultimately, this analysis will aid the Agency in its decision of whether to prepare a FONSI or environmental impact statement (EIS).

4.2. Approach to Assessment

As part of the overall process of developing an approach for the regulation of genetically engineered (GE)[7] animals, FDA commissioned the National Research Council (NRC) to evaluate "*food, animal, and environmental safety issues with bioengineering animals and cloning that would be appropriate to address in any science-based regulatory scheme developed for these products*." This resulted in a

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[25] See, e.g., Natural Resources Defense Council, Inc. v. Nuclear Regulatory Com., 647 F.2d 1345, 1366 (D.C. Cir. 1981); Consejo de Desarrollo Economico de Mexicali v. United States, 438 F. Supp. 2d 1207, 1234 (D. Nev. 2006), vacated and remanded on other grounds, 482 F.3d 1157 (9th Cir. 2007). CEQ has issued guidance on NEPA analyses for actions taking place within the US that may have transboundary effects extending across the border and affecting another country's environment. This does not apply here because would be no effects that cross the border from the US into other countries from AAS. https://ceq.doe.gov/docs/ceq-regulations-and-guidance/memorandum-transboundary-impacts-070197.pdf (accessed December 8, 2023). Canada exercises regulatory authority over ABT facilities in their country; see Section 2.3 of this assessment.

[26] Canada regulates AquAdvantage Salmon facilities in their country under their own authority. See DFO (2013) at 18 ("*[T]he risk to the Canadian environment associated with the manufacture and production of AAS is low with reasonable certainty*.").

[27] In this assessment, FDA only evaluates potential impacts to the US environment as required under NEPA. Evaluating potential impacts to the Canadian environment is the responsibility of the Canadian government. See fn 25 and 26.

report entitled *Animal Biotechnology: Science Based Concerns* (NRC, 2002). This report did not specify or describe a method of risk assessment for GE animals, but rather identified risk issues associated with products of animal biotechnology. In particular, when considering environmental risks and associated risk analysis, the NRC report adapted principles of risk assessment described in two previous NRC reports on risk (NRC, 1983; NRC, 1996). The 1996 NRC report provided two important definitions: ***Hazard***: an act or phenomenon that has the potential to produce harm, and ***Risk***: the likelihood of harm resulting from exposure to the hazard.

Risk [R], as described in the 2002 NRC report, is the joint probability of exposure [$P(E)$], and the conditional probability of harm given that exposure has occurred [$P(H|E)$]:

$$\text{Risk (R)} = P(E) \times P(H|E).$$

Inherent in these definitions is the concept that both exposure and harm (i.e., adverse effects) are required components of risk, i.e., Risk = Exposure × Effects. Without either component (exposure or effect), there can be no risk. In other words, risk is the probability of harm (likelihood of a prescribed undesired effect), which is dependent on the probability of exposure. Therefore, the lower the probability of exposure, the lower the risk (or probability) of harm.

In this context, NRC (2002) described the following steps in the risk analysis:

1. to identify the potential harms[28] regardless of likelihood (addressed in Risk-Related Question 4 in Section 9.3.1, below);
2. to identify the potential hazards that might produce those harms (addressed in Risk-Related Question 4 in Section 9.3.1, below);
3. to define what exposure means for a genetically engineered organism and the likelihood of exposure, $P(E)$ (addressed in Risk-Related Questions 1-3, see Section 9.2);
4. to quantify the likelihood of harm assuming[29] that exposure has occurred, $P(H|E)$ (addressed in Risk-Related Question 4, see Section 9.3.2); and
5. to multiply the resulting probabilities to characterize[30] risk (addressed in Risk-Related Question 5, see Section 9.4).

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[28] When conducting NEPA evaluations, the terms "effects or impacts" are used as defined under 40 CFR 1508.1(g) and can include both beneficial and adverse effects/impacts. However, in this assessment, the terms "harms" will be used because the focus of this EA is on adverse effects only. In addition, in the 2015 EA, the term "consequences" was used. However, herein the term "harm" is used to be consistent with the recommendations in the NRC (2002) and (2004a) reports, but is considered to be synonymous, or the same as, "consequence."

[29] NRC (2002) uses the term "given" that exposure as occurred. However, it was changed to "assume" herein to clarify the intention of this step.

[30] The NRC (2002) stated "to multiply the resulting probabilities to *prioritize* risk"; however, in this risk assessment we will characterize (i.e., qualitatively rank) all risks.

NRC published additional recommendations on conducting a risk assessment of GE organisms in 2004 (NRC, 2004a). NRC (2004a) includes "estimating severity of harm" as an additional consideration under Step 4.

NRC (2002) defined hazard, harm and exposure relative to their use in ecological risk assessment of GE animals. Hazard was defined as the GE organism itself "*because it is the agent that might cause negative impacts*" (NRC, 2002). Harm was "*defined as gene pool, species, or community perturbation resulting in negative impacts to community stability*." Exposure was defined as "*establishment of a GE organism in the community.*" However, NRC (2002) goes on to clarify that it does not mean that risk cannot occur without establishment. NRC (2002 and 2004a) notes that quantifying environmental risk of GE organisms is difficult, and in some cases may be impossible, at this time, but relative qualitative discrete rankings from high to low are possible based on available evidence for each category (NRC, 2004a).

For this assessment, we conceptually incorporated the NRC principles described above, as well as those from the US Environmental Protection Agency (EPA) approach to ecological risk assessment (EPA, 1992). Our approach in this amended EA is based on an evaluation of possible exposure pathways, and potential hazards and harms that may ultimately result in ecological risks. The qualitative risk of those harms occurring is evaluated by considering the likelihood of exposure of ABT salmon in the US environment [$P(E)$] and the likelihood of harms assuming exposure has occurred [$P(H|E)$]. The severity of the harms is also considered when characterizing risk (see Section 9.4.1.4 below).

In this assessment, we expanded the NRC (2002) definitions of hazard, harm and exposure to ensure that all potential harms of ABT salmon itself and the rDNA construct were evaluated. The definitions of these terms are provided below.

> Hazard is defined herein as the GE organism itself and any "*act or phenomenon that has the potential to produce harms or other undesirable effects"* (Kapuscinski *et al*., 2007a; NRC, 1996), which is similar to the definition in the 2015 EA. Thus, the hazards in this assessment are ABT salmon and its interactions with ecosystem components.

An expanded definition of harm was developed and used in this assessment:

> Harm is defined herein as an adverse effect to the environment due to the hazard (in this case, ABT salmon and its interactions with the ecosystem components).

This definition is meant to be inclusive and to encompass all potential harms at the population, community, and ecosystem levels, including "*gene pool, species, or community perturbation resulting in a negative impact to community stability*" (NRC, 2002), as well as "*ecosystem displacements, disruptions, or species extinctions*" (Devlin *et al*., 2006).

Note that in the 2015 EA, and as referenced in the November 2020 Court opinion, the term "consequence" was used when discussing effects or impacts to the US environment. However, in this assessment, the term "harm" was chosen to be consistent with the terminology used in the 2002 NRC report. Herein, "harm" is considered synonymous with adverse "consequence", "effect", or "impact." This definition would encompass any adverse impacts to endangered Atlantic salmon populations in the US.

Finally, the NRC (2002) definition of exposure was also expanded herein to include presence in addition to establishment because potential harms to the population, community, and ecosystem could also occur from the presence of the ABT salmon alone.

> Exposure is defined as establishment and/or presence of ABT salmon or its progeny in the environment.

This amended EA centers on the likelihood of any ABT salmon produced at ABT's PEI facilities escaping into and surviving in the Canadian environment, dispersing or migrating to the US environment (i.e., evaluating whether there is an exposure pathway to US), and subsequently establishing and/or causing an adverse outcome (harm) on the US environment. In addition, this amended EA evaluates the likelihood of pathogen and/or parasite transmission 1) via ABT salmon infected with a pathogen/parasite interacting with wild fish populations both in and outside the US; 2) from a pathogen/parasite discharged in the wastewater of a ABT facility; and 3) from the shipment of AAS eggs containing a pathogen/parasite to a US facility. These evaluations take into account the production of eyed-eggs on PEI (including shipment of eggs to the US for grow-out to market size), within the framework of a conceptual risk assessment model and a series of risk-related questions (see next section). These analyses and their outcomes are discussed in the Section 9 of this EA.

It is important to note that although the NRC (2002) report is 20 years old, the general risk assessment principles discussed therein are still currently used by many regulatory agencies, including FDA and the US EPA. In addition, more recent publications that discuss methodologies for assessing environmental risk of GE animals were also consulted and were found to use similar principles, including NRC (2004a), Kapuscinski (2005), Kapuscinski *et al*. (2007a), DFO (2013 and 2019), and EFSA (2013). When newer publications diverged from the NRC methodology, the newer methods were considered and incorporated when appropriate in this assessment.

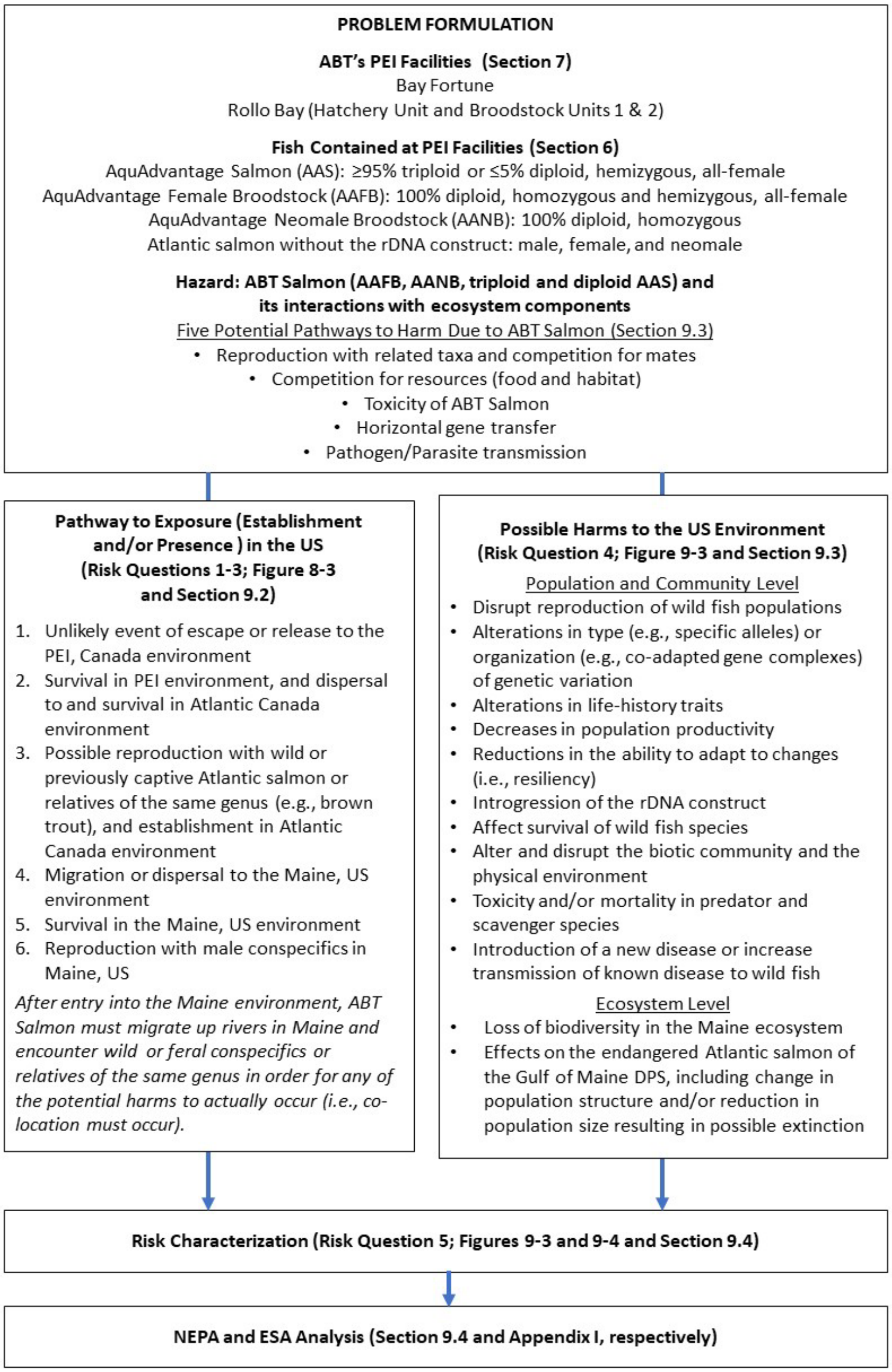
4.3. Problem Formulation and Conceptual Model

A problem formulation is the first step of a risk assessment, and it evaluates the hazard characteristics (i.e., ABT salmon, which contain the rDNA construct), and characterizes the potential exposure, ecological effects, and ecosystem potentially at risk from that stressor, with the key goal of developing a conceptual model that illustrates the relationships between these components (EPA, 1992). FDA previously presented a conceptual model in Figure 2 of the 2015 EA that generally illustrated the exposure pathways, hazards, effects, and risks based on the risk paradigm outlined in the 2002 NRC report. However, a new expanded conceptual model illustrated in Figure 4-1 has been developed to address the goal of this amended EA as described in Section 4.1, above.

The expanded conceptual model in Figure 4-1 captures the information in the previous conceptual model presented in the 2015 EA and provides additional detail on the potential exposure pathways, hazards, and harms in the highly unlikely event that ABT salmon escape the PEI facilities and establish and/or are present in the US environment. In addition, the conceptual model is expanded in sub-models to illustrate the details and complexity of the assessment, see Figure 8-4, Figure 8-5, and Figure 9-2. The information in Figure 4-1 is used in the next section (Section 4.4) to revise the series of risk-related questions outlined in the 2015 EA, and ultimately to characterize the risk of harmful consequences (harms) occurring in the US environment. The risk-related questions were presented in the 2015 EA and were revised herein to address the Court's concerns. The risk-related questions are listed in the next section.

As a reminder, this amended EA focuses only on the approved production of AAS at facilities in PEI, Canada. As of November 5, 2019, the Panama facility is no longer part of FDA's approval of NADA 141-454 and an EA and FONSI were prepared for the Indiana facility, which found that there is no valid exposure pathway for AAS raised at the Indiana facility to affect endangered Atlantic salmon in the US. Thus, these facilities are not evaluated herein (see Section 3 above). In addition, this amended EA only evaluates the potential impacts to the US environment, as required under NEPA (see Section 3).[25,26] As explained earlier, effects or impacts in Canada have not been explicitly evaluated by FDA, though exposure pathways originating in Canada have been because they are relevant to evaluating effects in the US environment.

Figure 4-1. Expanded Conceptual Model for Risk Assessment*



PROBLEM FORMULATION

ABT's PEI Facilities (Section 7)
Bay Fortune
Rollo Bay (Hatchery Unit and Broodstock Units 1 & 2)

Fish Contained at PEI Facilities (Section 6)
AquAdvantage Salmon (AAS): ≥95% triploid or ≤5% diploid, hemizygous, all-female
AquAdvantage Female Broodstock (AAFB): 100% diploid, homozygous and hemizygous, all-female
AquAdvantage Neomale Broodstock (AANB): 100% diploid, homozygous
Atlantic salmon without the rDNA construct: male, female, and neomale

Hazard: ABT Salmon (AAFB, AANB, triploid and diploid AAS) and its interactions with ecosystem components

<u>Five Potential Pathways to Harm Due to ABT Salmon (Section 9.3)</u>

- Reproduction with related taxa and competition for mates
- Competition for resources (food and habitat)
- Toxicity of ABT Salmon
- Horizontal gene transfer
- Pathogen/Parasite transmission

Pathway to Exposure (Establishment and/or Presence) in the US (Risk Questions 1-3; Figure 8-3 and Section 9.2)

1. Unlikely event of escape or release to the PEI, Canada environment
2. Survival in PEI environment, and dispersal to and survival in Atlantic Canada environment
3. Possible reproduction with wild or previously captive Atlantic salmon or relatives of the same genus (e.g., brown trout), and establishment in Atlantic Canada environment
4. Migration or dispersal to the Maine, US environment
5. Survival in the Maine, US environment
6. Reproduction with male conspecifics in Maine, US

After entry into the Maine environment, ABT Salmon must migrate up rivers in Maine and encounter wild or feral conspecifics or relatives of the same genus in order for any of the potential harms to actually occur (i.e., co-location must occur).

Possible Harms to the US Environment (Risk Question 4; Figure 9-3 and Section 9.3)

<u>Population and Community Level</u>

- Disrupt reproduction of wild fish populations
- Alterations in type (e.g., specific alleles) or organization (e.g., co-adapted gene complexes) of genetic variation
- Alterations in life-history traits
- Decreases in population productivity
- Reductions in the ability to adapt to changes (i.e., resiliency)
- Introgression of the rDNA construct
- Affect survival of wild fish species
- Alter and disrupt the biotic community and the physical environment
- Toxicity and/or mortality in predator and scavenger species
- Introduction of a new disease or increase transmission of known disease to wild fish

<u>Ecosystem Level</u>

- Loss of biodiversity in the Maine ecosystem
- Effects on the endangered Atlantic salmon of the Gulf of Maine DPS, including change in population structure and/or reduction in population size resulting in possible extinction

Risk Characterization (Risk Question 5; Figures 9-3 and 9-4 and Section 9.4)

NEPA and ESA Analysis (Section 9.4 and Appendix I, respectively)

* This new conceptual model is based on the conceptual model from the 2015 EA (Figure 2) and has been expanded to include details on the potential exposure pathways, hazards, and harms in the highly unlikely event that ABT salmon escape the PEI facilities and establish in the US environment. This conceptual model only covers the production of AAS at PEI facilities (see Section 3 above). It is important to note that the assumption that up to 5% of AAS are diploid is a worst-case assumption based on the requirements under the NADA (see 2015 FOI Summary, fn 12). According to testing data from 2017-2022, triploidy averaged 99.5% in eggs of the batches that were released for shipment.

Additional exposure pathways also exist that are not illustrated in Figure 4-1 above that relate to the transmission of pathogens and/or parasites via 1) ABT salmon interacting with wild fish populations, including endangered US Atlantic salmon, following escape from the PEI facilities, 2) discharge of pathogens/parasites from the PEI facilities via wastewater, and 3) a shipment of AAS eggs containing pathogens/parasites into the US. These potential pathways could lead to harms (e.g., disease) in US endangered Atlantic Salmon populations, as described in Figure 4-1 above. The pathogen/parasite transmission pathways are discussed in more detail in Sections 8.2.2 and 9.2.4, and the potential harms due to pathogen/parasite transmission are discussed in Section 9.3.

4.4. Risk-related Questions

Risk is the joint probability of exposure [$P(E)$] and the conditional probability of harm assuming that exposure has occurred [$P(H|E)$] (NRC, 2002), see Section 4.1, above. Based on the 2002 NRC Report and original conceptual model in Figure 2 of the 2015 EA, four risk-related questions were previously developed, evaluated and discussed in the 2015 and 2019 EAs. In some cases, these questions have been modified herein and new ones added based on the expanded conceptual model in Figure 4-1 above. FDA intended the original Risk-related Question 4 in the 2015 and 2019 EAs to evaluate the risk (i.e., likelihood of harm occurring) in the US from the production of ABT salmon on PEI;[31] however, we believe this question may have been subject to misinterpretation and/or was incomplete with respect to identification of the harms assuming that exposure occurred. Therefore, to separate the issues and build upon FDA's analyses in the 2015 and 2019 EAs, Risk-related Question 4 has been modified slightly and expanded. A fifth risk-related question has been developed for this amended EA to specifically evaluate the overall risk of harms (adverse consequences, effects, or impacts) occurring based on the probability of exposure (i.e., establishment and/or presence of ABT salmon) in the US environment.

1. What is the likelihood that AquAdvantage Salmon or AquAdvantage Broodstock will escape the conditions of confinement? (addressed in Section 9.2)
2. What is the likelihood that AquAdvantage Salmon or AquAdvantage Broodstock will survive and disperse if they escape the conditions of confinement? (addressed in Section 9.2)
3. What is the likelihood that AquAdvantage Salmon or AquAdvantage Broodstock will reproduce and establish if they escape the conditions of confinement, survive, and disperse? (addressed in Section 9.2)
4. What are the identified potential harms to, or effects on, the US environment if AquAdvantage Salmon or AquAdvantage Broodstock establish and/or are present? What is the likelihood of these potential harms occurring assuming exposure in the US environment has occurred? (addressed in Section 9.3)

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[31] The 2015 EA also evaluated impacts on the US from grow-out at the Panama facility, which has been closed since 2019. Thus, it is not included in this assessment.

5. What is the risk that the potential harms to, or effects on, the US environment would occur given the likelihood of exposure in the US environment? (addressed in Section 9.4)

Based on the Court's opinion (summarized in Section 2.4), this amended EA will re-evaluate Risk-related Question 4 by characterizing and explaining the harms (adverse consequences, effects, or impacts) to the US environment in the unlikely event that ABT salmon escape either of ABT's facilities located on PEI, Canada. We will also re-evaluate the risk of significant environmental harms to occur in the US environment[27] (new Risk-related Question 5), including impacts on endangered Atlantic salmon of the Gulf of Maine DPS and its critical habitat, based on the risk principles discussed in Section 4.2, above. The responses to Questions 1-3 from the 2015 and 2019 EAs are expanded herein (Section 9.2, below) to give context to the discussion in the risk characterization (see Section 9.4, below).

In addition to the above, this amended EA will also evaluate likelihood of pathogen and/or parasite transmission via (1) escaped ABT salmon interacting with wild fish populations, including wild Atlantic salmon, following escape from the PEI facilities, (2) discharge of pathogens/parasites from the PEI facilities via wastewater, and (3) import of AAS eggs containing pathogens/parasites into the US (see Sections 8.2.2 and 9.2.4). The potential harms and risk for this pathway will also be characterized in Sections 9.3 and 9.4, respectively.

5. ALTERNATIVES DESCRIBED IN 2015 EA

For major Federal actions, including an action to approve the NADA for AAS,[1] NEPA and its implementing regulations require that environmental documents include a brief discussion of the alternatives to the proposed action, as well as the environmental impacts of these alternatives. This section describes the reasonable range of alternatives considered by the agency in the 2015 EA, which includes the action (the preferred alternative) and "no action" alternative.

Section 4 of the 2015 EA describes the alternatives to the action being proposed at that time. Briefly, the preferred alternative was the approval of the NADA with the specific conditions for production and grow-out of ABT salmon described in Sections 2.1 and 2.2 (above), as well as the containment conditions described in the 2015 EA. The "no action" alternative considered the environmental ramifications of not approving the NADA for AAS.

On November 12, 2015, FDA decision makers chose the preferred alternative and prepared a FONSI. NADA 141-454 was subsequently approved on November 19, 2015, under specific conditions of the approval as described in the approval letter and Section 2.1, above. FDA continues to consider its original approval the preferred alternative. The only alternatives in the present context would be rescission or vacatur of the approval based on environmental concerns described in this EA that are both hypothetical and speculative. Neither is a preferred alternative for the reasons FDA stated in rejecting the original "no action" alternative in the 2015 EA.[3]

It is also important to note that regardless of the outcome of this EA, and even if FDA had previously adopted the no action alternative, the ABT facilities on PEI would continue to operate (but with no shipment of AAS eggs or food products to the US) because of prior, and subsequent, Canadian regulatory decisions, and commercial sale of food products from AAS in Canada (Section 2.3, above).

6. CHARACTERIZATION OF AQUADVANTAGE SALMON AND BROODSTOCK

6.1. AquAdvantage Salmon Genotype

The plasmid form of the AquAdvantage rDNA construct, known as the *opAFP-GHc2* construct (hereafter referred to as "the rDNA construct"), is a 6721 base-pair (bp) recombinant plasmid comprising 4061 bp of fish DNA and 2660 bp of vector backbone DNA derived primarily from the bacterial plasmid, pUC18. There were rDNA inserts from three different sources in the final rDNA construct. These sources included the 5′- and 3′-regulatory sequences from ocean pout, the growth hormone (GH) coding region from Chinook salmon, and small synthetic linkers to aid in assembly of the inserts and plasmid. An ampicillin resistance gene is present in the vector backbone and was used as a selectable marker. The rDNA construct was released from the bacterial pUC vector backbone DNA prior to microinjection of the eggs but was not removed from the delivery solution. However, ABT demonstrated that the pUC DNA is not inserted into the genomic DNA of AAS, and therefore, the ampicillin resistance gene is not present in the final animal. The construct has been shown to retain the molecular-genetic integrity required for GH expression in salmonid cells, which is what causes the rapid-growth phenotype. See 2015 FOI Summary[12] and Figure E.1 of the 2015 EA for additional information.

6.2. Phenotypic Characterization of AAS

Section 5.2 of the 2015 and 2019 EAs contains a discussion of the phenotype of AAS and diploid ABT salmon relative to farm-raised Atlantic salmon without the rDNA construct to help characterize its fitness. These discussions are included herein in Appendix F for ease of reference. In addition, new information on the phenotypic characterization since the publication of the 2019 EA is also provided in Appendix F.

6.3. Atlantic salmon Containing the *opAFP-GHc2* Construct

FDA analysis has found that AAS are Atlantic salmon.[32] Atlantic salmon with the rDNA construct only differ from wild Atlantic salmon in the that they contain one or more copies of the *opAFP-GHc2* construct that allow them to grow faster than Atlantic salmon without this rDNA construct. ABT produces four types of Atlantic salmon containing this rDNA construct on PEI, Canada:

- AquAdvantage Salmon (AAS): There are two types of ploidies of AAS that could exist at the ABT facilities:
    - <u>Triploid AAS:</u> All female Atlantic salmon that are hemizygous for the *opAFP-GHc2* rDNA construct. The AAS eggs are pressure-shocked to

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[32] FDA's FOI Summary (fn 12) for the Original NADA 141-454 for *opAFP-GHc2* rDNA construct in EO-1α lineage Atlantic salmon (AquAdvantage Salmon) explains on pages 66-68 that AAS meets FDA's standard for identity for Atlantic salmon under the criteria established in the Regulatory Fish Encyclopedia (RFE) by both Isoelectric Focusing (IEF) electrophoresis and mitochondrial DNA bar coding. FDA's RFE is a compilation of data that assists with the accurate identification of fish species; https://www.fda.gov/food/science-research-food/regulatory-fish-encyclopedia-rfe (accessed December 8, 2023).

    create triploid[33] salmon that are effectively sterile (see Figure 6-2). However, there is a very low possibility that some of the AAS will be diploid[34] and could reproduce naturally (see diploid AAS description below). Per ABT's Durability Plan all batches of eggs must be tested prior to shipment for grow-out. If, based on testing, triploidization in these eggs does not exceed 95% (based on the statistical 95% lower confidence limit), the batch of eggs is to be destroyed. ABT's success rate for achieving triploidy has been demonstrated to be quite high; between 2017 and 2022, triploidy averaged 99.5% in eggs of the batches that were released for shipment and ranged from 96.9% to 100% for each of 29 different batches.[35]
  - Diploid AAS: All female Atlantic salmon that are hemizygous for the *opAFP-GHc2* rDNA construct. Diploid AAS can reproduce naturally. As stated above, per ABT's Durability Plan, up to 5% of AAS in a batch can be diploid; however, testing to date has shown this percentage to be much smaller.[36] Thus, the term "diploid AAS" is used in this assessment to represent this small percentage of the population of AAS that could be diploid.

- AquAdvantage Female Broodstock (AAFB): Genotypically female Atlantic salmon that are either homozygous[37] or hemizygous[38] for the *opAFP-GHc2* rDNA construct. The AAFB are diploid and able to produce viable eggs. These fish are used to maintain the AquAdvantage broodstock, which are necessary to produce each new cohort of AAS (see Figure 6-1).

- AquAdvantage Neomale Broodstock (AANB): Genetically female but phenotypically male Atlantic salmon homozygous for the *opAFP-GHc2* rDNA construct. Diploid, homozygous AquAdvantage females are treated with 17α-methyltestosterone (MT) early in their development to render these fish phenotypically male with the ability to produce sperm, although they do not have a functioning sperm duct and cannot spawn naturally.

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[33] Triploid: Having three complete sets of chromosomes per cell; results in the fish being effectively sterile.

[34] Diploid: A cell, tissue, or organism having two complete sets of chromosomes, one from each parent.

[35] There has been only one batch that did not meet the 95% specification. That batch consisted of seven component groups, i.e., a component group represents a group of eggs pressure shocked at the same time. One of the seven component groups failed to meet specification for triploidy. After consultation with FDA, this component group was destroyed. In addition, ABT chose to destroy the two additional component groups that had been pressure shocked immediately after the failed group (even though those two additional component groups met specifications at 95 and 96% triploidy). The remaining four component groups had been pressure shocked at a different time and achieved an average 99.75% triploidy. The batch in this instance was redefined so that it included the four component groups that averaged 99.75% triploidy. This batch was then shipped to Indiana.

[36] Based on recent testing (2017-2022), it is estimated that on average only 0.5% (range of 0-3.1%) of AAS released for shipping are diploid. It is also important to note that, based on the test methods used, the eggs that are not determined to be triploid could potentially be diploid, haploid (1n) or classified as inconclusive. Therefore, the average number of diploid AAS could be less than 0.5%.

[37] Homozygous: The genetic status in which an individual inherits the same alleles for a particular gene from both parents; in this case, both alleles contain the rDNA construct

[38] Hemizygous: An individual having only one copy (or allele) of a given pair of genes instead of the usual two.

The sperm from the neomales are manually collected and used to fertilize eggs from Atlantic salmon without the rDNA construct to create the AAS (see Figure 6-2, below). These fish are also used to maintain the AquAdvantage broodstock (see Figure 6-1, below).

All four of these types of Atlantic salmon containing the rDNA construct are evaluated in this amended EA. For the purposes of this EA, the term ABT salmon[39] will be used when collectively discussing AAFB, AANB, and diploid and triploid AAS.

It is important to note that ABT will also maintain Atlantic salmon without the rDNA construct (including males, females, and neomales) at the PEI facilities for use in producing AAS and for commercial sale (see Figure 6-2).[40]

6.4. Production and Maintenance of AquAdvantage Salmon and Broodstock

Section 5.3.1.1 of the 2015 EA provides details regarding the development of AAS from the founder animal, including the creation of broodstock through gynogenesis. This process is no longer utilized. Information regarding the current procedures for maintenance of broodstock and production of AAS eyed-eggs is described below and illustrated in Figure 6-1 and Figure 6-2.

6.4.1. Maintenance of AquAdvantage Broodstock

Subsequent generations of AAFB and AANB can be derived from existing AAFB and AANB stocks. The technical details and logistics of maintaining AAFB and AANB are illustrated in Figure 6-1 and Figure 6-2, below.

Milt from AANB that are homozygous for the rDNA construct is manually collected and used to fertilize eggs from true females (i.e., AAFB) that are either homozygous (CC, XX) or hemizygous (C-, XX) for the rDNA construct. The offspring are AAFB homozygous (or hemizygous) for the rDNA construct. Part of the resulting homozygous progeny are kept as AAFB for continued production of broodstock. The rest of the resulting homozygous progeny will be sex-reversed with 17α-MT to produce AANB. All broodstock are graded, tagged, and subject to molecular-diagnostic confirmation of genotype prior to their qualification for use in future spawning.

6.4.1.1. Production of AquAdvantage Salmon eyed-eggs

The technical details and logistics of producing AAS eyed-eggs are illustrated in Figure 6-2, below.

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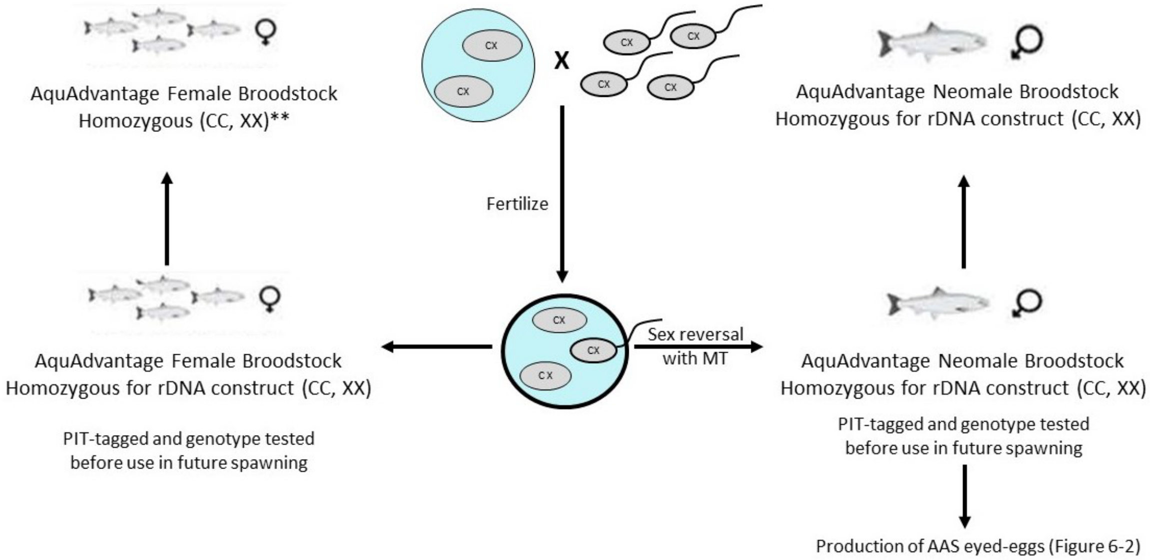
[39] ABT salmon are any Atlantic salmon containing the rDNA construct from the EO-1α lineage irrespective of ploidy, zygosity, or gender.

[40] Eggs of AAS and Atlantic salmon without the rDNA construct can be present in the same facility at the same time but will **not** be produced on the same day or held in the same incubators in order to eliminate the potential for co-mingling. All incubators are clearly labeled to identify the genetic nature of the eggs in the incubator, and all batches of eggs and/or fry of Atlantic salmon without the rDNA construct are tested with a defined and validated testing protocol to confirm absence of the rDNA construct before sale and shipment to 3rd parties.

Eggs from female Atlantic salmon without the rDNA construct (--, XX) will be fertilized with the milt from homozygous AANB (CC, XX) to produce AAS fertilized eggs that are all female, diploid, and hemizygous for the rDNA construct (C-, XX). The diploid AAS fertilized eggs (C-, XX) will be either (1) collected for grow-out as AAFB and used to add new genetics into the production of new AAFB and AANB (see Figure 6-2, below), or (2) pressure shocked to induce triploidy (C-, XXX), which will produce AAS eyed-eggs that are all female, triploid, and hemizygous for the rDNA construct, and reared to market size and harvested for processing for food use.

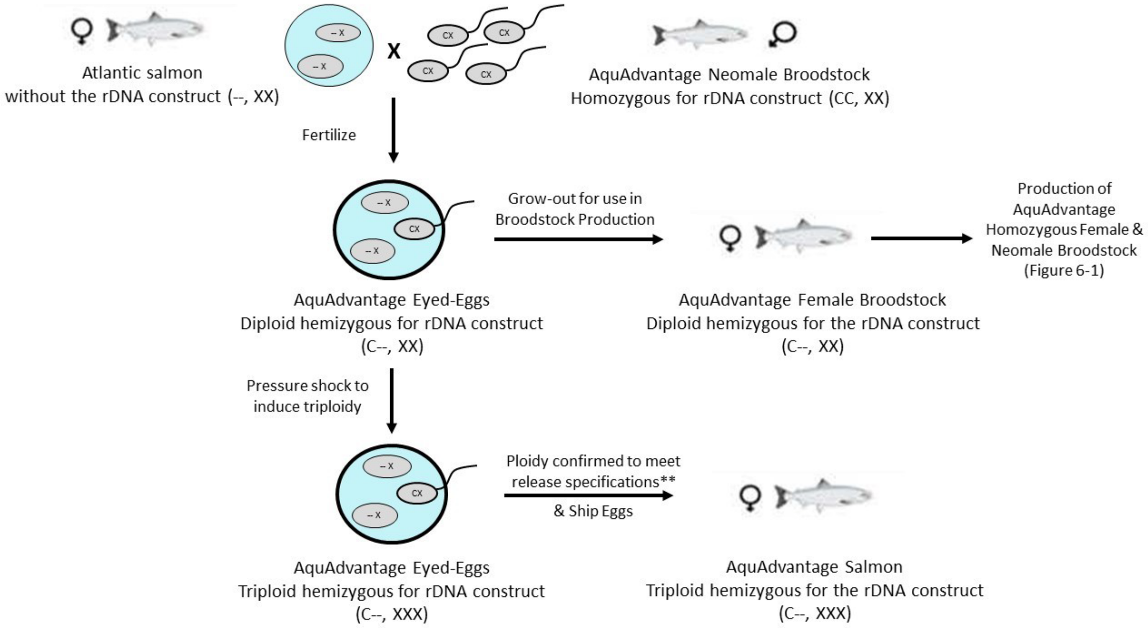
The AAS triploid eyed-eggs will be incubated in Heath stack incubators (~10,000 eggs/tray x 12-16 trays) or upwelling jars (100-200,000 eggs) for 325-400 °C-days, at which time batch-wise sampling will be performed to confirm the successful induction of triploidy via flow cytometry prior to shipment to AquaBounty-owned facilities for grow-out. As a condition of approval, if, based on testing, triploidization in these eggs does not exceed 95% (based on the statistical 95% lower confidence limit), the entire batch of eggs must be destroyed.

Figure 6-1. Maintenance of AquAdvantage Broodstock*



*Abbreviations: **CC**, homozygous for rDNA construct; **XX**, genetic female. Symbols next to the fish indicate phenotype (i.e., male or female) not their actual genotype, which is determined by chromosomes. **MT**, 17-methyl testosterone.
** Hemizygous AquAdvantage Female Broodstock (AAFB; C-, XX) could also be used for creation of homozygous AAFB and AANB (see how hemizygous AAFB are produced in Figure 6-2).

Figure 6-2. Production of AquAdvantage Salmon Eyed-eggs*



***Abbreviations: **CC**, homozygous for rDNA construct; **C-**, hemizygous for the rDNA construct; **XX**, genetic female; **XXX**, triploid female. Symbols next to the fish indicate phenotype (i.e., male or female) not their actual genotype, which is determined by chromosomes.

****** Each batch of AAS eyed-eggs is tested and must have >95% triploids in order to be released for shipment. Therefore, up to 5% of AAS could be diploid (or potentially haploid or inconclusive, see fn 36). If the batch does not meet this specification, then it is destroyed.

6.4.2. Production Numbers of ABT Salmon on PEI

As described in the sections above, there are many different types of salmon, both with and without the rDNA construct, required to maintain the AquAdvantage Broodstock and produce AAS eyed-eggs. The different types of salmon needed in ABT's production process are listed below. The zygosity, ploidy, sex by phenotype and genotype are also noted for each type of fish. In addition, ABT plans to produce Atlantic salmon without the rDNA construct for commercial sale during times at which they are not producing AAS eggs.[40]

ABT provided FDA with a projection of the maximum number of each type of fish that could be held at any one time throughout the year in all PEI facilities based on weight ranges (eggs, <100 g or ≥100 g). The maximum values below include current production capabilities, as well as projections due to the planned future expansion of new broodstock units at Rollo Bay (known as Broodstock Units 1 and 2, see Section 7.2 below). It is also important to note that ABT is planning in the future to construct a new incubation room within the current Bay Fortune facility to consolidate egg production there at a single location (see Section 7). This change is designed for enhanced operational efficiency but will not expand or increase the production of AAS eyed-eggs at the Bay Fortune facility. These numbers account for the eyed-eggs that will be supplied from the Rollo Bay facility to the Indiana facility and the new facility currently under construction in Pioneer, Ohio (see Section 9.4.3, below).

These maximum values are hypothetical and a conservative worst-case estimate because they are based on the maximum number of each size of fish that can be held in different tanks in ABT's PEI facilities. That is, these values represent the maximum number of fish that could be contained at any one time in all tanks designated for fish of that size range. Because fish are normally transferred from small tanks to larger tanks as they grow throughout the year, and some fish are normally removed from production (i.e., culled) when they are moved to larger tanks due to capacity limitations, the number of eggs and fish present in the PEI facilities at any particular time would be substantially less than those listed below.

Types, size,[41] and approximate maximum number of type of fish potentially housed at one time at all ABT facilities currently located on PEI, Canada:

- AAFB (diploid, all-female)
    - homozygous: ~ 200,000 eggs; 60,000 <100 g fish; and 1200 ≥100 g fish; and
    - hemizygous: ~ 50,000 eggs; 28,000 <100 g fish; and 350 ≥100 g fish
- AANB (diploid, neomale)
    - homozygous: ~ 70,000 <100 g fish; and 8,000 ≥100 g fish
- AAS (at least 95% triploid: up to 5% diploid, all-female)
    - hemizygous: ~30,000,000 eggs;[42] and 4,000 <100 g fish
- Atlantic salmon without the rDNA construct
    - Female: ~ 30,000,000 eggs; 200,000 <100 g fish; and 30,000 ≥100 g fish;
    - Male: ~ 20,000 eggs; 20,000 <100 g fish; and 3,000 ≥100 g fish; and
    - Neomale: ~65,000 <100 g fish; and 7,500 ≥100 g fish

These numbers include anticipated expansion (including the planned Broodstock Units 1 and 2 at the Rollo Bay facility, see Section 7 below). However, any new facilities under NADA 141-454 would require a supplemental approval (NADA), which would include a NEPA evaluation.

7. FACILITIES AND CONTAINMENT ON PEI

This section contains high-level descriptions of the Bay Fortune and Rollo Bay facilities, including their location, layout, water flow and discharge, and containment measures. Extensive, detailed descriptions of the Bay Fortune and Rollo Bay facilities are available in Section 5.4 of the 2015 EA and Section 5.6 of the 2019 EA, respectively. Schematics of the site layout and containment and water flow at the

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[41] The size delineations in this list were chosen based on the approved claim for AAS, "*Significantly more AquAdvantage Salmon grow to at least 100 g within 2,700 °C-days than their comparators*." In addition, as defined in the 2015 EA, smolt are defined by a weight of 100 g. These fish are assumed to be ready to go through the smoltification process, which is the physiological change that allows young salmon to adapt to saltwater conditions. Therefore, for this amended EA, pre-smolt is considered encompass fish up to 100 g and post-smolt is considered to be those fish weighing ≥100 g.

[42] Most AAS eggs will only be held at the facility for a short-period of time (~50-125 days) before being shipped to the US for grow-out. However, some AAS eggs may remain in PEI for grow-out for the Canadian market (although this is not routinely done at this time, but may occur in the future), as well as for quality assurance evaluations and use in research studies.

Bay Fortune and Rollo Bay facilities are presented in Appendices C and D, respectively, including planned future expansions and changes at each facility.

7.1. Facility Locations

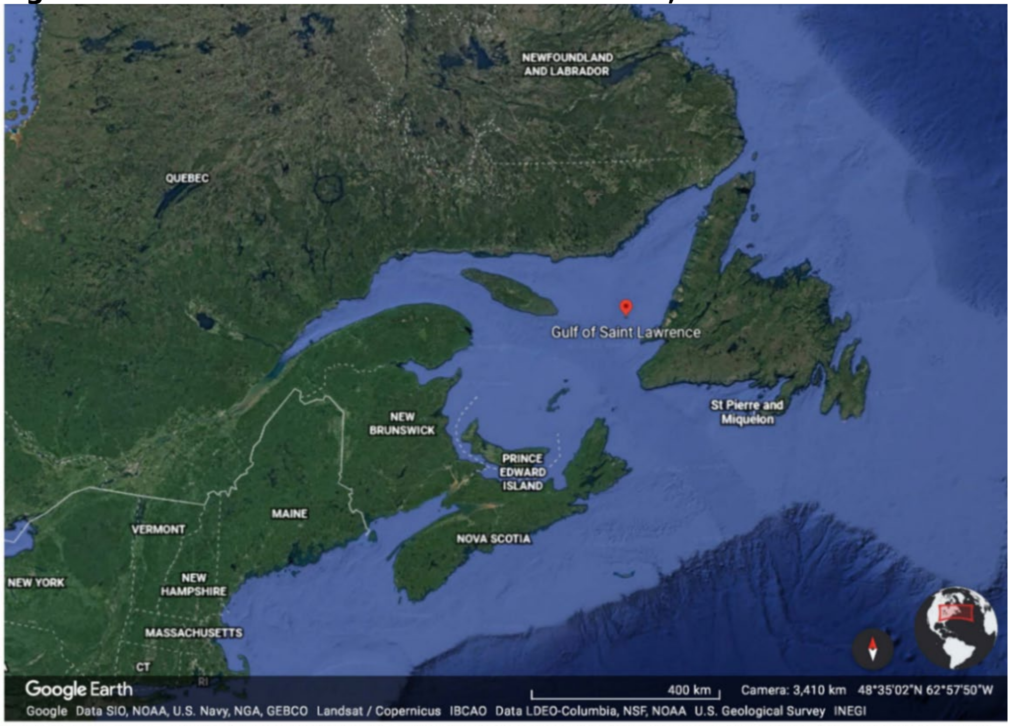
ABT currently produces ABT salmon at two facilities in PEI, Canada: Bay Fortune and Rollo Bay. Figure 7-1 (below) illustrates the location of PEI in relation to Canada and the US. Figure 7-2 (below) shows the location of the ABT facilities on PEI and in relation to the Northumberland Strait and the Gulf of St. Lawrence, and Figure 7-3 (below) illustrates the locations of the facilities to each other and Bay Fortune estuary and Rollo Bay.

The Bay Fortune facility is a land-based aquaculture facility situated on the northeast side of PEI, near a tidal (estuarine) portion of the Fortune River, i.e., Bay Fortune (Figure 7-3, below). The Bay Fortune facility is located approximately 1.6 km inland from the river’s confluence at a southern portion of Rollo Bay. This bay in turn connects with the Northumberland Strait (illustrated in Figure 7-2, below), which ultimately connects to the Gulf of St. Lawrence (illustrated in Figure 7-1, below) and the Atlantic Ocean.

The Rollo Bay facility is also a land-based aquaculture facility located in eastern PEI on 70 acres in a predominantly agricultural area (Figure 7-3, below). It is about 1 km north of coastal waters, Rollo Bay, and about 12 km north from the Bay Fortune facility. A small stream with variable flow, Rollo Bay Brook, runs through the property and travels approximately 1.5 km from the property before entering Rollo Bay, which ultimately connects to the Northumberland Strait, the Gulf of St. Lawrence (illustrated in Figure 7-1 and Figure 7-2) and the Atlantic Ocean.

Descriptions of these facilities and their containment are provided in the next sections (Sections 7.2 and 7.3).

Figure 7-1. Location of Prince Edward Island, Canada.



https://earth.google.com/web/search/Gulf+of+St+Lawrence/@48.58399334,-62.96406285,-254.17498257a,3410201.25384092d,35y,-0h,0t,0r/data=CigiJgokCWuFrLPvE0ZAETT54At80kRAGWTYMQc6…

Figure 7-2. Locations of Bay Fortune and Rollo Bay, PEI, Canada. The white circle indicates the area where the two facilities are located.

https://earth.google.com/web/@46.46686655,-63.52817583,97.46370584a,437931.56824864d,35y,-0h,0t,0r

Figure 7-3. Locations of ABT’s Bay Fortune and Rollo Bay Facilities. The white circles indicates the areas where the two facilities are located.

7.2. Description of PEI of Facilities

The layout of and effluent discharge from the Bay Fortune and Rollo Bay facilities are summarized below. Site plans and schematics of water flow at each facility are illustrated in Figure C-1 and Figure D-1 of Appendices C and D, respectively, including planned future expansions and/or changes at each facility.

7.2.1. Bay Fortune

The Bay Fortune facility consists of one building containing aquaculture operations, laboratory, office, living space, a storage facility and several ancillary structures. Aquaculture operations are conducted in two principal Areas:

1. Early-Rearing Area (ERA) for production of AAS eggs, and rearing of alevin and fry (size ranges of 0.1 up to 100 g); and
2. Grow-Out Area (GOA) for rearing of alevin, fry and smolt (see definitions of these life stages in Section 8.1, below), as well as longer-term cultivation of juveniles and broodstock (size ranges of 0.1 to greater than 100 g).

The incubator trays in the ERA operate with 100% recirculation of water during early egg incubation but are transitioned to continuous flow-through operation once the eggs hatch. The rest of the aquaculture system operates with approximately 97% recirculation of water. A schematic of these Areas, including the containment and water flow, is provided in Figure C-2 in Appendix C.

In June 2023, ABT informed FDA that they are planning to construct a new incubation room within the Bay Fortune facility in the future. This new incubation room will replace egg incubation locations currently in the ERA D-Room and Main ERA. This future planned change will not expand the number of eggs produced at the Bay Fortune facility (i.e., egg numbers reported in Section 6.4.2 are accurate), rather it will consolidate egg care into one location at this facility. The planned location of the future new incubation room is illustrated in Figure C-3 of Appendix C, and the planned physical containment components for the new incubation room are depicted in Figure C-4 of Appendix C. The physical containment is planned to be equal to that currently in place but would be verified in a future review under NEPA as this change requires a supplemental approval under the NADA. The incubation room will operate under RAS.

All effluent streams are combined and pass through a single containment sump before being discharged to a nearby drainage ditch (see Bay Fortune site plan in Figure C-1 in Appendix C), which ultimately empties into the Fortune River estuary, then discharges to Rollo Bay which is connected to the Northumberland Strait, the Gulf of St. Lawrence, and the Atlantic Ocean (see Figure 7-2 and Figure 7-3, above). Fish wastes (biosolids) from the facility are subject to extensive treatment prior to discharge to the local estuary (see Section 7.5 below).

7.2.2. Rollo Bay

The Rollo Bay facility consists of three buildings each containing one aquaculture Unit: Hatchery, Broodstock Unit 1, and Broodstock Unit 2 (see site plan in Figure D-1 of Appendix D).

As of the date of this amended EA, only the Hatchery Unit is approved under NADA 141-454. Construction of Broodstock Unit 1[43] has been completed, but production of AAS at this Unit has not yet been proposed for approval under a supplemental NADA.[44] However, ABT has notified FDA that they do plan to submit this Unit for approval under a supplemental NADA in the future. With reference to Broodstock Unit 2, as of the date of this amended EA, a building shell has been constructed and a floor plan has been created, but the containment plans have not been finalized and an operation date has not been set. ABT also plans to submit Broodstock Unit 2 for approval under a supplemental NADA in the future. If a supplement(s) to the NADA is submitted for production of AAS in either Broodstock Unit 1 or 2, or both, a supporting EA(s) will need to be prepared.

The Hatchery Unit is used to produce AAS eyed-eggs; to produce and house AAFB and AANB; for breeding of improved AAS lines; and for other research activities. The Hatchery Unit may also be used to produce eggs of Atlantic salmon without the rDNA construct. AAS eggs and eggs of Atlantic salmon without the rDNA construct will not be produced (i.e., fertilized) in the Hatchery Unit on the same day or held in the

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[43] In the 2019 EA for the approval of production of AAS eyed-eggs at the Rollo Bay facility, three Units at the Rollo Bay facility were named: Hatchery, Grow-Out, and Broodstock. Since that time, ABT has decided to change the Grow-Out Unit into another unit that will hold broodstock, now known as Broodstock Unit 1. Broodstock 2 is a shell building and has not yet been constructed for use.

[44] Broodstock Unit 1 is currently approved by Canada for grow-out of AAS (DFO, 2019).

same incubators (i.e., within the same set of Heath stack trays or the same upwelling chamber) to eliminate the possibility of comingling. However, eggs of AAS and Atlantic salmon without the rDNA construct may be reared in the same Unit at the same time. ABT ensures that comingling will not occur through proper labeling and training of staff. All batches of eggs and/or fry of Atlantic salmon *without* the rDNA construct are tested with a defined and validated testing protocol to confirm *absence* of the rDNA construct before sale and shipment to conventional Atlantic salmon producers. Likewise, all batches of AAS eggs are also tested to confirm presence of the rDNA construct before shipment to ABT grow-out facilities. This testing is conducted to ensure that the proper eggs are shipped to the correct operators.

The Hatchery Unit contains two Areas: Early Rearing Area (ERA) for production of eggs and rearing of alevin (size ranges up to 30 g), and Advanced Rearing Area (ARA) (also known as the Grow-Out Area or GOA) for rearing of fry, smolt and broodstock (size ranges from 10 to greater than 100 g). All aquaculture activities at the Rollo Bay site operate on Recirculating Aquaculture Systems (RAS) designed to operate at a 99.7% recirculation rate (i.e., with 0.3% make-up water being added continuously). A schematic of the Hatchery Unit and Rollo Bay Site Plan, including the containment and water flow, are provided in Figure D-1 and Figure D-2 of Appendix D.

Broodstock Unit 1 consists of three Areas: ERA, ARA or GOA, and Conditioning, which are depicted in a schematic of the floor plan in Figure D-3 of Appendix D. Similar to the Hatchery Unit, production of eggs and rearing of alevin (size ranges up to 30 g) will occur in the ERA, while rearing of fry, smolt and broodstock (size ranges from 10 to greater than 100 g) will occur in the ARA. The Conditioning area is used for purging of adult fish prior to harvest; however, that area will likely only be used to hold immature Atlantic salmon without the rDNA construct now that the Unit has been converted from a grow-out facility to a broodstock production facility. The ERA and ARA in Broodstock Unit 1 will operate at a 99.7% recirculating rate, while the Conditioning area will be operated in flow-through mode only. The containment schematics for the ERA, ARA and Conditioning for Broodstock Unit 1 are provided in Figure D-4 and Figure D-5 of Appendix D. Broodstock Unit 1 is also discussed in depth in the 2019 Rollo Bay EA, including pictures of some of the containment barriers. In the 2019 Rollo Bay EA, Broodstock Unit 1 is referred to as the Grow-Out Unit, but since that time, ABT has changed the use of this facility to a broodstock unit. Although the use has changed, the layout and containment has remained the same.

ABT also plans in the future to construct and operate a second broodstock unit called Broodstock Unit 2. This unit is illustrated on the Rollo Bay site plan in Figure D-1 of Appendix D. At this time, a building shell has been constructed; however, the building plans, including containment schematics, have not been finalized. However, Broodstock Unit 2 will have containment similar to the Hatchery and Broodstock Unit 1.

All effluent from the Hatchery Unit passes through a polishing pond before entering Rollo Bay Brook. Effluent from both Broodstock Units will be discharged to a stone wash-out and vegetative strip before also entering Rollo Bay Brook. All solids from Broodstock Unit 1 are collected in either 1) radial flow separators located within Early and Advanced Rearing Areas and sent to a closed septic tank for solid storage, or 2) in the waste treatment building where solids from the Advanced Rearing/Purge area are de-watered and stored in a tank. Collected solids will either be transported to offsite waste treatment or used for agricultural purposes (land application), and water removed during solid waste processing will either be discharged to Rollo Bay Brook or pass into an underground leaching field. The water from the leaching field will filter through the ground similar to rainwater. The Rollo Bay Brook flows downstream to Rollo Bay, which is connected to the Northumberland Strait and ultimately the Atlantic Ocean (see Figure 7-1 and Figure 7-2, above). The effluent treatment for Broodstock Unit 2 is expected to be similar to Broodstock Unit 1.

7.3. Description of Containment Measures

Table 7-1 below summarizes security and containment at the Bay Fortune and Rollo Bay facilities. Additional detailed descriptions of the containment measures used at the Bay Fortune and Rollo Bay facilities are available in Section 5.4 of the 2015 EA and Section 5.6 of the 2019 EA, respectively.

There are four general types of containment measures employed by ABT at the PEI facilities:

- **Physical containment** refers to measures or barriers implemented on-site to prevent the movement or escape of fish from the facility. Containment measures can include the use of mechanical devices, either stationary or moving (e.g., tanks, screens, filters, covers, nets, etc.), or in some cases, the use of lethal temperatures or chemicals (e.g., chlorine pucks) to prevent uncontrolled escape. An important component of physical containment is the implementation of policies and procedures to ensure that the devices and chemicals are used as prescribed (see procedural containment below). The containment methods and locations at the ABT facilities have been designed specifically for the operations and life stages that will be present in each area of each facility.
- **Geographical and geophysical containment** is defined as the presence of inhospitable conditions in the surrounding environment that would preclude or significantly reduce the probability of survival, dispersal, and/or long-term establishment should an animal escape confinement at its site of production (e.g., hatchery) and/or rearing (e.g., grow-out).
- **Biological containment** includes limiting the reproduction of the fish within the culture system, preventing reproduction of the fish once they enter the receiving environment, or preventing the expression of the genes of concern (e.g., the transgene) in the event of an escape (Mair *et al*., 2007).
- **Procedural containment** includes security measures, equipment, and Standard Operating Procedures (SOPs) important to 1) control normal movement of authorized personnel; 2) prevent unauthorized access to the site; and 3) eliminate access of predators that could potentially carry ABT

salmon offsite (ABRAC, 1995).[45] Multiple and redundant forms of security are present at the ABT facilities to prevent malicious activities and unauthorized access to operational structures and ABT salmon.

Table 7-1. Description of Facility Containment

Containment Measure	Bay Fortune Facility	Rollo Bay Facility
Physical Containment	• All areas have at least 4 independent, sequential forms of physical containment on their water systems. Some areas have 5 to 6 levels of containment. • Containment is illustrated in Figure C-2 and is listed in Table C-1 in Appendix C. • Detailed description available in Section 5.4.3 of the 2015 EA.	<u>Hatchery Unit</u> • A minimum of 6 independent levels of physical containment are in place in the Early-Rearing Area of the Hatchery Unit where eggs and young fish are reared. • A minimum of 4 independent levels of physical containment are in place in the Advanced-Rearing Area where adult fish (>10 g) are reared. • Some units have 10 or more levels of containment. • Containment in the Hatchery Unit is illustrated in Figure D-2 and is listed in Table D-1 in Appendix D. • Detailed description available in Section 5.6.4 of the 2019 EA. <u>Broodstock Units 1 and 2</u> • A minimum of 6 levels of independent physical containment are in place in the ERA of Broodstock Unit 1 where eggs and young fish are reared. • A minimum of 8 levels of independent containment are in place in the ARA (also known as

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[45] The U.S. Department of Agriculture's (USDA) Agricultural Biotechnology Research Advisory Committee (ABRAC) was established in 1987 as a federal advisory committee composed of scientific experts with the purpose of providing advice to the Secretary of Agriculture on the conduct of biotechnology research. In 1991, the ABRAC recommended a set of "Guidelines for Research Involving Planned Introduction into the Environment of Genetically Modified Organisms" (Document No. 91-04, USDA Office of Agricultural Biotechnology, Washington D.C.) [see https://www.readcube.com/articles/10.1038%2Fnbt1195-1142a (accessed on December 8, 2023)]. ABRAC also prepared specific Performance Standards for safely conducting research with genetically modified fish and shellfish, which was published in 1995 (ABRAC, 1995). Although these standards are over 25 years old, they still offer useful recommendations for controls for GE organisms.

Containment Measure	Bay Fortune Facility	Rollo Bay Facility
		the GOA) of Broodstock Unit 1 where adult fish are reared. • A minimum of 6 levels of independent containment are in place in the Conditioning Area of Broodstock Unit 1 where adult fish are purged. • Containment schematics for each area of Broodstock Unit 1 are illustrated in Figure D-4 and Figure D-5 in Appendix D. • Broodstock Unit 2 will have containment similar to the Hatchery and Broodstock Unit 1. • These Units are not approved under an NADA and will require approval of a supplemental NADA that will undergo a NEPA evaluation to ensure proper containment prior to any approval.
SOPs on Physical Containment	• The sponsor has developed and employs an extensive number of SOPs that govern physical containment, as well as every other significant activity that occurs at each facility (see Procedural Containment below). • All containment equipment is inspected by facility staff on a daily basis and observations documenting the results of this inspection are recorded at each facility.	
Geographical/ Geophysical Containment	• Salinity in Fortune River estuary is typically in the range of 21 ppt (and up to ~30 ppt at times), which would preclude survival of all pre-smolt stages of Atlantic salmon (generally <100 g). • Salinity in the Northumberland Strait near Rollo Bay has been reported to range from 23 to 29 ppt during the summer months (Weldon *et al*., 2008). These salinity levels would also preclude survival of all pre-smolt stages of Atlantic salmon. • The generally shallow depth of Rollo Bay causes strong tidal currents, water turbulence and a high concentration of suspended red silt and clay. High sediment loads, high summertime water temperatures and low dissolved oxygen, and high nitrate concentrations would make the waters of the Rollo Bay unfavorable long-term establishment of ABT salmon.	
Biological Containment	• AAS and AAFB are 100% female. • AANB are neomales that lack a sperm duct and cannot reproduce naturally. • Greater than 95% of AAS are functionally sterile, produced by induction of triploidy. Triploid females rarely produce eggs (i.e., do	

<table>
<tr><th>Containment Measure</th><th>Bay Fortune Facility</th><th>Rollo Bay Facility</th></tr>
<tr><td></td><td colspan="2">not reach ovulation), and if they do, the eggs usually are very few, undeveloped and unfertilizable (Piferrer *et al.*, 2009). Per the Durability Plan, if, based on testing of batches, triploidization in these eggs does not exceed 95% (based on the statistical 95% lower confidence limit), the batch of eggs is to be destroyed. ABT’s success rate for achieving triploidy has been demonstrated to be quite high. Between 2017 and 2022, triploidy was achieved in 99.5% of fish on average and ranged from 96.9% to 100% for each of 29 different batches.</td></tr>
<tr><td>Procedural Containment*</td><td colspan="2">• Commitment by top management
• Written plan for implementing backup measures in case of failure, including documentation, monitoring, and remediation, including a disaster preparedness plan
• Training of employees
• Dedication of permanent staff to maintain continuity
• Use of SOPs for implementing redundant confinement measures
• Periodic audits by independent agency
• Periodic internal review and adjustment to allow adaptive modifications
• Reporting to an appropriate regulatory body
• See additional details in Section 5.7 of the 2015 EA</td></tr>
<tr><td>Security</td><td>• There is an eight-foot-high, heavy-gauge, galvanized chain-link fence of commercial quality that surrounds the property.
• Outside entryways are secured at all times with deadbolts and bars on windows.
• The primary well and pumping facilities are enclosed in steel containment structure.
• There are interior and exterior cameras and sensors that are professionally monitored 24/7, as well as environmental alarms that notify the staff remotely.
• Detailed description in Section 5.4.4 of the 2015 EA.</td><td>• All exterior doors are kept locked and secondary access requires a key.
• Visitor entrances only provide access to the administrative areas.
• There are exterior motion-activated cameras that record and store the video. A commercial security service continuously monitors various motion detectors, door magnets, and environmental sensors.
• The wells are secured inside concrete buildings with tamper-proof metal covers.
• Sponsor-employed personnel are present on-site 24/7.</td></tr>
</table>

* Meets the recommendations for an integrated confinement system for GE organisms discussed in NRC (2004a) and Kapuscinksi (2005).

7.4. Description of Containment During Shipping

Under the approved NADA, ABT is allowed to ship AAS eyed-eggs from the production facilities on PEI to any ABT approved grow-out site. Eyed-eggs are the life stage most efficiently, effectively, and safely transported. Since receiving FDA approval in 2015, ABT has established SOPs for packaging and shipping eyed-eggs from both PEI facilities.

The product would be packaged in a manner consistent with, but more rugged than, the Styrofoam egg crate typical of industry practice. AAS eyed-eggs would be packed in a hard-plastic insulated cooler containing alternating trays of eggs and wet-ice; the cooler would be bound with packing straps and further secured in a heavy-cardboard shipping container.

A bilingual (English and Spanish) FDA Product Label printed on tear- and water-resistant paper would be affixed to both the egg crate and shipping container; this label shows the product name and provides information on the product identity, claim, limitations, warnings, and handling instructions of immediate importance to the end-user (see Appendix B). A bilingual Package Insert comprising detailed handling recommendations and important information regarding performance, animal safety, and environmental considerations also would be included. The shipment would be identified as "Eggs & Fry"[46] that is "Not for Resale." The following additional warnings (or facsimile thereof) would also appear on the Product Label:

- Rear only in a physically-contained freshwater culture facility as specified in an FDA- approved application;
- Must not be reared in conventional sea cages or net-pens;
- Dispose of morbid or dead fish in a manner consistent with local regulations.

ABT works with Canadian authorities to ensure eyed-eggs meet the receiving country's biosecurity requirements for the transport of living salmonid eggs and obtains required health certificates to export eggs from Canada to the receiving country, including the US. Product prepared for shipment would be transported by motor vehicle to a local international airport by ABT staff, where direct control would be assumed (through prior arrangement) by a freight-forwarder. The freight-forwarder would arrange, manage, and personally monitor air-freight shipment of the product to the US (inclusive of permits & customs requirements), where control would be returned to ABT personnel waiting on the ground.

During handling, transport, and opening, the container would be maintained in an upright position; and upon receipt, egg temperature would be determined to assess the need for equilibration to the receiving temperature if the difference between the two exceeds 4 ºC. The equilibrated eggs would be held in fresh water at 2-8 ºC and ≥ 7 mg/L DO.

All tanks holding AAS at any ABT grow-out facilities would be required to be marked with the product label.

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[46] Although eyed-eggs are the product in commerce identified in the product definition, it is anticipated that some eyed-eggs may hatch in transit; hence, the label on the shipping container includes the phrase "Eggs and Fry."

7.5. Disease Surveillance and Status

7.5.1. Canada

7.5.1.1. Aquatic Animal Health Management by Canadian Authorities

The Government of Canada has developed a National Aquatic Animal Health Program (NAAHP) to bring Canada into compliance with international aquatic animal health management standards. The Canadian Food Inspection Agency (CFIA) and Fisheries and Oceans Canada (DFO) share responsibilities for federal components of NAAHP. CFIA, as the lead agency for the NAAHP, provides program direction under the authority of the Health of Animals Act.[47] CFIA is also responsible for aquaculture health surveillance. DFO is primarily responsible for providing scientific support for implementation of NAAHP. The authority for international movement of fish (including salmonids) in Canada falls within the domain of the CFIA. DFO continues to regulate all interprovincial movement of salmonids. CFIA is responsible for certification of the health status of aquatic animal exports with respect to the risk of introduction or movement of an aquatic animal disease into a receiving country; however, it is important to note that it is not CFIA, but rather the importing country that sets the conditions for importation (see Section 7.5.2 below for the US requirements for importation of live fish and eggs). CFIA will assess and determine if the Canadian aquatic animals are eligible for export (i.e., whether they meet the importing country’s conditions). If import requirements can be met, the CFIA will issue an export certificate allowing the animals or products to be exported to the importing country. The US also separately assesses whether the AAS eggs have met requirements for importation. Anyone who owns or works with aquatic animals and knows of or suspects a reportable disease is required by Canadian law to notify CFIA.

7.5.1.2. Disease Surveillance and Status at the Bay Fortune Facility

An outbreak of infectious salmon anemia (ISA) occurred in the Bay Fortune, PEI facility during the third quarter of 2009 (see Section 5.4.2 of the 2015 EA for additional details). Prior to this, the PEI facility had been considered “disease free” for many years based on periodic inspections and testing by Canadian authorities. The ISA outbreak was first detected in fish in the GOA and later spread to fish in parts of the ERA. Once the presence of the ISA virus (ISAV) was confirmed, ABT notified DFO. CFIA was notified shortly thereafter. ABT responded to the ISA outbreak by implementing standard Atlantic salmon mitigation strategies appropriate for this disease in its facility (e.g., extirpation of all affected fish and eggs, and implementation of an ISA detection and monitoring program). No animal drugs, such as antibiotics, were used in response to this outbreak. All year classes of fish produced since the 2009 ISA outbreak have tested negative for ISAV when assayed using the most sensitive quantitative real time polymerase chain reaction (qPCR) diagnostic assay available, and all new year classes will continue to be tested.

Since the 2009 outbreak, ABT has implemented disease prevention and surveillance strategies to prevent disease introduction, outbreak, and transmission in the Bay

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[47] https://laws-lois.justice.gc.ca/eng/acts/H-3.3/ (accessed December 8, 2022)

Fortune facility. For example, the two production areas in the Bay Fortune facility, the ERA and GOA, have been separated into two distinct, biosecure facilities (i.e., outer coats and boots must be changed when moving between areas, and boot washing and hand sanitizing are required before entering production areas) with separate access points. Ultraviolet (UV) lights were installed to disinfect both the incoming well water as well as the recirculated water within both the ERA and GOA. Ozone treatment was added to disinfect water recirculated within the ERA. All mortalities in the GOA are necropsied and examined for signs of ISAV. No mortalities with clinical signs of ISAV have been observed. A proportion of the population of fry to smolt are collected randomly throughout the year and tested for ISAV. No ISAV positive samples (fry, whole blood, or mortalities) have been detected by any method in the ERA since the 2009 outbreak.

Periodic inspections by the DFO Fish Health Unit (2010 through 2014) and by Canadian Food Inspection Agency (2012 through July 2023) detected no notifiable diseases or disease agents for finfish per Canadian or international (World Organization for Animal Health (OIE)) requirements at the Bay Fortune, PEI facility. Pathogens encompassed by these inspections are shown in Table 7-2 and include several viruses and filterable replicating agents, such as ISAV, plus other common fish pathogens. The CFIA, the Federal organization responsible for monitoring health status of aquaculture facilities in Canada, considers Bay Fortune to be free of the pathogens included in the CFIA compartment program and no longer conducts routine tests for all pathogens. CFIA inspects the Bay Fortune facility at a minimum of every 4 months. CFIA tests for Infectious Pancreatic Necrosis three times per year and tests for ISAV and ISAV strain HPR0 two times per year. CFIA conducts annual inspections that include a review of biosecurity protocols. CFIA conducted its most recent inspection of the Bay Fortune facility in July 2023 and found the facility’s biosecurity plan to be adequate. The US Title 50 and Provincial clearances require re-testing every six months.

The Bay Fortune facility is recognized as an approved Compartment in the CFIA Compartmentalization Program for Atlantic salmon. According to CFIA (2020),[48] “*Recognition by the CFIA as a compartment for international trade means that the premises has an aquatic animal health management system that is consistent with international standards and provides assurance of the health status of aquatic animals originating from these premises.*” The Bay Fortune facility is officially recognized as free from the diseases listed in Table 7-2 below under the “CFIA Compartment Program” column heading.[49]

The Bay Fortune facility is also part of the Atlantic Canada provincial Certificate of Fish Health Transfer program and undergoes routine inspection of fish health by Provincial authorities. The provincial Certificate of Fish Health Transfer certifies that cultured finfish from an aquaculture facility have been tested for certain pathogens,

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[48] https://inspection.canada.ca/animal-health/aquatic-animals/diseases/compartmentalization/eng/1345164530104/1345164735083 (accessed December 8, 2023)

[49] https://inspection.canada.ca/animal-health/aquatic-animals/exporting-aquatic-animals/compartments-for-international-trade/eng/1524061917801/1524061918488 (accessed December 8, 2023)

and obtained negative results, prior to transfer between and within the Atlantic provinces. The list of pathogens included in that testing are included in Table 7-2 below under the column heading “Provincial Certificate of Health.” In order to obtain these Certificates, a facility must have a minimum of three veterinary visits in winter/spring, spring/summer, and winter/fall and the visits must occur at least 60 days apart.

Table 7-2. Pathogens Included in Inspections by Canadian and US Authorities[a] as Indicated by an X

Disease	Provincial Certificate of Health	CFIA Compartment Program	DFO FHPR Program	US Federal Title 50	Indiana DNR
Bacterial Kidney Disease	X	-	-	-	X
Infectious Haematopoietic Necrosis	X	X	X	X	X
Viral Hemorrhagic Septicemia	X	X[b]	X	X	X
Infectious Salmon Anemia	X	X	-		X
Infectious Pancreatic Necrosis	X	X	X	X	X
Epizootic Haematopoietic Necrosis	-	X	-	-	-
Oncorhynchus Masou Virus Disease	-	X	-	X	-
Salmon alphavirus	-	X[b]	-	-	-
Myxobolus cerebralis (Whirling disease)	-	X	X	-	X
Ceratomyxa shasta	-	X	X	-	X
Gyrodactylus salaris	-	X		-	-
Aeromonas salmonicida	X	-	X	-	-
Yersinia ruckeri	X	-	X	-	X
Salmonid Rickettsial Septicaemia	-	-	-	-	X
Other filterable replicating agents	-	-	X	-	X

[a] This table was originally presented in Section 5.4.5 of the 2019 EA and has been modified for presentation in this amended EA

[b] For export to Brazil

ABT maintains several SOPs that outline procedures for ensuring prevention of pathogens/parasites at the PEI facilities, including procedures for cleaning, disinfection, and handling of equipment, footwear, eggs, fish, etc. For example, prior to fertilization of eggs, there are several steps that are taken to prevent disease transmission to the eggs. All areas and equipment (including footwear) used during the procedures are disinfected. Following fertilization and water hardening,[50] eggs

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[50] Fertilized eggs are placed in pathogen-free water for at least one hour to water harden before being disinfected and placed in the incubators.

are submerged and disinfected in an Ovadine (iodophor; 100 ppm iodine) solution for a minimum of 10 minutes, and then are immediately transferred to the ERA to reduce introduction of pathogens. Following disinfection, the eggs are rinsed for 30-60 seconds in a ~0.9% saline, pathogen-free solution. Following transfer of eggs to incubators, the floor in the ERA is cleaned and disinfected. In addition, AAS eggs are disinfected a second time with an Ovadine solution and saline solution immediately prior to shipping to grow-out facilities.

In addition, it is important to emphasize that ABT does not import any milt, eggs or fish from outside (third) parties. ABT may move eggs between Bay Fortune and the Rollo Bay facilities. If eggs from one facility must be incubated at another facility, they are surface disinfected according to ABT SOPs using Ovadine (as described above). If a fish health problem is suspected at one of the ABT facilities, no fish will be transferred to another facility. Upon receipt at receiving facility, eggs which are hatched for further rearing are kept in early rearing areas until they are large enough to be moved to the grow-out areas. ABT does not routinely transfer milt between facilities.

The Bay Fortune facility has not used antibiotics, antiparasitics, antivirals, or pesticides to treat AquAdvantage broodstock or AAS since the original NADA approval in 2015. As discussed in Section 6.3, ABT uses 17α-MT for producing neomale broodstock. In addition, ABT uses Ovadine (PVP Iodine) (which provides 1% available iodine) and formalin prophylactically to prevent infection of fish eggs, salt (sodium chloride) treatments for disinfection, and MS-222 (tricane methyl sulfonate) for anaesthetizing fish for handling and euthanizing fish. All drug and/or chemical use at the facility is consistent with Canadian regulations.

7.5.1.3. Disease Surveillance and Status at the Rollo Bay Facility

To date, the Rollo Bay facility has not had any disease outbreak since it began operation in 2018. The Rollo Bay facility follows the same or more robust SOPs for cleaning, disinfection, and handling of equipment, footwear, eggs, fish, etc. as used at the Bay Fortune facility (see Section 7.5.1.2 above). All of the fish present at the Rollo Bay facility originated from the nearby ABT Bay Fortune facility which is recognized as disease-free by CFIA (see Section 7.5.1.2 above). The Rollo Bay facility contains state-of-the-art equipment for water treatment further reducing the likelihood of disease transmission. For example, the Rollo Bay facility disinfects incoming water and recirculating water via UV light, treats recirculating water with ozone, and removes and collects solid waste via drum filters (see Figure D-2; i.e., only water is discharged) and radial flow separators. Solids are then applied to land. The Rollo Bay facility is also managed using strict biosecurity protocols, such that personnel must wash boots and cover clothes before entering each area of the facility.

The Rollo Bay facility is also periodically inspected by provincial authorities under the Certificate of Fish Health Transfer program and undergoes required pathogen testing as part of the US Title 50 Certification program when shipping AAS eggs to the US facilities (see description under Section 7.5.1.2 above). In addition, Rollo Bay will likely begin to undergo compartmentalization inspections by CFIA starting in 2024, which will require inspections a minimum of every 4 months. Similar to the Bay Fortune facility, all year classes of fish held at the Rollo Bay facility are assayed for ISAV using the most sensitive qPCR diagnostic assay available. All mortalities in the

ARA are necropsied and examined for signs of ISAV. A proportion of the population of fry to smolt are collected randomly throughout the year and tested for ISAV. No ISAV positive samples (fish, fry, eggs, whole blood, or mortalities) have been detected by any method. In addition, similar to Bay Fortune, ABT does not import any milt, eggs or fish from outside (third) parties. However, ABT may move eggs between the Bay Fortune and the Rollo Bay facilities. As described in the section above, the eggs are disinfected and held in the ERA until grow-out. If a fish health problem was suspected at one of the ABT facilities, no fish would be transferred to another facility.

Similar to the Bay Fortune facility, the Rollo Bay facility has not used antibiotics, antiparasitics, antivirals, or pesticides to treat AquAdvantage broodstock or AAS since the supplemental NADA approval in 2019. As discussed in Section 6.3, ABT uses 17α-MT for producing neomale broodstock. In addition, ABT uses Ovadine (PVP Iodine) (which provides 1% available iodine) and formalin prophylactically to prevent infection of fish eggs, salt (sodium chloride) treatments for disinfection, and MS-222 (tricane methyl sulfonate) for anaesthetizing fish for handling and euthanizing fish. All drug and/or chemical use at the facility is consistent with Canadian regulations.

7.5.2. United States

To date, there is only one ABT facility approved by FDA to receive AAS eggs and grow-out AAS to harvest in the US, a facility located in Albany, Indiana (see Section 2.2 above, and the 2018 EA[14]). However, in 2021, ABT announced that it plans to build a new facility in Pioneer, Ohio[51] to receive AAS eggs and grow-out AAS to harvest. If constructed, this facility will need FDA approval under a supplemental NADA in order to receive AAS eggs for grow-out.

In order to import salmon eggs into the US, there are Federal and State requirements to ensure the eggs are disease-free prior to importation. Title 50 CFR Part 16.13 outlines the Federal government requirements promulgated by the US Fish and Wildlife Service (FWS) to import live or dead fish, mollusks, and crustaceans or their eggs into the US. According to 50 CFR 16.13(a)(3), live fertilized eggs of salmonid fish are prohibited from entering the US except by direct shipment accompanied by a certification that the fish lots have been sampled and tested using acceptable methods outlined in 50 CFR 16.13(e), and the following diseases have not been detected: *Oncorhynchus masou* virus and the viruses causing viral hemorrhagic septicemia, infectious hematopoietic necrosis, and infectious pancreatic necrosis.

Furthermore, a written certification must accompany the import certifying that the requirements of 50 CFR 16.13 were met (50 CFR 16.13(b)(1)); this is known as a Title 50 Certification.[52] The Title 50 Certification must be signed by a qualified fish pathologist designated as a certifying official by the FWS. With every shipment of AAS eggs to the US, ABT works with Canadian authorities to obtain a Title 50 Certification. Furthermore, 50 CFR 16.13(a)(4) requires disinfection of all live fish

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[51] If approval is sought for the Pioneer, Ohio facility through a supplemental NADA, the environmental impacts of that action will be evaluated in an EA.

[52] https://www.fws.gov/media/us-title-50-certification-form-3-2274 (accessed December 8, 2023)

eggs of salmonid fish within 24 hours prior to shipment to the US via immersion for 15 minutes in a 75 mg/L solution of iodophor followed by a rinse in water free of fish pathogens. This disinfection requirement is included in ABT's shipping SOPs.

In addition, each State in the US has separate fish health requirements before allowing import of eggs into that State. For example, the Indiana Department of Natural Resources requires a pre-entry permit[53] be obtained from the Board of Animal Health before importation of live fish or their gametes into the State (312 Indiana Administrative Code (IAC) 9-10-15). Fish samples must also test negative for all of the diseases listed under the column heading "Indiana DNR" in Table 7-2 above.[54] ABT ensures that all AAS eggs shipped to the US meets the requirements of the State in which they are received and held.

ABT also has several protocols in place at the Indiana facility to prevent and minimize the risk of introducing pathogens/parasites. ABT plans to implement similar SOPs at any facility operated in the US that receives AAS eggs. All ABT facilities use strict biosecurity protocols (e.g., boots and clothing dedicated to a specific area of the facility; boots and hands are disinfected before entering and after leaving fish areas; limiting movement between different areas of the facility) to prevent introduction of pathogens/parasites into the facility or between areas within the facility. Upon receipt of AAS eggs, the exterior packaging is disinfected for 10 minutes with Ovadine then rinsed with freshwater. All eggs are inspected and disinfected (100 ppm free iodine solution for 10 minutes) prior to incubation followed by a rinse with incubation water. The area in the Hatchery where eggs are received is disinfected after movement of eggs to incubators. Each batch of fish is sampled and screened for disease prior to transferring fish from the nursery to the pre-grow out area, and fish are not transferred without a veterinary certificate. In addition, fish are constantly monitored by ABT employees for signs of distress/morbidity, mortalities are removed daily, and necropsies are performed where mortality signs are abnormal or of concern, comparatively to typical mortality characteristics at that life stage and time. Necropsies are performed by trained staff and if there is any concern about the cause of death, fish are sent to a veterinary laboratory for additional analysis. ABT also has a stepwise process outlined in an SOP for the employees to follow in the unlikely event of a suspected infectious disease emergency, including contacting management and state officials, quarantining affected tanks, designating specific equipment and personnel for quarantined tanks, and potentially culling diseased fish, among other measures.

8. EXPOSURE PATHWAY ANALYSIS

As described in Section 7 above, ABT's PEI facilities have multiple, independent, and redundant forms of physical and procedural containment to reduce the likelihood of escape or malicious release of ABT salmon. In addition, in the highly unlikely event of escape or release, there are biological and geographical/geophysical containment

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[53] https://www.in.gov/dnr/fish-and-wildlife/fishing/commercial-fish-suppliers/ (accessed December 8, 2023)

[54] https://www.in.gov/boah/species-information/aquaculture/indiana-fish-entry-requirements/ (accessed December 8, 2023)

measures that would prevent most ABT salmon from surviving, reproducing, and establishing in the environment. Therefore, FDA previously concluded in the 2015 and 2019 EAs that the likelihood of exposure of ABT salmon to the local environment of PEI is very low, which was consistent with the findings of Canadian authorities (DFO, 2013; DFO, 2019). Given the likelihood of escape, survival and establishment in the local environment of PEI was very low, FDA concluded that it was highly unlikely that ABT salmon could disperse and migrate such that there would be a complete exposure pathway to the environment of the US.

However, in this amended EA, FDA is addressing the Court's considerations by assuming that the ABT salmon will escape, survive, migrate to, and establish in the US environment (even if this scenario is highly unlikely). Therefore, this section will outline the exposure pathways ABT salmon could possibly take to establish and/or be present in the US environment. This section will also describe the potential pathways for transmission of pathogens and/or parasites from ABT salmon and the production facilities on PEI. More specifically, this section will describe 1) the biology of the Atlantic salmon, including their habitat range and migration routes, 2) hypothetical exposure pathways to the US environment, 3) accessible environments in which the ABT salmon could survive and establish, and 4) the current status of wild Atlantic salmon in those accessible environments (Atlantic Canada and Maine, US). This information will be used in the exposure assessment (Section 9.2, below) and the risk characterization (Section 9.4, below) to characterize the likelihood of ABT salmon being present in the US environment and causing potential harms.

8.1. Biology of Atlantic salmon

Appendix E contains detailed background information on the biology, ecology, life history, and distribution/status of wild Atlantic salmon. This information was previously presented in Appendix A of the 2015 and 2019 EAs. A summary of relevant information for the current assessment is provided below. This information is critical to predicting the likelihood of ABT salmon to survive (and possibly establish) in the Canadian environment and their potential to disperse/migrate to and establish in the US environment. Additional information on the biology and ecology of wild Atlantic salmon is contained in an Organization of Economic Cooperation and Development (OECD) Consensus Document (OECD, 2017). The OECD document also presents extensive information on the biology and rearing of domesticated farmed Atlantic salmon and the genetics of Atlantic salmon, some of that information is also contained in Appendix E, below.

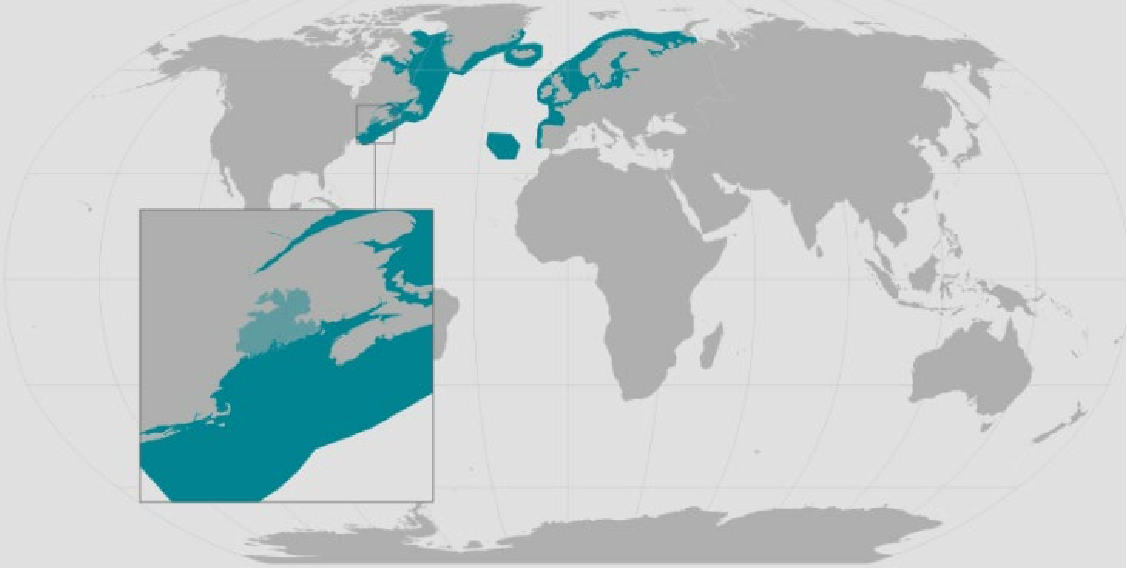
8.1.1. Native Distribution

Atlantic salmon have historically inhabited the North Atlantic Ocean and associated coastal drainages as illustrated in Figure 8-1, below. Globally, there are three groups of native Atlantic salmon: North American, European, and Baltic (NOAA, undated(a)). These groups spawn in freshwater rivers of northeastern North America, Iceland, Europe, and northwestern Russia and then migrate through the North Atlantic Ocean to summer feeding grounds off Greenland (NOAA, undated(a)).

The North American group of Atlantic salmon are of greatest relevance to this assessment given the location of the PEI facilities and will be discussed herein. The North American group is native to the northeastern US and Atlantic Canada. Atlantic

Canada includes the Maritime provinces (New Brunswick, Nova Scotia, and PEI) and the eastern provinces of Newfoundland and Labrador (Figure 7-1, above).

Figure 8-1. World Map Providing Approximate Representation of Atlantic Salmon's Range*



* Obtained from NOAA (undated(a)), https://www.fisheries.noaa.gov/species/atlantic-salmon-protected (accessed December 8, 2023)

In the US, the North American Atlantic salmon group historically ranged in river systems and marine waters from the Hudson River in New York state northward to the Canadian border. In Canada, Atlantic salmon were found in all of Atlantic Canada, including the Bay of Fundy, throughout the Gulf of St. Lawrence, and along the whole coast of Newfoundland and Labrador north to the Fraser River. Self-sustaining native populations no longer exist in most of the historical rivers at the southern distributional limits in the eastern US and the adjacent Maritime Provinces of Canada, including New Brunswick, Nova Scotia, and PEI (Webb *et al*., 2007). Native populations have also become extinct in the upper St. Lawrence River, including Lake Ontario.

Currently, native populations of Atlantic salmon in the US only exist in Maine (NOAA, undated(a)), and populations are greatly depressed and frequently supported by supplemental stocking programs. Atlantic salmon are extinct in 84 percent of the rivers in New England that historically supported salmon (Knapp *et al*., 2007). They are in "critical condition" in the remaining 16 percent (Knapp *et al*., 2007). In 2000, the NOAA's National Marine Fisheries Service (NMFS; also known as NOAA Fisheries) and FWS listed the Gulf of Maine DPS of Atlantic salmon as "endangered" under the ESA (NOAA, undated(b)). That designation was extended in 2009 to include Atlantic salmon in several additional rivers in Maine (NOAA, undated(b)). Additional information on the current status of native Atlantic salmon populations in Atlantic Canada and the US is provided in Section 8.4.

8.1.2. Life History

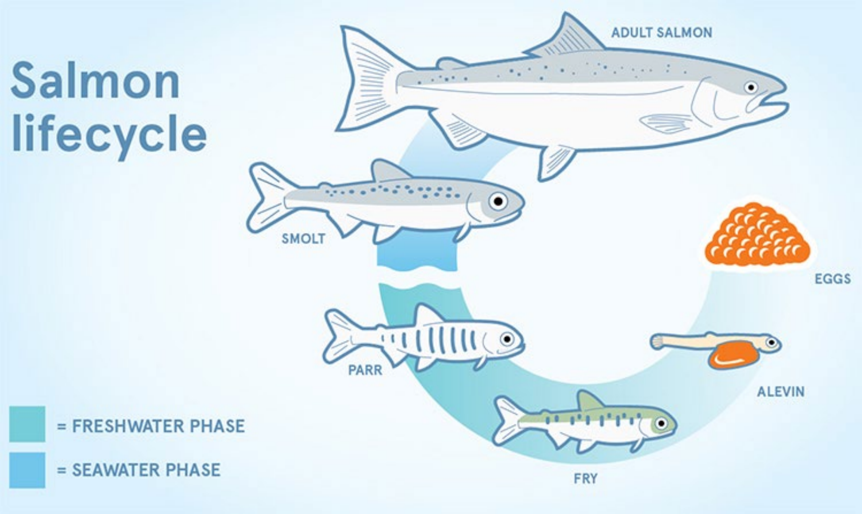
Atlantic salmon are anadromous (i.e., living in salt water and spawning in fresh water); therefore, they can live in fresh and saltwater depending on their life stage.

However, Atlantic salmon populations exhibit diverse physiological, anatomical, and behavioral characteristics that derive in part from local genetic adaptation. In populations for which seaward migration is not prevented by physical barriers, females are usually anadromous; however, males often reproduce after living 1-4 years in freshwater, after which they may or may not migrate to sea. Anadromous populations also exhibit considerable variation in the type of freshwater habitat chosen for rearing (estuarine or lacustrine), the total duration of their seawater habitation (20-50% of lifetime), and the timing of spawning migration (spring or fall). Some Atlantic salmon complete their entire life cycle in freshwater.

The developmental phases of Atlantic salmon include the following and are illustrated in Figure 8-2:

- Alevin: A newly hatched fish in the larval stage that has not yet emerged from the nesting area and is dependent upon a yolk sac for its nutritional requirements
- Fry: An alevin that has fully absorbed its yolk sac and must hunt for, and consume, live food
- Parr: A young salmon in fresh water that has developed a characteristic skin coloration known as "parr marks"
- Smolt: A young salmon that has undergone the physiologic adaptation necessary for transition to saltwater
- Grilse: An adult salmon returning to freshwater one year after migrating to the sea
- Kelt: An adult salmon after spawning

Figure 8-2. Atlantic Salmon Lifecycle*



*Obtained from Maine Department of Marine Resources (https://www.maine.gov/dmr/fisheries/sea-run-fisheries/programs-and-projects/salmon-restoration-and-conservation-program-asrcp; accessed December 8, 2023)

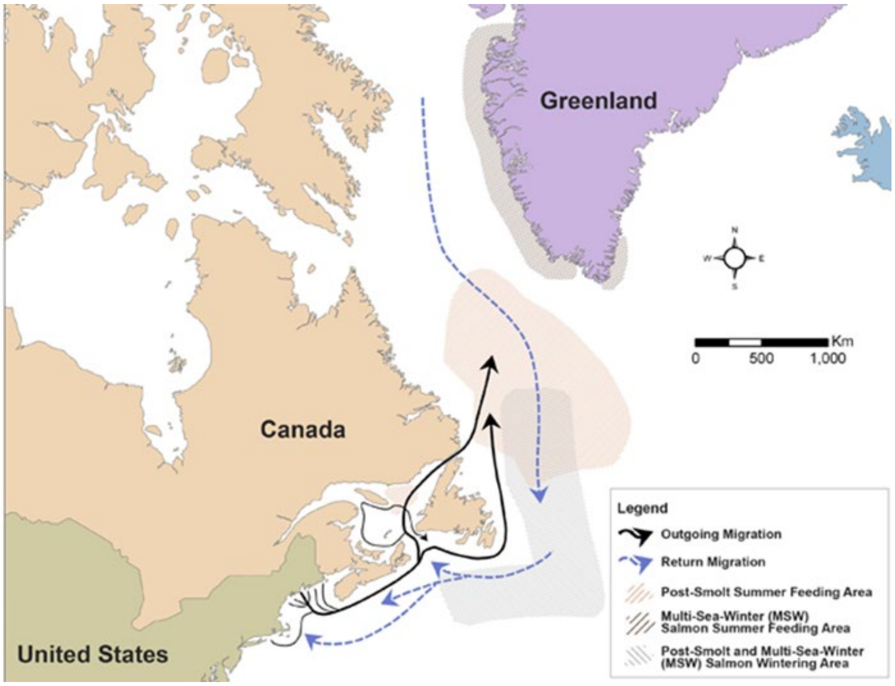
The Atlantic salmon is iteroparous, meaning it may spawn repeatedly. Typically, Atlantic salmon spawn during October to February, with the peak of spawning usually occurring in late October and November. The nesting site, or redd, is chosen by the female, and is usually a gravel-bottom riffle upstream from a pool (Bigelow, 1963; Scott and Crossman, 1973). The ecomorphological demands of the spawning grounds are stringent and include the following: water descent of 0.2-3%; water depth of 50 to 90 cm; running speed of 0.3 to 0.7 m/s; gravel size of 3 to 5 cm; and nest size of 1 to 2 m (MUNLV, 2001).

The female rests after digging a depression in the gravel with her tail and then repeats the operation, creating a new redd for depositing more eggs, and resting again until spawning is complete. The male continues to guard the female, and to drive away competitors aggressively defending the spawning site. Several males, including precocious parr, may be in the vicinity of the female and will continue to engage in spawning behavior until she has completed making redds and depositing her eggs. This may take as long as a week and require the building of up to seven redds to deposit her nearly 7,500 eggs. Thereafter, the post-spawn adult fish, or kelt, may return to the ocean without delay, move to a pool downriver for a period of rest, or over-winter in the nursery river and return to sea in the spring. Many kelt do not survive the first mating; some survive to mate twice, but very few mature male or female salmon survive to spawn three or more times. Only about 9-20% of the fertilized eggs in the redds survive to develop over the winter and, depending on temperature and water conditions, will usually hatch in April.

Hatchlings are referred to as alevin, which develop to fry and then parr. Parr may spend between one and six years (usually two to three years in the Canadian Atlantic and Maritimes and Gulf of Maine) in their natal streams; at some point, if they are not in land-locked lakes, they begin their downstream migration and prepare for life in the sea. The seaward migration involves a change in physiology which allows the young salmon to adapt to saltwater conditions. This transformation in physiology is referred to as "smoltification" and the young fish that migrate to the sea are called "smolts." In general, smolts tend to live for a while in brackish (part salt) water, such as bays and estuaries while they complete their adaptation to salt water. It is thought that the "imprinting" of the natal river occurs during smoltification (NOAA, undated(b)).

At the end of the spring, during which they have adapted to living in saltwater, the smolt generally swim to sea. For example, Atlantic salmon leave Maine rivers sometime in April or May and can be found in the waters off Labrador and Newfoundland by mid-summer. They then migrate to take advantage of available food supplies and generally spend their first winter at sea off the coast of Greenland. The typical migration patterns of North American Atlantic salmon are illustrated in Figure 8-3 below. Salmon typically form schools after they enter the sea and may travel with or be mistaken for herring, mackerel or other pelagic fish, since post-smolts occur as by-catch in these fisheries according to the North Atlantic Salmon Conservation Organization (NASCO, 2007). Post-smolts follow ocean currents, feeding as they migrate. After two years at sea, an adult salmon can weigh about 8-15 pounds, and be up to 76 cm (30 inches) long. During their time in the open sea, which can last from one to several winters, the fish become sexually mature.

Figure 8-3. North American Atlantic Salmon Migration Patterns (NOAA, 2022)



A few non-anadromous salmon never make the migration to saltwater environments because they spend their entire lives in landlocked freshwater lakes. In addition, a small percentage of the males become sexually mature in fresh water as parr and are referred to as "precocious males." Rather than migrating to sea, these small, young males establish residence in the water in which mature salmon spawn. When the females release their eggs, the precocious males dart in and deposit their milt before the sexually mature large males can. Because they are small, the precocious males are not recognized as threats by the larger mature males and are generally not the object of their aggression. Precocious parr are thought to make up approximately 2-100 percent of the males, depending on the specific Atlantic salmon population, but may end up fertilizing up to 11-65 percent of the total eggs that are released by females (OECD, 2017). Precocious males may also subsequently migrate to sea and return as large anadromous males. This reproductive strategy shortens the generation time and also partly compensates for low returns of anadromous males.

8.1.3. Survival and Return Rates for Wild-Reared Atlantic Salmon [*i.e.*, without the rDNA construct] in the Natural Environment

Survival of wild-reared Atlantic salmon in freshwater from egg to smolt varies from 0.1-6.5% (O'Connell *et al*., 2006; Hutchings and Jones, 1998). Chaput *et al*. (1998) report values of <0.5% have been observed frequently in populations in eastern Canada. Survival in the sea from smolt to return as grilse varies from 1.3-17.5% (Hutchings and Jones, 1998). Chaput (2012) states that Atlantic salmon have unusually high annual rates of mortality at sea ranging as high as 65-95% (equating to a survival at sea of 5-35%). Most wild-reared Atlantic salmon (70-80%) survive spawning and migrate to sea a second time as kelt, but only about 10% of them return to spawn a second time (Fleming, 1998). Jensen *et al*. (2016) report mean return rates from marine to fresh water as 2.35% (range = 0.77-5.40%) for wild-reared Atlantic salmon.

8.2. Exposure Pathways

This section will outline the hypothetical pathways and steps required for ABT salmon and their progeny to result in an exposure in the US environment. As discussed in Section 4.2 (above), for this assessment, we have defined exposure as establishment and/or presence in the US environment. As described in Section 8.1 (above), native populations of Atlantic salmon in the US currently only exist in Maine, including the endangered Atlantic salmon that make up the Gulf of Maine DPS. Therefore, this analysis will primarily focus on the hypothetical exposure pathways and steps required for ABT salmon and their progeny to establish and/or be present in the Maine environment (Section 8.2.1, below). Pathogen/parasite transmission pathways are also identified due to the potential for ABT salmon or the PEI facilities to spread pathogens/parasites to the natural environment (Section 8.2.2, below).

8.2.1. Pathways to Establishment and/or Presence of ABT Salmon in the US Environment

ABT salmon includes AAFB, AANB, diploid AAS, and triploid AAS. There are two different hypothetical exposure pathways that could theoretically result in the presence and establishment of ABT salmon and their progeny in Maine. The pathways and their associated steps are illustrated in Figure 8-4 and Figure 8-5. The first two steps of the pathways (escape/release from ABT facilities on PEI and survival and dispersion in PEI and Atlantic Canada) are the same for all ABT salmon. However, the steps required after survival and dispersion in PEI and Atlantic Canada (i.e., Steps 3 through 6) are dependent upon the specific type of ABT salmon being considered and their ability to reproduce in the natural environment.

As discussed in Section 6.3 (above), only AAFB and diploid AAS are capable of reproducing naturally on their own, whereas AANB and triploid AAS are not. Therefore, for the purpose of the pathway analysis, AAS salmon are divided into two subgroups, diploid AAS (≤5% of AAS) and triploid AAS (>95% of AAS),[55] in order to show different hypothetical pathways for AAS depending on their ability to reproduce.

Figure 8-4 illustrates the hypothetical pathways and steps for AAFB and diploid AAS. Figure 8-5 illustrates the same pathways and steps for AANB and triploid AAS, except red “X” marks are included in Figure 8-5 to illustrate the pathways that are not possible, i.e., AANB and triploid AAS cannot reproduce and establish. An expanded description of the possible pathways and steps is provided in the text under Figure 8-4 and Figure 8-5.

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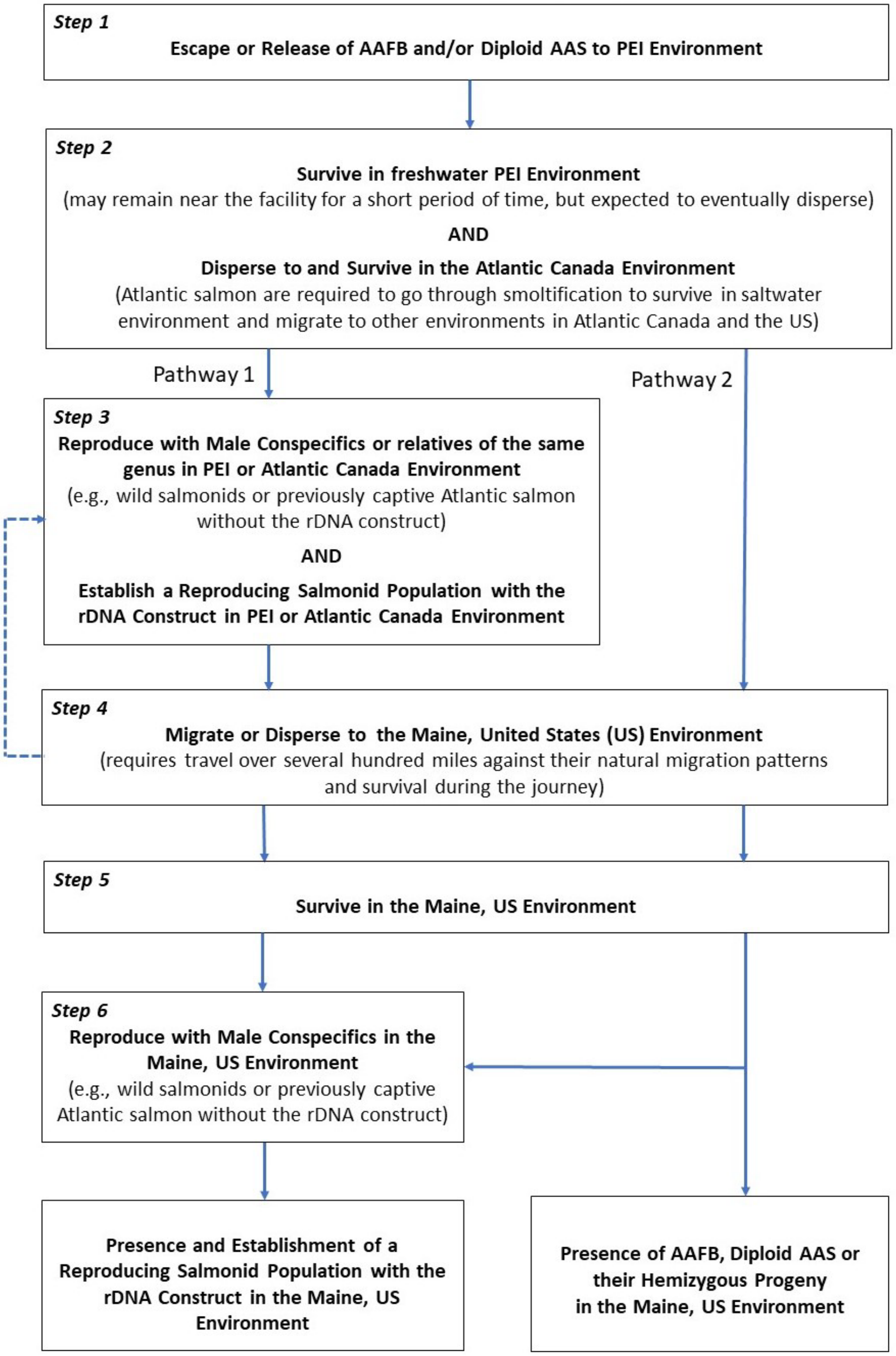
[55] Between 2017 and 2020, triploidy was achieved in 99.5% of AAS on average and ranged from 96.9% to 100% (i.e., on average only 0.5% AAS would be diploid).

Figure 8-4. Possible Exposure Pathways and Steps Required for AquAdvantage Female Broodstock (AAFB)*, Diploid AquAdvantage Salmon (AAS) and Their Progeny to be Present and Establish in Maine, United States**



Step 1

Escape or Release of AAFB and/or Diploid AAS to PEI Environment

Step 2

Survive in freshwater PEI Environment

(may remain near the facility for a short period of time, but expected to eventually disperse)

AND

Disperse to and Survive in the Atlantic Canada Environment

(Atlantic salmon are required to go through smoltification to survive in saltwater environment and migrate to other environments in Atlantic Canada and the US)

Step 3

Reproduce with Male Conspecifics or relatives of the same genus in PEI or Atlantic Canada Environment

(e.g., wild salmonids or previously captive Atlantic salmon without the rDNA construct)

AND

Establish a Reproducing Salmonid Population with the rDNA Construct in PEI or Atlantic Canada Environment

Step 4

Migrate or Disperse to the Maine, United States (US) Environment

(requires travel over several hundred miles against their natural migration patterns and survival during the journey)

Step 5

Survive in the Maine, US Environment

Step 6

Reproduce with Male Conspecifics in the Maine, US Environment

(e.g., wild salmonids or previously captive Atlantic salmon without the rDNA construct)

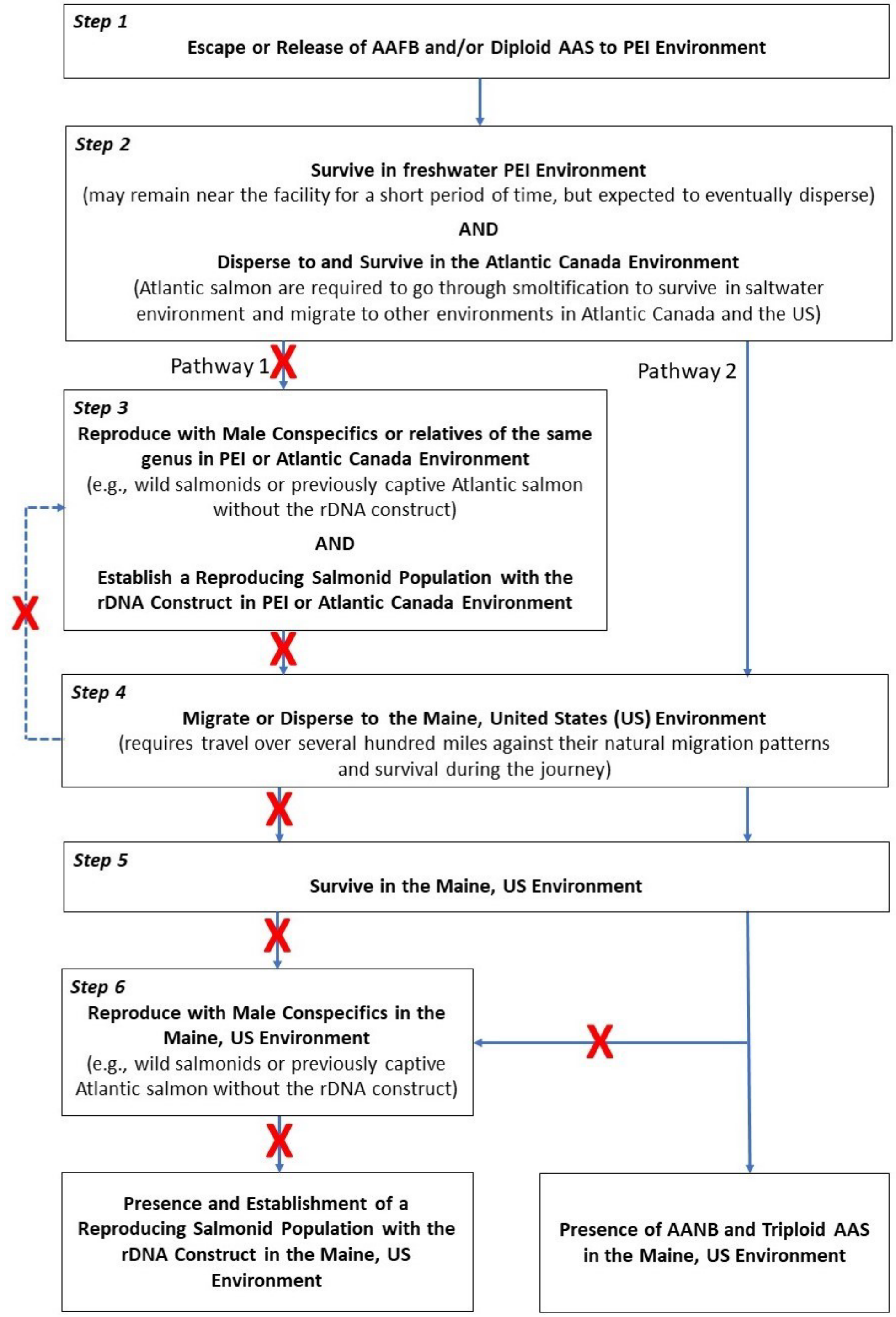
Presence and Establishment of a Reproducing Salmonid Population with the rDNA Construct in the Maine, US Environment

Presence of AAFB, Diploid AAS or their Hemizygous Progeny in the Maine, US Environment

* AAFB are diploid; 100% female; homozygous or hemizygous for rDNA construct; and are capable of reproducing naturally.

** AAS are >95% triploid and ≤5% diploid; 100% female; hemizygous for the rDNA construct; triploid are sterile, and diploid are capable of reproducing naturally.

Figure 8-5. Hypothetical Pathways and Steps Required for AquAdvantage Neomale Broodstock (AANB)* and Triploid AquAdvantage Salmon (AAS) to be Present in Maine, United States**



* AANB are diploid; 100% neomale; homozygous for the rDNA construct; and are NOT capable of reproducing naturally

** AAS are >95% triploid and ≤5% diploid; 100% female; hemizygous for the rDNA construct; triploid are sterile, and diploid are capable of reproducing naturally

8.2.1.1. Escape, Survival, and Dispersal in PEI and Atlantic Canada (Steps 1 and 2)

As stated above, Steps 1 and 2, escape (or release) from the ABT facilities on PEI and survival and dispersion in PEI and Atlantic Canada, are the same for all ABT salmon (AAFB, AANB, triploid AAS and diploid AAS). ABT employs multiple independent levels of physical containment and stringent procedures in all of their facilities to ensure that ABT salmon do not escape and are not unintentionally released. Table 7-1 (above) lists the physical and procedural containment and security in place at both ABT facilities. In order for ABT salmon to escape these facilities, they would have to overcome a minimum of 4, and in some areas more than 6, independent levels of containment at the Bay Fortune facility, and a minimum of 6 to 8, in some areas more than 10, independent levels of containment at the Rollo Bay facility. Alternatively, they could be released due to malicious intent.

In the highly unlikely event that ABT salmon escaped or were released, they would enter either the drainage ditch beside the Bay Fortune facility or Rollo Bay Brook beside the Rollo Bay Facility. ABT salmon would first need to survive in the immediate PEI environment, including adapting to changes in environmental conditions (e.g., temperature, salinity), avoiding predators, finding food, and finding a suitable habitat (including spawning habitat). If any of these factors are not suitable, ABT salmon may disperse to other areas on PEI or in Atlantic Canada to find adequate resources. In order to disperse, the ABT salmon would need to overcome many physical (e.g., low water levels and other obstructions) and environmental (e.g., appropriate environmental conditions to allow for smoltification) barriers to reach Rollo Bay and Northumberland Strait. If the ABT salmon were able to survive the PEI environment and need to disperse to other parts of PEI/Atlantic Canada, they would need to undergo smoltification, which is required to survive in the saltwater conditions that they would encounter in Rollo Bay, Northumberland Strait, Gulf of St. Lawrence and eventually the Atlantic Ocean. Salmon require certain environmental conditions to smoltify (e.g., salinity gradient as they moved from fresh to saltwater), so those would need to occur in drainage ditch and Rollo Bay Brook before entering salt water. Typically, once Atlantic salmon smoltify they migrate[56] to other parts of Atlantic Canada or Greenland to feed before returning to their natal waters to reproduce (see Section 8.1 and Figure 8-3, above, for details on the life history traits of Atlantic salmon).

8.2.1.2. Reproduction, Migration, and Establishment in Atlantic Canada and the US (Steps 3 through 6)

After survival and dispersion in PEI and Atlantic Canada, the steps to exposure, i.e., presence and/or establishment of ABT salmon, in the US are dependent upon the ABT salmon's ability to reproduce in the natural environment. The steps in the pathway analyses are described below in more detail for each type of ABT salmon.

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[56] For this assessment, migration is defined as travel over long distances (hundreds of kilometers) with no establishment of new populations along the way. That is compared to dispersion, which is travel over shorter distances with the potential for establishment and further spread.

a. AAFB and Diploid AAS (Figure 8-4)

AAFB are all female fish that are homozygous or hemizygous for the rDNA construct. The AAFB are diploid and would be able to reproduce in the natural environment.

AAS are all female fish that are hemizygous for the rDNA construct. As discussed above, there is a slight possibility that some AAS may be diploid (≤5%). Diploid AAS are capable of reproducing naturally and could potentially produce viable offspring in the natural environment. All possible pathways and steps for AAFB and diploid AAS are illustrated in Figure 8-4, above.

If AAFB and diploid AAS were able to survive in the PEI and/or the Atlantic Canada environment and develop to sexually mature smolt, then there are two hypothetical pathways to exposure in Maine, which are discussed below.

Pathway 1 (Figure 8-4): Reproduction of AAFB and diploid AAS in the Atlantic Canada environment, and then dispersion and spread of their hemizygous progeny to the US environment (Steps 3 through 6)

One possible pathway to enter the Maine environment would be for AAFB and diploid AAS that survive and disperse in PEI/Atlantic Canada to reproduce with local male conspecifics[57] (such as previously captive Atlantic salmon without the rDNA construct released from the ABT facility) or relatives of the same genus and establish a reproducing salmonid population with the rDNA construct in PEI/Atlantic Canada (denoted as Pathway 1 in Figure 8-4). This population could continue to reproduce and then disperse (or spread) to other areas in Atlantic Canada. If this continued with a series of short-distanced dispersals and establishments of salmonids with the rDNA construct down the eastern coastline of Nova Scotia, the salmonids with the rDNA construct could eventually enter the Maine environment and establish a reproducing population. Pathway 1 is illustrated as arrows from Step 2 → Step 3 → Step 4 → Step 5 → Step 6 in Figure 8-4.

In order to establish a reproducing population in PEI/Atlantic Canada (Step 3), AAFB and diploid AAS would need to find a suitable mate, which would need to be a male conspecific (i.e., wild Atlantic salmon or previously captive Atlantic salmon without the rDNA construct[58]) or relatives of the same genus (e.g., brown trout) in PEI and/or Atlantic Canada. In order to reproduce with the mate, the AAFB and diploid AAS would need to find an unoccupied suitable spawning habitat that follows stringent guidelines: generally a gravel-bottom riffle upstream from a pool, water descent of 0.2-3%; water depth of 50 to 90 cm; running speed of 0.3 to 0.7 m/s; gravel size of 3 to 5 cm; and nest size of 1 to 2 m. AAFB and diploid AAS would also need to outcompete wild Atlantic salmon for mates and spawning sites (see Section

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[57] A conspecific is an organism (plant or animal) of the same species. Herein, the term includes wild or native Atlantic salmon, as well as salmon that may have been intentionally introduced or stocked into the environment.

[58] A "previously captive Atlantic salmon without the rDNA construct" would include any Atlantic salmon without the rDNA construct that has different genetics than the wild Atlantic salmon. This would include the Atlantic salmon without the rDNA construct that could escape the Rollo Bay facility or those held in net pens in the northeast. As a reminder, Atlantic salmon without the rDNA construct (male, female, and neomale; see Section 7.4.2) are also reared the ABT facilities.

9.3.2.2, below). If fertilization of the diploid AAS and AAFB eggs were successful and the eggs survived, it is expected that 50 and 100% of the offspring (F1 generation), respectively, would be hemizygous for the rDNA construct, and if the conditions were optimal, the offspring could potentially continue to reproduce with each other and/or relatives and establish a reproducing salmonid population with the rDNA construct in Atlantic Canada.

This hemizygous population could eventually slowly spread south, establishing populations along the coast of Nova Scotia, and eventually (after many generations) could disperse to the Maine environment (Step 4). Once in Maine, they would need to survive (Step 5), including adapting to changes in environmental conditions, avoiding predators, and finding suitable food and habitat. If they survived the Maine environment, then their presence alone in the Maine environment could potentially result in interactions, including competing with native salmon for food, habitat and mates, that could result in harmful effects (see Section 9.3 and Figure 9-2, below).

In order to establish a reproducing population (Step 6), the hemizygous progeny would need to migrate up rivers and streams in Maine and find suitable mates and spawning habitat (see discussion in paragraph above).

Pathway 2 (Figure 8-4): Direct migration of AAFB and diploid AAS to the US environment (Steps 4 through 6)

The second pathway to enter the Maine environment would for AAFB and diploid AAS to disperse and migrate from PEI or Atlantic Canada directly to the Maine environment, which is illustrated by arrows from Step 2 → Step 4 → Step 5 (→ Step 6, if possible) denoted as Pathway 2 in Figure 8-4. Migration (Step 4) would require the AAFB and diploid AAS to travel hundreds of kilometers against their normal migration patterns. Female Atlantic salmon smolt typically return to their natal waters to spawn. In the case of ABT salmon, that would likely be considered the rivers of northeast PEI because "imprinting" of the natal river usually occurs during smoltification (NOAA, undated(b)), which would occur prior to the AAFB and diploid AAS entering the Northumberland Strait. Regardless, if AAFB and diploid AAS were able to migrate to Maine, then they would need to survive the migration by avoiding predation, avoiding being caught by fisherman, and finding food.

If they survived the migration, they would then need to survive in the Maine environment (Step 5), as described in the description of "Pathway 1" above, and similarly to Pathway 1, their presence alone would potentially result in harms. However, in order to establish a reproducing population in Maine (Step 6), AAFB and diploid AAS would also need to migrate up rivers and streams and find a suitable mate and suitable spawning habitat in Maine as described in "Pathway 1" above. AAFB and diploid AAS would also need to outcompete wild Atlantic salmon for mates and spawning sites (see Section 9.3.2.2, below). If fertilization of the AAFB and diploid AAS eggs were successful and the eggs survived, it is expected that 50-100% of the offspring (F1 generation) would be hemizygous for the rDNA construct, and if the conditions were optimal, the offspring could potentially continue to reproduce with relatives and establish a reproducing salmonid population with the rDNA construct in Maine.

b. AANB and Triploid AAS (Figure 8-5)

AANB are all neomale fish that are genotypically female but phenotypically male. These fish are homozygous for the rDNA construct. The AANB are diploid but are not capable of reproducing on their own because they lack a sperm duct.

AAS are all female fish that are hemizygous for the rDNA construct. Most of the AAS are triploid (>95%). Triploid AAS are sterile and cannot produce viable offspring.

All pathways and steps for AANB and triploid AAS are illustrated in Figure 8-5, above, and are discussed below.

Pathway 1 (Figure 8-5): Reproduction of AANB and triploid AAS in the Atlantic Canada environment, and then migration of their hemizygous progeny to the US environment (Steps 3 through 6)

Because AANB and triploid AAS cannot reproduce naturally on their own, there are no potential pathways for them to establish a reproducing salmonid population with the rDNA construct in PEI, Atlantic Canada or Maine, i.e., Steps 3 and 6 are not possible for AANB and triploid AAS as represented by the red “X” marks shown in Figure 8-5 above.

Pathway 2 (Figure 8-5): Direct migration of AANB and triploid AAS to the US environment (Steps 2 through 5)

Although AANB and triploid AAS cannot reproduce or establish in Maine, they could be present in the Maine environment. Presence in the environment could result in harms, including competition with native salmon for food, habitat, and mates (see Section 9.3 below). The steps for this pathway are identical to those described for AAFB and diploid AAS in Section 8.2.1.2.a “Pathway 2” above, except they cannot complete Step 6 and reproduce or establish in Maine.

8.2.1.3. Conclusions

Only AAFB and diploid AAS could, hypothetically, establish a reproducing population of salmonids with the rDNA construct in Maine (Figure 8-4, above). AANB and triploid AAS cannot reproduce naturally, and therefore, cannot establish a new population of salmonids with rDNA construct (Figure 8-5, above). However, if any of the ABT salmon or their hemizygous progeny were present in the Maine environment, they could potentially cause other harms to the Maine environment, such as competing with native Atlantic salmon for food, habitat, and mates. Section 9.3 provides a more thorough discussion of the potential harms due to the presence of ABT salmon and establishment of a reproducing population with the rDNA construct in the Maine environment. Table 8-1 below summarizes the outcome of the analysis for pathways to establishment and/or presence of ABT salmon in the US environment.

Table 8-1. Types of ABT Salmon that Could Possibly be Present or Establish a Reproducing Population of Salmonids with the rDNA Construct in the US Environment if ABT Salmon Could Escape Confinement in PEI Canada are Indicated by an X

ABT Salmon Type	Could be Present in US Environment	Could Reproduce & Establish in US Environment
AquAdvantage Female Broodstock (AAFB)	X	X
AquAdvantage Neomale Broodstock (AANB)	X	-
Triploid AquAdvantage Salmon (AAS) (>95% of AAS)	X	-
Diploid AAS (≤5% of AAS)	X	X

8.2.2. Pathogen/Parasite Transmission Pathways

Harms to the US environment could also occur through transmission of pathogens and/or parasites from ABT salmon and/or ABT's production facilities on PEI. Herein, a pathogen includes a bacterium, virus, parasite, or other microorganism that could result in harms (e.g., disease). In addition, the potential transmission of parasites that do not directly cause disease in fish is also considered herein because these parasites can act as vectors for disease transmission/spread and cause physical damage to the fish. Any type of ABT salmon (AAFB, AANB, diploid or triploid AAS) and life stage (egg to smolt) is potentially capable of transmitting pathogens/parasites; therefore, they will be discussed collectively below.

In order for pathogens/parasites to be transmitted by ABT salmon or from ABT's PEI facilities, they first need to be introduced into the ABT facility or to ABT salmon in the facility and be spread within the facility. Introduction could occur through 1) egg/milt/fish carrying a pathogen/parasite being brought into an ABT facility, 2) ABT personnel carrying disease into the facility, or 3) a contaminated water source. Pathogens/parasites can then be spread within a facility via circulation of water between tanks, movement of fish between tanks or areas, equipment exchange between tanks and areas, and ABT personnel cross-contaminating tanks. As described in Section 7.5 above, ABT has implemented extensive disease prevention and surveillance strategies to prevent disease introduction, outbreak, and transmission at both PEI facilities, including not importing eggs/milt/fish from outside (third) parties into any ABT facility. This information along with the transmission pathways described below will be used in Section 9.2.4 to determine the likelihood of pathogen/parasite transmission occurring from ABT salmon and the ABT PEI facilities to the US endangered Atlantic salmon and the US environment.

According to Jones *et al*. (2015), pathogen/parasite transmission from an aquaculture facility may occur through the following mechanisms:

1) Water (including mucus and feces),
2) Fish-to-fish contact (including escaped fish and egg movement),
3) Biological vectors (including parasites, and other organisms), and
4) Equipment and personnel.

In the unlikely event of a pathogen/parasite occurring in ABT salmon or the ABT PEI facilities, four potential pathways of pathogen/parasite transmission from ABT salmon and the PEI facilities to US wild Atlantic salmon are identified and discussed below. These transmission pathways are also illustrated in Figure 8-6 and Figure 8-7.

1) Transmission of pathogens/parasites directly from escaped ABT salmon to US wild Atlantic salmon [Figure 8-6, ABT salmon → US wild Atlantic salmon],
2) Transmission of pathogens/parasites from escaped ABT salmon to PEI wild Atlantic Salmon then to US wild Atlantic salmon [Figure 8-6, ABT salmon → PEI wild Atlantic salmon or other migratory wild fish (directly or via other wild fish) → US wild Atlantic salmon],
3) Transmission of pathogen/parasites from wastewater discharged from ABT's PEI facilities [Figure 8-7, ABT PEI facility discharge → PEI wild Atlantic salmon (direct contact, via other wild fish, or from other aquaculture operations) → US wild Atlantic salmon], and
4) Transmission via shipment of AAS eggs that contain a pathogen/parasite from an ABT PEI facility to a US facility [Figure 8-7, ABT PEI facility water/fish → Shipment of AAS eggs to US → ABT US facility discharge → US wild fish (including US wild Atlantic salmon)].

Figure 8-6. Potential Transmission Pathways for Pathogens/Parasites from AquaBounty Technologies (ABT) Salmon to US Wild Atlantic Salmon

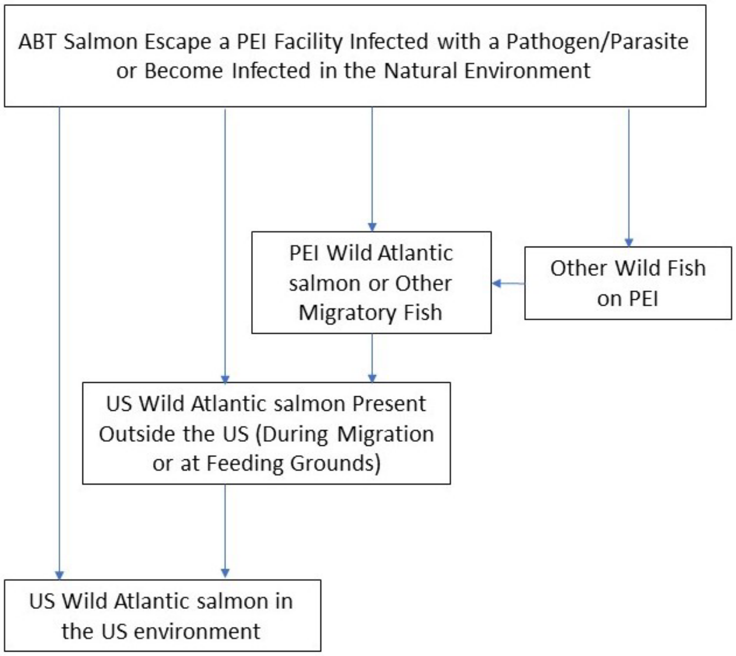
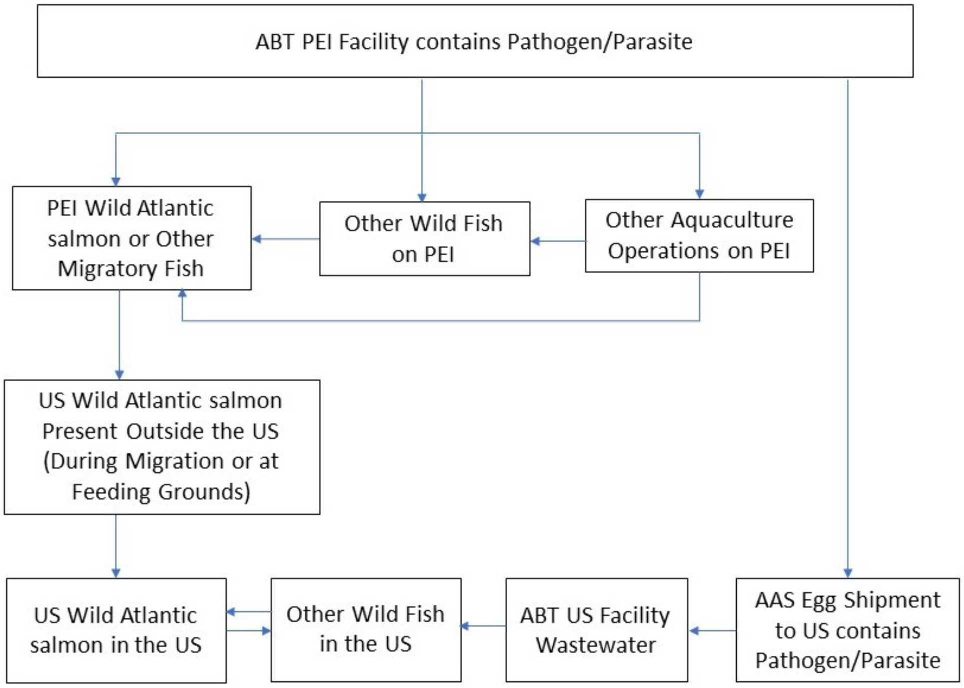


Figure 8-7. Potential Transmission Pathways for Pathogens/Parasites from AquaBounty Technologies (ABT) Facilities to US Wild Atlantic Salmon



8.2.2.1. Transmission of Pathogens/Parasites Directly from Escaped ABT Salmon to US Wild Atlantic Salmon

In the highly unlikely event that ABT salmon were able to escape the ABT facilities on PEI, they could be infected with a pathogen/parasite from the PEI facilities, or alternatively, they could become infected in the natural environment after escape.[59] Transmission could then occur in the US environment if ABT salmon were to disperse or migrate to the US environment (see Section 8.2.1, above) and transmit the pathogen/parasite directly to US wild Atlantic salmon populations via fish-to-fish contact or biological vectors (parasites). It is also possible for AAFB and diploid AAS to transmit some type of pathogens/parasites from mother to offspring if they are able to reproduce with endangered US Atlantic salmon in the US environment.

It could also potentially occur due to comingling of ABT salmon with US wild Atlantic salmon during migration along the eastern coast of Canada or at feeding grounds in Atlantic Canada or Greenland (see Figure 8-4 above). If US wild Atlantic salmon were infected outside the US, they could carry the pathogen/parasite back to the US environment and further transmit it to endangered wild Atlantic salmon in the US environment. The transmission of pathogens/parasites in the US environment could

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[59] The potential for ABT salmon to become infected in the environment and transmit pathogens/parasites is evaluated herein because it is unknown whether ABT salmon has a greater susceptibility to pathogen/parasite infections. See additional discussion on susceptibility of ABT salmon to pathogen/parasite infections in Section 9.3.2.2.e below.

result in disease, and possibly mortality, in the endangered Atlantic salmon population.

These transmission pathways are displayed in Figure 8-6 [ABT salmon → US wild Atlantic salmon (in the US environment, during migration or at feeding grounds)].

8.2.2.2. Transmission of Pathogens/Parasites from Escaped ABT Salmon to PEI Wild Atlantic Salmon or Other Migratory Fish

Transmission of pathogens/parasites from escaped ABT salmon to US wild Atlantic salmon could also occur via PEI wild Atlantic salmon or other migratory fish. ABT salmon could be infected prior to escape from the PEI facilities or could become infected in the natural environment following escape. There are two possible pathways in which this could occur. First, the escaped ABT salmon could transmit a pathogen/parasite to wild Atlantic salmon or other migratory fish on PEI via fish-to-fish contact or biological vectors. The second, alternative pathway of transmission would be via escaped ABT salmon transmitting the pathogen/parasite to other wild fish on PEI that, in turn, transmit it to wild Atlantic salmon or other migratory fish on PEI. PEI wild Atlantic salmon or other migratory fish could then comingle with US wild Atlantic salmon during migration along the eastern coast of Canada or at feeding grounds and transmit the pathogen/parasite to US wild Atlantic salmon. As stated under Section 8.2.2.1 above, the US wild Atlantic salmon could carry the pathogen/parasite back to the US environment and further transmit it to endangered wild Atlantic salmon in the US environment.

These transmission pathways are displayed in Figure 8-6 [ABT salmon → PEI wild Atlantic salmon or other migratory wild fish (directly or via other wild fish) → US wild Atlantic salmon].

8.2.2.3. Transmission of Pathogens/Parasites from Wastewater Discharged from ABT's PEI Facilities

Transmission of a pathogen/parasite to the environment could also potentially occur via wastewater discharged from ABT's PEI facilities. If in the highly unlikely event a pathogen/parasite was introduced to ABT's facilities on PEI, it could be spread throughout the facility via water, fish-to-fish contact, and equipment and personnel (although, as described in Section 7.5.1 above, ABT has employed many biosecurity procedures to ensure that introduction and spread of a disease would not occur in its PEI facilities). If pathogens/parasites were in the water discharged from ABT's facilities on PEI, this could potentially lead to infection of US wild Atlantic salmon via infected PEI Atlantic salmon or other migratory fish (similar to the pathways described in Section 8.2.2.2 above).

PEI wild Atlantic salmon could become infected via three theoretical pathways. First, wild Atlantic salmon on PEI could become infected by direct interaction with the water discharged from the ABT facilities on PEI. Second, other wild fish on PEI could become infected due to the discharge and transmit the pathogen/parasite to PEI wild Atlantic salmon via fish-to-fish contact or a biological vector. Third, following introduction into the PEI environment, other aquaculture operations on PEI could become infected with the pathogen/parasite and spread it to other aquaculture operations on the island, effectively increasing the pathogen/parasite load in the environment as well as the geographic scope of the infected area. These other aquaculture operations could in turn transmit the pathogen/parasite to wild fish and/or Atlantic salmon on PEI. PEI Atlantic salmon or other migratory fish could then infect US wild Atlantic salmon during migration or at feeding grounds and US wild Atlantic salmon could spread the pathogen/parasite in the US environment.

These transmission pathways are displayed in Figure 8-7 [ABT PEI facility discharge → PEI wild Atlantic salmon (direct contact, via other wild fish, or from other aquaculture operations) → US wild Atlantic salmon].

8.2.2.4. Transmission Via Shipment of AAS Eggs that Contain a Pathogen/Parasite from a ABT PEI Facility to a US Facility

Transmission of pathogens/parasites to the US environment from the ABT PEI facilities could also potentially occur if a shipment of AAS eggs (either the eggs, water, or packaging) to the ABT facilities in the US contains a pathogen/parasite. The pathogen/parasite could be spread within ABT's US facilities via water, fish-to-fish contact, equipment, and personnel (as described in Section 7.5.2 above, ABT has employed many biosecurity procedures to ensure that introduction and spread of a disease would not occur in its US facilities). If pathogens/parasites were in the water discharged from ABT's facilities in the US, it could lead to infection of wild fish in the US environment, which could potentially result in infection of endangered Atlantic salmon if the infection spread throughout the US. In addition, pathogens/parasites could be transmitted from AAS Salmon should they escape from the Indiana facility. The potential for AAS Salmon to escape from the Indiana facility was evaluated in the 2018 EA and was found to be extremely low; therefore, the transmission pathway is considered incomplete (i.e., it will not occur) and will not be evaluated herein.

This transmission pathway is displayed in Figure 8-7 [ABT PEI facility water/fish → Shipment of AAS eggs to US → ABT US facility discharge → US wild fish (including US wild Atlantic salmon)].

8.3. Potentially Accessible Environments

As described in Section 8.2 above, in the highly unlikely event of escape, ABT salmon could possibly be present in PEI, Atlantic Canada, and Maine, US (Figure 7-1). All three environments are capable of supporting a population of Atlantic salmon and are within the species native range (Figure 8-1), but wild Atlantic salmon populations have been declining for decades in each of these areas (see Section 8.4 for more information). These potentially accessible environments are discussed in more detail below.

8.3.1. PEI

Production of AAS will only occur at the land-based, freshwater aquaculture facilities located in Bay Fortune and Rollo Bay on the northeast side of PEI as illustrated in Figure 7-2 and Figure 7-3 (above).

The Bay Fortune facility is located near the northeast coast of the island, close to Bay Fortune, a tidal coastal estuary of the Fortune River. The facility is located approximately 1.6 km inland from the river's confluence with Rollo Bay, a bay which is connected to the Northumberland Strait and the Gulf of St. Lawrence (Atlantic Ocean); see Figure 7-2 and Figure 7-3. All effluent from the Bay Fortune facility passes through an external containment sump before discharging to a small drainage ditch which empties into Fortune River estuary. The Bay Fortune facility sits at an elevation of approximately 6-7 m above water level, and the distance to the Fortune River estuary from the Bay Fortune discharge point is approximately 36-37 m.

The Rollo Bay facility is also located on the northeast side of the island about 1 km north of coastal waters, Rollo Bay, and about 12 km north from the Bay Fortune facility. The effluent from the Rollo Bay facility passes through a polishing pond or stone wash-out before discharging to Rollo Bay Brook. Rollo Bay Brook is a small stream with variable flow that runs through the property and travels approximately 1.5 km from the property before entering Rollo Bay. At the lowest point, the site is located approximately 19 m above sea level, and to our knowledge, there has never been a storm surge greater than 2.1 m with the sea level rising to 4.23 m on the south shore of PEI.[60] Due to the local topography and the location of the site in the Northumberland Strait drainage, water will drain away from the facilities and flooding is not a concern in the area.

Ultimately, the effluent from both facilities will enter Rollo Bay, which connects to the Northumberland Strait, and eventually the Gulf of St. Lawrence (Atlantic Ocean). It is important to note that Rollo Bay, the Northumberland Strait, the Gulf of St. Lawrence, and the Atlantic Ocean are all interconnected in terms of water flow, with no physical boundaries separating them (see Figure 7-1 and Figure 7-2, above). Therefore, they will often be discussed together herein. Thus, many of the potentially accessible environments for both facilities on PEI are the same and include the Fortune River estuary, Rollo Bay Brook, Rollo Bay, the Northumberland Strait and the Gulf of St. Lawrence (Atlantic Ocean). These ecosystems are described below. The environment of the Gulf of St. Lawrence is expected to be substantially the same as the north Atlantic Ocean, and therefore, is not described separately below.

8.3.1.1. Local Conditions in Northeastern PEI near ABT's Facilities

The climate on the northeast side of PEI, where both facilities are located, is generally damp with an average yearly rainfall of 87 cm and an average yearly snowfall of 340 cm; average air temperature is -7 ºC in January and 19 ºC in July. Water temperatures across three Eastern PEI sampling sites in the vicinity of the facilities (Basin Head, Montague and Brudenell River, and Murray River) ranged from 8.2 °C to 22.0 °C, salinity ranged from 23 to 29 ppt, and dissolved oxygen (DO) ranged from 5.8 to 9.9 mg/L (Weldon *et al.*, 2008).

The local environment near the ABT facilities has numerous shallow bays, broad estuaries, and short rivers that contain an abundance of favorable habitat for diadromous fishes, those species that use both marine and freshwater habitats at some time during their lifecycle. Fish common to the area include the following: mackerel; herring; eel; gaspereau (e.g., alewife & blueback herring); silverside; smelt; and salmonids. The salmonid fishes found on PEI include the following: Atlantic salmon (*S. salar*) and brook trout (*Salvelinus fontinalis*), which are native to the region; and, rainbow trout (*O. mykiss*), which were introduced into the region in 1925. Brown trout (*Salmo trutta*), though not native to North America but widely

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[60] https://climatlantic.ca/ (accessed December 8, 2023)

introduced into Canada and the US, do not occur on PEI (DFO, 1988). Commercially important crustaceans include lobster and snow crab; bivalves (e.g., mussels, oysters, soft-shelled & bar clams, quahogs) are also fished.

Although PEI is frequently affected by outcomes such as power outages, rain, and snowstorms from December until April, it has rarely been subject to significant weather-related damage. In 2004, 1 meter (40 inches) of snow fell in one day causing serious impacts to the area. Flooding and severe storm surges occur regularly in the vicinity of Charlottetown on the south side of PEI (Figure 7-2); however, in the vicinity of the two facilities on the northeast side of PEI, this is much less common. Only four major hurricanes were reported in PEI prior to 2000. In 2003, high winds (about 90 mph) from a hurricane caused impacts to the area. In late September 2022, post-tropical storm Fiona caused widespread wind and water damage on PEI, including a power loss to most of its residents. This storm carried heavy rains and high winds in excess of 170 km/h (105 mph). Damage included downed trees, significant beach erosion on north-facing shores, and structure loss. The ABT facilities on PEI lost primary power during this storm, but backup generators continued to supply power until primary power was restored. The ABT facilities did not sustain any other significant damage and normal operations were maintained throughout and after the event.

Two tsunamis have been reported east of Nova Scotia in the vicinity of southern Newfoundland and the Grand Banks (Figure 7-1), and one tornado has been reported in coastal New Brunswick northwest of Moncton (Figure 7-1). No avalanches, earthquakes, forest fires, hailstorms, landslides, or volcanic eruptions have been reported for PEI or the Canadian Maritime Provinces.

8.3.1.2. Description of Drainage Ditch Adjacent to the Bay Fortune Facility and Fortune River Estuary

Any effluent from the Bay Fortune facility, which hypothetically could include ABT salmon and pathogen/parasites, would discharge to a drainage ditch located adjacent to the Bay Fortune Facility. This drainage ditch is less than a meter in width. The drainage ditch begins west-southwest of the facility on a farm then flows east-northeast towards the Fortune River estuary, collecting surface water runoff from areas adjacent to the facility as well as the effluent from the Bay Fortune facility. This drainage ditch may be suitable for salmon survival for a short period of time; however, the ditch does not contain adequate food or habitat for long-term salmon survival. The drainage ditch ultimately empties into the Fortune River estuary situated approximately 36-37 m (120 ft) to the northeast of the facility discharge point.

During the spring, summer and fall, temperatures in the Fortune River estuary are suitable for salmon survival; however, water temperatures during the winter months are typically very low, with surface ice being common. The temperature of the Fortune River estuarine waters ranges from -2 to 2 ºC in the winter, with a typical ice cover of 0.3-0.6 m. Salinity in the Fortune River estuary varies with the tide, distance from the outflow, and time of year. Despite these variations, the water remains quite saline, with common salinity values exceeding 21 ppt (and up to ~30 ppt).

8.3.1.3. Description of Rollo Bay Brook Adjacent to the Rollo Bay Facility

Effluent from the Rollo Bay facility, which hypothetically could include ABT salmon and pathogens/parasites, discharges to Rollo Bay Brook, a small stream less than a meter in width with variable flow that runs through the Rollo Bay property. The brook travels approximately 1.5 km before entering Rollo Bay (which is an offshoot of the Northumberland Strait), the name of the tidal water body located between PEI and the coast of eastern New Brunswick and northern Nova Scotia (see Figure 7-1 and Figure 7-2 and additional description below). When the brook reaches Rollo Bay, it spreads into a mini-delta and crosses a sandbar prior to entering Rollo Bay and the Strait. The shallow water depth at the sandbar would provide some barrier inhibiting larger fish from entering the marine environment, particularly at low tide and during periods of low water flow from the brook.

Rollo Bay Brook is a freshwater brook until it reaches Rollo Bay where salinity increases. Water levels in the brook are quite shallow except during periods of heavy rain. ABT has measured water flows through the brook downstream of the Hatchery building ranging from 1086 L/minute to greater than 8500 L/minute during heavy rain events. Water temperatures are generally cool enough to support salmonids and a population of brook trout (*Salvelinus fontinalis*) is established in the brook, including the area near the Hatchery. Therefore, Rollo Bay Brook is suitable to support younger, smaller life stages of non-migrating salmonids for a short period of time; however, older and larger life stages are less likely to be able to migrate and survive the journey to Northumberland Strait via the Rollo Bay Brook due to shallow water in many locations, the presence of culverts and other obstacles, and a significant drop in elevation (i.e., waterfall) downstream of the culvert carrying water flow under Route 2.

8.3.1.4. Description of Rollo Bay/Northumberland Strait

The Northumberland Strait is a tidal water body that separates PEI and the coast of eastern New Brunswick and northern Nova Scotia (Figure 7-1 and Figure 7-2). The strait extends 225 km west-northwest to east-southeast from Richibucto Cape, New Brunswick, to Cape George, Nova Scotia. The Strait has a width between 13 to 43 km and a depth of 68 m at the eastern end to less than 20 m over a large central area. The Strait's generally shallow depth is associated with strong tidal currents, water turbulence, and a high concentration of suspended red silt and clay. "*Shallowness is also largely responsible for the warmest summer water temperature in eastern Canada (July, 20 °C or higher)*" (Brookes, 2018). Dissolved oxygen (DO) levels decline as temperatures increase, and water temperatures greater than 25 °C and anoxic conditions have been reported in the Northumberland Strait near Souris, PEI (see Figure 7-2) during the month of September (Coffin *et al*., 2013). Summer conditions (late July through September) at the sampling site near Souris included DO levels below 5 mg/L on numerous occasions (Coffin *et al*., 2013; van den Heuvel *et al*., 2017). Agriculture is an important industry in PEI and a source of high nitrogen loading in the estuarine waters of the province (van den Heuvel *et al*., 2017).

The Northumberland Strait could be a suitable environment to support Atlantic salmon smolts; however, the salinity is too high for younger stages of salmon (eggs to parr) to survive. In addition, there are many other undesirable environmental conditions that may preclude salmon from surviving in the Strait and adjacent waterways (e.g., warm summer temperatures, low DO, high nitrate concentrations), but those conditions would only occur during certain times of the year (mainly summer). These conditions would make the waters of the Northumberland Strait unfavorable for long-term survival of ABT salmon. The salinity in the Northumberland Strait near Rollo Bay has been reported to range from 23 to 29 ppt during the summer months (Weldon *et al*., 2008). Consequently, only smolt, juvenile, or adult ABT salmon (i.e., those with the ability to osmoregulate) would have any prospect of surviving the ambient salinity in Rollo Bay and Northumberland Strait.

8.3.2. Atlantic Canada

In the highly unlike event that ABT salmon were able to escape the facilities on PEI and enter the Gulf of St. Lawrence, they could migrate to other locations in Maritime and Atlantic Canada. The Gulf of St. Lawrence is surrounded by the Atlantic provinces in Canada. Maritime Canada includes the provinces of New Brunswick, Nova Scotia, and PEI. Atlantic Canada includes the Maritime provinces plus the eastern provinces of Newfoundland and Labrador (Figure 7-1, above).

Maritime Canada (New Brunswick, Nova Scotia, and PEI) has a mean winter air temperature range of -8 to -2 ºC and mean summer temperatures ranging from 13 to 15.5 ºC (Vasseur and Catto, 2008). The mean annual precipitation for this region is 800-1500 mm. The interior areas have a more continental climate and the coastal areas along the Bay of Fundy and PEI have cool summers and mild winters. The other parts of Atlantic Canada that surround the Gulf of St. Lawrence (Newfoundland and southeastern Labrador) have winter air temperatures ranging from -20 to -1 ºC, and mean summer air temperatures ranging from 8.5 to 12.5 ºC (Vasseur and Catto, 2008). The annual precipitation ranges from 900-2000 mm. Overall, Atlantic Canada has shown changes in temperatures due to global climate change, including an increase in temperatures in spring, summer, and fall, and slightly colder winters (Vasseur and Catto, 2008). The temperatures in Maritime Canada, where PEI is located, are projected to increase 2-4 ºC in summer and 1.5-6 ºC in winter by 2050, but the changes in temperature would be less pronounced in coastal areas (Vasseur and Catto, 2008). In addition, precipitation is also expected to increase. Newfoundland and Labrador will differ from Maritime Canada because their climate is influenced by the North Atlantic Oscillation. Recently, the trends have supported temperatures at or below average for this region (Vasseur and Catto, 2008).

The Gulf of St. Lawrence includes both estuarine and marine ecosystems and flows into the Atlantic Ocean (Figure 7-1). The coastal estuaries and the Gulf of St. Lawrence have a surface area of over 240,000 square km and the marine ecosystem receives more than half of the freshwater inputs from the Atlantic coast of North America, including drainage from the Great Lakes (DFO, 2012). The Gulf of St. Lawrence is expected to have similar temperatures to that of the northern Atlantic Ocean. The Gulf of St. Lawrence has the furthest extension of sea ice in the North Atlantic during the winter, but also has the warmest surface water temperatures in Atlantic Canada during the summer (DFO, 2012). The coastal waters of Atlantic Canada vary in salinity but are reported to be around 33 to 34 ppt (Butler *et al*. 1996). The Gulf of St. Lawrence ecosystem supports a diverse and productive biological community, including estuarine, marine, and sub-tropic to arctic species (DFO, 2012). The Gulf of St. Lawrence has known feeding grounds for Atlantic salmon off the shore of Labrador and Newfoundland, and Atlantic Canada supports many native populations of Atlantic salmon. Therefore, Atlantic Canada and the Gulf of St. Lawrence would provide suitable habitat for ABT salmon survival and possible establishment.

It is important to note that there are commercial Atlantic salmon aquaculture operations, both land-based and net pen, in Atlantic Canada. According to 2016 Canadian Government report: "*PEI does not have marine finfish grow-out sites (net cages). The estuaries that surround the Island are shallow and are not conducive to marine cage aquaculture due to seasonal temperature extremes. For this reason, the finfish aquaculture sector occurs in pond cages and land-based tank systems. Currently, the finfish aquaculture sector in PEI consists of five hatcheries and one grower, predominantly located in the eastern and central part of the province*" (Standing Senate Committee on Fisheries and Oceans, 2016). The closest known salmonid aquaculture operation is a land-based hatchery near Cardigan, PEI (see Figure 8-10(a) below). This hatchery produces Atlantic salmon smolt that are shipped to net pen farms off the coast of Newfoundland. The Center for Aquaculture Technology operates research facilities in Souris and Victoria, PEI. Both locations conduct fish health research, and both are containment certified and have BSL3 facilities. In addition, there is also land-based production of salmonids in Nova Scotia. Net-pen operations with Atlantic salmon currently exist off the coast of Newfoundland, and also off the coast of Nova Scotia (primarily on its southeastern shore). The largest concentration of net pen operations in Atlantic Canada occurs off the coast of New Brunswick, where in 2012 there were 92 marine finfish grow-out sites in southwestern part of the province, all in the Bay of Fundy (Standing Senate Committee on Fisheries and Oceans, 2016). At that time, forty-five of these sites were actively growing salmon, one was used for research, and the remaining 46 were fallow.[61]

8.3.3. Maine, US

The most likely location in the US where ABT salmon would migrate is the state of Maine because it is the closest US location to PEI that supports Atlantic salmon populations. In addition, Maine is currently the only state where native Atlantic salmon populations still exist (NOAA, 2021). It is surrounded by New Hampshire to the west and south, the Canadian provinces New Brunswick and Quebec to the north, and the Gulf of Maine and Atlantic Ocean to the southeast (Figure 7-1).

According to NOAA's National Centers for Environmental Information (2022), Maine has over 5600 km (3,500 miles) of coastline. Maine has a continental climate with mild, humid summers and cold, snowy winters. In the winter, the average air temperature ranges from -4 °C in the south to less than -9 °C in the northern and interior portions, and in the summer, the average air temperature range from 15°C in the far north to 21°C in the south (NOAA, National Centers for Environmental Information, 2022). The average annual precipitation is 106 cm, and average accumulated snowfall ranges between 101 and 203 cm, with the northern tip of the

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[61] https://salmonbusiness.com/atlantic-canada-to-maintain-status-quo-amidst-headwinds-in-bc-salmon-farming/ (accessed December 8, 2023)

state receiving up to 254 cm (NOAA, National Centers for Environmental Information, 2022).

The Gulf of Maine is surrounded by Massachusetts, New Hampshire, Maine, New Brunswick, and Nova Scotia (Figure 7-1). It is a semi-enclosed sea that includes the Bay of Fundy, the Northeast Channel, and George's Bank. The Gulf of Maine watershed (Figure 8-8) encompasses a total land area of 179,008 square km (69,115 square miles) and extends into Maine and parts of Canada (Thompson, 2010). The watershed is divided into 27 major watersheds and the major river drainages include Merrimack, Androscoggin, Kennebec, Penobscot, St. Croix, Petitcodiac, Shubenacadie, and Saint John rivers (Benoy *et al*., 2016). The Gulf of Maine's waters cover 93,000 sq km of ocean and 12,000 km of coastline (Gulf of Maine Council on the Marine Environment, undated). The average depths of portions of the Gulf of Maine include 150 m deep in the inner lowland area, 40 m deep in George's Bank, and 190 km deep in the Bay of Fundy (Thompson, 2010). According to Thompson (2010), "*The temperature, salinity, density, and nutrient content of the water across the Gulf of Maine vary enormously depending on the location, time of year and water depth.*" All of these factors are heavily influenced by the Labrador Current and Gulf Stream (Thompson, 2010). It has been reported that the temperatures and water levels in the Gulf of Maine have increased over the years due to climate change (Thompson, 2010; Benoy *et al*., 2016).

The cold waters, extreme tidal mixing, and diverse geography and hydrology of the gulf make it one of the most diverse and productive marine environments in the world (Sherman and Skjoldald, 2002 as cited in Thompson, 2010), and it furnishes habitat for over 3000 marine species and birds (Gulf of Maine Council on the Marine Environment, undated). As stated above, Maine is the only state in the US where native Atlantic salmon populations still exist; therefore, ABT salmon could, hypothetically, survive in Maine's waters, if they were able to escape and migrate there. In addition to Atlantic salmon, Maine's environment supports populations of many other fish species, including many piscivorous species such as introduced brown trout, largemouth and smallmouth bass, chain pickerel, lake trout, and rainbow trout. Predators include cormorants, harbor seals and gray seals (Baum, 1997).

Figure 8-8 Map of Gulf of Maine Watershed*

* Map created by Richard D. Kelly, Jr., Maine State Planning Office, for The Gulf of Maine Council on the Marine Environment; http://www.gulfofmaine.org/2/resources/maps-and-images-of-the-gulf/ (accessed December 8, 2023)

8.4. Status of Atlantic Salmon in the Accessible Environments

The historical range of the North American Atlantic salmon (found in eastern Canadian and northeastern US waters) ranged from northern Quebec to Newfoundland, and southwest to Long Island Sound (Figure 8-1, above). At one time, they could be found in almost every river northeast of the Hudson River in the State of New York (NOAA, undated(a)). However, populations in this region have declined to critically low levels, with many of the Atlantic salmon populations in this region either extirpated (locally extinct) or at risk. The historical and current status of Atlantic salmon in Atlantic Canada, PEI, and Maine is described below. This information will be used in the risk assessment to discuss the likelihood that ABT salmon could harm native North American Atlantic salmon populations in Maine.

8.4.1. Status of Atlantic Salmon in Atlantic Canada

Atlantic salmon inhabit the rivers and bays along eastern Canada and range from the St. Croix River at the border of Maine, US, to the outer Ungava Bay in Quebec (Figure 8-9, COSEWIC, 2010). Historically, Atlantic salmon once occupied at least 700 rivers in eastern Canada, as well as Lake Ontario (COSEWIC, 2010), but populations in the southern part of eastern Canada have been declining (Knapp *et al*., 2007).

In the 1970s, approximately 1.5 million salmon returned to their natal rivers in Eastern Canada; by the early 2000s, that number had dropped to approximately 350,000 (Knapp *et al*. 2007). Between 1971 and 1985, the estimated abundance of Atlantic salmon grilse (1 year at sea or 1SW) in North America fluctuated between 0.8-1.7 million fish annually; between 1995 and 2006, the estimated abundance declined to about 0.4-0.7 million fish (DFO, 2009). According to DFO (2009), "*When pronounced declines in abundance were observed in the 1980s, a wide range of management measures were introduced for conservation purposes. The closures of commercial fisheries, which began in 1972 in strategic intercepting and terminal fisheries were expanded in 1984 to include all the commercial fisheries of the Canadian Maritime Provinces and portions of Québec. Also, in 1984, mandatory catch and release in the recreational fisheries of all large salmon was introduced in the Maritime Provinces and insular Newfoundland*." Closure of all commercial fisheries for Atlantic salmon was expanded to all of eastern Canada in 2000.

Atlantic salmon status and abundance in Canada has been assessed by the Committee on the Status of Wildlife in Canada (COSEWIC). "*COSEWIC was created in 1977 to provide a single, scientifically-sound classification of wildlife species at risk of extinction. Each year it meets to assign risk categories for all native mammals, birds, reptiles, amphibians, fish, arthropods, mollusks, vascular plants, mosses, and lichens included in its current mandate. As an independent, arms-length advisory panel to the Minister of Environment and Climate Change Canada, members are wildlife biology experts drawn from academia, government, non-governmental organizations and the private sector.*"[62] The Canadian Species at Risk Act (SARA) established COSEWIC as an independent, non-governmental advisory body in 2003. The government of Canada takes their designations into consideration when establishing the country's official list of wild species at risk. COSEWIC's results are reported to the government and public in assessment reports, and wildlife species that have been designated by COSEWIC may qualify for legal protection and recovery under SARA. It is important to note that a wildlife species may be designated by COSEWIC as endangered, but not be designated as such under SARA.

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[62] https://cosewic.ca/index.php/en/about-us.html (accessed December 8, 2023)

In the most recent COSEWIC assessment of Atlantic salmon populations in Canada prepared in 2010, 16 Designatable Units (DUs)[63] of Atlantic salmon in eastern Canada were defined based on genetic data and broad patterns in life history variation, environmental variables, and geographic separation (COSEWIC, 2010). The 16 DUs are shown on Figure 8-9, below. These DUs were defined by geographical boundaries, and each was assessed for its status to the level of risk of extirpation (extinct, extirpated, endangered, threatened, special concern, not at risk, or data deficient). The next COSEWIC assessment of the status of Atlantic salmon was under revision at the time is amended EA was prepared.[64] The current status of aquatic species at risk in Canadian waters can be found at: http://www.dfo-mpo.gc.ca/species-especes/sara-lep/identify-eng.html (accessed December 8, 2023).

The northern most populations of Atlantic salmon (inhabiting northern Newfoundland and Labrador) are currently the only populations in Canada that are considered "not at risk" according to COSEWIC's 2011 evaluation (except for Nunavick (DU1), which is considered data deficient). Of the other DUs, five were assessed as "endangered", one as "threatened" and four as "special concern." However, only the Inner Bay of Fundy population (DU15 in Figure 8-9) was legally listed by the Canadian Government as endangered under SARA (see current status at link provided in paragraph above), and the landlocked Lake Ontario population (DU11) is considered extinct as of May 2006 (COSEWIC, 2010).

COSEWIC's 2010 designations for each DU are shown in Table 8-2 below. The Atlantic salmon population on PEI is part of DU12, Gaspé-Southern Gulf of St. Lawrence population segment, and is designated as special concern, which is defined as "*a wildlife species that may become a threatened or an endangered species because of a combination of biological characteristics and identified threats*" (COSEWIC, 2010). DU12 is listed as population segment 5 in Table 8-2 (below). Additional information on the status of Atlantic salmon on PEI is discussed in the next section.

There are four DUs that reside between PEI and the US border; all four are considered endangered according to the COSEWIC 2010 assessment, including DU13 (Eastern Cape Brenton), DU14 (Nova Scotia Southern Upland); DU15 (Inner Bay of Fundy); and DU16 (Outerbay of Fundy). COSEWIC defines endangered as "*a wildlife species facing imminent extirpation or extinction*" (COSEWIC, 2010). These are listed as population segments 1-4 in Table 8-2 (below). The 2010 COSEWIC assessment states that DU 14, 15 and 16 are at or near their lowest abundance on record.

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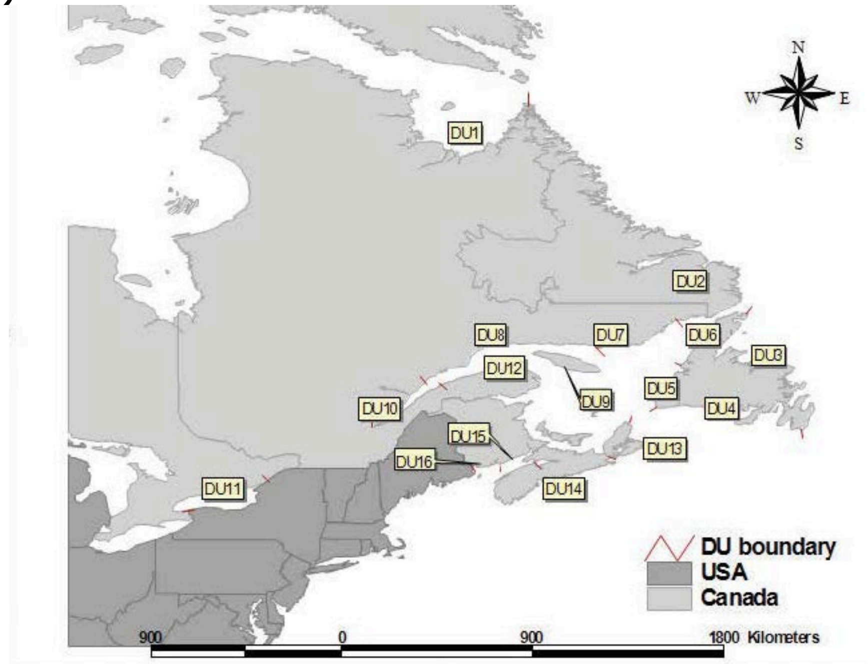
[63] "*[A] population or group of populations may be recognized as a DU if it has attributes that make it 'discrete' and 'evolutionarily' significant relative to other populations*." (COSEWIC, 2010)

[64] COSEWIC reassesses species previously designated in a category of risk every 10 years (or earlier if warranted); https://cosewic.ca/index.php/en-ca/status-reports.html (accessed December 8, 2023).

Figure 8-9. Map Illustrating the 16 Designatable Units (DUs) Defined in the 2011 COSEWIC Assessment Report* (names of the DUs are listed below figure)



* Figure obtained from Figure 9 (page 23; COSEWIC, 2010)
Key to DUs: (1) Nunavik; (2) Labrador; (3) Northeast Newfoundland; (4) South Newfoundland; (5) Southwest Newfoundland; (6) Northwest Newfoundland; (7) Quebec Eastern North Shore; (8) Quebec Western North Shore; (9) Anticosti Island; (10) Inner St. Lawrence; (11) Lake Ontario; (12) Gaspé-Southern Gulf of St. Lawrence; (13) Eastern Cape Brenton; (14) Nova Scotia Southern Upland; (15) Inner Bay of Fundy; (16) Outer Bay of Fundy.

Table 8-2. Status of Atlantic Salmon Populations in Atlantic Canada Assigned by COSEWIC (2011)

Population Segment	Location	Status
1	Outer Bay of Fundy	Endangered
2	Inner Bay of Fundy	Endangered
3	Southern Uplands	Endangered
4	Eastern Cape Breton	Endangered
5	Gaspé-Southern Gulf of Lawrence	Special Concern
6	Inner St. Lawrence	Special Concern
7	Quebec Western North Shore	Special Concern
8	Quebec Eastern North Shore	Special Concern
9	Anticosti Island	Endangered
10	South Coast Newfoundland	Threatened
11	Southwest Newfoundland	Not at Risk
12	Northeast Newfoundland	Not at Risk
13	Labrador	Not at Risk
14	Nunavik (Ungava Bay)	Data Deficient
15	Lake Ontario	Extinct

*Atlantic Salmon Federation illustrates this in a figure on page 5 of the June 2018 State of the North American Atlantic Salmon Population report.

8.4.2. Status of Atlantic Salmon on PEI, Canada

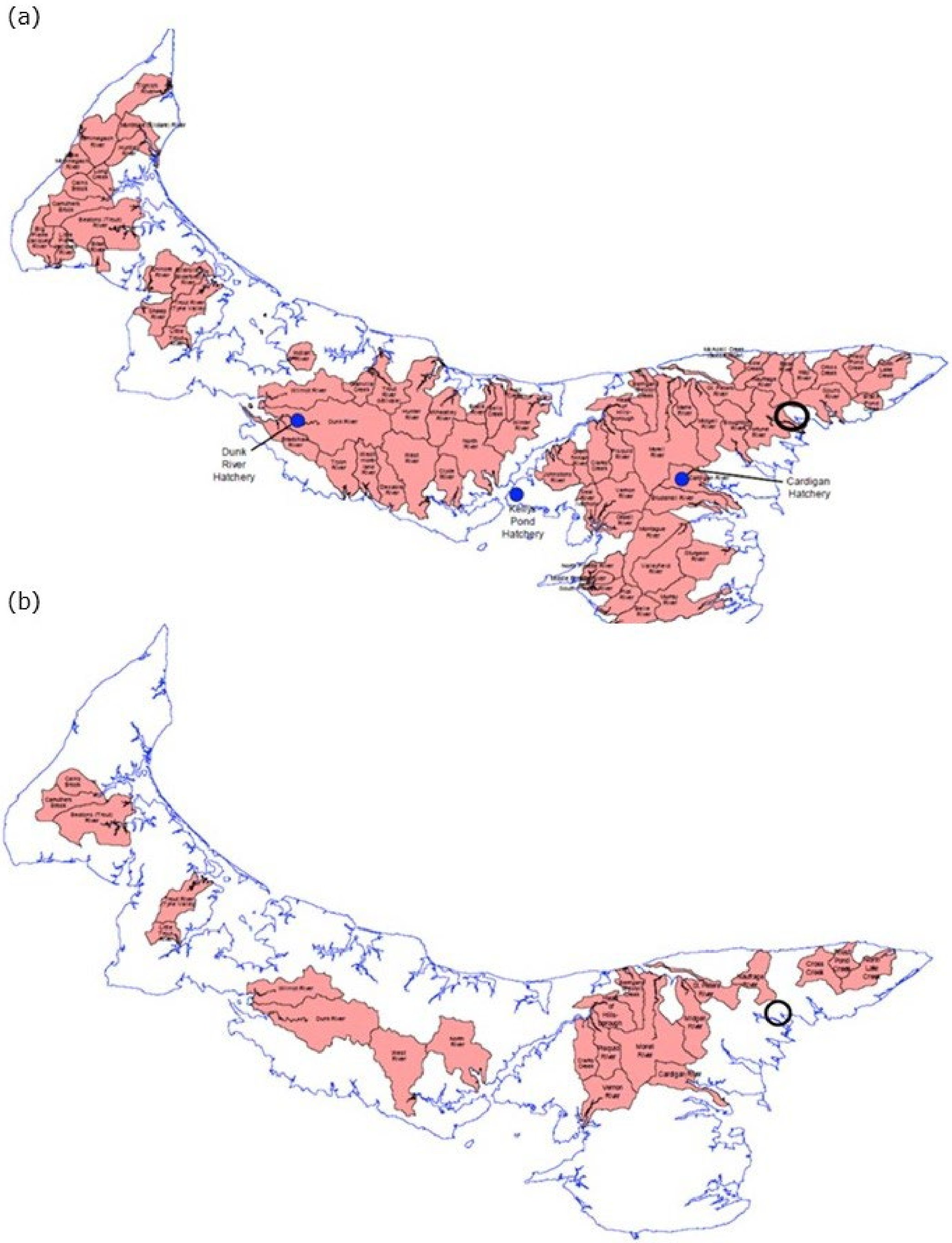
As stated above, PEI is part of the Gaspé-Southern Gulf of St. Lawrence population segment (DU12) designated as "special concern" in the 2011 COSEWIC assessment (see Figure 8-9, and #5 in Table 8-2). This DU encompasses the entire southern Gulf of St. Lawrence and PEI. It extends from the Ouelle River in western Gaspé to the northern tip of Cape Brenton (COSEWIC, 2010). In this DU, there are 78 rivers, spread over four provinces (Quebec, PEI, Nova Scotia and New Brunswick), that contain salmon populations (COSEWIC, 2010). COSEWIC (2011) reports an abundance of 103,149 adult spawners for this DU in 2007. COSEWIC (2011) notes that juvenile Atlantic salmon are sparse, and densities are low on PEI; and notes that of particular concern in PEI is habitat degradation due to land use issues. On PEI, Atlantic salmon populations are listed as "may be at risk" (Guignion *et al*., 2010).

According to Cairns *et al*. (2010), salmon likely inhabited all rivers on PEI at one time except smaller creeks that could not provide adequate habitat for spawning or rearing. Guignion (2009) reports that prior to European settlement, it is believed that there were approximately 70 rivers on PEI that contained suitable habitat for Atlantic salmon. By 2008, only 22 large streams on PEI still supported Atlantic salmon, with seven of those populations considered to be precariously low (Guignion, 2009; Cairns and MacFarlane, 2015). Salmon restocking and habitat enhancement have been pursued with some success in the Province, and in 2013, salmon occupied approximately 26 rivers on PEI (Cairns and MacFarlane, 2015), and number of returning salmon continues to improve (Oak Meadows, Inc., 2017). The historic watersheds on PEI that contained Atlantic salmon at the beginning of European settlement are illustrated in Figure 8-10(a), and the watersheds that still contained Atlantic salmon as of 2007-2008 are illustrated in Figure 8-10(b). The location of ABT's two PEI facilities, Bay Fortune and Rollo Bay, are identified in these figures by the black circle.

Based on Cairns *et al*. (2010), Atlantic salmon populations never inhabited the watershed in which the Rollo Bay facility resides. However, they did inhabit the Fortune River watershed, in which the Bay Fortune facility resides, but have not been found there since prior to the 2000s. The Atlantic salmon status in the waterbodies that sit adjacent to ABT's PEI facilities, Fortune River and Rollo Bay Brook, are discussed in more detail below.

<u>Fortune River and Estuary:</u> Atlantic salmon are no longer found in the Fortune River and estuary. Although reported to be present there naturally in the late 1800s and stocked in this river periodically from 1907 to at least 1937, and perhaps later (Cairns *et al*., 2010), they disappeared at some point thereafter and were not present in surveys conducted there in the 1980s, in 2001, and in 2008 (Cairns *et al*., 2010; Guignion, 2009; Guignion *et al*., 2010). According to Guignion *et al*. (2010), "*The Fortune River was once well known for its salmon run, but during the 1980s when more than 150 beaver dams were removed, no evidence of Atlantic salmon were found over three years of sampling*." Although present in 19 other streams on PEI, rainbow trout are also not reported to occur in the Fortune River, although brook trout are (Guignion *et al*., 2010).

Figure 8-10. Watersheds of PEI that Likely Contained Atlantic salmon (a) at the Beginning of European Settlement and (b) Based on 2007 or 2008 Surveys (Cairns *et al*., 2010)*



* A black circle surrounds the locations of ABT's PEI facilities on the northeastern shore of PEI.

<u>Rollo Bay Brook and Rollo Bay:</u> According to the information in Cairns *et al*. (2010), it appears that the watershed surrounding the Rollo Bay facility never contained Atlantic salmon, except for one time when it was stocked with 30,000 advanced fry

in 1933. The two rivers closest to Rollo Bay that have been sampled, the Souris and Fortune,[65] do not currently contain resident Atlantic salmon populations (Cairns and MacFarlane, 2015).

The Bay Fortune and Rollo Bay facilities are approximately 64 nautical miles (NM)[66] via water from Cheticamp, Nova Scotia, the approximate closest point within the area for the Gaspé-Southern Gulf of St. Lawrence population of Atlantic salmon, which is listed as an area of special concern; ~400 NM via water from the South Coast of Newfoundland population segment that is listed as threatened; and ~580 NM via water from the Inner Bay of Fundy population which is listed as endangered.

Over-exploitation, competition from non-native rainbow trout, and other factors have contributed to the elimination of natural Atlantic salmon runs in the environs of the PEI production sites; however, the current primary limitations to population recovery are believed to be stream sedimentation caused by agriculture and other land-use activities and blockages from beaver dams (Cairns *et al*., 2010; Guignion, 2009). Man-made and beaver blockages in the Grovepine branch of the Fortune River have caused summer temperatures to exceed tolerable levels for salmonids and oxygen levels to likewise fall below minimum accepted concentrations (Guignion, 2009). As a result, water quality is compromised in much of the main branch of the Fortune River, down to the head of tide. Restocking and habitat enhancement in PEI streams and rivers have been attempted with limited success. As a practical matter, few, if any, native salmon populations remain, and future returns of salmon to local rivers are dependent on hatchery stocking of smolts raised semi-naturally in open impoundments.

8.4.3. Status of Atlantic Salmon in Northeastern US and the State of Maine

Historically, Atlantic salmon were native to most US rivers from the Hudson River north to the Canadian border (NOAA, 2022); however, populations began to decline rapidly in the 19th century (NRC, 2004b). In the 1800s, Atlantic salmon became extinct in the Connecticut (CT), Merrimack (MA), and Androscoggin (NH, ME) rivers mostly likely due to the results of dam building to harness the energy of the water (NRC, 2004b). These dams blocked access of the fish to their natal streams, and thus, their spawning areas. Industrial pollution also contributed to the decrease in populations, as did commercial overfishing and climate changes that affected the temperature of the water in the ocean at the depths at which Atlantic salmon are found (2-10 m below the surface) (NOAA, undated(a)). (Atlantic salmon need clear, sediment-free water and cold temperatures to survive.)

Table 8-3 lists the current locations of populations of Atlantic salmon in the Northeastern US. Atlantic salmon are no longer found in 84% of the rivers in New England that historically supported salmon (Knapp *et al.,* 2007). They are in "critical condition" in the remaining 16% (Knapp *et al.*, 2007). Currently, the Atlantic salmon

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[65] The Souris watershed was sampled in 2000, 2001, 2002, 2005, 2006, 2008 and 2012 and Fortune watershed was sampled in 2001 and 2008.

[66] Approximate distances derived from electronic chart data information.

populations in the US are grouped into three distinct populations segments (DPS): Long Island Sound, Central New England, and the Gulf of Maine (NRC, 2004b). The native populations in the Long Island Sound and Central New England DPS are extirpated; however, non-native populations exist in these waters due to reintroduction programs in the Connecticut and Merrimack Rivers (NOAA and FWS, 2020). Federal stocking programs were ended in 2013, but there are still state-supported programs to maintain these stocks. Therefore, currently, the Gulf of Maine DPS contains the only native Atlantic salmon populations in the US (NOAA and FWS, 2020).

Table 8-3. Locations of Atlantic Salmon Populations in the US Northeast

River	State	Status	Date Listed
Aroostook	Maine	Not Listed	N/A
Meduxnekeag	Maine	Not Listed	N/A
Prestile Stream	Maine	Not Listed	N/A
Saint Croix	Maine	Not Listed	N/A
Dennys	Maine	Endangered	2000
East Machias	Maine	Endangered	2000
Machias	Maine	Endangered	2000
Pleasant	Maine	Endangered	2000
Narraguagus	Maine	Endangered	2000
Tunk Stream	Maine	Not Listed	N/A
Union	Maine	Not Listed	N/A
Penobscot	Maine	Endangered	2009
Cove Brook	Maine	Endangered	2000
Ducktrap	Maine	Endangered	2000
Sheepscot	Maine	Endangered	2000
Kennebec	Maine	Endangered	2009
Androscoggin	Maine, New Hampshire	Endangered	2009
Saco	Maine, New Hampshire	Not Listed	N/A
Cocheco	Maine, New Hampshire	Not Listed	N/A
Lamprey	Maine, New Hampshire	Not Listed	N/A
Merrimack	New Hampshire	Not Listed	N/A
Pawcatuck	Rhode Island	Not Listed	N/A
Connecticut	Connecticut, Massachusetts, New Hampshire and Vermont	Not Listed	N/A

N/A = not applicable

Today, very few rivers in Maine support wild Atlantic salmon. In 2000, the NOAA’s NMFS and the US FWS listed Atlantic salmon in the Gulf of Maine DPS as “endangered” under the ESA (65 FR 69459, November 17, 2000).[67] This listing included naturally reproducing populations of Atlantic salmon in eight river systems ranging from the Kennebec River (downstream of the former Edwards Dam site) and northward to the mouth of the St. Croix River (NOAA and FWS, 2020), see Table 8-3 (above). That designation was expanded in 2009 to include nine distinct breeding

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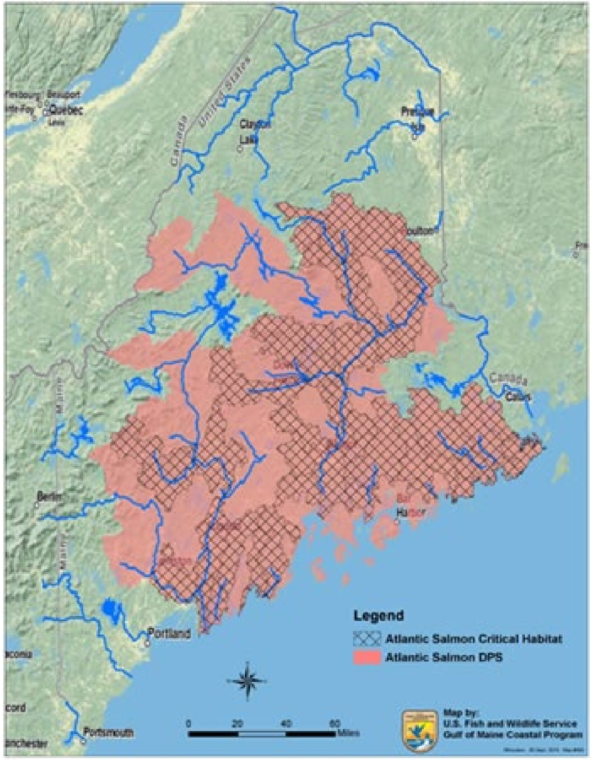
[67] 65 FR 69459. (2000, November 17). Endangered and Threatened Species; Final Endangered Status for a Distinct Population Segment of Anadromous Atlantic Salmon (*Salmo salar*) in the Gulf of Maine.

populations in the watersheds extending from the Androscoggin River northward to the Denny's River (74 FR 29344, June 19, 2009)[68] and its critical habitat (74 FR 29300, June 19, 2009).[69] Figure 8-11 (below) shows the geographic boundary of the Gulf of Maine DPS and it's designated critical habitat, and Table 8-3 (above) shows the locations of rivers that support the endangered Gulf of Maine DPS. The marine range of this designation extends from the Gulf of Maine throughout the Northwest Atlantic Ocean to Greenland (NOAA and FWS, 2020). The 2009 rule also listed four major threats to the Gulf of Maine DPS, including dams, inadequacy of regulatory mechanisms related to dams, low marine survival, and a number of secondary stressors (NOAA and FWS, 2020). The threats to this species have expanded to now include road stream crossings that impede fish passage, international intercept fisheries, and climate change (NOAA and FWS, 2020).

Figure 8-11. The Geographic Boundary of the Gulf of Maine Distinct Population Segment of Atlantic Salmon and Designated Critical Habitat*



* US FWS, Gulf of Maine Coastal Program. This map has since been replaced by an interactive map on US FWS website, https://www.fws.gov/species/atlantic-salmon-salmo-salar (accessed December 8, 2023).

The endangered designation for the Gulf of Maine DPS was upheld in the most recent review by NOAA NMFS and FWS in 2020 (NOAA and FWS, 2020). This review found

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[68] 74 FR 29344. (2009, June 19). Endangered and Threatened Species; Determination of Endangered Status for the Gulf of Maine Distinct Population Segment of Atlantic Salmon.

[69] 74 FR 29300. (2009, June 19). Endangered and Threatened Species; Designation of Critical Habitat for Atlantic Salmon (*Salmo salar*) Gulf of Maine Distinct Population Segment.

that the Atlantic salmon populations in the Gulf of Maine DPS have been at "*critically low abundance*" (NOAA and FWS, 2020). The 2020 assessment reported a 10-year average return of 1247 Atlantic salmon adults to the three Salmon Habitat Recovery Units (SHRU)[70] in the Gulf of Maine DPS.[71] This total included both wild/naturally reared adult Atlantic salmon and hatchery reared adults. Seven of the eight locally adapted populations in the DPS are "*supported by conservation hatcheries to buffer the extinction risk*", and the eighth population is "*at very high risk of extirpation*" (NOAA and FWS, 2020). Of the adults that that returned, 84% were hatchery-reared adults (1048 of 1247) and the remaining 16% were wild/naturally-reared (199 of 1247). In addition, the average 10-year return of wild/naturally-reared Atlantic salmon in each of the three SHRUs is below 100 adult spawners, which is well-below the threshold needed to down list their status to threatened (i.e., 500 adult spawners return to each of the three SHRUs). NOAA and FWS (2020) found that "*These very low populations can significantly increase risk to genetic fitness, loss of adaptive traits and reduced ability to withstand catastrophic events*." Of the three SHRUs monitored, Penobscot Bay had the greatest 10-year average returns, accounting for 85% of the total returns of both hatchery and wild/naturally reared Atlantic salmon. However, the 10-year average return for wild/naturally-reared adult salmon for Penobscot Bay was still only 98 adults (in contrast, the 10-year average return for hatchery reared adult was 973).

In the 2020 review, NOAA and FWS also found the population growth rate has improved in all SHRUs and the quantity of suitable and accessible habitat for spawning and juvenile rearing in Maine has increased due to dam removals and improvements in fish passage. According to NOAA and FWS (2020), these two criteria could support down listing to "threatened" if the abundance level was not so low. Figure 8-12 illustrates the distribution of accessible habitats with respect to dams (note: it does not account for stream segments that may be blocked by culverts).

The US Atlantic Salmon Assessment Committee (USASAC) reported similar findings for the 2020 annual salmon return rates at meeting held in 2021 (USASAC, 2021). In 2020, a total of 1715 Atlantic salmon returned to US rivers. This number was estimated by returns to traps and redd counts for three meta-population areas: Long Island Sound (0 returns), Central New England (10 returns), and Gulf of Maine (1705 returns). Of these, 78% (1322) were of hatchery-stocked origin. Overall, the returns remain critically low and have not met the interim recovery target of 500 naturally reared returns per SHRU.

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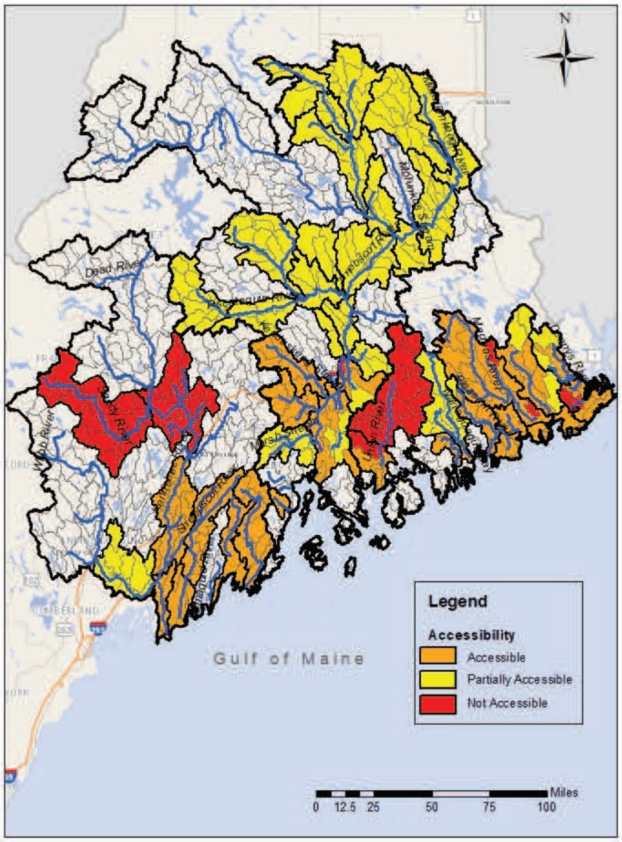
[70] The DPS is divided into three SHRUs, which include Merrymeeting Bay (the Androscoggin and Kennebec Rivers east to the St. George River), Penobscot Bay (the Penobscot basin extending west to the Ducktrap River), and Downeast Coastal SHRU (all coastal watersheds from the Union River east to the Dennys River); see Figure 8-12.

[71] Population status is estimated by counting the number of adults that return to spawn. They are counted using traps, weirs, net surveys or modeling. Yearly assessment biomass estimates can be accessed at NOAA Fisheries, Stock SMART (Status, Management, Assessments & Resource Trends), https://www.st.nmfs.noaa.gov/stocksmart?stockname=Atlantic%20salmon%20-%20Gulf%20of%20Maine&stockid=10498 (accessed December 8, 2023).

Figure 8-12. Distribution of Accessible Habitats within the Designated Critical Habitat*



* NOAA and FWS. November 23, 2020. Atlantic salmon (*Salmo salar*) 5-Year Review: Summary and Evaluation. https://www.fisheries.noaa.gov/resource/document/atlantic-salmon-5-year-review (accessed December 8, 2023). Note: This map shows accessibility with respect to dams but does not account for stream segments that may be blocked or impeded by culverts.

US regulations also prohibits the domestic harvest of Atlantic salmon. Specifically, possession of wild Atlantic salmon caught (even incidentally) in federal waters is prohibited, and those "*caught incidentally in other fisheries must be released in a manner that ensures maximum probability of survival*" (NOAA, undated(b)). In addition, only farm-raised Atlantic salmon are sold in US markets as of 1948 (NOAA, undated(b)).[72] However, there are no international laws or regulations prohibiting the harvest of Atlantic salmon of US origin from the Atlantic Ocean; therefore, these fish could be harvested in the West Greenland and St. Pierre and Miquelon fisheries during migration to or at winter feeding grounds (NOAA and FWS, 2020). International Council for the Exploration of the Sea (ICES) has recommended to the

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[72] Farmed Atlantic salmon are reared in net pens at several locations in northeastern Maine. These sites are licensed by the State of Maine, and aquaculture permits for these sites prohibit the rearing of GE Atlantic salmon. In addition, the FDA-approved AAS label also prohibits the rearing of AAS in net pens. Therefore, ABT salmon could not be raised in these net pens.

North Atlantic Salmon Conservation Organization (NASCO)[73] that no Atlantic salmon of US origin be harvested, and the US is engaging through NASCO to eliminate these harvests (NOAA and FWS, 2020). Other factors contributing to reduced marine survival of Atlantic salmon remain largely unknown, although significant factors affecting survival in fresh water include acid rain, poaching, habitat alteration, and agricultural activities.

9. RISK ASSESSMENT

This amended EA was prepared to address the Court's determinations discussed in its November 5, 2020, order, regarding FDA's NEPA and ESA evaluations for the 2015 approval of NADA 141-454 concerning AAS. The Court held that "*on remand the FDA must":*

1. *"…complete the final step of its own risk analysis by addressing the consequences that would result from the engineered salmon successfully establishing a persistent population outside of captivity*", and
2. *"…reconsider its "no effect" determination under the ESA together with its revised NEPA evaluation*."

This section re-evaluates under NEPA the risk of significant environmental impacts occurring in the US environment using the information provided in the sections above, the 2015 and 2019 EAs, and new literature published since the 2019 EA was prepared. In addition, the potential effect on the endangered Atlantic salmon of the Gulf of Maine DPS is also re-evaluated under the ESA in light of this revised and expanded NEPA evaluation. FDA's ESA determination is presented in Appendix I.

It is important to note that the current risk assessment is expanded from the assessment completed in the 2015 EA. It not only considers the production of AAS eyed-eggs at the Bay Fortune facility, which was the subject of the 2015 EA and November 2020 Court order, but also expands that analysis to include approved egg production in the Hatchery Unit at the Rollo Bay facility, which was evaluated in the 2019 EA. Further, this assessment also includes the planned expansion at the Rollo Bay facility of two new broodstock units there to be known as Broodstock Unit 1 and 2, as well as planned future changes to consolidate egg incubation at the Bay Fortune facility within a new egg incubation room (see Section 7 above). As a reminder, this EA does not evaluate production at the former Panama facility or the Indiana facility (see Section 3 above for additional explanation).[2,24]

9.1. Scope and Approach to the Risk Assessment

The approach to this assessment was outlined previously in Section 4. This assessment follows the recommended risk assessment principles from NRC (2002

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[73] NASCO is an international organization established in 1984 with members from the US and other North Atlantic Nations (Canada, Denmark, European Union, Norway, the Russian Federation, and the United Kingdom) to conserve, restore, enhance and rationally manage Atlantic salmon through international cooperation and the best available scientific information (https://nasco.int/, accessed December 8, 2023). ICES is a research unit of NASCO that provides scientific advice on the conversation of North Atlantic salmon stocks.

and 2004a), which describe risk [R] as the joint probability of exposure [*P(E)*], and the conditional probability of harm given that exposure has occurred [*P(H|E)*]; R = *P(E)* × *P(H|E)*. Herein, *exposure* is defined as establishment and/or presence of ABT salmon or their progeny in the environment; *harm* is defined as an adverse effect to the environment due to the hazard (in this case, ABT salmon). Herein, the term "harm" is considered synonymous with adverse consequence, effect, or impact.

Using the recommendations in NRC (2002 and 2004a) and the problem formulation presented in Figure 4-1 (above), five risk-related questions were developed in Section 4.4. As discussed in Section 4.4, the risk-related questions from the 2015 and 2019 EAs were re-evaluated and revised herein following the Court's remand decision. The five risk-related questions used in this assessment are repeated below and addressed in the following sections.

1. What is the likelihood that AquAdvantage Salmon or AquAdvantage Broodstock will escape the conditions of confinement? (addressed in Section 9.2)
2. What is the likelihood that AquAdvantage Salmon or AquAdvantage Broodstock will survive and disperse if they escape the conditions of confinement? (addressed in Section 9.2)
3. What is the likelihood that AquAdvantage Salmon or AquAdvantage Broodstock will reproduce and establish if they escape the conditions of confinement, survive and disperse? (addressed in Section 9.2)
4. What are the identified potential harms to, or effects on, the US environment if AquAdvantage Salmon or AquAdvantage Broodstock establish and/or are present? What is the likelihood of these potential harms occurring assuming exposure in the US environment has occurred? (addressed in Section 9.3)
5. What is the risk that the potential harms to, or effects on, the US environment would occur given the likelihood of exposure in the US environment? (addressed in Section 9.4)

Questions 1-3 address the likelihood of exposure, *P(E),* of ABT salmon or their progeny in the US environment by evaluating the likelihood of escape, survival dispersion, reproduction, presence, and establishment. Questions 1-3 are answered in Section 9.2 (Exposure Analysis) based on the information provided in the 2015 and 2019 EAs and were also updated based on new information, including the new exposure pathway analysis provided in Section 8.2 and Figure 8-4 and Figure 8-5.

Revised Question 4, related to *P(H|E)*, is addressed in Section 9.3 (Harms Identification and Analysis) by characterizing and explaining the potential harms (adverse consequences, effects, or impacts) to the US environment assuming that ABT salmon escape ABT's facilities located on PEI, Canada, and establish and/or are present in the Maine environment. Finally, in Section 9.4 (Risk Characterization), the risk of significant environmental impacts occurring in the US environment, including impacts on endangered Atlantic salmon in the Gulf of Maine and coastal areas, is re-evaluated based on the answers to Questions 1-4 and the risk principles from NRC (2002 and 2004a), R = *P(E)* × *P(H|E)*.

In addition, the likelihood of pathogen and/or parasite transmission via the pathways outlined in Section 8.2.2 above will be evaluated in Section 9.2.4. The potential harms and risk for this pathway will also be characterized in Sections 9.3 and 9.4, respectively.

As a reminder, NEPA does not require analysis of effects on the environment in foreign sovereign countries, such as Canada (see Section 3).[25,26] In this section, we have considered the potential for survival, dispersal, reproduction, establishment, and pathogen/parasite transmission in Canada, but only in the context that these events are involved in the pathways that could potentially result in exposure and effects in the US environment. Effects in Canada are not considered herein; however, an independent environmental evaluation was conducted by the Canadian government prior to approval of the NSN that determined that with the containment measures in place at the facilities, the AAS were considered "non toxic" (see Section 2.3).

Further, the FDA action is limited to a NADA approval under a specific set of conditions of use, i.e., production of AAS eggs at the Bay Fortune and Rollo Bay facilities on PEI Canada. As previously stated, any modifications that the sponsor may propose to the conditions established in the original and supplemental NADAs require notification to FDA. Major and moderate changes require the filing, review, and approval of a supplemental NADA. Approvals of such supplemental applications constitute agency actions (21 CFR 25.20(m)) and trigger additional environmental analyses under NEPA. For example, two additional EAs were prepared for the supplemental approvals of the Indiana facility in 2017 and the Rollo Bay facility in 2019 under NADA 141-454 (see Section 2.2). Therefore, any future changes to the NADA, including the addition of Broodstock Units 1 and 2 at the Rollo Bay facility and new incubation room at the Bay Fortune facility, would require additional filings and reviews under NEPA and the FD&C Act, and potentially under the ESA.

9.2. Exposure Analysis

As stated above in Section 9.1, Risk-related Questions 1-3 evaluate the likelihood of exposure, *P(E)*, of ABT salmon or its progeny in the US environment. Herein, exposure was defined as establishment and/or presence of ABT salmon or their progeny in the environment (Section 4.1, above). This assessment is primarily focused on the potential for exposure in the Maine environment as explained in Section 8.2.1. The assessment also evaluates the potential for pathogen/parasite transmission from ABT salmon and ABT's PEI facilities as described in Section 8.2.2 above. The likelihood of disease transmission will be evaluated separately under Section 9.2.4, below.

The possible exposure pathways and steps required for ABT salmon and their progeny to be present and establish in the Maine environment are presented in Section 8.2, above, and Figure 8-4 and Figure 8-5. The pathway analysis shows that for ABT salmon to be present in the Maine environment the following steps need to occur: ABT salmon need to escape or be released from the PEI facilities (Step 1), survive and disperse in the PEI/Atlantic Canada environment (Step 2) with the potential for reproduction and establishment in PEI/Atlantic Canada (Step 3). Following dispersal from PEI, ABT salmon would need spread or migrate to and survive in the Maine environment (Steps 4 and 5). In addition to these steps, in order for establishment to occur in the Maine environment, the ABT salmon or their progeny also need to reproduce with conspecifics or relatives of the same genus in the Maine environment (Step 6). It is important to note that presence must occur for establishment or pseudo-establishment[74] to occur, and likewise, establishment is necessary for long-term, multi-generational presence in the environment. However, presence alone is also important because even if establishment through reproduction does not occur, the presence of ABT salmon in the Maine environment could still potentially result in harms through direct interactions with ecosystem components (e.g., competition for food, habitat, mates). Genetic introgression through reproduction is not necessary for these types of harms to occur (see Figure 8-5, above, and Section 9.3, below).

The likelihood of establishment and/or presence of ABT salmon or their progeny in the US environment is dependent upon whether there is a complete pathway to exposure. This was determined by evaluating the likelihood for each step in the pathway analysis to occur using Risk-related Questions 1 through 3. The NRC (2002 and 2004a) noted that quantifying the environmental risks of GE organisms is difficult, and in some cases may be impossible, but relative qualitative rankings from high to low are possible based on available evidence for each category. Kapuscinski (2005) also recommended this approach and Canada's DFO used a similar qualitative approach in their risk assessments of the Bay Fortune and Rollo Bay facilities (DFO, 2013 and 2019, respectively). DFO (2019) defined the rankings for the likelihood of exposure to the Canadian environment (see Table 1 of the DFO Summary Report) and categorized the severity of biological effects (Table 4 of the DFO Summary Report). DFO then integrated the final rankings for exposure and effects in a risk matrix (Figures 2 and 3 of the DFO Summary Report) to categorize the risk to the Canadian environment as either negligible, low, moderate, or high. For ease in explaining the likelihood of occurrence and risk, a similar approach was used herein. In order to rank the likelihood of exposure occurring in the US environment, definitions for each likelihood ranking for exposure of ABT salmon or its progeny were developed *a priori* and are listed in Table 9-1 below.

Table 9-1. Likelihood Rankings for Exposure Analysis

Ranking	Definition
Negligible[75] likelihood	Extremely unlikely or not reasonably foreseeable occurrence
Low likelihood	An event or situation not occurring very often and not occurring in large numbers; isolated occurrence; ephemeral presence
Moderate likelihood	Occurs at certain times of the year or in isolated areas or under certain conditions
High likelihood	Often occurs at all times of the year and/or in diffuse areas

These rankings will be used in Sections 9.2.1 through 9.2.3 below to define the likelihood for each step in the exposure pathway to occur, as well as the overall likelihood of a complete exposure pathway to the Maine environment. These rankings

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[74] In addition to establishment through reproduction, a type of pseudo-establishment could potentially occur if successive waves of large numbers of salmon escaped confinement and entered the local environment, with each wave replacing or supplementing the former as fish die off or disperse (Kapuscinski and Brister, 2001).

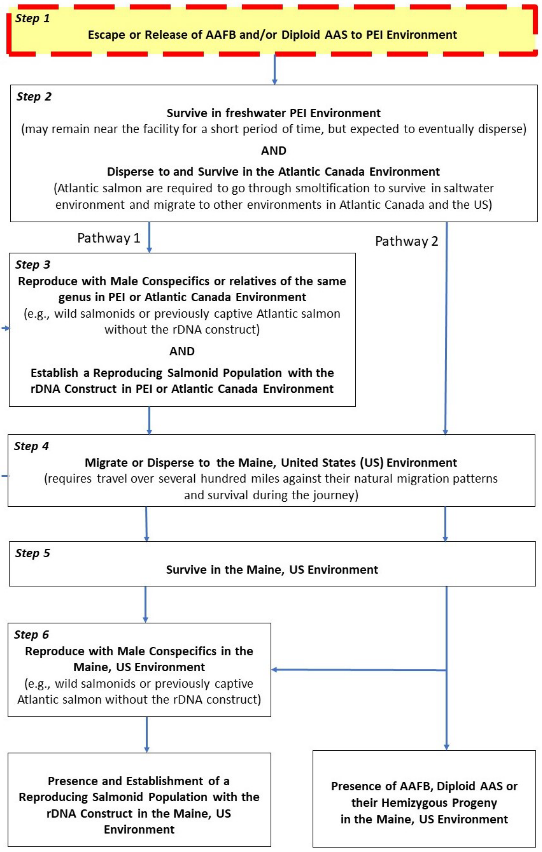
[75] Herein, negligible is synonymous with minimal, *de minimis*, very low, and extremely low.

will also be used when evaluating the likelihood of pathogen/parasite transmission in Section 9.2.4 below. A similar qualitative ranking process will be established and used for the harms analysis (Risk-related Question 4) under Section 9.3.2 below. The exposure and harms rankings will then be compared in the Risk Analysis (Risk-related Question 5) under Section 9.4 to determine the ultimate risk to the US environment.

9.2.1. Question 1: What is the Likelihood that AquAdvantage Salmon or AquAdvantage Broodstock will Escape the Conditions of Confinement?

The possible exposure pathways and steps required for ABT salmon and their progeny to be present and establish in Maine are presented in Figure 8-4 and Figure 8-5 in Section 8.2 above. The first step (Step 1 in Box 1) in the pathway analysis is escape or release of ABT salmon to the PEI environment. The potential for ABT salmon to escape or be intentionally released from the Bay Fortune facility and Hatchery Unit at the Rollo Bay facility was previously evaluated in Section 7.2 of the 2015 and 2019 EAs. In both circumstances, it was concluded that likelihood of escape of any life stage of AAS or diploid ABT salmon is extremely low due to multiple and redundant forms of effective physical containment at these facilities. These conclusions are consistent with DFO's risk assessment, based in part on a Failure Modes Analysis, in which DFO concluded that the potential for an acute or chronic failure of physical containment at these facilities is negligible (DFO, 2013 and 2019).

Box 1: Possible Pathways to Exposure (Figure 8-4)



Question 1 is re-evaluated herein. The information and conclusions from the 2015 and 2019 EAs are summarized below, and any new information is also considered, including the planned future expansions at the Rollo Bay facility and the planned changes at the Bay Fortune facility. Based on this analysis, FDA continues to conclude that, as a result of multiple and redundant forms of effective physical and procedural containment at the Bay Fortune and Rollo Bay facilities, the likelihood of escape of any life stage or type of ABT salmon is negligible.[76] The following discussion provides the reasoning for this conclusion.

9.2.1.1. Physical and Procedural Containment at the PEI Facilities

The likelihood of escape would depend primarily on the extent and adequacy of physical containment at the facilities. Procedural containment measures, such as SOPs and security, are also important as they augment physical containment. In addition, life stage will also affect the likelihood of escape. For example, the likelihood of escape increases with the smaller life stages, like eggs and fry, because they are small, can be difficult to contain, and may be impossible to re-capture if they escape. They can also be highly mobile if the aquatic environment is sufficiently hospitable. These factors generally oblige the use of redundant, multi-level physical containment strategies. GE fish are considered to pose little risk to native populations if they are adequately contained (Mair *et al*., 2007; Wong and Van Eenennaam, 2008). Confinement of GE fish in closed land-based facilities is considered optimal to ensure an acceptably low risk of escape (Mair *et al*., 2007). Such is the case for the Bay Fortune and Rollo Bay facilities.

Physical containment refers to measures implemented on-site, such as the use of mechanical devices, either stationary or moving (e.g., tanks, screens, filters, covers, nets, etc.), or the use of lethal temperatures or chemicals (e.g., chlorine puck) to prevent uncontrolled escape. An important component of physical containment is the implementation of procedural containment measures, such as policies and procedures, to ensure that the devices and chemicals are used as prescribed (Mair *et al*., 2007). Security measures and plans are also important to prevent unauthorized access, control movement of authorized personnel, and prevent access by predators.

Information on the Bay Fortune and Rollo Bay facilities and their containment is available in the following locations in this EA:

- Sections 7.1 and 7.2 contain information on the location and descriptions of the Bay Fortune and Rollo Bay facilities,
- Figure C-1 and Figure D-1 in Appendices C and D contains the site plans for the facilities,
- Table 7-1 in Section 7.3 contains a summary of the containment measures implemented at the Bay Fortune and Rollo Bay facilities,
- Figure C-2, Figure C-3, Figure D-2, Figure D-3, and Figure D-4 in Appendices C and D, respectively, illustrate the physical containment measures at the Bay Fortune facility (including the planned incubation room) and in the Hatchery and Broodstock 1 and 2 Units at Rollo Bay, and
- Additional in-depth details on containment are also provided in Section 5.4 of the 2015 EA for egg production in the Bay Fortune facility and Section 5.6 of the 2019 EA for egg production in the Rollo Bay facility.

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[76] In the 2015 EA, FDA concluded that the likelihood of escape from the Bay Fortune facility was very low. In the 2019 EA, the likelihood of escape from the Rollo Bay facility was defined as extremely low. For this assessment, very low and extremely low likelihoods are considered to be synonymous with negligible likelihood, see fn 75.

The main physical and procedural containment and security measures employed at each facility are also summarized below. These multiple and redundant containment measures prevent the escape and unintended release of any life stages of ABT salmon from these facilities, and, to date, no ABT salmon have escaped the Bay Fortune facility (owned by ABT since 1996) or the Rollo Bay Facility (open since 2018).

a. Physical Containment

The US Department of Agriculture's (USDA) Agricultural Biotechnology Research Advisory Committee (ABRAC) has prepared Performance Standards for safely conducting research with genetically modified fish and shellfish (ABRAC, 1995). These Performance Standards are conceptual in nature and neither require nor recommend specific types and/or numbers of containment measures. For risk management, the Performance Standards state that although the number of independent containment measures[77] is site- and project-specific, they should generally range from three to five. As described below, both ABT facilities on PEI meet or exceed these standards and have many more levels of containment than traditional aquaculture facilities.

All areas of the Bay Fortune facility, including the newly planned incubation room, have at least 4 independent forms of physical containment. The areas of highest concern with respect to potential escape/release, i.e., the youngest and smallest life stages (egg incubation and fry rearing tanks), have 5-6 separate, independent forms of physical containment. All containment barriers use materials and equipment that are durable and appropriately sized for the life stage in that unit. The containment used in the Bay Fortune facility is illustrated in Figure C-2 and Figure C-4 in Appendix C and includes (but is not limited to): screened trays for incubation of eggs, screens over standpipes and catchment boxes, sock filters on drainpipes, stainless steel perforated basket filters in containment sumps, floor drain covers, tank covers, slotted standpipes, overflow screens, and chlorine pucks in floor drains (during spawning of fish). Specific information regarding the containment level, component and details of the containment for each area (except the newly planned incubation room) of the Bay Fortune facility is listed in Table C-1 of Appendix C. In addition, all effluent streams, including that for the newly planned incubation room, are combined and pass through a single containment sump that has 3 stainless steel perforated basket filters/screens (a minimum of 2 of these baskets remain in the sump at all times during cleaning) prior to discharge to a drainage ditch.

It should be noted that DFO (2013) has previously conducted a Failure Modes Analysis (FMA) of the physical containment in the ERA and GOA at the Bay Fortune facility following guidance from Stamatis (2003) and McDermott *et al.* (2009). The details on that analysis were not available publicly until November 2021, see McGowan *et al.* (2021). According to McGowan *et al.* (2021), "*The FMA was intended to identify potential weaknesses along all pathways of entry into the environment. The FMA also provide a systematic method to examine and assess each and every*

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[77] The term "barriers" is used in the Performance Standards when discussing similar containment measures. The term includes physical, chemical, mechanical, and biological barriers.

element of physical containment. Therefore, the effectiveness of each barrier, the operational procedures in place to maintain and ensure the proper use of each barrier, and the potential consequences of a failure at each barrier were all taken into consideration." DFO conducted an FMA on the mechanical and operational processes of physical containment at the Bay Fortune facility, and provides specific details on how the FMA was conducted in McGowan *et al.* (2021). "*Each element of physical containment is ranked according to the severity of a failure (based on the redundancy of downstream containment), its likelihood of occurrence (based on incident records provided by AquaBounty), and the mitigation measures in place to prevent a potential failure (based on SOPs and oversight documentation provided within the notification).*" (McGowan *et al.,* 2021). Severity (S), occurrence (O), and mitigation (M) are given a rank of 1, 2 or 3 based on specific criteria set *a priori*. "*The product of the three rankings generates a risk priority number (RPN) that is used to identify where potentially severe failure modes are most likely to occur, assess the consistency of containment across all entry pathways, and indicate where a recommendation of additional mitigation may be required*" (McGowan *et al.,* 2021). The RPNs were categorized as Low (1 to 3), Medium (4 to 9), or High (10 to 27) concern for each entry pathway. These rankings are considered a "*qualitative estimate of the likelihood of an unintentional release through the examination of every element of physical containment for each life stage of AAS along all pathways of entry*" (McGowan *et al.,* 2021). DFO ranked all potential pathways of entry to the environment for all life stages that will be produced and reared at the Bay Fortune facility, including AAS gametes, embryos (eggs), fry, parr, smolts, post-smolt juveniles and adults. Using the RPNs and other information, DFO determined the likelihood of that life stage entering into the environment by means of that pathway. DFO (2013) identified a total of 16 potential pathways of entry for ABT salmon from the Bay Fortune facility, and using an FMA, they examined all containment components and 294 failure modes. The RPNs ranged from low to medium for all the entry pathways and life stages, with the majority of RPNs ranking low. Thus, the likelihood of any life stage entering into the environment was found to be negligible with high uncertainty, except for AAS gamete collection. AAS gamete collection was found to have a medium to high concern due to limited physical containment during gamete collection; however, McGowan *et al.* (2021) found that this was mitigated through procedural containment and oversight leading to a low likelihood of entering the environment. Overall, DFO (2013) concluded that the potential for acute and chronic release of AAS from the Bay Fortune facility ERA and GOA is negligible with reasonable to high certainty.

At the Rollo Bay facility, the ERA of the Hatchery Unit, where eggs and young fish are housed and handled, has the highest level of containment with a minimum of 8 independent levels of physical containment. The ARA where larger fish (>10 g) are reared has 4 or more independent levels of containment in place for all effluent flow paths. Some areas of the Hatchery Unit have up to 10 or more levels of containment. The containment barriers are similar to those used at the Bay Fortune facility (see examples listed in paragraph above), constructed of durable materials (e.g., stainless steel, polyester, PVC) and sized for the life stage present in each area, including screened incubator systems and appropriately sized nets covering all tanks. The containment for the Hatchery Unit is illustrated in Figure D-2 and specific information regarding the containment level, component and details of the containment for the Hatchery Unit is listed in Table D-1 of Appendix D. In the Hatchery Unit, all effluent is discharged from one main containment sump after first passing through 3 independent inline stainless steel perforated basket filters/screens (a minimum of 2 of these baskets remain in the sump at all times during cleaning). The sump effluent discharges to an outdoor polishing pond (where ABT salmon could be preyed upon) before finally being discharged to Rollo Bay Brook. The polishing pond is a freshwater man-made pond in which all life stages of ABT salmon could survive for a short period of time and potentially escape to Rollo Bay Brook. The polishing pond was not intended to act as a containment measure; however, there are predators in and around the pond (predatory fish, birds, etc.) that would reduce the likelihood of escape (and survival) of ABT salmon.

Currently, the Hatchery Unit is the only unit at the Rollo Bay facility that is approved by FDA for the production of AAS under NADA 141-454. Another building on the property is currently used for rearing AquAdvantage broodstock, referred to as Broodstock Unit 1 (see the site plan in Figure D-1 and the floor plan in Figure D-3 of Appendix D), which was described in the 2019 EA as the Rollo Bay "Grow-Out Unit." Broodstock Unit 1 is not currently approved under the NADA. It is approved by Canada for producing AAS eggs and fish (for shipment to Canadian markets). The containment flow for the Broodstock Unit 1 is provided in Figure D-4 and Figure D-5 of Appendix D, and is the same as that presented for the "Grow-Out Unit" in the 2019 EA. The Broodstock Unit 1 ERA has a minimum of 6 levels of independent containment, while the ARA has 8 levels and the Conditioning Area has 6 levels.

DFO has also conducted an FMA on the Hatchery Unit and Broodstock Unit 1 (formally "Grow-Out Unit") at Rollo Bay to facilitate its assessment of physical containment. DFO (2019) used the same FMA methods as those used in McGowan *et al.* (2021; see above). DFO has provided high-level details regarding this analysis in DFO (2019), with additional specifics provided in McGowan and Leggatt (2020). DFO (2019) identified 4 potential pathways of entry into the environment for ABT salmon (including gametes, embryos, fry, juveniles and adults) from the Hatchery Unit at the Rollo Bay facility, and using an FMA, they examined 44 elements of containment (e.g., screens, filters, chlorine pucks, etc.) and 88 potential failure modes (DFO, 2019). DFO found that "*there must be simultaneous failure of at least six independent containment measures along a single pathway of entry*" for ABT salmon in the Hatchery Unit to reach the outside environment (DFO, 2019). For the Broodstock Unit 1 (referred to as "The Grow-Out Building" in DFO (2019)), 4 potential pathways of entry into the environment were identified, 34 elements of containment and 72 potential failure modes were examined using FMA. DFO (2019) found that "*there must be simultaneous failure of at least five independent containment measures along a single pathway of entry."*

In addition, a second broodstock unit may be constructed in the future and, if it is constructed, will be known as Broodstock Unit 2, but currently only a building shell is present. Because Broodstock Unit 2 is expected to be completed in the future, the specifics of the containment for this unit is unknown at this time. However, ABT has indicated to FDA that the containment for Broodstock Unit 2 will be similar to, and no less than, that currently in the Hatchery Unit. FDA will evaluate the containment of the broodstock units prior to any future supplemental NADA approval(s) covering these units, and any shipment of AAS eyed-eggs from those units to the US, to ensure that the containment in these units is similar to, or no less than, the minimum containment levels in the Hatchery Unit. It is important to note that the effluent flow and external containment measures are expected to be the same for the future broodstock units and are discussed below and depicted in Figure D-1 of Appendix D.

For the future broodstock units, all solid waste will be pumped to permanent concrete storage tanks where it will be held until the solid waste is moved to an offsite waste treatment facility or used for agricultural purposes (land application). Discharged water will flow into a stone out-wash followed by a vegetative strip approximately 140 m west of the future Broodstock Unit 1 (see Figure D-1 in Appendix D). The water will be filtered through the stone and will flow approximately 40 m across a natural vegetative strip with trees and undergrowth before eventually entering the Rollo Bay Brook. It is not possible for any life stage of ABT salmon to survive the solids collection, dewatering, land application, and leaching field. In addition, the likelihood of any life stage of ABT salmon surviving the stone washout or vegetative strip is extremely low.

Finally, the production process at both the Bay Fortune and Rollo Bay facilities is conducted within self-contained buildings (i.e., fish are not contained in outside tanks or ponds), so there is no risk of escape or movement of fish through predation by wildlife.

b. Recirculating Aquaculture System (RAS)

Both PEI facilities are land-based and have RAS, which in itself is an additional level of physical containment. Most of the aquaculture system at the Bay Fortune facility operates with approximately 97% recirculation of water (i.e., with 3% new water being added continuously). The Heath Stacks in the ERA operate with 100% recirculation during egg incubation but are transitioned to continuous flow-through after the eggs hatch. Likewise, at the Rollo Bay facility, the Hatchery Unit and Broodstock Unit 1 operate as a 99.7% RAS (i.e., with 0.3% new water being added continuously), and it is expected that the future Broodstock Unit 2 will also operate as a RAS. These conditions mean that the discharge of water, and concomitant potential for fish escape, is minimal.

c. Procedural Containment and Security

ABT has developed and employs an extensive number of SOPs that govern physical containment and other significant activities that occur at the PEI facilities, including disaster preparedness plans. Operational protocols and procedures are in place for twice-daily inspections of critical containment barriers, for responding to emergencies (such as an interruption of the water supply, natural disaster, catastrophic events, etc.), and there is a contingency plan in place to address the unlikely possibility of a fish escape. Additionally, there are SOPs in place controlling the movement of eyed-eggs from the PEI facilities to the Charlottetown airport when eggs are shipped to Indiana.

Furthermore, both facilities have several security measures in place to prevent access of unauthorized persons. At the Bay Fortune facility, there is an eight-foot-high chain-link fence that surrounds the property, locks on all exterior entryways into the building and primary well and pumping facility, and interior and exterior cameras and sensors that are professionally monitored at all hours. At Rollo Bay, all exterior entryways are locked and secondary access requires a key; there are exterior motion-activated cameras and sensors that are professionally monitored at all hours; and a sponsor-employee is on-site at all times. In addition, there are environmental sensors at both facilities that alert personnel at any hour to mechanical issues (e.g., issues with water flow, oxygen).

d. FDA and Canadian Inspections

All PEI facilities covered by an approval or supplemental approval have been inspected by FDA at least twice, and the Bay Fortune facility has been inspected multiple times. A primary aspect of these inspections was to ensure that the physical and procedural containment described in the 2015 and 2019 EAs, and herein, is in place and operational. The Bay Fortune facility had two pre-approval inspections in 2008 and 2012 (additional information on these inspections is available in Section 7.2 and Appendix F of the 2015 EA), and one post-approval inspection in 2019. The Rollo Bay facility has had one pre-approval inspection in 2019. In addition, as part of the evaluation for this amended EA, FDA conducted an additional post-approval inspection of both the Bay Fortune and Rollo Bay Hatchery facilities in June 2023. During all inspections, the FDA inspector(s) was accompanied by experts in aquaculture and biotechnology from CVM. The primary goals of the inspection were to (1) examine and evaluate the physical containment equipment within the PEI facilities to prevent the escape of all life stages of ABT salmon; (2) to examine facility records and SOPs to ensure they are adequate to maintain physical containment and security and biosecurity; and (3) to examine the security measures in place to ensure unauthorized persons cannot enter the facility.

All levels of physical containment and security were verified by FDA and found to be in place (as illustrated in Figure C-2 and Figure D-2, and Table C-1 and Table D-1) and in proper operating condition at both the Bay Fortune facility and Hatchery Unit at the Rollo Bay facility. The SOPs and records were found to contain appropriate detail and adequate policies and procedures to ensure physical and biological containment of all life stages of ABT salmon. In addition, the biosecurity protocols were adequate to prevent introduction and transmission of disease, and the physical security was found to be adequate to prevent unauthorized persons from entering the facilities. Overall, we verified the facilities were in compliance with the NADA application and applicable FDA regulations, and no Form FDA 483[78] was issued at the conclusion of these inspections. In addition, CVM experts also conducted a site visit of the Broodstock Unit 1 at Rollo Bay following the inspections. CVM verified that all levels of physical containment were in place as illustrated in Figure D-4 and Figure D-5 and were in good working condition.

These facilities are also subject to periodic inspections by Canadian authorities. Since 1996, the ABT facilities on PEI have been subject to oversight by DFO and ECCC[17] for the use of these facilities in research and development involving GE fish, and more recently by the CFIA with respect to its compartmentalization program (see Section 7.5.1.1 above). Canadian government inspections of these facilities for various purposes over the past 20+ years have shown it to be compliant with appropriate containment practices. Following inspection of the Bay Fortune facility, DFO characterized the facility as being "*as escape-proof as one can reasonably expect*"[79] and, based on a qualitative Failure Modes Analysis, DFO concluded that the potential

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[78] Form FDA 483 is issued to the sponsor at the conclusion of an inspection when FDA investigators have observed any conditions that in their judgement may constitute violations of the FD&C Act.

[79] Memorandum from M.I. Campbell (Inspector) to I.M Price (Director) dated March 2, 2001, in re: *Visit to Aqua Bounty Farms Transgenic Research Facility*.

for an acute failure of physical containment at the Bay Fortune Facility is negligible with reasonable certainty. DFO also concluded that the potential for chronic release of any life stage of ABT salmon from the Bay Fortune facility is negligible with high certainty. Likewise, following a pre-approval inspection of the Rollo Bay site and facilities in June 2018 by DFO and ECCC, the Canadian regulators determined there was *"[a] high degree of certainty associated with the physical, biological and operational containment of EO-1α Salmon*[80] *results from available information that adequately demonstrates the efficacy and redundancy of mechanical barriers, and the efficacy of SOPs and operational oversight*" and concluded with low uncertainty that the likelihood of EO-1α Salmon exposure to the Canadian environment was low to negligible (DFO, 2019).

These facilities will continue to be subject to future inspections by both FDA and Canadian authorities to ensure adequate physical and procedural containment and security remains in place.

9.2.1.2. Issues Affecting Containment and Security

There are three hypothetical methods of escape or unintended release of ABT salmon from the PEI facilities, including:

1. a major malfunction in physical containment and/or security,
2. human error in care of fish or maintenance of containment;
3. a natural disaster that compromises physical containment (e.g., hurricane, flooding), and/or
4. malicious intentional release or disruption of operations.

None of these hypothetical methods of escape or release is expected to occur at either of the facilities on PEI due to the redundant, multiple-level physical containment, well-established procedural containment (e.g., SOPs, security) and management oversight employed at both facilities. The reasoning for this is discussed in further detail below.

a. Malfunction in Physical Containment and Security

As stated above in Section 9.2.1.1, both ABT facilities on PEI have multiple and redundant levels of containment; with a minimum of 4 independent levels of physical containment at Bay Fortune, a minimum of 8 independent levels in the ERA (area with highest likelihood of escape of eggs and fry) and a minimum of 4 independent levels in the ARA of the Hatchery Unit at Rollo Bay, and a minimum of 6 independent levels in all areas of Broodstock Unit 1 at Rollo Bay. The future planned Broodstock Unit 2 at Rollo Bay is expected to have similar containment to the Hatchery and Broodstock Unit 1. In addition, some areas of these facilities have more than these minimum levels; up to 6 levels at Bay Fortune and 10 levels at Rollo Bay. The number of levels of physical containment at these facilities reflects the considerations in the ABRAC Performance Standards (ABRAC, 1995) and exceeds the number of

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[80] This is the term that Canada uses for AquAdvantage Salmon and those salmon containing the rDNA construct. See fn 18.

levels of containment of traditional aquaculture facilities. It is important to note that although the eggs have the greatest likelihood of escape/release due to their small size and large numbers, most AAS eggs are only expected to be at the Bay Fortune and Rollo Bay facilities for a short period of time until they reach their eyed-egg stage (approximately 50-125 days depending on water temperature) before being shipped to a grow-out facility in the US, which reduces the likelihood of escape/release from the PEI facilities for this life stage. However, some AAS eyed-eggs may remain at the PEI facilities for grow-out to supply the Canadian market.

There are also procedural containment measures in place to augment the physical containment. This includes operational protocols and procedures for twice-daily inspections of critical containment barriers (which are recorded), routine internal audits (which are also recorded), and occasional inspections by FDA and Canadian authorities. In addition, there are SOPs in place to address emergency response procedures for catastrophic events that could potentially cause mass mortality and/or escape of fish (e.g., electrical and pump failures), and there are contingency plans in place to address the unlikely escape of fish. Further, strong adherence to these procedures is ensured with comprehensive training of employees. Therefore, in the unlikely event of a malfunction at some point in the physical containment, or for instances where containment equipment is removed for repair or routine cleaning, there are several additional levels of physical containment in place to ensure adequate containment of all life stages of ABT salmon, as well as comprehensive procedures in place to identify and address and/or repair a malfunction in containment quickly to minimize the likelihood of escape. For example, in the case of a total or partial electrical failure, a pump failure, etc., there are specific steps outlined in an internal SOP for the employees to remedy the problem. In addition, there are specific procedures to notify management of a catastrophic event.

The ABRAC Performance Standards (ABRAC, 1995) call for security measures to (a) control normal movement of authorized personnel, (b) prevent unauthorized access to the site, and (c) eliminate access of predators that could potentially carry fish off-site (for outdoor projects). The Performance Standards also mention the possible need for alarms, stand-by power, and an operational plan (including training, traffic control, record keeping, and an emergency response plan). The physical security measures in place at the PEI facilities are extensive (see Table 7-1) and were verified by FDA during the two to four PEI facility inspections and/or through subsequent submissions from ABT. Measures include perimeter fencing, remote monitoring systems (surveillance cameras), redundant locking systems, etc. FDA considers these security measures to be adequate to address the concerns listed above with respect to unauthorized entry; access by predators is not an issue at these facilities as they are totally enclosed. ABT is aware that unauthorized access to these sites may represent a potential hazard and has taken appropriate steps to reduce the possibility this will occur. In addition to the physical security measures, ABT has written operational plans for each facility and SOPs in place at the PEI facilities to address security issues.

b. Human Error in Care of Fish or Maintenance of Containment

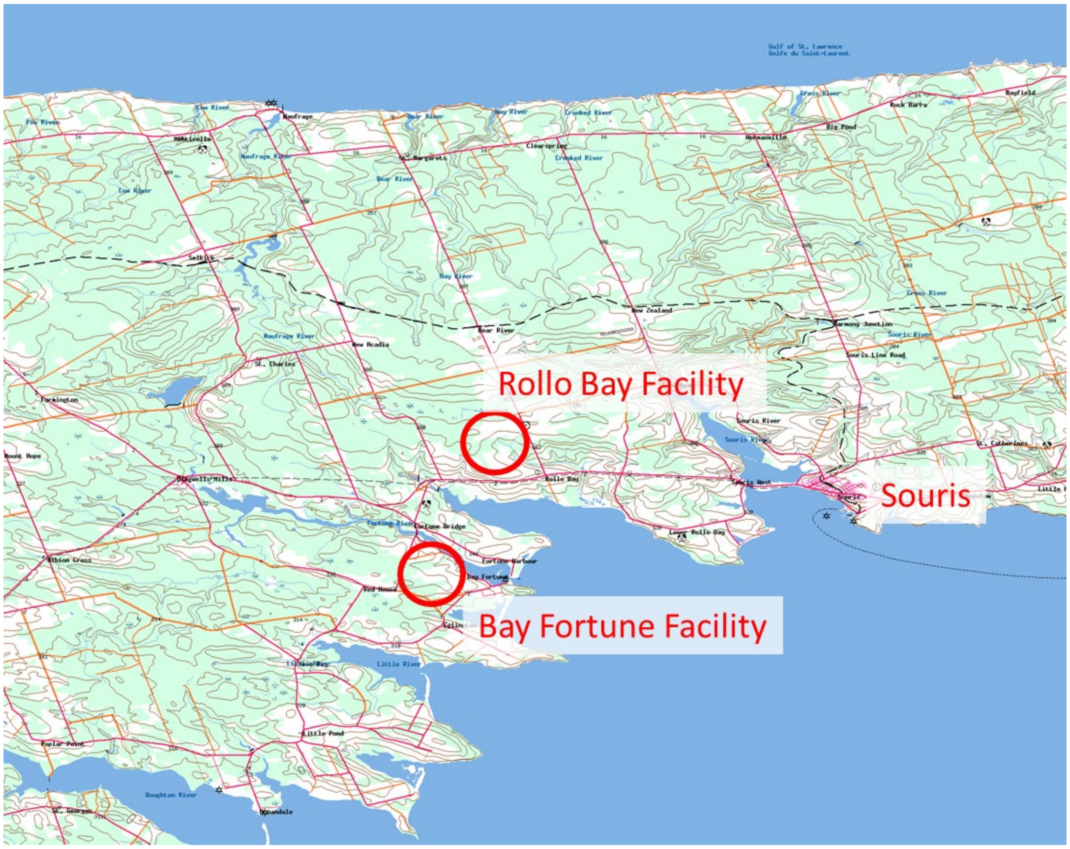
The containment of ABT salmon is dependent upon the staff's attention to care of the physical and procedural containment measures, as well as security. As stated above, staff are comprehensively trained to adhere to containment procedures. In the event of human error that results in a loss at some point of physical containment (e.g., not adhering to protocols during cleaning of containment areas), redundant additional levels of physical containment are in place to help ensure adequate containment of all life stages of ABT salmon. Also, there are comprehensive procedures in place to address any error quickly to minimize the likelihood of escape. In addition, if ABT salmon eggs or fish are dropped on the floor due to human error during handling, there are strict protocols for recovery or destruction of the eggs/fish. Critical containment points are required to be inspected two times a day to ensure the containment is in place and functioning and to check for any fish that may have entered the drains. There are specific procedures to notify management in the event of human error or if fish are observed in, on, or outside containment barriers. To date, no fish have escaped from either of ABT's PEI facilities.

c. Natural Disaster

In some cases, containment may be adversely affected by natural disasters such as floods, storms, earthquakes, etc.; therefore, it is important to consider the potential for these events to occur and take them into account when locating and designing facilities for fish with IGAs. Information on the potential occurrence of natural disasters (e.g., hurricanes, storm surge, floods, tsunamis, and tornados) in the vicinity of PEI was presented in Section 8.3.1.1. Based on history, these are all rare or extremely rare events. Storm surges and flooding have been reported elsewhere on PEI, particularly in the vicinity of Charlottetown, but flooding has not been an issue in the specific area where the ABT facilities are located on the northeast side of the island. The Bay Fortune facility is situated approximately 7.6 m above sea level and sits approximately 36.5 m inland from Fortune River estuary. The Rollo Bay facility is approximately 19 m above sea level at its lowest point and sits approximately 1 km inland from Rollo Bay. There is no report of a storm surge greater than 1.37 m and the highest sea level rising was 4.23 m on the south shore of PEI (https://climatlantic.ca/, accessed December 8, 2023).

It is highly unlikely that storm- or hurricane-induced surges or tidal waves would directly impact the Bay Fortune facility or subject it to flooding as there are rip-rap (rock) barriers across much of the river mouth at its confluence with Rollo Bay (Figure 7-3), which is approximately 1.6 km away. The Rollo Bay facility is approximately 12 km by highway from the Bay Fortune facility and the risk of a catastrophic event occurring at Rollo Bay is, if anything, lower than the risk at Bay Fortune due to its higher elevation and increased distance to Rollo Bay compared to Bay Fortune. In addition, due to the local topography and the locations of the sites (see topography map for Rollo Bay in Figure 9-1), water is expected to drain away from the facilities and flooding is not expected to be an issue. Even in the remote event that flooding was to occur in the area, all of the fish tanks at both PEI facilities are located indoors within steel-structured buildings, are above-ground, and have top netting, which would further preclude the escape of fish. The conditions at both facilities are in general conformance with recommendations in the ABRAC Performance Standards for research facilities holding GE fish and shellfish.

Figure 9-1. Coastal Topography Near the Rollo Bay Facility. Approximate Location of the Facilities is Indicated with the Red Circles.*



* Obtained by AquaBounty Technologies, Inc. from Canada Topographic Maps Online at https://www.canmaps.com/ (accessed October 24, 2022)

In addition, the facilities at Rollo Bay have been constructed to withstand the weather extremes that are common to PEI, including high winds and heavy snow loads. Thus, damage to the physical structure of the Rollo Bay facilities from these causes is unlikely. In the event of a sustained power outage, backup electrical generating capacity is in place at both facilities to allow full operation of the facilities indefinitely as long as fuel is available (on-site fuel reserves would allow the generators to run for 96 hours). During the power loss caused by post-tropical storm Fiona in late September 2022, the back-up generators at both ABT facilities supplied power until primary power was restored. The facilities maintained normal operations throughout and after that event even though the storm packed winds in excess of 170 km/h (105 mph). However, it should be noted that even if a complete power failure occurred at one of the ABT facilities, containment would not be compromised because most of the containment barriers, e.g., stainless steel screens, boxes, and filters, do not require electrical power for operation. In the event of partial damage to either of the facilities, the presence of multiple, redundant containment measures (see discussion above) makes it highly unlikely fish could escape the facilities. In the event of weather severe enough to damage the entirety of one or both facilities, it is highly unlikely that the fish would be able to survive very long due to a degradation in water quality (i.e., appropriate DO and/or temperature) in the tanks in which they are kept. For example, without supplemental oxygenation, DO levels will quickly deplete to lethal levels. In addition, there are SOPs in place that address emergency response procedures for catastrophic events (i.e., disaster preparedness plans) that could potentially cause mass mortality and/or escape of fish (e.g., electrical and pump failures), and all personnel are trained on these procedures.

d. Malicious Intent

Given the redundancy in physical containment measures and the low probability of occurrence of natural disasters in the area, the more likely event leading to introduction of ABT salmon to the environment surrounding the PEI facility would be an intentional malicious release or intentional malicious disruption of operations by unauthorized individuals or authorized staff. As described in Table 7-1 in Section 7.3 above, there are extensive security measures, equipment, and contingency plans in place to prevent unauthorized access to both of the PEI facilities. There are redundant passive and active measures, such as perimeter fences, locked doors, alarm, key control and 24/7 security monitoring, to prevent illegal access to the PEI facilities. There is also automated environmental monitoring of culture conditions; therefore, if an individual maliciously attempted to disrupt operations, staff would be alerted at any hour. In addition, ABT has experienced staff at PEI that are properly trained in SOPs and other procedures, and any incidents of non-compliance with SOPs are documented and routine compliance audits are conducted on a regular basis. Further, ABT staff that is responsible for transport of AAS eggs to and from the airport during shipment have gone through extensive background checks. To date, ABT has not experienced any disruptions at the PEI facilities due to malicious intent by authorized or unauthorized individuals. Therefore, it is highly unlikely that a malicious act would occur at either of the PEI facilities.

9.2.1.3. Other Concerns Regarding Containment

a. Transportation of Eggs

Most AAS eggs are expected to be at the Bay Fortune and Rollo Bay facilities for only a short period of time (50-125 days) before shipment to the US, reducing the likelihood of escape from those facilities. Some AAS eggs may remain at the PEI facilities for hatching and grow-out to supply the Canadian market (grow-out of AAS at the PEI facilities is not currently approved by FDA but is approved by Canada).[81] However, there is also a risk of escape during transportation to the US. As described in Section 7.4, AAS eyed-eggs are currently shipped from the Bay Fortune and Rollo Bay facilities to the US via air freight with subsequent ground-shipment to the grow-out facility. When shipped, multiple containment measures are in place for AAS eggs. Eggs are shipped in coolers, sealed with tape, and bound with packing straps, which are then placed in a sealed heavy cardboard shipping container. In addition, the eggs are in control of ABT personnel on the way to the airport in Canada and upon landing in the US. Unintentional escape of AAS eggs during shipment is therefore particularly unlikely. This exposure pathway was further discussed in the 2015 and 2019 EAs.

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[81] Because grow-out of AAS at the PEI facilities is not approved by FDA, ABT can only ship AAS eggs to the US. ABT cannot ship fish or tissues of the fish (e.g., fillets) to the US.

b. Disposal of Fish and Fish Wastes

Disposal of ABT salmon (including non-viable eggs, mortalities, and culls) and the non-viable waste material associated with the production, processing, and consumption of AAS (e.g., feces, fish pieces) would not require handling that is different from that used for wild or domesticated fish without the IGA: the rDNA gene construct added to this fish is stably integrated into the genome; it is not infectious, communicable, or transmissible from these materials; and will degrade in the same manner (i.e., rapidly) as other DNA in the environment.

In PEI, mortalities and culls requiring disposal will be stored frozen until they are incinerated offsite in a local facility. Fish wastes (biosolids) from the PEI facilities are subject to extensive treatment prior to discharge to the local estuary. For example, biosolids from the Hatchery Unit at the Rollo Bay facility are collected from stainless steel baskets located in the Hatchery ARA containment sump and frozen. Frozen waste can be incinerated at a Provincial incinerator or used for land application in compliance with Canadian and Provincial laws. Dead fish will be collected, frozen and stored for incineration at a Provincial incinerator or used for land application in compliance with Canadian and Provincial laws.

9.2.1.4. Conclusions for Question 1 (Step 1)

For ABT salmon, the production of eyed-eggs is to be conducted *only* in land-based facilities with redundant physical containment measures and with point-to-point control of shipping and land-based materials transfer. There are multiple and redundant physical barriers in place in the water systems at the PEI facilities to prevent the accidental release of eggs and/or fish to nearby aquatic environments. These barriers have been designed specifically to prevent the escape of different life stages of ABT salmon. Both facilities have a minimum of 4 barriers in place for all internal flow streams that release water to the environment. Areas of these facilities that contain the youngest life stages, eggs and fry, have 4 to 8 (sometimes more) barriers in place. This level of containment is consistent with or exceeds recommendations in the ABRAC Performance Standards and has been verified by an FDA inspection in June 2023.

Based on the definitions presented in Table 9-1, FDA considers the likelihood that any life stage of ABT salmon could escape or be released from confinement at these sites (Step 1 of the pathway analysis in Figure 8-4 and Figure 8-5, see Box 1) to be negligible. In addition, FDA has made the determination that physical and procedural containment and security to prevent intentional releases of salmon due to natural disasters or intentional releases due to malicious activities are acceptable at both sites. The containment measures described above include strictly physical measures (e.g., screens, covers, filters), as well as chemical measures (e.g., chlorine).

ABT also employs SOPs that govern physical containment, as well as every other significant activity that occurs at these sites. In addition, strong operations management plans are in place, comprising policies and procedures that meet the recommendations for an integrated confinement system for GE organisms as summarized in Table 9-2 below. Any significant failure in these measures would be highly unlikely because of the following factors: the sponsor’s use of multiple types of containment; use of experienced, properly trained staff operating under established plans and procedures; automated monitoring of culture conditions and unauthorized intrusion; redundant passive and active measures to ensure physical security; and continued inspections by local and US officials.

The combination of all of these factors results in a negligible likelihood that any eggs or fish of any life stage of ABT salmon could escape into the wild and cause effects on the environment of the US.

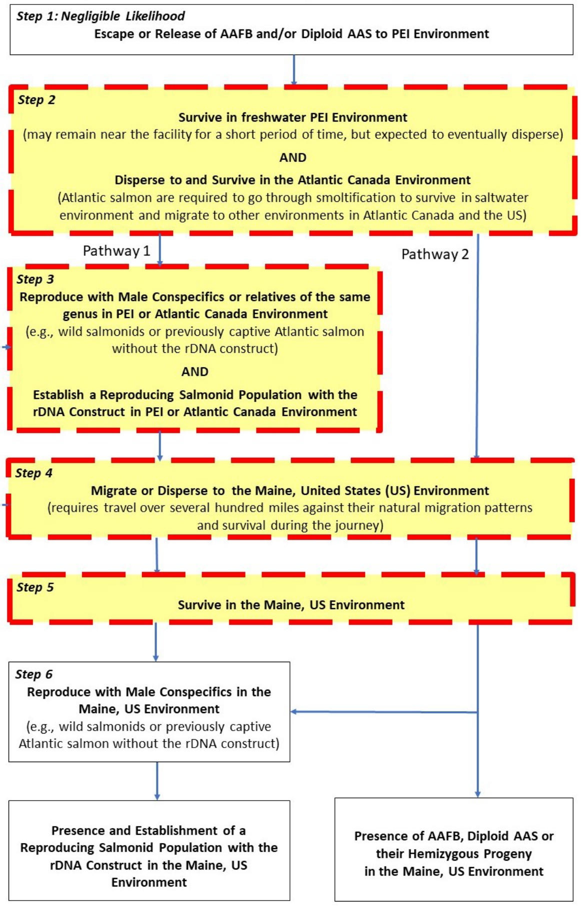
Table 9-2. Implementation of an Integrated Confinement System for ABT Salmon*

Recommended Element	**Bay Fortune**	**Rollo Bay**
Commitment by top management	X	X
Written plan for implementing backup measures in case of failure, including documentation, monitoring, and remediation	X	X
Training of employees	X	X
Dedication of permanent staff to maintain continuity	X	X
Use of SOPs for implementing redundant confinement measures	X	X
Periodic audits by independent agency	X	X
Periodic internal review and adjustment to allow adaptive modifications	X	X
Reporting to an appropriate regulatory body	X	X

* After NRC (2004a) and Kapuscinski (2005)

9.2.2. Questions 2 and 3: What is the Likelihood that AquAdvantage Salmon or AquAdvantage Broodstock will Survive and Disperse if they Escape the Conditions of Confinement? What is the Likelihood that AquAdvantage Salmon or AquAdvantage Broodstock will Reproduce and Establish if they Escape the Conditions of Confinement, Survive, and Disperse?

Box 2: Possible Pathways to Exposure (Figure 8-4)



Risk-related Question 2 evaluates the next steps of the pathway analysis, which includes the likelihood of survival and dispersal in the PEI, Atlantic Canada and Maine environments (Steps 2, 3 and 4 in Box 2). Without survival, dispersal and migration, there can be no exposure (establishment and/or presence) in the US environment. Risk-related Question 3 evaluates the likelihood of reproduction and establishment in Maine (Step 5 in Box 2).

Because each of these steps must occur in order for there to be an exposure (establishment and/or presence) in the US, the evaluation of Risk-related Questions 2 and 3 was combined herein. This approach will allow for the determination of whether there is a complete pathway to exposure in the US environment from PEI, and to determine the likelihood exposure would occur in the US environment (see Section 9.2.3, below). The likelihood for each of the steps in the pathway analysis (see Box 2) to occur will be evaluated Sections 9.2.2.2 to 9.2.2.5 below.

9.2.2.1. Background on Factors Affecting Survival, Dispersal, and Reproduction in PEI/Atlantic Canada and Maine (US)

In the highly unlikely event of escape, the likelihood of survival and dispersal in the natural environment is mainly dependent on two parameters: (1) the phenotype and fitness of the fish (e.g., physiologic tolerance to physico-chemical parameters such as temperature and DO) and (2) the specific geographical and geophysical containment in the accessible environment, which are a function of the specific location and environmental conditions at the site of escape or release. In addition, the dispersal of ABT salmon would depend on how many eggs/fish escaped and survived, their fitness and physiological characteristics, and their reproductive potential. The establishment of the ABT salmon in the environment is also dependent upon biological containment and their ability to reproduce in the natural environment. This section discusses factors that affect the survival, dispersal, and reproduction of ABT salmon in the natural environment. This information will be used in Sections 9.2.2.2 to 9.2.2.5 below to evaluate the likelihood of Steps 2-5 occurring (see Box 2).

a. Geographical/Geophysical Containment

Geographical and geophysical containment is defined as the presence of inhospitable conditions in the surrounding environment that would preclude or significantly reduce the probability of survival, dispersal, and/or long-term establishment should an animal escape confinement at the site of rearing. Unless deemed to be 100% effective under all reasonably foreseeable circumstances, containment of this type would normally be considered to be secondary to other containment measures. As stated above, geographical and geophysical containment is a function of the location of the facility and the environmental conditions of the area surrounding the facility; therefore, the geographical and geophysical containment at each facility is discussed separately below. A description of the location of the PEI facilities is provided in Section 7.1 and illustrated in Figure 7-3, a description of the immediate accessible environment on PEI is provided in Section 8.3.1, and a description of the geographical/geophysical containment on PEI is listed in Table 7-1.

In addition, the life stage of the ABT salmon at the time of escape/release also plays a role in survival and dispersal at the immediate site of release. Atlantic salmon are anadromous; they hatch and live in freshwater when younger, then migrate to saltwater as adults (post-smolts) after undergoing the physiologic process of smoltification (see Figure 8-2 in Section 8.1, above). Therefore, depending on the life stage at the time of escape, the environment at the site of introduction may be inhospitable to the egg/fish and result in rapid mortality (e.g., if a younger, pre-smolt life stage is introduced into salt water). Furthermore, in order for escapees to survive, the accessible ecosystem must meet their needs for food, habitat, environmental conditions (e.g., temperature, salinity and water quality), and environmental cues for reproduction.

The geographical and geophysical containment measures that exist at each of the PEI facilities are described in more detail below.

i. Bay Fortune

The Bay Fortune facility lies on the southern shore of Fortune River, a tidal river, close to its confluence with Rollo Bay and the Gulf of St. Lawrence (Atlantic Ocean) on the northeast side of PEI (see Figure 7-3). The site plan for the Bay Fortune facility is illustrated in Figure C-1 of Appendix C. ABT salmon are reared in freshwater conditions at both PEI facilities; ABT salmon are not contained in salt water at any stage of their lifecycle. Fresh water from the facility, including effluent from all floor drains, fish tanks and egg incubators, discharges to a drainage ditch located adjacent to the facility. The drainage ditch originates at a farm west-southwest of the facility. The drainage ditch is about a meter in width, and the water level in the ditch at the location where the Bay Fortune effluents enters it is very shallow (7.6-10.2 cm). Further downstream, the ditch ranges in depth from 66-157 cm to allow for runoff from the farm. This drainage ditch also collects surface water runoff from adjacent areas and eventually discharges to the Fortune River estuary at a distance of approximately 36 meters from the facility. This freshwater drainage ditch may be suitable for the early life stages of salmon (eggs, fry, pre-smolt) for a short period of time. However, the shallow ditch does not contain adequate food or suitable habitat (e.g., gravel bottom for laying eggs) for long-term salmon survival, and rain events that cause high water flow would likely push any eggs or early life stages downstream into Fortune River estuary.

The environmental conditions of Fortune River estuary would not be conducive to early life stages of these fish although they are generally conducive to adult (post-smolt) Atlantic salmon. The water has a relatively high salinity, in the range of 21 ppt,[82] and during the winter months, water temperatures are typically very low (less than 0 ºC). Therefore, it is highly unlikely that early life stages of any ABT salmon at the Bay Fortune facility would be able to survive the high salinity, and colder winter months, if they were able to escape the multiple levels of physical containment in place. Although not as applicable to older fish, it is still unlikely that adults raised entirely in fresh water in the Bay Fortune facility would be able to survive the sudden, abrupt transition from their low salinity, freshwater environment to the moderately high salinity, brackish water environment of the Fortune River estuary.

As a result of intentional stocking efforts, hatchery-reared Atlantic salmon inhabit the ocean waters surrounding PEI and several watersheds on the island (Cairns *et al*., 2010), although they are not known to currently populate the waters near the Bay Fortune facility (Guignion, 2009); see Figure 8-10(b) and Section 8.4.2 for information on the current status of wild Atlantic salmon on PEI. In fact, the particular watershed in which the Bay Fortune facility is located has not had populations of Atlantic salmon (either wild-type or hatchery-reared salmon) since prior to 2001 (Cairns *et al*., 2010; Guignion *et al*., 2010; Cairns and MacFarlane, 2015). Thus, although the local environment of Fortune River estuary might provide a suitable temporary habitat for at least some life stages of ABT salmon during part of the year, environmental conditions do not appear to be suitable for the long-term survival and establishment of populations in the area.

ii. Rollo Bay

The Rollo Bay facility is surrounded by farmland, forest and pasture and the only aquatic access to the local marine environment is via the freshwater Rollo Bay Brook (see Section 8.3.1.3). The site plan for the Rollo Bay facility is illustrated in Figure D-1 of Appendix D. Water flow through the brook can vary by season or as a result of specific weather events. The PEI Provincial Department of Environment requires ABT to discharge water into the brook during operations to ensure adequate recharge of the aquifer. The required minimum discharge volume varies by season and has been set at 364 L/minute from July through September and at 546 L/minute the rest of the year. There are no limits on the maximum discharge volumes. In test operations, discharge of the required minimum volumes has had minimal effect on flow rate or depth of the brook. Water temperature and apparent water quality of the Rollo Bay Brook (no data are available on water quality) are sufficient to support a

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[82] For comparison, the salinity of ocean water typically ranges from 28 to 32 ppt; freshwater has a salinity of less than 1 ppt.

local population of brook trout and, therefore, could also potentially support escaped ABT salmon. The likelihood of survival would be affected by the environmental conditions at the time of escape and the life stage of fish that escaped.

Rollo Bay Brook flows into Rollo Bay, which is an extension of the Northumberland Strait, both of which are described in Section 8.3.1.4. Rollo Bay is located approximately 1.5 km downstream from the Rollo Bay facility (Figure 7-3). The generally shallow depth of Rollo Bay and the Strait causes strong tidal currents, water turbulence, and a high concentration of suspended red silt and clay, conditions adverse to the general requirement of salmonids for clear water. Water temperatures >25 °C and low DO concentrations (including anoxic conditions) have been reported during summer months in the Northumberland Strait near Souris, PEI, and as documented in Appendix F, conditions such as those are much less than optimal for Atlantic salmon in general and more specifically for GH modified Atlantic salmon, which have higher metabolic rates and oxygen requirements.

The existing presence of conspecifics (i.e., an organism of the same species) or species closely related to the ABT salmon in accessible ecosystems indicates that a suitable environment does exist (provided that the fitness of the escapee does not differ significantly from conspecifics or closely related species in that environment) (Kapuscinski *et al*., 2007a). Brook trout and rainbow trout do occur in streams in the general vicinity of the Rollo Bay facility on PEI (Guignion *et al*., 2010). However, Atlantic salmon are not currently present in the Rollo Bay watershed or any nearby watersheds (Cairns and McFarlane, 2015); see Figure 8-10(b). This information suggests that the local environment is potentially suitable for survival of salmonid species, but the lack of existing Atlantic salmon populations suggests that it is likely unfavorable for long-term survival and establishment.

b. Phenotype and Fitness of ABT Salmon

The term “fitness” refers to all of the phenotypic attributes of an animal that affect survival and reproduction, and ultimately how the individual’s genetics contribute to future generations of the animal’s population. In general, animals adapt to a specific niche in the ecosystem (i.e., habitat and ecological role) and exhibit maximal “fitness” for that environment. For example, native North American Atlantic salmon have evolved over centuries to adapt to and thrive in waters of the northeastern US and Atlantic Canada. In terms of population and community dynamics, if escaped GE animals have a greater overall net fitness than other animals occupying the same niche in the receiving environment (including wild relatives or farmed domesticated animals of the same species), they may eventually replace them and become established in that community. On the other hand, if the GE animals are less fit, they will either not survive in the receiving environment, or the engineered trait will eventually be removed (by virtue of selection) from the receiving population. For purposes of assessing risk associated with GE animals, it is critical to characterize the fitness of the GE animals in relation to the appropriate comparator animal(s), whether wild or domesticated, and compare the two in the context of expected environment(s) in which either population of animals can be or will be found.

A key factor affecting the fitness of a GE animal is the nature of the introduced trait, and its effects on survival, reproduction and establishment. For example, an introduced trait could either improve or reduce the adaptability of an organism to a wider range of environmental conditions, allow it to obtain nutrition from previously indigestible sources, or limit the extent to which existing food sources provide adequate nutrition. Information on the phenotypic characterization of ABT salmon and their relatives that contain the EO-1ɑ construct is contained in Appendix F. In addition, information on the phenotype of other GE salmonids, most notably GE coho salmon, is contained in Appendices F and G and can be used to make inferences about the effects of growth-promoting constructs on the fitness of salmonids.

Detailed analyses of the phenotype of the various life stages of ABT salmon (see Appendix F) indicate that the introduction of the EO-1ɑ construct did not have deleterious effects on the health of the salmon. Fitness and associated endpoints (e.g., oxygen requirements, swimming speed, metabolic scope, etc.) was not explicitly evaluated in the studies conducted by ABT and submitted to the agency in support of phenotypic characterization and animal safety. Peer-reviewed scientific studies investigating fitness characteristics in GH transgenic Atlantic salmon containing the EO-1ɑ construct (described in Appendix F), however, generally indicate that changes in the observed phenotype of these fish result in neutral or decreased fitness characteristics. Decreased fitness would be expected to reduce the chances for establishment should ABT salmon escape from commercial production facilities. This potential reduction in fitness of adult animals, however, is not expected to be compromised to such an extent that survival would be affected greatly, at least on a short-term basis.

In an optimized production environment where food is not a limiting resource, Atlantic salmon expressing a GH gene, including AAS relatives (Du *et al*., 1992) and EO-1ɑ salmon (Tibbetts *et al*., 2013), will have a growth advantage over wildtype Atlantic salmon. However, it is unclear how much of a fitness advantage, if any, the rapid early growth exhibited by EO-1ɑ salmon would provide to salmon that have escaped the confines of a production system and entered the natural environment where food availability and other conditions are not as optimal. Several studies have noted the absence of enhanced growth in EO-1ɑ first feeding fry, the growth stage at which rapid growth might be expected to provide a significant advantage in a natural setting (Levesque, *et al*., 2008; Moreau, 2014). There is similar evidence from ABT salmon relatives (Cook *et al*., 2000a), coho salmon (Devlin *et al*., 2004a), and rainbow trout (Crossin *et al*., 2015).

The growth-enhancing and metabolic effects of the EO-1ɑ transgene are delayed until after first feeding is underway (Moreau, 2014). Subsequently, GH transgenic salmonids, including AAS relatives, exhibit aggressive foraging behavior even in the presence of predators and, as a result, suffer more severe predation than wildtype comparators (Abrahams and Sutterlin, 1999; Crossin and Devlin, 2017; Crossin *et al*., 2015; Devlin *et al*. 2015). This increased predation would be expected to negatively affect the potential survival and establishment of GH transgenic salmon populations. In addition, in food constrained environments, GH transgenic salmonids, including EO-1ɑ salmon, do not always exhibit superior growth relative to wildtype comparators (Crossin *et al*., 2015; Leggatt *et al*., 2017b; Moreau *et al*., 2011b), showing a lack of enhanced growth fitness at times under conditions expected to more representative of those in the real world.

The development of EO-1ɑ salmon has been undertaken with the express purpose of developing a genotype of salmon that will grow more rapidly and more efficiently in an aquaculture setting (i.e., a food-rich, predator-free environment). The physiological effects and phenotypic characteristics that accompany the rapid growth achieved in these fish are not optimized for success in a natural environment. In their paper on early life consequences of GH-transgenesis in rainbow trout, Crossin *et al*. (2015) noted that the growth and survival effects of GH-transgenesis exhibited by the rainbow trout in their studies could "*impose a significant fitness cost at an early life-history stage of salmonids when selection pressure is naturally high*." They concluded that the growth enhancements arising from expression of a GH transgene in nature would "*likely be constrained by the interacting effects of low resource availability and high predation risks*" (Crossin *et al*., 2015). Leggatt *et al*. (2017b) came to a similar conclusion in their study of the fitness of GH coho salmon reared in marine-like mesocosms, stating "*the current and previous data do not provide evidence that overall increased performance of GH [coho salmon] relative to wildtype coho salmon would arise in the marine environment*."

Introducing GH genes through transgenesis has been shown to have multiple effects on the transgenic salmonids beyond the targeted increase in growth. EO-1α salmon have been shown to have increased cardiovascular capacity but no increase in total metabolic scope; elevated oxygen requirements in adult EO-1α; lower critical swimming speed than the strain of wildtype salmon used to develop the EO-1α salmon; and elevated responses to stress factors (Cnaani *et al*., 2013; Deitch *et al*., 2006; Levesque *et al*., 2008; Polymeropoulos *et al*., 2014). Given that any one of these effects could reduce overall fitness, it is reasonable to theorize the combined effect of multiple factors would have an even more severe impact on the fitness of EO-1α salmon.

In addition to the physiological and behavioral factors that reduce the fitness of EO-1α salmon, genetic factors associated with the requirement to rear EO-1α salmon in contained aquaculture systems likely further mitigate the risk that escapees would become established. Multiple studies of escaped farmed salmonids and released wild strains of salmon have shown that even a single generation of rearing in captivity is sufficient to reduce fitness relative to wild conspecifics (Abrantes *et al*., 2011; Glover *et al*., 2012; Milot *et al*., 2013; Rodewald *et al*., 2011; Salvanes, 2017; see Appendix E). ABT salmon have been bred and reared in captivity for 14 generations. Although there are no data to show that 14 generations reduce the fitness of ABT salmon more than described in the studies above, it can be inferred that the risk of survival and dispersal posed by an escape would be reduced overall.

c. Survival and Return Rates for Escaped Farmed Atlantic salmon without the rDNA Construct in the Natural Environment

There are no specific data addressing survivability of ABT salmon should they escape confinement in PEI and enter nearby estuarine and marine environments. However, general information on the survival of farmed Atlantic salmon in the natural environment can be helpful to understand the likely survival/return rates for ABT salmon. Survival rates for wild-reared Atlantic salmon are presented in Section 8.1 of this assessment.

In terms of feeding and survival, farmed Atlantic salmon that have escaped ocean net pens often remain in the vicinity of the fish farm from which they have escaped and continue to feed on feed pellets that pass through the pen netting (Soto *et al*., 2001). Further, although not extensively studied to date, the survival of escaped and released farmed salmon has been found to be low (Hansen, 2006; Whoriskey *et al*., 2006), supported by the fact that marine survival rates for hatchery origin Atlantic salmon are also very low, 0.04 to 0.5%, and well below those of wild salmon (ICES, 2009). Jensen *et al*. (2016) found that, when released into the natural environment, hatchery-reared stocked Atlantic salmon had reduced growth, age of maturity and survival compared to wild-reared Atlantic salmon. For example, the mean return rate of post-smolt Atlantic salmon from marine to fresh water was 2.35% (range = 0.77% - 5.40%) for wild-reared Atlantic salmon and 0.98% for hatchery-reared Atlantic salmon (range = 0.44% - 2.69%) (Jensen *et al*., 2016).

The low survival and return rates may be due, at least in part, to the hypothesis that farmed fish fail to adapt to feeding on live prey after they have escaped from net pens in which they are fed artificial feeds, and thus, starve to death (Muir, 2004). In support of this, Olsen and Skilbrei (2010) simulated salmon escape from net pens and found the stomachs of recaptured fish were generally empty in the first few weeks after release. Using lipid analysis, they also found that none of the fish recaptured many months later near the release site had switched to wild prey diets. The previous work by Hislop and Webb (1992) found that that 65% of the escaped farmed salmon on the west coast of Scotland had empty stomachs, while only 35% had switched to natural prey. Similarly, Soto *et al*. (2001) found that approximately 60% of recaptured escaped Atlantic salmon in southern Chile had empty stomachs. Because ABT salmon are raised on pelleted synthetic diets similar to those fed to farmed salmon in ocean net pens and cages, this collective information suggests that in the highly unlikely event they were to escape the PEI facilities, many, if not most, of these ABT salmon would have difficulty transitioning to a wild prey diet, and thus, would be highly susceptible to starvation and early mortality.

In addition to feeding deficiencies, Morera *et al*. (2021) discussed deficient smoltification as a primary cause of mortality in Chilean Atlantic salmon aquaculture, with 11.2% of deaths attributed to fish experiencing problems with osmoregulation. The change in smoltification and salinity tolerance associated with genetic differences arising from generations of breeding decreases the likelihood that farmed salmon will survive seawater migration (Handeland *et al*., 2003; McCormick, 1994).

Behavioral changes such as decreased predator awareness may also be a factor resulting in increased mortality in farmed fish. In experiments with artificial conditions, farmed salmon have displayed decreased predator awareness compared to wild-reared salmon, attributed to the effects of breeding in an environment without predator selection pressure (Glover *et al*., 2017). Under controlled hatchery conditions, farmed fish spend less time in refuges than their wild-type counterparts and demonstrate a smaller reduction in growth than wild salmon in the presence of a predator, potentially leaving farmed salmon more vulnerable to predation (Glover *et al*., 2017). Studies conducted under seminatural conditions with artificial predators, however, have not shown a clear relationship between domestication and predator risk (Glover *et al*., 2017; Solberg *et al*., 2015).

Skaala *et al*. (2019) reported the relative survival of domesticated, F1-hybrid, and wild-reared Atlantic salmon, introduced as eggs to the River Guddalselva in Western Norway. Egg-to-smolt survival of domesticated salmon was about half of the other cohorts and showed significantly reduced survival in both freshwater and marine environments. The authors concluded that the relative fitness of domesticated fish was about 21 to 30% of the lifetime fitness of wild Atlantic salmon. In cohorts with greater salmon abundance, the survival difference between domesticated and wild salmon was more significant, attributed to increased competition (Skaala *et al*., 2019). Hybrid salmon also showed reduced survival compared to wild salmon in the freshwater stage, but similar survival in marine environments (Skaala *et al*., 2019). Depending on the type of data used, egg-to-returning adult ratios for wild, hybrid, and domesticated fish were 1:0.76:0.30 or 1:0.44:0.21 respectively (Skaala *et al*., 2019). Previous studies conducted in the environment estimated the lifetime fitness of domesticated Atlantic salmon to be 2-16% of that of wild salmon (Glover *et al*., 2017).

d. Biological Containment

The ability of ABT salmon to reproduce and subsequently establish in the natural environment is largely a function of the extent and adequacy of biological containment in the fish that escape. Biological containment includes limiting the reproduction of the fish within the culture system, preventing reproduction of the fish once they enter the receiving environment, or preventing the expression of the genes of concern (e.g., the transgene) in the event of an escape (Mair *et al*., 2007). Biological containment can serve as an effective risk mitigation measure by both (1) preventing any possibility of reproduction at the grow-out site, thus greatly reducing the risk of escape and/or release of gametes, embryos, or larval stages, and (2) greatly reducing or eliminating the possibility of reproduction of the GE organisms if they accidentally escape. The biological containment methods used by ABT at the PEI facilities are listed in Table 7-1 and presented below in Table 9-3. Each biocontainment measure used by ABT is discussed in detail below.

Table 9-3. Biological Containment for Each Type of AquaBounty Technologies (ABT) Salmon held at Facilities on Prince Edward Island is Designated by an X

Type of ABT Salmon	All-Female[c]	Triploid	Cannot Reproduce Naturally
AquAdvantage Salmon (AAS)—triploid[a]	X	X	X
AAS—diploid[a]	X	-	-
AA Female Broodstock (AAFB)	X	-	-
AA Neomale Broodstock (AANB)[b]	X	-	X

[a] Per the Durability Plan, triploidization of AAS eggs must exceed 95% (based on the statistical 95% lower confidence limit) or the batch of eggs is to be destroyed. Therefore, it is possible that ≤5% of the AAS will be diploid and capable of reproducing naturally.

[b] Neomales are genotypically female, but phenotypically male, and lack a sperm duct. Therefore, they cannot reproduce on their own.

[c] There are no genotypic male ABT salmon raised at the PEI facilities. However, there may be genotypic male Atlantic salmon without the rDNA construct reared the PEI facilities (see Sections 6.3 and 6.4.2).

Biological confinement is ensured in AAS through the use of triploidy and the production of all-female populations for grow-out. As discussed further below, although very highly effective (99.5% in the case of AAS), the current process used for inducing triploidy is not perfect (i.e., not 100% effective). Therefore, a second inherent form of reproductive containment, which ensures that ABT salmon (not just AAS) are all females through the design of a production process using gynogenesis, and which in this case is 100% effective (i.e., production of males is not possible), is required. Although all ABT salmon are genetically female, only AAS are triploid. The female broodstock used for the production of AAS eyed-eggs are diploid, and therefore, could hypothetically reproduce naturally. In addition, because the methods to induce triploidy are not perfect, up to 5% of the AAS may also be diploid and capable of reproducing naturally.[36] A description of each ABT salmon's reproductive potential is provided below and is used in the following sections (Sections 9.2.2.2 through 9.2.2.5) to determine the likelihood of reproduction and establishment in the natural environment.

i. Female, Mono-Sex Populations

As described in Section 5.3.1.1 of the 2015 EA, in the initial development of AAS, ABT used a complex production process involving gynogenesis and neomales (sex-reversed females)[83] to ensure that a monosex, all-female population of AAS would be produced for grow-out. Using gynogenesis[84] as part of the production process, rather than chemically induced sex-reversal alone, not only eliminated the time and labor that would be needed to distinguish neomales from true males following androgen treatment, but also essentially ensured 100% effectiveness in producing a genetically all-female population with a full complement of maternal DNA. When combined with a sterilization technique, such as triploidy, the production of all-female populations of ABT salmon ensures a highly effective form of biological containment, which is the reason that production of all-female triploids has often been discussed in relation to GE fish (NRC, 2004a; Devlin *et al*., 2006; Mair *et al*., 2007). It is important to note that the gynogenesis process is no longer utilized in the production process of AAS because it is no longer needed to maintain an all-female population (see Section 6.4, above). Although the production method could have been designed to produce an all-male fish population, the production of females is preferred because triploid males, although sterile, can still engage in spawning behavior with diploid females in the wild, thereby leading to the reduced reproductive success of the wild females. Therefore, all ABT salmon are genotypically female fish. They would need to find a male conspecific (e.g., wild male Atlantic salmon) or a relative in the same genus (e.g., brown trout) to reproduce, i.e., ABT salmon cannot reproduce with one another.

In addition, AANB are genetically female but phenotypically male. AANB are treated with 17α-MT early in their development to create genotypically female fish that can produce sperm. AANB do not have a functioning sperm duct and cannot spawn naturally (see Section 6, above). The inability of neomales, in general, to release milt on their own would further preclude potential reproduction should an escape or release of broodstock occur. In salmonids, sexual development is usually disrupted in neomales such that they usually have less well-developed testes, and most individuals characteristically lack functional sperm ducts (also known as gonopores or gonoducts) (Fitzpatrick *et al*., 2005; Geffen and Evans, 2000; Johnstone *et al*., 1978; Tsumura *et al*., 1991). As a result, in the hatchery, spermatozoa (milt) must usually be collected directly from the testis by sacrificing the fish. In order to produce crosses resulting in AAS, the neomales are sacrificed and their milt is

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[83] Genetic (XX) females that have been treated with an androgen (17-methyltestosterone) during early development produce milt and have the other sexual characteristics of a male fish. Crossing milt from neomales with eggs from true females can produce only genetically female offspring.

[84] The process of gynogenesis involves the destruction of the genetic component in fish sperm, use of those "empty" sperm for egg activation, and restoration of a diploid state in the activated egg by forced retention of the second polar body.

manually removed in order to fertilize eggs. Therefore, AANB are not able to reproduce in the natural environment.

To ensure the future validity of the production process in making all-female populations, the NADA approval requires additional genotypic post-approval monitoring of the AANB as part of its Durability Plan (see FOI Summary for NADA 141-454). The Durability Plan involves periodic testing and annual reporting on this (and other) processes. Records kept by ABT on these and other processes are subject to validation by ABT and inspection by the agency.

ii. Triploidy

All AAS eggs sold or distributed for grow-out are subjected to pressure treatment shortly after fertilization to induce triploidy. The induction of triploidy is the only accepted method currently available for sterilizing fish on a commercial scale. Triploid fish have three sets of chromosomes in their somatic cells, rather than the two sets in the normal diploid state. Benfey (2001) describes two fundamental effects of triploidy on fish physiology: (1) the size of the somatic cells increases to accommodate the extra genetic material, but the number of cells decreases so that triploid fish are no larger overall than diploids, and (2) gametogenesis and gonadal development is so severely impaired that triploids are sterile. Triploidy is generally induced by either thermal or hydrostatic pressure shock of the eggs within the first hour after fertilization. Hydrostatic pressure shock is more easily controlled and therefore preferred (Benfey, 2001); this is the method that is used to generate triploid AAS. Pressure shock treatment that occurs at 5-10 ^{0}C for 5 mins approximately 30-60 mins after fertilization has been used successfully to induce triploidy in five year-classes of Atlantic salmon in New Brunswick, Canada (O'Flynn *et al*., 1997). Following pressure treatment, the AAS eggs are water-hardened. The very high efficiency of the induction process (> 99%) ensures that very few diploid eggs with possible future reproductive potential would be shipped for grow-out.

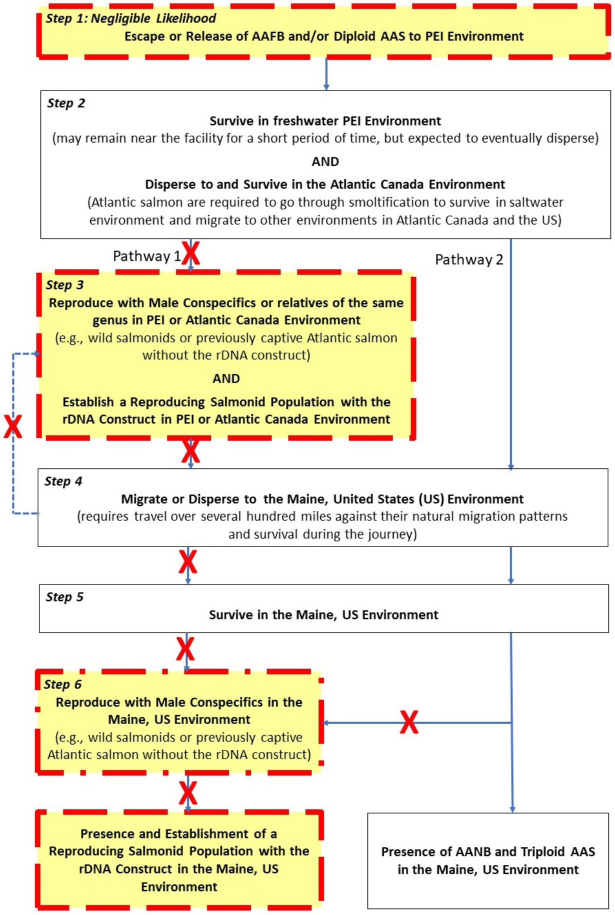
As part of the Durability Plan to which ABT has committed, ploidy testing will be conducted on a subset of all batches of fertilized eggs intended to be sold or distributed. Per the Durability Plan, if, based on testing, triploidization in these eggs does not exceed 95% (based on the statistical 95% lower confidence limit), the batch of eggs is to be destroyed. ABT's success rate for achieving triploidy has been demonstrated to be quite high. Between 2017 and 2022, triploidy was achieved in 99.5% of AAS eyed-eggs on average and ranged from 96.9% to 100%.[36] The validation of ABT's pressure shocking method to induce triploidy and cause sterilization in fish was extensively discussed in Section 7.4.1.1 of the 2015 EA. Because the testing methodology used for verifying triploidy results in egg destruction, it would be impossible to verify 100% triploidy in all of the eggs actually used for grow-out through testing. Therefore, there is a slight possibility that ≤5% of the AAS will be diploid and could hypothetically reproduce naturally.

AAS have been described in the 2015 and 2019 EAs as being "sterile" or "effectively sterile" or "functionally sterile." The common characterization in the fisheries and aquaculture scientific literature is that "triploidy" equals "sterility," in other words, the major consequence or outcome of triploidy is gonadal sterility (Piferrer *et al*., 2009). Because of this, the two words are often used interchangeably. Although adequate demonstration of triploidy has been provided to FDA, there are no specific data demonstrating that triploid AAS are indeed sterile, that is, incapable of producing viable offspring; however, as discussed below, there are several reasons why this is believed to be the case, which is described in Appendix H.9.

iii. AANB and Triploid AAS Cannot Reproduce

As stated in Section 8.2, Figure 8-5 and Table 9-3, and explained in detail in the sections above, AANB and triploid AAS cannot reproduce naturally on their own, and therefore, they cannot establish a population in the natural environment. Thus, AANB and triploid AAS cannot complete Steps 3 and 6 of the pathway analysis (see Box 3); i.e., AANB and triploid AAS cannot reproduce or establish a population in either PEI/Atlantic Canada or Maine. However, AANB and triploid AAS could potentially still cause harms simply by being present in the Maine environment, that is, without being able to reproduce. This possibility is discussed further under Section 9.3, below.

Box 3: Possible Pathways to Exposure for AANB and triploid AAS (Figure 8-5)



e. Reproduction of GH Transgenic Fish in the Natural Environment

AAFB and diploid AAS are reproductively competent and homozygous or hemizygous for the rDNA construct, and therefore, could hypothetically reproduce with male conspecifics (e.g., wild Atlantic salmon) or relatives of the same genus (e.g., brown trout) to establish a population of salmonids with the rDNA construct in PEI/Atlantic Canada and/or Maine (see paragraph above and Section 8.2).

In order to evaluate the likelihood of this occurring, it is important to understand whether AAFB and diploid AAS could reproduce with conspecifics or relatives of the same genus in the natural environment. ABT has not conducted any laboratory studies with their fish to determine their reproductive capabilities, but based on the production process, it is known that AAFB can produce viable eggs. Also, because

AAFB and diploid AAS have not escaped or been released from any ABT facilities, it is unknown whether they could reproduce in the natural environment. However, there is published literature studying the potential for GE or transgenic salmon with similar GH constructs to reproduce in the natural environment. Those studies are summarized below and used in Sections 9.2.2.3 (Step 3) and 9.2.2.5 (Step 5) to help evaluate the likelihood of AAFB and diploid AAS to reproduce in PEI/Atlantic Canada and Maine.

Changes in the age at maturation, fecundity, and sterility could alter population and community dynamics and interfere with the reproduction of related organisms (ABRAC, 1995). Due to their enhanced growth rate, diploid ABT salmon could be expected to achieve reproductive maturity in a shorter time frame than their siblings without the rDNA construct. Because many animals, including Atlantic salmon, select mates based upon male body size, it has been theorized that diploid GE males exhibiting larger-than-average body size potentially might have an advantage over their wild counterparts. This could contribute to enhanced reproductive performance (fitness).

Contrary to what has been theorized, however, the research conducted to date on GH transgenic Atlantic salmon, particularly under simulated natural conditions, generally does not indicate that these fish have a reproductive advantage compared to their non-GE counterparts (Appendix F). In fact, studies with two alternative male reproductive phenotypes of Atlantic salmon (i.e., large anadromous adults that have migrated to the sea and returned to their natal streams, and small precocial parr that have matured in freshwater, having never been to sea) indicate that GH transgenic salmon display reduced breeding performance relative to non-transgenics (Moreau *et al*., 2011a; Moreau and Fleming, 2012). In pair-wise competitive trials with a naturalized stream mesocosm, wild anadromous (i.e., large, migratory) males outperformed captively reared GH transgenic counterparts in terms of nest fidelity, quivering frequency, and spawn participation (Moreau *et al*., 2011a). In addition, captively reared non-transgenic mature parr were superior competitors to their GH transgenic counterparts with respect to nest fidelity and spawn participation. The non-transgenic parr also had higher overall fertilization success than GH transgenic parr, and their offspring were represented in more spawning trials. Similarly, for precocial males with an alternative (small, non-migratory) phenotype, GH-transgenesis did not influence male maturation in the first year of life, despite facilitating growth to sizes typical of mature wild-type parr, and in the second year, the number of maturing transgenic parr was only half that of the non-transgenic individuals (Moreau and Fleming, 2012).

Similar reproductive studies on GH transgenic coho salmon, although not necessarily representative of diploid GH transgenic Atlantic salmon, also indicate they are out-competed by wild-reared coho salmon in semi-natural mating arenas within a contained facility (Fitzpatrick *et al*., 2011). In competitive spawning experiments, GH transgenic coho salmon performed fewer courtship and aggressive defense behaviors (male salmon defend mates from other males during breeding) than coho salmon from nature and sired less than 6% of offspring. These and additional study findings led the study authors to suggest that there is "*limited potential for the transmission of transgenes from cultured GH transgenic coho salmon through natural matings should they escape from a contained culture facility into nature and reproductively interact with a local wild coho salmon strain*." These study results corroborate those of previous studies by Bessey *et al*. (2004) on GH transgenic coho salmon in which fewer transgenic females spawned than hatchery females under experimental conditions, and transgenic females displayed consistently low levels of courtship behavior. See Section 9.3.2.2 and Appendices F and G for additional information on the likelihood of harms occurring due to reproduction of GH transgenic salmon.

Oke *et al*. (2013) reported on the hybridization of diploid GH transgenic Atlantic salmon with closely related wild diploid brown trout (*Salmo trutta*). Experimental crosses produced in the laboratory using gametes from diploid fish resulted in transgenic hybrids (i.e., hybrids with the GH EO-1α transgene) that were viable[85] and grew more rapidly than GE salmon and other non-transgenic crosses in hatchery-like conditions. In stream mesocosms designed to emulate natural conditions, transgenic hybrids appeared to express competitive dominance and suppressed growth of transgenic and non-transgenic salmon. The researchers did not investigate the fertility of the transgenic hybrids or the viability of any progeny resulting from hybrid backcrosses[86] to either Atlantic salmon or brown trout; however, they did identify and discuss several lines of evidence from the literature that combine to suggest that introgression of the transgene into the brown trout genome via backcrossing is unlikely. The implications of these observations (i.e., viable hybrids) for risk of establishment and further introgression are mitigated; however, as it has long been observed that progeny resulting from backcrosses of Atlantic salmon X brown trout hybrids are either non-viable, or triploid and therefore effectively sterile (Galbreath and Thorgaard, 1995). Thus, there is virtually no potential for any further introgression of the transgene into brown trout or Atlantic salmon genomes via backcrossing.

Collectively, these results suggest that in the event of an escape, AAFB and diploid AAS would have compromised reproductive performance, that is, reduced fitness compared to wild Atlantic salmon.

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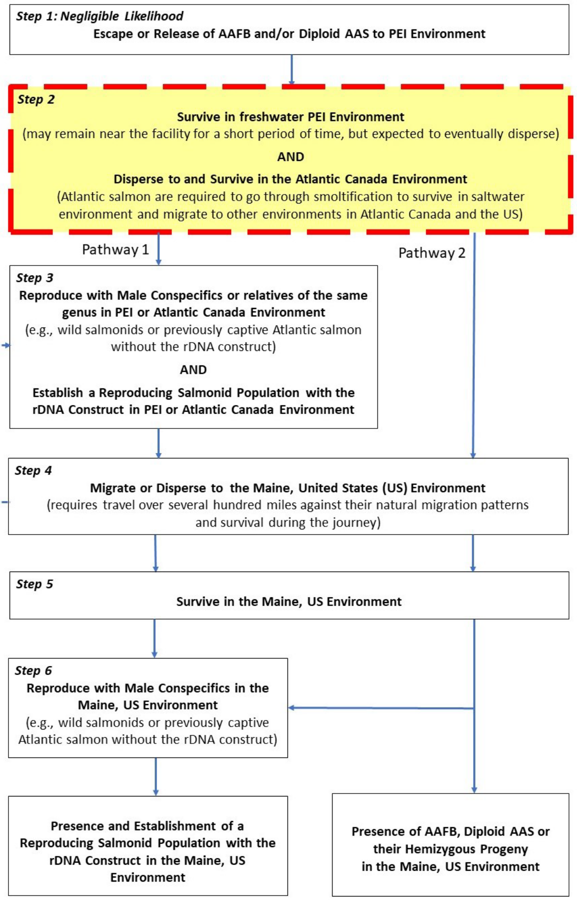
[85] This is not the first time that viable offspring (hybrids) have been produced by crossing diploid Atlantic salmon with diploid brown trout; these species are closely related and others have demonstrated hybridization both in wild populations through natural hybridization (Verspoor, 1988; Hurrell and Price, 1991; Jansson *et al*., 1991; McGowan and Davidson, 1992) and in the laboratory through artificial fertilization (Refstie and Gjedrem, 1975; Chevassus, 1979; Gray *et al*., 1993). This study differs from the others, as it appears to be the first report of production of viable hybrids from a cross of *transgenic* diploid Atlantic salmon with diploid brown trout. One clear implication is that transgenic Atlantic salmon are no different from non-transgenics with respect to this characteristic.

[86] Backcrosses are the result of a crossing of a hybrid with one of its parents or an individual genetically similar to its parent, in order to achieve offspring with a genetic identity which is closer to that of the parent.

9.2.2.2. Step 2: Likelihood of Survival and Dispersal in PEI and Atlantic Canada

This section of the assessment will use the information presented in Sections 9.2.2.1.a-e (above) to evaluate the potential for escaped ABT salmon to survive and disperse in PEI and Atlantic Canada. This is Step 2 in the exposure pathway analysis illustrated in Figure 8-4 and Figure 8-5 (see Box 4). The analysis for Step 2 applies to all types of ABT salmon, including diploid and triploid AAS, AAFB and AANB.[87]

Box 4: Possible Pathways to Exposure (Figure 8-4)



The likelihood of ABT salmon surviving and dispersing in the immediate PEI environment following a highly unlikely escape from the Bay Fortune and Rollo Bay facilities was previously evaluated under Risk-related Question 2 in Section 7.3 of the 2015 and 2019 EAs. Herein, survival and dispersal are re-evaluated considering future planned expanded production at the Rollo Bay facility, including the rearing of Atlantic salmon without the rDNA construct, and new information published in the literature since the 2019 EA.

In the unlikely event of escape, the survival of ABT salmon on PEI would be a function of the life stage(s) escaping, their fitness, and the location in which escape occurred. In order for escapees to survive, the accessible ecosystem must meet their needs for environmental conditions (salinity, temperature, water quality, etc.), food, habitat, and environmental cues for reproduction. In order to disperse and begin their migration to sea as adults, ABT salmon would need a waterway clear of obstructions (e.g., beaver dams, culverts) and with access to the estuarine/marine environment, and to have optimal environmental conditions that allow for the salmon to undergo smoltification (the gradual change in physiology to adapt to saltwater

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[87] For reference, Section 8.3 contains a detailed description of the potentially accessible environments, including PEI, Atlantic Canada, and Maine, and Section 8.4 contains the status of wild Atlantic salmon in these environments.

conditions). These factors are discussed below when determining the likelihood of survival and dispersal of ABT salmon in PEI and Atlantic Canada (Step 2 of Figure 8-4 and Figure 8-5, see Box 4).

a. Survival in the PEI Environment

Survival of ABT salmon in the natural environment could be either short- or long-term depending upon the specific situation. Because there are a variety of possible situations that ABT salmon may encounter following escape, the potential length of survival is variable and, therefore, both possibilities (short- and long-term survival) are discussed herein. For this assessment, short-term survival is defined as acute, meaning weeks to months, and less than one generation. Long-term survival is defined as years. It is important to clarify that long-term survival does not necessarily mean establishment has occurred; therefore, long-term survival and establishment will be discussed separately.

PEI is within the natural range of wild Atlantic salmon. However, the life stage at the time of escape and the location of escape (e.g., fresh versus salt water) are critically important to determining both short-term and long-term survival of ABT salmon. The most likely location of escape would be in waterways adjacent to the facilities on PEI. Because the location of escape is an important factor when considering likelihood of long-term survival, survival of escapees in the immediate environment surrounding the Bay Fortune and Rollo Bay facilities will be discussed separately below.

i. Bay Fortune

As described above in Section 9.2.2.1.a, the Bay Fortune facility discharges to a small freshwater drainage ditch that flows approximately 36-37 m downhill until it discharges into Fortune River estuary, which eventually connects to Rollo Bay and the Northumberland Strait (Figure 7-3). Although embryos and early life stages (i.e., alevin) could survive for a short time in this drainage ditch, the small, shallow ditch does not contain adequate food or habitat (including spawning habitat) for long-term salmon survival, reproduction, and establishment. They would likely move quickly down the ditch a short distance and into the Fortune River either on their own or with the push of water flow during a storm event. Once there, the embryos and early life stages of ABT salmon would not be expected to survive the conditions of high salinity (21 ppt) found there.

The potential fate of post-smolt ABT salmon in the environment surrounding the Bay Fortune facility is less clear. Based on the information provided in Section 9.2.2.1 above, it is quite possible that juvenile and adult ABT salmon[88] would not be able to survive in the marine/estuarine environment of Fortune River estuary and Rollo Bay due to (1) the sudden, abrupt transition from their low salinity (1 ppt), freshwater environment to a moderately high salinity (21 ppt), brackish water environment, (2) failure to transition to a wild prey diet (see Section 9.2.2.1.c, above), and (3) a higher likelihood of being preyed upon (Sundström *et al.*, 2004b). However, it cannot

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[88] Juveniles and adults include the life stages that may have smoltified (smolt and post-smolt), see Figure 8-2.

be concluded with certainty that some of the juvenile and adult ABT salmon would not survive the salinity conditions of the Fortune River estuary environment. Depending on their age at the time, a portion of juveniles and adult ABT salmon may have smoltified, thus their physiologic status may allow them to tolerate the salinity there.

Thus, depending upon the life stage at the time of escape/release, the likelihood of survival in Fortune River estuary following the escape from the Bay Fortune facility is ranked as negligible for eggs, fry, and parr (i.e., extremely unlikely or not reasonably foreseeable occurrence) to moderate for smolt and older life stages (i.e., occurs at certain times of the year or in isolated areas or under certain conditions).

ii. Rollo Bay

If ABT salmon survive the polishing pond, stone wash-out, and vegetative strip outside the effluent discharge pipes at the Rollo Bay facility (see Sections 7.2 and 9.2.1.1, above), then they would enter the Rollo Bay Brook. The Rollo Bay Brook is a small freshwater stream less than a meter in width with variable flow that runs through the Rollo Bay property. The brook flows downstream approximately 1.5 km before discharging to Rollo Bay, an offshoot of Northumberland Strait (which is the same estuarine/marine environment that the Fortune River estuary discharges to, see Figure 7-3).

In the highly unlikely event escaped fish were to enter the Rollo Bay Brook, it is quite possible that any life stage of escaped ABT salmon could survive there, at least in the short term, based on the current presence of brook trout in this stream. However, older and larger life stages are less likely to be able to disperse (migrate) downstream and survive the journey to Northumberland Strait via the Rollo Bay Brook due to shallow water in many locations. In the unlikely event that some ABT salmon were able to migrate downstream and exit the brook, they would reach Rollo Bay (Figure 7-3). When the brook reaches Rollo Bay, it spreads into a mini-delta and crosses a sandbar prior to entering Rollo Bay and the Strait. The shallow water depth at the sandbar would provide some barrier inhibiting larger fish from entering the marine environment, particularly at low tide and during periods of low water flow from the brook. Further, the Northumberland Strait is turbid and can be highly inhospitable to salmonids in the summer months due to high temperatures (>25 °C) and low DO levels (<5 mg/L) (Coffin *et al*. 2013; and van den Heuvel *et al*., 2017; see Section 8.3.1.4, above) that are potentially lethal to salmon.

Consequently, while the receiving environment of Rollo Bay Brook may allow short-term survival, it is not a highly favorable environment and does not appear to be a habitat suitable for reproduction and establishment as evidenced by the absence of Atlantic salmon in the watershed where the facility resides and despite stocking and attempts in the past to establish populations in the area. However, because it cannot be concluded with certainty that ABT salmon would not survive in Rollo Bay Brook and Rollo Bay, the likelihood of survival in Rollo Bay Brook following the escape from the Rollo Bay facility is ranked as low (i.e., an event or situation not occurring very often or not occurring in large numbers; isolated occurrence; ephemeral presence) to moderate (i.e., occurs at certain times of the year or in isolated areas or under certain conditions) depending on which life stage(s) is present at the time of escape.

The conclusions for the likelihood of survival and dispersal of ABT salmon in PEI is similar to the conclusions from the previous 2015 and 2019 EAs, and to the Canadian government's evaluations of these facilities (DFO 2013 and 2019).

b. Dispersal to and Survival in Atlantic Canada

As discussed above in Section 8.4.2 (above), the Bay Fortune estuary has not supported wild Atlantic salmon populations since prior to 2001 (Guignion *et al*., 2010; Cairns *et al*., 2010; see Section 8.4.2), and Atlantic salmon have never been reported in the Rollo Bay watershed (Figure 8-10(a),(b)), indicating that these immediate environments are impaired and/or lack the necessary resources for long-term salmon survival and/or establishment. If ABT salmon survive the initial introduction into the PEI environment, there is nothing to preclude them from eventually dispersing to other areas on PEI or in Atlantic Canada in search of food, habitat, and mates. Although drastically reduced from historic levels (see Section 8.4.2), there are still some suitable habitats on PEI (see Figure 8-10(b)) and in other parts of Atlantic Canada close to PEI (New Brunswick and Nova Scotia) that could potentially support Atlantic salmon populations.

There is nothing specifically precluding dispersal from the Bay Fortune estuary to Northumberland Strait and beyond. However, there are some physical impediments in the Rollo Bay Brook such as shallow water, snags and downed trees, a road culvert and a sandbar at the mouth of Rollo Bay that could impede some ABT salmon life stages from dispersing downstream and into Rollo Bay (Section 8.3.1.3). In general, as they mature, escaped farmed Atlantic salmon of hatchery origin show a strong tendency to migrate into rivers in the vicinity of the site of escape (Ferguson *et al*., 2007). If ABT salmon behave similarly, and they would be expected to because of their domesticated genetic background, then they should remain in the general vicinity of the PEI facilities in the event of an escape or release. Thus, more than likely, the ABT salmon would disperse to other areas on PEI close to the two ABT facilities that are suitable for Atlantic salmon survival. However, there is a possibility they could also disperse to other coastal areas of Atlantic Canada such as New Brunswick and Nova Scotia along the Northumberland Strait. Thus, the likelihood of dispersing and surviving in the local PEI and Atlantic Canada environment is ranked as moderate (i.e., occurs at certain times of the year or in isolated areas or under certain conditions).

When assessing likelihood of survival in Atlantic Canada, it is also important to consider their survival when migrating to feeding grounds in Atlantic Canada or Greenland (see Section 8.1.2 and Figure 8-1 and Figure 8-3, above). If escaped ABT salmon survived the PEI environment, and if they behaved as wild Atlantic salmon post-smolts normally do, then they may migrate to other parts of Atlantic Canada or Greenland (via the Northern Atlantic Ocean) to feed before eventually returning to their natal waters on PEI to reproduce (Figure 8-3). Atlantic Canada and the Northern Atlantic Ocean are within the historical natural range of Atlantic salmon (see Section 8.1.2 and Figure 8-1 and Figure 8-3, above). However, in order to survive the migration from PEI to Atlantic Canada and the Northern Atlantic Ocean, they would need to avoid predation, avoid being caught by fishing vessels, and find sufficient food. Based on information in the literature, survival and return rates for wild and farmed Atlantic salmon during their migration at sea are small and vary greatly from year to year, likely due to annual differences in food availability and environmental conditions. As described in Section 8.1, marine survival rates of wild

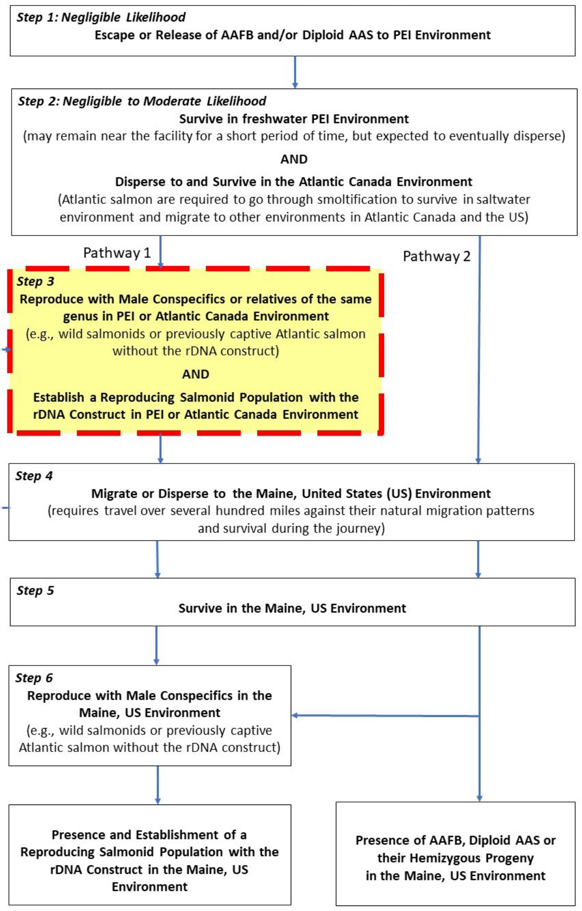
Atlantic salmon smolts have been reported as: 1.3 to 17.4% (Hutchings and Jones, 1998) and 5 to 35% (Chaput, 2012), with <10% surviving a second season at sea (Fleming, 1998). Marine survival rates for farmed Atlantic salmon are even lower than those for wild salmon, only 0.04 to 0.5% (ICES, 2009). Similarly, Jensen *et al.* (2016) found low mean return rates (marine to freshwater) for post-smolt wild-reared Atlantic salmon (2.35%, range = 0.77-5.40%), and even lower return rates for hatchery-reared Atlantic salmon (0.98%, range = 0.44-2.69%). Thus, marine survival for ABT salmon, a domesticated farmed salmon, in Atlantic Canada are not expected to occur in large numbers (see Section 9.2.2.1.c above). Therefore, it can be concluded that the likelihood of escaped ABT salmon surviving marine migration in Atlantic Canada is expected to be low (i.e., an event or situation not occurring very often and not occurring in large numbers; isolated occurrence; ephemeral presence).

c. Conclusion for Step 2

Based on the information above, there are many physical and environmental barriers to survival and dispersal of ABT salmon at all life stages from the environment surrounding the PEI facilities. However, Atlantic salmon have historically resided in PEI waters (see Section 8.4.2, above), although not found naturally in the Rollo Bay watershed, and therefore, there is nothing that would specifically preclude some life stages from surviving in Fortune River and Rollo Bay Brook environments. If they are able to survive in these environments, then they could potentially disperse to Rollo Bay and move on to Northumberland Strait and the Atlantic Ocean. Although marine survival and return rates for farm-reared Atlantic salmon that escape are low (<1%), these environments are within the natural range of wild Atlantic salmon, and thus, hypothetically some ABT salmon could survive. Therefore, it is concluded that the combined likelihood of survival on PEI and dispersal and survival in Atlantic Canada (including other areas of PEI) and the Atlantic Ocean is ranked as negligible to moderate depending on life stage at time of escape and location of escape (see Table 9-1 for definition of rankings).

9.2.2.3. Step 3: Likelihood of Reproduction and Establishment of ABT Salmon in PEI/Atlantic Canada

Box 5: Possible Pathways to Exposure (Figure 8-4)



Step 3 of the exposure pathway analysis illustrates the potential for ABT salmon to reproduce with conspecifics or relatives of the same genus in PEI and/or Atlantic Canada (see Box 5). This could result in establishment of local populations of salmonids containing the rDNA construct. Theoretically, these populations could continue to disperse and spread slowly (over many generations) down the eastern coast of Atlantic Canada and eventually reach the Maine environment resulting in presence or establishment.

As discussed in Section 8.4.2, above, the watersheds surrounding the PEI facilities do not currently have established populations of wild Atlantic salmon (see Figure 8-10(b)). Therefore, the likelihood of Step 3 occurring is dependent upon the ability of the escaped salmon to disperse to areas with suitable spawning habitats and mates, as well as their ability to reproduce in the natural environment (including competing for mates).

a. Reproductive Capability

As stated above in Section 9.2.2.1.d, AANB and triploid AAS cannot reproduce naturally, and therefore, cannot complete Step 3 (see Figure 8-4). Thus, only AAFB and diploid AAS[89] could hypothetically reproduce with conspecifics or relatives of the same genus in PEI and/or Atlantic Canada (see inset Box 5), which is a substantially smaller population compared to that of triploid AAS (see Section 6.4.2). For the purpose of this assessment, conspecifics include wild Atlantic salmon and previously

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[89] AAFB are all female and homozygous or hemizygous for the rDNA construct, and diploid AAS are all female and hemizygous for the rDNA construct. Per the Durability Plan, up to 5% of AAS could be diploid. However, based on recent testing (2016-2020), it is estimated that on average only 0.5% (range of 0-3.1%) of AAS are diploid (see Section 6.3). Thus, the "diploid AAS" evaluated in this assessment represent this small population of AAS that could be diploid.

captive Atlantic salmon without the rDNA construct, including those held at the PEI facilities as part of the production process.[90] AAFB and diploid AAS could also hypothetically mate with a relative in the same genus, such as brown trout, and produce hybrid salmonids with the rDNA construct.

In addition, AAFB and diploid AAS are all-female fish, and therefore, would need to find a male with which to mate, along with a suitable mating habitat. The nesting site (known as a redd) is chosen by the female, and has stringent requirements, including: generally a gravel-bottom riffle upstream from a pool (Bigelow, 1963; Scott and Crossman, 1973), water descent of 0.2-3%; water depth of 50 to 90 cm; running speed of 0.3 to 0.7 m/s; gravel size of 3 to 5 cm; and nest size of 1 to 2 m (MUNLV, 2001), see Section 8.1, above.

Furthermore, the reproductive fitness of AAFB and diploid AAS would also impact their reproductive success in the natural environment. As discussed in Section 9.2.2.1.e above, based on studies conducted in GH transgenic Atlantic salmon, it does not appear that the GH transgene imparts any reproductive advantage compared to non-transgenic counterparts. In fact, literature suggests that AAFB and diploid AAS may have compromised reproductive performance compared to wild Atlantic salmon. However, there is nothing to preclude some of the AAFB and diploid AAS from reproducing if the conditions are suitable.

Therefore, in order to reproduce, the escaped all-female AAFB and diploid AAS would need to find a suitable male conspecific or relative of the same genus, a suitable nesting site, and would need to outcompete wild females for both. Without each of these variables occurring, reproduction and establishment of a salmonid population with the rDNA construct in PEI/Atlantic Canada cannot occur.

b. Establishment in Different Environments in Atlantic Canada

Atlantic Canada, including PEI, is within the natural range of the North American Atlantic salmon (see Figure 8-1), and therefore, it may provide suitable habitat for survival, reproduction, and establishment of salmonids with the rDNA construct. The most likely location for this to occur in Atlantic Canada would be environments close to the ABT facilities on PEI. In addition, for this assessment, reproduction and establishment of salmonids with the rDNA construct in New Brunswick (along Northumberland Strait) and Nova Scotia also need to be evaluated due to their close proximity to Maine (see Figure 7-1), and in the case of Nova Scotia, its location intermediate between PEI and Maine. Establishment in New Brunswick (along Northumberland Strait) or Nova Scotia, in particular, could hypothetically lead to the dispersal and spread of salmonids with the rDNA construct down the eastern Canadian coastline which could eventually result in exposure in Maine (although, it would likely require many generations and would take many years, if not decades). Therefore, the suitability of each of these habitats for establishment of a salmonid population with the rDNA construct is separately considered below.

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[90] As a reminder, male Atlantic salmon without the rDNA construct may be reared at the Rollo Bay facility. See fn 40.

i. PEI

Although once prevalent in PEI waters and currently inhabiting 26 other rivers on PEI (Cairns *et al*., 2010, and Cairns and MacFarlane, 2015), wild Atlantic salmon populations (or those resulting from stocking efforts on the island) are no longer found in the Fortune River basin/estuary where the Bay Fortune facility is located or in any of the other rivers in the area (Guignion *et al*., 2010; Cairns *et al*., 2010), see Section 8.4.2, above. In addition, wild Atlantic salmon populations have never been reported in Rollo Bay Brook or the waters in the Rollo Bay watershed where the Rollo Bay facility is located (Figure 8-10(a),(b)). This strongly suggests that the local aquatic ecosystems near the PEI facilities are not suitable for reproduction and establishment of Atlantic salmon. In addition, the Atlantic salmon populations on PEI are designated as "special concern" according to COSEWIC (2011; see Table 8-2 in Section 8.4.1), and, on PEI, Atlantic salmon populations are listed as "may be at risk" (Guignion *et al*., 2010). Several serious threats to salmon populations on PEI have been identified, including stream sedimentation, pesticide runoff and associated kills, and blockage to fish passage, among others (Cairns *et al*., 2010). Therefore, AAFB and diploid AAS would likely have difficulty finding male wild Atlantic salmon to mate with and suitable nesting sites on PEI, especially in the areas surrounding the PEI facilities.

However, it is also important to consider the potential for AAFB and diploid AAS to reproduce with Atlantic salmon without the rDNA construct that will be reared in the Bay Fortune and Rollo Bay facilities. Atlantic salmon without the rDNA construct are needed in the production process, but ABT also plans to produce eggs of Atlantic salmon without the rDNA construct for commercial sale at the Rollo Bay facility.[40,58] Because there are limited wild conspecifics on PEI, escape or release of ABT's male Atlantic salmon without the rDNA construct would increase the likelihood of AAFB and diploid AAS finding a suitable mate on PEI. As a result, the potential for reproduction and establishment with these fish needs to be considered in the event of an escape.

As discussed previously, Atlantic salmon have not been found in the Fortune River since before the year 2001 and have never been found in Rollo Bay Brook or in the Rollo Bay watershed (see Section 8.4.2; Guignion, 2009; Cairns *et al*., 2010; Guignion *et al*., 2010). This indicates that if Atlantic salmon without the rDNA construct escaped from the ABT facilities on PEI, it is highly unlikely that they would be able to survive long-term and establish in areas around the facilities (i.e., if native Atlantic salmon have not established in these areas, then there is no reason to expect escaped farmed-Atlantic salmon to do so). In addition, ABT plans to primarily rear *female* Atlantic salmon without the rDNA construct (see Section 6.4.2 above). Although there will be some male Atlantic salmon without the rDNA construct[40,58] used for production of Atlantic salmon without the rDNA construct at these facilities, the number will be substantially lower compared to the females (Section 6.4.2), which further lowers the probability that an all-female AAFB and diploid AAS would find a suitable male with which to mate. Further, given that GH transgenic Atlantic salmon in general do not have a reproductive advantage compared to non-transgenic Atlantic salmon, and sometimes are disadvantaged (see Section 9.2.2.1.e; Moreau *et al*., 2011a; Moreau and Fleming, 2012), it is expected that a significant number of fish would need to escape in order for there to be any potential chance of reproduction and establishment. As previously discussed in Section 9.2.1, there is a negligible likelihood of escape occurring at the ABT facilities due to the many levels of containment, which has been confirmed by Failure Modes Analysis conducted by Canadian authorities (DFO, 2013 and 2019; McGowan and Leggatt, 2020; McGowan *et al.*, 2021). However, because the escape or release of male Atlantic salmon with the rDNA construct could occur at the same time and location as AAFB and diploid AAS, and there is some uncertainty with regards to survival, dispersal, and reproduction of the AAFB and diploid AAS on PEI (see Section 9.2.2.2), there is an increased likelihood that female AAFB and diploid AAS could mate with ABT's male Atlantic salmon without the rDNA construct.

AAFB and diploid AAS could also hypothetically mate with a relative in the same genus, such as brown trout. Although widely occurring in many other parts of Canada and North America, there are no brown trout present on PEI (DFO, 1988; see Section 8.3.1.1). Thus, although Atlantic salmon (*Salmo salar*), including AAFB and diploid AAS, could hypothetically interbreed with brown trout (*Salmo trutta*), which are of the same genus, to produce viable hybrids (see Section 9.2.2.1.e), interactions and genetic introgression with brown trout will not occur because the two species will not be present together in the same location on PEI. In addition, as shown by Galbreath and Thorgaard (1995), progeny resulting from backcrosses of Atlantic salmon and brown trout hybrids are either non-viable, or triploid and therefore effectively sterile. These results preclude the potential for any further introgression of the transgene into brown trout or Atlantic salmon genomes via backcrossing and indicate that any highly unlikely hybrid populations will die out after a single generation, which eliminates the likelihood of establishment of a hybrid Atlantic salmon x brown trout with the rDNA construct from further consideration in this assessment.

Aside from Atlantic salmon, two other salmonids species, brook trout (*S. fontinalis*) and non-native rainbow trout[91] (*O. mykiss*) are found in PEI streams (Guignon *et al*., 2010). Of these, only brook trout are found in the Fortune River where the Bay Fortune facility is located. Brook trout are also found in the Rollo Bay Brook adjacent to the Rollo Bay facility. Laboratory crosses of male brook trout with female Atlantic salmon have been shown to produce small numbers of viable fry (1-5%), but the reciprocal crosses (female brook trout crosses with male Atlantic salmon) are unsuccessful (Sutterlin *et al*., 1977; Gray *et al*., 1993). More importantly, FDA is unaware of any reports of natural hybridization between these two species in the wild despite the fact that they have coevolved in North America and often coexist, at least as juveniles, within habitats where their native ranges overlap (Fausch, 1998). Therefore, the likelihood of establishment of a hybrid Atlantic salmon x brook trout with the rDNA construct is also eliminated from further consideration herein.

ii. New Brunswick and Nova Scotia

The most recent COSEWIC assessment in 2011 designated Atlantic salmon populations in PEI, New Brunswick and parts of Nova Scotia as "special concern," which is defined as "*a wildlife species that may become a threatened or an endangered species because of a combination of biological characteristics and identified threats*"; see Table 8-2 and Section 8.4.1 above. In addition, the Atlantic

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[91] The rainbow trout is a native of western North America. It was introduced into PEI in 1925 (DFO, 1988).

salmon populations on the eastern coast of Nova Scotia, including Eastern Cape Brenton, Nova Scotia Southern Upland, Inner Bay of Fundy, and Outer Bay of Fundy, are considered "endangered" according to the COSEWIC (2011). COSEWIC defines endangered as "*a wildlife species facing imminent extirpation or extinction*" (COSEWIC, 2010). Thus, like PEI, New Brunswick and Nova Scotia also do not appear to currently have favorable environments for the reproduction and establishment of a salmonid population with the rDNA construct. With small or no local populations of Atlantic salmon present, the likelihood of reproduction of all-female AAFB and diploid AAS would be low.

c. Conclusions for Step 3

In order to have reproduction and establishment on PEI or in Atlantic Canada, AAFB and diploid AAS must find a suitable male conspecific, suitable spawning site, and be capable of reproducing (e.g., outcompete wild Atlantic salmon). Wild Atlantic salmon populations in PEI, New Brunswick, and Nova Scotia have substantially declined over the past century and are currently listed as "special concern" or "endangered." This suggests that AAFB and diploid AAS would likely have difficulty finding a suitable male wild Atlantic salmon to mate with, as well as a suitable nesting site. In addition, in the unlikely event they did find a wild male, studies suggest that transgenic fish, like AAFB and diploid AAS, would have reduced reproductive performance when competing for mates and reproducing.

However, because ABT plans to also rear Atlantic salmon without the rDNA construct at the Rollo Bay facility, the likelihood of finding a suitable male conspecific with which to reproduce increases. Hypothetically, all-female AAFB and diploid AAS could escape at the same time and place as male Atlantic salmon without the rDNA construct reared at ABT facilities and, therefore, could possibly result in reproduction on PEI. This is a highly unlikely scenario that would require failure in redundant multi-level physical and procedural containment and security (which has a negligible likelihood of occurring, Section 9.2.1) to allow for escape or release, and the number of AAFB, diploid AAS, and male Atlantic salmon without the rDNA construct that escaped would have to be high enough to offset natural mortalities. Further, these fish would have to find suitable habitat to reproduce and establish in watersheds that have not supported a wild Atlantic salmon population in decades or would have to disperse over longer distances on PEI to find such habitats. All of these situations occurring in succession or simultaneously appears highly unlikely; however, because there is some uncertainty associated with this step, it will be assigned a low (i.e., an event or situation not occurring very often and not occurring in large numbers; isolated occurrence; ephemeral presence) to moderate (i.e., occurs at certain times of the year or in isolated areas or under certain conditions) likelihood of occurrence.

In addition, if Step 3 is unlikely to occur on PEI, then the likelihood of reproduction and establishment occurring in New Brunswick (along the Northumberland Strait) or Nova Scotia is also considered low because it is unlikely that AAFB and diploid AAS and male ABT Atlantic salmon without the rDNA construct, or their progeny, would migrate to these locations, find a suitable habitat, and establish.

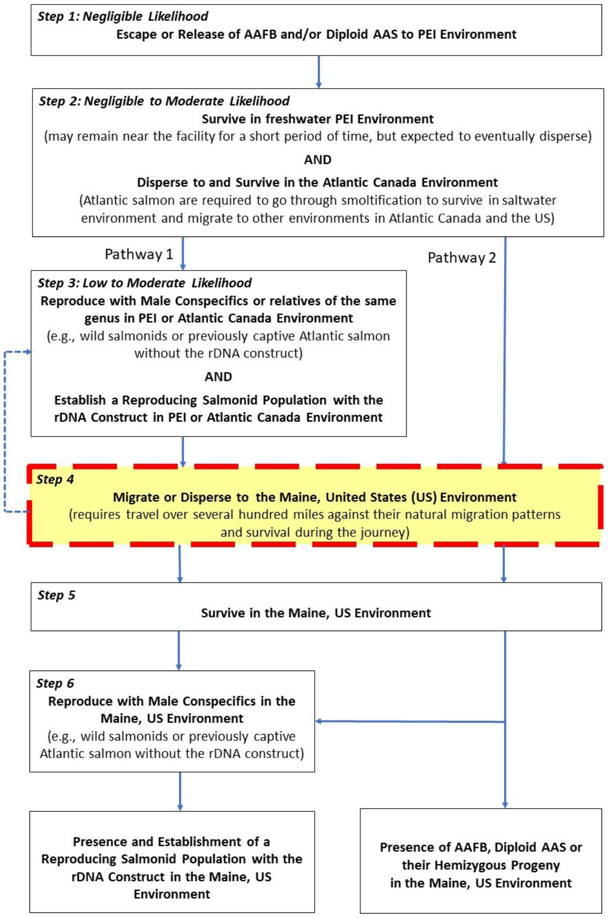
Therefore, the combined ranking for the likelihood of reproduction and establishment of AAFB and diploid AAS in PEI and/or Atlantic Canada is low to moderate depending on whether Atlantic salmon with the rDNA construct escape from the PEI facilities at the same time as AAFB and diploid AAS. Furthermore, as stated above, the progeny of a cross between AAFB and diploid AAS and a relative of the same genus (brown trout or brook trout) would not be viable or could not produce viable offspring when backcrossed; thus, this likelihood is ranked as negligible.

9.2.2.4. Step 4: Likelihood of Migration or Dispersal to the Maine (US) Environment

Step 4 of the pathway analysis illustrates the potential for ABT salmon to migrate or disperse to the Maine, US, environment (see Figure 8-4, Box 6). For the purposes of this assessment, migration is defined as travel over long distances (hundreds of kilometers) with no establishment of new populations along the way. Dispersion (or spread) is travel over shorter distances with the potential for establishment, similar to that described for New Brunswick and Nova Scotia above in Step 3 (Section 9.2.2.3 above).

There are two hypothetical pathways by which migration to Maine could occur: (1) direct migration of ABT salmon or their progeny from PEI to Maine as illustrated in Pathway 2 with an arrow directly from Step 2 → 4 in Figure 8-4 and Figure 8-5 (see inset Box 6); and (2) a series of short-distance dispersals with establishment of populations of salmonids with the rDNA construct (i.e., progeny of ABT salmon) at locations down the eastern coastline of Nova Scotia and eventually entering the Gulf of Maine, illustrated in Pathway 1 with an arrow from Step 3 → 4. The likelihood of these pathways occurring are discussed further below.

Box 6: Possible Pathways to Exposure (Figure 8-4)



ABT salmon could possibly migrate directly to the Maine environment without establishing any populations in PEI/Atlantic Canada, which is illustrated as Step 2 → Step 4 in Figure 8-4 (see Box 6). In order to migrate directly to the Maine environment, the surviving ABT salmon would not only need to complete a significant long-distance migration (hundreds of kilometers) but would also need to overcome a natural tendency through a process known as imprinting to return to the waterways in which they originated. Female Atlantic salmon smolt typically return to their natal waters to spawn (see Section 8.1, above), which for ABT salmon would likely be considered the rivers of northeast PEI (Fortune River and Rollo Bay Brook) because "imprinting" of the natal river usually occurs during smoltification (NOAA, 2022). In addition, there is no reason to expect any escaped ABT salmon to undertake a migration to waters of the US given that these fish are produced from domesticated hatchery stocks, as are farmed Atlantic salmon. As previously discussed in Step 2 (Section 9.2.2.3 above), ABT salmon are expected to migrate into rivers in the vicinity of the site of escape as do escaped farmed Atlantic salmon of hatchery origin (Ferguson *et al*., 2007). Therefore, ABT salmon are expected to remain around the PEI facilities, and it is considered to be highly unlikely that ABT salmon (or salmonids with the rDNA construct) would migrate directly to Maine (Figure 8-3, Step 2 → Step 4, see Box 6) because it would require them to act counter to their typical behaviors and travel hundreds of kilometers against their normal migration patterns.

Even if ABT salmon were to undertake such a migration, it is unlikely that any significant numbers would survive the journey. Similar to the analysis for PEI/Atlantic Canada above, the Northern Atlantic Ocean and Maine are well-within the historical natural range of Atlantic salmon (see Figure 8-3), but in order to survive the migration to Maine, escaped ABT salmon would need to avoid predation, avoid being caught by fishing vessels, and find suitable food. Based on recent survival and return rate data for US and Canadian Atlantic salmon stocks, marine survival rates for wild origin Atlantic salmon are very low (0.16 to 6.1%) and those for hatchery origin Atlantic salmon are even lower, 0.04 to 0.5% (ICES, 2009). Similarly, Jensen et al. (2016) found low mean return rates (marine to freshwater) for post-smolt wild-reared Atlantic salmon (2.35%, range = 0.77-5.40%), and even lower return rates for hatchery-reared Atlantic salmon (0.98%, range = 0.44-2.69%). Triploidy has been shown to further reduce survival/recapture rates of salmon in the field (O'Flynn *et al*., 1997; Cotter *et al*., 2000a; Wilkens *et al*., 2001, see additional information in Appendix H). Mortality rates for AAFB and AANB would be expected to be at least as high and perhaps higher (>99%) because of their higher metabolism and food requirements, susceptibility to predation, and adaptation to feeding on synthetic aquaculture diets.

In addition, for the same reasons as discussed for direct migration to Maine, the likelihood of salmonids with the rDNA construct dispersing to Maine from established populations in New Brunswick (along the Northumberland Strait) or Nova Scotia (Step 3 → 4) via a series of short-distance dispersals is also considered to be unlikely. The likelihood of establishment of a new population of salmonids with the rDNA construct in New Brunswick and Nova Scotia was found to be low in Step 3. Thus, the likelihood of continual short-distance dispersions followed by establishing local populations, then additional short-distanced dispersions until a population eventually enters Maine waters, is even lower. This type of dispersion would require many generations to accomplish and likely take decades.

However, Atlantic salmon are known to routinely complete migrations over long distances, and thus, it is hypothetically possible for them to migrate the long distance to Maine or other areas of Atlantic Canada. Thus, based on the information above, the likelihood of ABT salmon migrating or dispersing to the US environment is ranked as negligible (i.e., extremely unlikely or not reasonably foreseeable occurrence) to low (i.e., an event or situation not occurring very often and not occurring in large numbers; isolated occurrence; ephemeral presence).

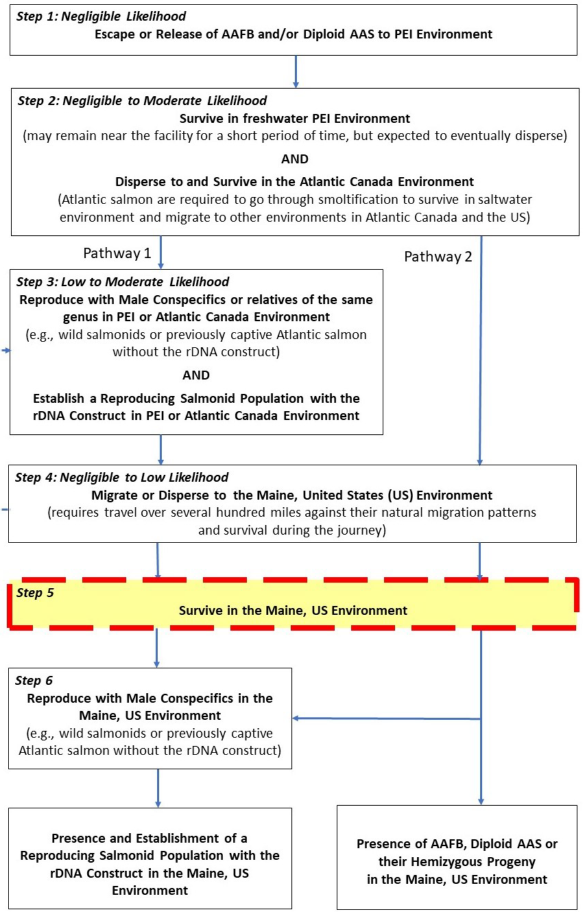
a. Conclusion for Step 4

The ABT salmon and progeny with the rDNA construct are not expected to migrate directly to Maine because of their biological instinct to return to their natal streams due to imprinting during smoltification. Imprinting would occur in Fortune River or Rollo Bay Brook; therefore, in the highly unlikely event that ABT salmon were able to escape or be released and disperse, it is expected they would return to PEI. In addition, domesticated Atlantic salmon, like ABT salmon, are likely to migrate into the rivers near the site of escape on PEI, not to those in Maine. Further, marine survival and return rates for farm-raised Atlantic salmon are very low; likely due to farm-raised fish's failure to adapt to feeding on live prey and high risk of predation (including fishing). Marine survival rates are expected to be even lower for triploid Atlantic salmon such as AAS. In addition, the likelihood of entering Maine via a series of short-distance dispersals and establishments of salmonids with the rDNA construct (i.e., progeny of ABT salmon) down the eastern coastline of Nova Scotia (Step 3 to 4) is expected to be low considering the low likelihood of establishment in Nova Scotia and the very low abundance of wild Atlantic salmon populations in this region (see Step 3, Section 9.2.2.3). However, because Atlantic salmon are known to routinely complete migrations over long distances, it is hypothetically possible for them to migrate the long distance to Maine waters. For these reasons, the likelihood of migration to Maine was assigned a range from negligible to low.

9.2.2.5. Step 5: Likelihood of Survival in the Maine Environment

Step 5 of the pathway analysis illustrates the potential for ABT salmon to survive in the Maine, US, environment (see Box 7). The factors for survival discussed under Step 2 are also applicable to this step, i.e., suitable environmental conditions, food, spawning habitat, and mates. This is discussed further below.

Box 7: Possible Pathways to Exposure (Figure 8-4)



Maine is within the natural range of Atlantic salmon. In the past, the northeastern US supported many populations of native Atlantic salmon (Section 8.1, above); however, due to dams, industrial pollution, climate change, overfishing, and other reasons (see Section 8.4.3), most of the native Atlantic salmon in the northeastern US have been extirpated. Some small remnant populations remain in some of the large rivers in New England. However, the endangered Gulf of Maine DPS is the only remaining native Atlantic salmon population in the US (NOAA and FWS, 2020), and the majority (85%) of the native and hatchery-reared Atlantic salmon only return to one river, the Penobscot River in Maine. This information suggests that critical habitat for long-term survival and establishment of Atlantic salmon may not be currently suitable or accessible in many areas in Maine as evidenced by the absence of Atlantic salmon in many of the local watersheds despite years of stocking and attempts in the past to establish populations in the area. However, ongoing habitat restoration efforts (e.g., dam removals and improvements to fish passage) are improving access to critical habitat and could improve the current situation.[92] Thus, the likelihood of survival in the Maine environment is ranked as low (i.e., an

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[92] NMFS published a "Recovery Plan (2019) for the Gulf of Maine Distinct Population Segment of Atlantic Salmon (*Salmo salar*)" in 2019. "*The goal of this 2019 recovery plan is the remove of a Gulf of Maine DPS of Atlantic salmon from the federal list of endangered and threatened wildlife and plants.*" https://www.fisheries.noaa.gov/resource/document/recovery-plan-2019-gulf-maine-distinct-population-segment-atlantic-salmon-salmo (accessed December 8, 2023).

event or situation not occurring very often or in large numbers; isolated occurrence; ephemeral presence) to moderate (i.e., occurs at certain times of the year or in isolated areas or under certain conditions).

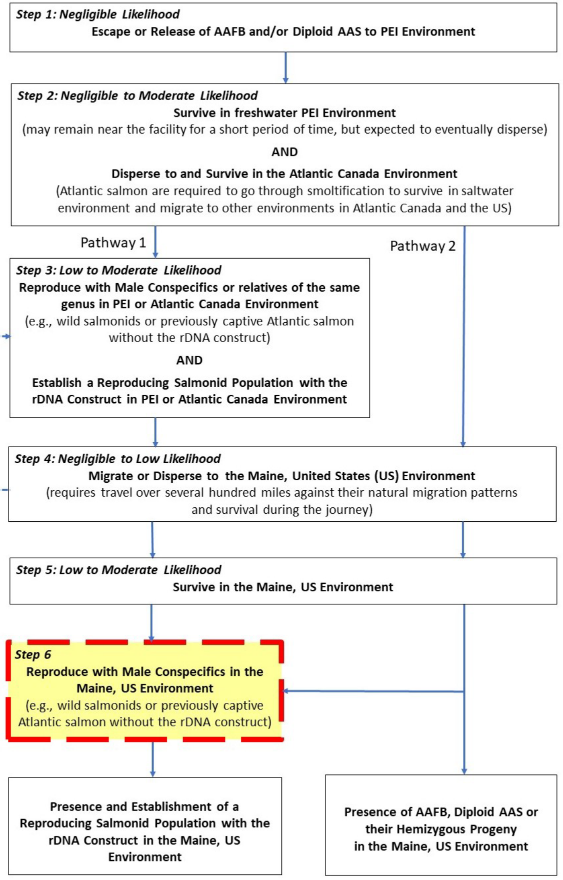
a. Conclusion for Step 5

Wild Atlantic salmon populations have decreased substantially in the northeastern US; see Section 8.4. The decline of Atlantic salmon populations in these regions suggests that these environments are not currently favorable for long-term survival or establishment. However, efforts are underway in the state of Maine focused on the restoration of habitat critical to the survival of Atlantic salmon in that region. Thus, the likelihood of survival in the Maine environment is ranked as low to moderate.

9.2.2.6. Step 6: Likelihood of Reproduction in the Maine Environment

Step 6 of the pathway analysis illustrates the potential for AAFB and diploid AAS or their progeny to reproduce with conspecifics[93] in the Maine, US, environment (see Box 8). This could result in the establishment of a new population of salmonids with the rDNA construct in Maine. Similar to reproduction in PEI/Atlantic Canada under Step 3 above (Section 9.2.2.3), Step 6 is dependent upon the ability of ABT salmon or salmonids with the rDNA construct to find suitable mates, suitable spawning sites in Maine, and be capable of reproducing, including outcompeting wild Atlantic salmon for mates. Without each of these variables occurring, reproduction and establishment of a salmonid population with the rDNA construct in Maine cannot occur.

Box 8: Posible Pathways to Exposure (Figure 8-4)



As discussed in Sections 9.2.2.1.d and 9.2.2.3 (Step 3), only AAFB and diploid AAS[89] can reproduce naturally on their own. Therefore, Step 6 cannot

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[93] Conspecifics include wild Atlantic salmon and previously captive Atlantic salmon without the rDNA construct, including those held at the PEI facilities as part of the production process.

occur for AANB or triploid AAS (see Figure 8-4). In addition, as described in Step 3 (Section 9.2.2.3), AAFB and diploid AAS are both all-female populations, and therefore, would need to find a male conspecific, wild Atlantic salmon or previously captive Atlantic salmon, with which to reproduce. Relatives of the same genus, such as brown trout and brook trout, were eliminated from further consideration under Step 3 (Section 9.2.2.3 above) because the progeny of Atlantic salmon and brown trout and Atlantic salmon and brook trout are either non-viable or triploid (effectively sterile) (Galbreath and Thorgaard, 1995) and would die out after a single generation. In addition, as discussed in Section 9.2.2.1.e above, literature suggests that AAFB and diploid AAS may have compromised reproductive performance compared to wild Atlantic salmon. However, there is nothing to preclude some of the AAFB and diploid AAS from reproducing with wild counterparts if the conditions are suitable.

As stated in Step 5 above (Section 9.2.2.4), Maine is within the historical natural range of Atlantic salmon (see Figure 8-1), and therefore, in theory it should provide suitable habitat for survival, reproduction, and establishment of salmonids with the rDNA construct. However, most native Atlantic salmon populations in the northeastern US have been extirpated due to dams, industrial pollution, climate change, overfishing, and other reasons (see Section 8.4.3, above). Some small remnant populations still exist in some large rivers in New England; however, the endangered Gulf of Maine DPS is the only remaining native Atlantic salmon population in the US. According to NOAA and FWS's most recent ESA assessment for the Gulf of Maine DPS conducted in 2020, this population is at "critically low abundance" with 10-year average return of 1247 Atlantic salmon adults (NOAA and FWS, 2020). Of these returning adults, 84% were hatchery-reared and the remainder were wild/naturally reared adult Atlantic salmon. Thus, most of the populations of the Maine DPS are supported by conservation hatcheries (NOAA and FWS, 2020). In addition, the majority (85%) of the native and hatchery-reared Atlantic salmon only returned to one Maine river, the Penobscot River.

The absence of wild Atlantic salmon, despite years of stocking and attempts in the past to establish populations in the area, indicates that the aquatic environment of the northeastern US, including Maine, is not currently favorable for reproduction and establishment of Atlantic salmon. However, as discussed in Step 5 above, ongoing habitat restoration efforts (e.g., dam removals and improvements to fish passage) are improving access to critical habitat. These improvements could allow for greater accessibility to spawning habitats in Maine, and thus, increase the abundance of suitable environments in Maine for AAFB and diploid AAS to reproduce and establish. Thus, the likelihood of reproduction and establishment in the Maine environment is ranked as low (based on current habitat conditions in Maine) to moderate (based on predicted restoration of Atlantic salmon habitat in Maine).

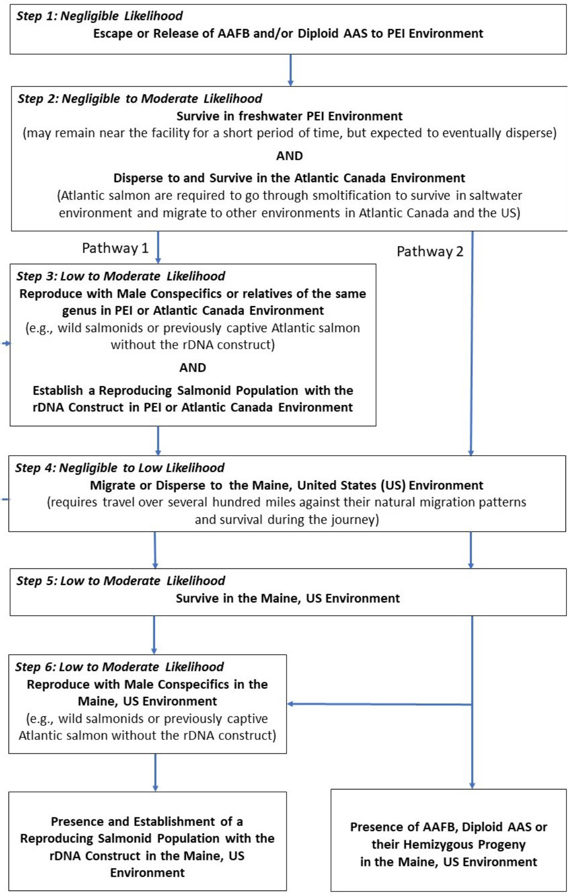
a. Conclusion for Step 6

In order to reproduce and establish in the northeastern US, including Maine (US), AAFB, diploid AAS or their progeny must migrate up rivers containing mature Atlantic salmon, find suitable male Atlantic salmon, suitable nesting sites and be capable of reproducing, including outcompeting wild female Atlantic salmon. Although wild Atlantic salmon populations were once abundant in the northeastern US these populations are mostly extirpated today. Although there are some remnants of Atlantic salmon in some large New England rivers, the only wild Atlantic salmon population that currently resides in Maine is listed as endangered under the ESA, known as the Gulf of Maine DPS, and is at critically low numbers. This information indicates that it would be difficult for AAFB, diploid AAS, and their progeny to find suitable mates and spawning sites in the current Maine environment. However, on-going restoration efforts could improve and increase accessible spawning habitats and increase the likelihood of AAFB and diploid AAS reproducing and establishing in the Maine environment. Therefore, the likelihood of AAFB, diploid AAS and their progeny to reproduce in Maine (US) is low (i.e., an event or situation not occurring very often or not occurring in large numbers; isolated occurrence; ephemeral presence) to moderate (i.e., occurs at certain times of the year or in isolated areas or under certain conditions).

9.2.2.7. Conclusion for Questions 2 and 3

In the highly unlikely event that escape occurred, ABT salmon would need to complete many sequential steps and overcome many physical and environmental barriers to survive, disperse, reproduce and establish in the natural environment, including: (1) surviving in the PEI environment and overcoming potential physical obstacles (e.g., shallow waters) to disperse from the PEI environment; (2) finding suitable mates and spawning sites and outcompeting wild and farm-reared Atlantic salmon without the rDNA construct (i.e., those that have escaped net pens or other aquaculture facilities) to reproduce and establish in PEI or other parts of Atlantic Canada (New Brunswick or Nova Scotia); (3) ignoring the imprinting of their natal streams and migrating directly to Maine, and surviving the migration, as well as surviving in the Maine environment (e.g., find food, avoid predation); and (4) migrating upstream in Maine, finding suitable mates and spawning sites and outcompeting wild Atlantic salmon to reproduce and establish in the Maine environment.

Box 9: Possible Pathways to Exposure (Figure 8-4)



The likelihood of each step occurring was evaluated in Sections 9.2.2.2 through 9.2.2.6 and the findings are presented in Box 9. The likelihoods for the steps range from negligible to moderate. The results of these analyses are highly variable due to the many situations that could occur in the natural environment after ABT salmon escape. The qualitative likelihood ranking for exposure (establishment and/or presence) occurring in Maine is further discussed in Section 9.2.3 below.

9.2.3. Conclusion on Likelihood of Exposure

In this section, we will determine whether there is a complete pathway to exposure in the US environment and determine the qualitative likelihood ranking for exposure in the Maine environment. For the purpose of this assessment, exposure has been defined as establishment and/or presence of ABT salmon or their progeny in the Maine (US) environment (Section 4.2). The hypothetical pathways and steps required to result in an exposure in the Maine environment were outlined in Section 8.2 and Figure 8-4 and Figure 8-5, and the likelihood of occurrence of each step was analyzed and assigned a qualitative ranking of likelihood in Sections 9.2.1 and 9.2.2 above. The steps and final likelihood ranking for each step is illustrated in the figure in Box 9 in Section 9.2.2.7 above and are listed in Table 9-4 below. The rankings for the likelihood of exposure (i.e., negligible, low, moderate, and high) are defined in Table 9-1.

As stated in Section 4.2, we are using the principles from NRC (2002 and 2004a) to evaluate risk from production of ABT salmon at two facilities on PEI, Canada. NRC (2002) describes risk (R) as the joint probability of exposure [$P(E)$], and the conditional probability of harm given that exposure has occurred [$P(H|E)$]: Risk (R) $= P(E) \times P(H|E)$. With that in mind, the probability of exposure [$P(E)$], i.e., establishment and/or presence in Maine, is the product of the probabilities of occurrence of each sequential step required for that exposure. Based on the exposure pathway analysis illustrated in Section 8.2 (Figure 8-4 and Figure 8-5), the establishment of a reproducing salmonid population with the rDNA construct in Maine could only occur for AAFB and diploid AAS.[89] Therefore, the probability of establishment in Maine by AAFB and diploid AAS can be described as:

$$\mathrm{P(E_{establishment}) = P(Step\ 1) \times P(Step\ 2) \times P(Step\ 3) \times P(Step\ 4) \times P(Step\ 5) \times P(Step\ 6)}$$

It is important to note that Step 3, reproduction and establishment of a salmonid population with the rDNA construct in PEI and/or Atlantic Canada, is not required to occur in order for establishment to occur in Maine. Alternatively, AAFB and diploid AAS could possibly migrate directly to Maine, and then reproduce with male conspecifics in the Maine environment, which is represented as Pathway 2 in Figure 8-4 (Step 2 → Step 4 → Step 5 → Step 6, and is discussed in Sections 8.2.1.2.a and 9.2.2.4). If this alternate pathway occurred, the probability for establishment in Maine by AAFB and diploid AAS can be described as:

$$\mathrm{P(E_{establishment}) = P(Step\ 1) \times P(Step\ 2) \times P(Step\ 4) \times P(Step\ 5) \times P(Step\ 6)}$$

Because the presence of ABT salmon in the Maine environment does not require the fish to reproduce or establish (i.e., Steps 3 and 6 of the pathway analysis), the probability of the presence of all types of ABT salmon (AAFB, AANB, triploid AAS and/or diploid AAS) in the Maine environment can be described as:

$$\mathrm{P(E_{presence}) = P(Step\ 1) \times P(Step\ 2) \times P(Step\ 4) \times P(Step\ 5)}$$

Some of the steps in the pathway analysis are limited by the occurrence of other steps. For example, the likelihood of reproduction is limited by the likelihood of survival, which is limited by the likelihood of escape. Thus, the overall likelihood of exposure would be determined by the step with the lowest ranked likelihood, i.e., if any of the steps of the pathways are found to be negligible, then the likelihood of that exposure, either presence or establishment, occurring would also be negligible.

The likelihood of each step occurring and the overall likelihood of establishment or presence in the Maine environment is presented in Table 9-4 below, along with the requirements for each step to occur. The overall likelihood of establishment was evaluated separately from the likelihood of presence in Table 9-4 because presence can occur without reproduction and establishment, and presence alone can potentially cause harms through direct interactions in the Maine environment (e.g., competition for food and habitat). In addition, the likelihood of presence and establishment was also separately ranked for those types of ABT salmon that can reproduce naturally, AAFB and diploid AAS, and those that cannot, AANB and triploid AAS, because those that cannot reproduce, cannot establish. The overall likelihood of presence and establishment are displayed below Table 9-4.

Table 9-4. Summary of Likelihood Rankings and Requirements for Each Step in the Exposure Pathway.

Step	Description	Requirements for Steps to Occur	Likelihood Rankings[a] of AAFB and Diploid AAS	Likelihood Rankings[a] of AANB and Triploid AAS
1	Escape or release into the PEI environment (Section 9.2.1)	• Malfunction in physical containment and security • Human error • Natural disaster • Malicious Intent	Negligible	Negligible
2	Long-term survival in PEI environment, and dispersal to and survival in Atlantic Canada environment (Section 9.2.2.2)	• Must have optimal environmental conditions (e.g., salinity, temperature, suitable habitat) • Food must be available, and the fish must be able to compete for and consume live prey • Must be able to avoid predation (e.g., other animals, fishing vessels) • Must escape in large numbers to offset natural mortality • Must smoltify to disperse in Northumberland Strait and Atlantic Ocean • Must have waterway free of obstructions to disperse	Negligible to Moderate (depending on life stage at escape and location of escape)	Negligible to Moderate (depending on life stage at escape and location of escape)
3	Reproduce with male conspecifics or relatives of the same genus in PEI or Atlantic Canada environment, and establish a reproducing salmonid population with the rDNA construct in PEI or Atlantic Canada (Section 9.2.2.3)	• Must disperse to other areas of PEI and/or Atlantic Canada • Only AAFB and diploid AAS are capable of reproduction • Must have male conspecifics (wild or previously captive Atlantic salmon) nearby or be capable of migrating to mates • Must find suitable spawning site (with stringent guidelines, see Step 3, Section 9.2.2.3 above) • Must be able to outcompete wild female Atlantic salmon for mate and nesting site	Low to Moderate for conspecifics (if Atlantic salmon without the rDNA construct escape from the PEI facilities) Negligible for relatives of the same genus	Not Possible

Step	Description	Requirements for Steps to Occur	Likelihood Rankings[a] of AAFB and Diploid AAS	Likelihood Rankings[a] of AANB and Triploid AAS
4	Migrate or disperse to the Maine (US) environment (Section 9.2.2.4)	• Must be able to complete and survive significant long-distance migration (hundreds of kilometers) or complete a series of short-distanced dispersals and establishments down the eastern coastline of Nova Scotia • Must ignore imprinting of native river and natural tendency to remain near site of escape • Must survive migration, including finding suitable environmental conditions, food, and to avoid predation	Negligible to Low	Negligible to Low
5	Survive in Maine environment (Section 9.2.2.5)	• Needs suitable environmental conditions, including accessible food and habitat, and to avoid predation (see Step 2, above)	Low to Moderate	Low to Moderate
6	Reproduce with male conspecifics in the Maine (US) environment (Section 9.2.2.6)	• Must migrate up an accessible river in Maine and encounter a male Atlantic salmon. • All of the bullets in Step 3	Low to Moderate (depending on habitat restoration)	Not Possible

[a] See definition of each likelihood ranking (negligible, low, moderate and high) in Table 9-1.

Based on the likelihoods presented in Table 9-4 above, the overall likelihood for establishment and presence are shown below for AAFB/diploid AAS and AANB/triploid AAS:

Overall Likelihood of Establishment in Maine:

P(E) = P(Step 1) × P(Step 2) [× P(Step 3)] × P(Step 4) × P(Step 5) × P(Step 6)
For AAFB/diploid AAS, $P(E_{establishment})$ = Negligible
For AANB/triploid AAS, $P(E_{establishment})$ = Not Possible

Overall Likelihood of Presence in Maine:

P(E) = P(Step 1) × P(Step 2) × P(Step 4) × P(Step 5) × P(Step 6)
For AAFB/diploid AAS, $P(E_{presence})$ = Negligible
For AANB/triploid AAS, $P(E_{presence})$ = Negligible

NRC (2002) identified three variables that are important for determining the likelihood of establishment for a GE animal, and these variables also apply to determining the likelihood of presence of a GE animal:

1. the effect of the transgene on the “fitness” of the animal within the ecosystem into which it is released (i.e., survival and reproduction within the ecosystem);
2. the ability of the GE animal to escape and disperse into diverse communities; and
3. the stability and resiliency of the receiving community.

The likelihood of presence and establishment is dependent on all three parameters; however, the ability of the GE animal to escape is considered the most important of these because without escape (or intentional release) there can be no presence or establishment in the environment, and thus, no resulting impacts.

Escape/release was Step 1 of the pathway analysis presented in Figure 8-4 and Figure 8-5. Hypothetically, if the ABT salmon were to escape or be released, the scale and frequency of introductions in a particular environment would have a large influence on the potential ecological risk. Any introductions would have to involve a critical mass that could offset natural mortality and be of sufficient frequency and in proper season to allow for long-term survival and establishment. If the scale and frequency of the escapes (i.e., introductions to the environment) are small, the chances of becoming established in the natural setting are extremely low (Kapuscinski and Hallerman, 1991).

In Section 9.2.1 above (Question 1 and Step 1), the likelihood that ABT salmon will escape or be released from the Bay Fortune or Rollo Bay facilities was found to be negligible due to multiple redundant forms of physical containment and strong procedural containment and security. The multiple redundant forms of containment substantially reduce any possibility of a large number of ABT salmon escaping at the same time. For example, ABT has internal SOPs in place that outline specific steps for employees to remedy emergency situations, such as loss of power, pump failure, etc., including procedures to notify management of a catastrophic event. The only time a large-scale release could occur would be due to a devastating natural disaster event, such as a tsunami, where there was loss of a major portion of the physical containment and ABT personnel could not respond to the site to enact procedural controls. Up to this point, there have been no reports of tsunamis on PEI (see Section 8.3.1.1). In addition, the Rollo Bay facility, which will house the majority of ABT salmon, is located 1 km inland and 19 m above sea level and is unlikely to be subjected to and damaged by a tsunami. Thus, escape of a large number of ABT salmon is extremely unlikely and not expected to occur. Furthermore, it is important to mention that the number of eggs or fish contained at the Bay Fortune and Rollo Bay facilitates reported in Section 6.4.2 are considered hypothetical conservative yearly worst-case estimates (i.e., the maximums that would be present there at any time during the year even if for only a very short time). Most of the time during the year, these number would be lower, and for eggs, substantially lower (if not zero). A reduction in the number of fish held would also reduce the likelihood of a large-scale release. Section 6.4.2 above discusses how these numbers were determined.

In addition to establishment through reproduction, a type of pseudo-establishment could potentially occur if successive waves of large numbers of salmon escaped confinement and entered the local environment, with each wave replacing or supplementing the former as fish die off or disperse (Kapuscinski and Brister, 2001). This scenario would require the periodic escape or release of large numbers of fish, such as sometimes occurs from salmon farms with net pens in the marine environment. This type of pseudo-establishment is not a realistic possibility for the Bay Fortune or Rollo Bay facilities for the reasons described in the paragraph above (i.e., due to the highly redundant containment and security measures employed at the site).

Because the likelihood of escape from the PEI facilities is considered negligible, the overall likelihood of presence of all types of ABT salmon (including AAFB, AANB, triploid and diploid AAS) in the Maine environment is considered negligible, and the overall likelihood of establishment of AAFB, diploid AAS and their progeny in the Maine environment is also considered to be negligible. Therefore, the overall likelihood of exposure in Maine can be concluded to be negligible. This conclusion is similar to that made by Canadian authorities in their 2013 and 2019 authorizations for the Bay Fortune and Rollo Bay facilities. DFO (2013 and 2019) concluded that the likelihood of exposure of ABT salmon to the Canadian environment due to the production at the Bay Fortune and Rollo Bay facilities is negligible due to containment (both physical and biological).[94] In addition, because many of the limiting steps in the exposure pathways are considered a negligible to moderate likelihood of occurrence, including the most important step of escape/release (Step 1), it can be concluded that there is a negligible likelihood of a complete exposure pathway to Maine.

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[94] DFO (2019) concluded that the likelihood of exposure to the Canadian environment increased from negligible to low if the Atlantic salmon without the IGA would be reared at the same time as ABT salmon in the Rollo Bay facility. DFO was concerned that AAS eggs could be accidentally mixed with eggs of Atlantic salmon without the IGA and be released into net pens. However, DFO outlined risk management strategies to mitigate this occurring, including, among other things, not allowing the eggs of AAS and Atlantic salmon without the IGA to be produced at the same time in the same location.

All of the other steps in the exposure pathway occurring after escape were also ranked between negligible to moderate for likelihood (Table 9-4, above). Thus, although the likelihood of establishment and presence in the US is considered negligible due to the likelihood of escape being negligible alone, the likelihood of exposure is even further reduced by the negligible to moderate likelihood of survival and dispersal in PEI and Atlantic Canada (Step 2), the negligible to low likelihood of migration/dispersal to the Maine environment (Step 4), the low to moderate likelihood of survival in the Maine environment (Step 5), as well as the low to moderate likelihood of reproduction in the Maine environment (Step 6). The product of these together is smaller than negligible given that the probability of escape alone is negligible.

$P(E_{\text{establishment}}) = P(\text{Step 1}) \times P(\text{Step 2}) \times P(\text{Step 4}) \times P(\text{Step 5}) \times P(\text{Step 6})$

$P(E_{\text{establishment}})$ = negligible × negligible to moderate × negligible to low × low to moderate × low to moderate

and,

$P(E_{\text{presence}}) = P(\text{Step 1}) \times P(\text{Step 2}) \times P(\text{Step 4}) \times P(\text{Step 5})$

$P(E_{\text{presence}})$ = negligible × negligible to moderate × negligible to low × low to moderate

Thus, the overall likelihood of establishment and presence in the US environment could be characterized as extremely negligible or infinitesimal (extremely small or a value approaching zero). This is true even if escape were to occur because the likelihood of all of these steps occurring sequentially is so small.

9.2.4. Likelihood of Pathogen/Pathogen Transmission

This section of the assessment will evaluate the likelihood of transmission of pathogens/parasites from escaped ABT salmon or the ABT PEI facilities to endangered US Atlantic salmon and the US environment. Four transmission pathways for pathogens/parasites to spread from ABT salmon and the ABT facilities on PEI to endangered US Atlantic salmon were identified in Section 8.2.2 and illustrated in Figure 8-6 and Figure 8-7. These are shown again in Boxes 10 and 10 below. The likelihood for transmission to occur via each of these pathways is discussed further in Sections 9.2.4.2 and 9.2.4.3 below. However, before doing so, we examine in Section 9.2.4.1 the likelihood of introduction of a pathogen/parasite to ABT salmon and the ABT PEI facilities, and the likelihood of spread within the ABT PEI facilities. The likelihood of introduction, spread, and transmission occurring is evaluated using the likelihood rankings provided in Table 9-1.

9.2.4.1. Likelihood of Introduction and Spread of Pathogens/Parasites in the ABT PEI Facilities

In order for pathogens/parasites to be transmitted by ABT salmon or ABT's PEI facilities to the natural environment, they first need to be introduced into the ABT facility or to ABT salmon in the facility and be spread within the facility. As described in Section 8.2.2, a pathogen and/or parasite could be introduced via 1) eggs/milt carrying a pathogen/parasite being brought into an ABT facility, 2) ABT personnel carrying disease into the facility, or 3) contamination of the water source for the facility. It could then be spread within the facility via water, movement of fish within the facility, equipment, and ABT personnel. The pathogen/parasite could then be transmitted to the environment via wastewater discharged from the PEI facility or via infected ABT salmon escaping from the facility. However, if pathogen/parasite introduction is prevented within the ABT facility, or quickly mitigated in the unlikely event it was to occur, then the likelihood of transmission to the natural environment, and subsequently to endangered US Atlantic salmon and the US environment, would be negligible.

The Bay Fortune facility has been disease free since the 2009 ISAV outbreak (discussed in Section 7.5.1.2) and Rollo Bay has not had any disease outbreak since it began operation in 2018. The Bay Fortune facility has been recognized as an approved Compartment in the CFIA Compartmentalization Program for Atlantic salmon and is considered to be free from the diseases listed in Table 7-2 (under the "CFIA Compartment Program" column heading). The Rollo Bay facility will undergo compartmentalization inspections by CFIA in 2024. These facilities remain disease free due to the comprehensive disease prevention and surveillance measures employed by ABT to prevent introduction, outbreak, and transmission of pathogens and/or parasites at all ABT facilities on PEI and in the US. These measures were discussed in detail in Section 7.5.1 above and are summarized below:

- ABT maintains several SOPs that outline procedures for employees to prevent the introduction and mitigate and eliminate spread of disease at the PEI and US facilities and in between facilities, including procedures for cleaning, disinfection, and handling of equipment, footwear, eggs, fish, etc. See Sections 7.5.1.2 and 7.5.1.3 above. These procedures further reduce the likelihood of introduction and spread of pathogens/parasites in all ABT facilities.
- ABT no longer introduces eggs, milt, or fish from the outside (third) parties into the ABT PEI facilities (see Section 7.5.1.2 above). This completely eliminates the most likely route of introduction of pathogens/parasites into the ABT facilities.
- The production areas in the Bay Fortune facility (the ERA and GOA) and Rollo Bay facility (ERA and ARA) have been physically separated into two distinct, biosecure areas. Personnel are required to cover clothing and disinfect boots before entering production areas. This reduces both the likelihood of introduction to, and spread of disease between, these areas.
- Both PEI facilities operate on freshwater only, which eliminates the possibility of a saltwater pathogen/parasite, that affects the post-smolt Atlantic salmon life stage, from being introduced to the facility. In addition, both facilities obtain their water from a well, which is less likely to be contaminated with a pathogen or parasite than surface water.
- ABT has installed UV lights to disinfect both the incoming water as well as the recirculated water in all areas of both facilities. In addition, the recirculating water in the ERA at the Bay Fortune facility and all areas of the Rollo Bay facility is disinfected via ozone treatment. These water treatment operations should reduce or eliminate pathogen loads in the water at both facilities, thereby substantially reducing the spread within the facility via the water.
- Disease surveillance occurs on a daily basis at both PEI facilities via observations by ABT personnel. In addition, all mortalities in the GOA at the Bay Fortune facility and the ARA at the Rollo Bay facility are necropsied and examined for signs of disease and a proportion of the fish population (fry to pre-smolt) at both facilities are randomly sampled for ISAV. This constant surveillance allows for ABT to identify a pathogen/parasite infection at the

early stages and to take measures to mitigate and eliminate the spread within the facility.

- Both facilities are routinely inspected by Canadian authorities to ensure adequate biosecurity measures are in place and to conduct disease surveillance. At the Bay Fortune facility, CFIA inspects a minimum of every 4 months (most recently, in July 2023), Atlantic Canada provincial authorities inspect routinely as part of the Certificate of Fish Health Transfer program, and testing is conducted every six months as required for the US Title 50 Certificate and provincial clearances. The Rollo Bay facility is also inspected routinely by Atlantic Canada provincial authorities, routinely completes the pathogen testing requirements under the US Title 50 Certification process and will begin inspections by CFIA under the compartmentalization program in 2024. As stated in the paragraph above, this surveillance program will result in early detection of pathogens and parasites, which allows for ABT to initiate mitigation strategies to eliminate spread of pathogens/parasites.

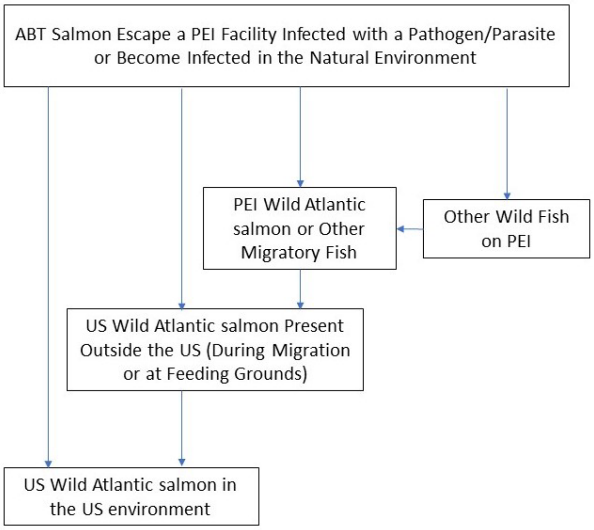
Many of the pathogen surveillance and biosecurity measures listed above (e.g., early detection, controlled movement of fish, disinfection) are widely used in the aquaculture industry and have been demonstrated to be effective at minimizing the spread of pathogens (Jones *et al*., 2015).

Based on the procedures and mechanisms in place at all ABT facilities, and considering their routine inspection by Canadian authorities, the likelihood of introduction of a pathogen/parasite into the ABT facilities is considered to be negligible (i.e., extremely unlikely or not reasonably foreseeable occurrence). This ranking is especially supported by the fact that one of the three potential routes of introduction of a pathogen/parasite will not occur, i.e., eggs/milt/fish from outside will not be brought into the PEI facilities. In the highly unlikely event of an introduction of a pathogen/parasite, ABT’s comprehensive surveillance program at all facilities would identify the pathogen/parasite early enough to implement mitigation strategies that would slow or eliminate spread in the facility (including reducing the load in wastewater discharge). Thus, the likelihood of spread of a pathogen/parasite within the ABT facility is also considered to be negligible.

9.2.4.2. Likelihood of Transmission of Pathogens/Parasites from Escaped ABT Salmon to US Wild Atlantic Salmon

In this section, the likelihood of transmission of pathogen/parasites from escaped ABT salmon to US wild Atlantic salmon is evaluated. In the unlikely event that ABT salmon escape the ABT facilities in PEI infected with a pathogen/parasite or are infected in the natural environment,[59] there are several potential pathways through which a pathogen/parasite could be transmitted. These pathways are illustrated in Figure 8-6 (see Box 10) and described in Sections 8.2.2.1 and 8.2.2.2.

Box 10: Pathogen/Parasite Transmission Pathways from Escaped ABT Salmon (Figure 8-6)



In order for any of these transmission pathways to occur, ABT salmon must escape the PEI facilities. As discussed under Section 9.2.1, the likelihood of escape is considered to be negligible due to multiple levels of physical and procedural containment and security at all ABT facilities. In the highly unlikely event of escape, the ABT salmon would need to be infected with pathogen/parasite either in the facility or natural environment, and then disperse/migrate to other areas on PEI, the feeding grounds in Atlantic Canada or Greenland, or the US environment to interact with and transmit the pathogen/parasite to naïve (non-infected) wild fish, including endangered US Atlantic salmon. As discussed in Section 9.2.3, the likelihood of dispersal and migration of ABT salmon is negligible to low but cannot be entirely ruled out if escape were to occur; thus, the likelihood of pathogen/parasite transmission in these scenarios is evaluated below.

There are three modes of pathogen transmission in the aquatic environment: horizontal, vertical, and vector-born (Grant and Jones, 2010). Horizontal pathogen transmission occurs via direct movement of a pathogen through the water column or physical contact from an infected fish to a non-infected (naïve) fish (Grant and Jones, 2010). Vertical pathogen transmission occurs via transmission of a pathogen from parent to offspring; whereas, vector-born transmission occurs via spread by a third host, such as a parasite, other animals, etc. (Grant and Jones, 2010). The most likely route of transmission of a pathogen/parasite from ABT salmon to US wild Atlantic salmon would be via the horizontal or vector-born transmission; although, vertical transmission is possible for some types of pathogens and ABT salmon that are able to reproduce with US wild Atlantic salmon (i.e., AAFB and diploid AAS).

There are several steps that need to occur for horizontal and vector-born transmission of a pathogen/parasite. Grant and Jones (2010) list criteria to establish a causal link between exposure to a marine pathogen and signs of disease. These criteria are also necessary for pathogen/parasite transmission from an infected fish to naïve fish. Thus, this list has been modified below and applied to this assessment of pathogen/parasite transmission from ABT salmon to naïve wild fish. The requirements for transmission of a pathogen/parasite from ABT salmon to other wild fish, including US wild Atlantic salmon, includes:

1. ABT salmon must contain a pathogen/parasite;
2. Pathogen/parasite must remain present in or on the host ABT salmon;
3. Surrounding water must contain susceptible hosts (i.e., susceptible wild fish);
4. Pathogen/parasite must survive the environment;
5. Pathogen/parasite must be exposed to a susceptible host by a route that allows infection;
6. Pathogen/parasite must be present in biologically significant numbers to initiate a new infection; and
7. Infection must spread to other hosts (i.e., wild fish).

As stated in Section 9.4.1.1 above, the likelihood of escaped ABT salmon containing a pathogen/parasite is negligible because the likelihood of introduction into the PEI facility is negligible (Step 1 above). However, it is possible for ABT salmon to become infected with a pathogen/parasite in the natural environment if they were to escape. Regardless, in the highly unlikely event that infected ABT salmon are present in the natural environment, then there is a potential for them to transmit the pathogen/parasite to local wild fish populations located in the freshwater environment on PEI (including PEI wild Atlantic salmon) via horizontal or vector-born transmission (Steps 2 and 3 above). This could result in wild fish populations on PEI being infected with a pathogen/parasite and resulting in further geographical spread.

All of these steps occurring are also dependent upon the conditions being optimal for pathogen/parasite survival and transmission in the natural environment. The transmission of a pathogen/parasite in the natural environment is complex and is dependent upon many factors. For example, horizontal transmission "*is dependent upon the frequency of contact between individuals, the susceptibility of the host (general health and immune ability) and the transmission coefficient (ability of the pathogen to invade, replicate and disperse)*" (Grant and Jones, 2010). In addition, the likelihood of transmission is also dependent on the specific characteristics of the pathogen/parasite (virulency, infectivity, pathogenicity, concentration, and bioavailability), the host (species, age, immunity, stress, density, nutrition, and health status), and the environment (temperature, salinity, water quality, contaminants, currents, and other hosts and carriers) (Grant and Jones, 2010; Oidtmann *et al*., 2013). It is impossible to accurately predict any of these factors due to the many unknowns, and these factors could vary substantially depending on the pathogen/parasite and disease, compounding the uncertainty. For example, how many infected ABT salmon would escape; could the infected ABT salmon come into close enough contact with wild fish on PEI and frequently enough to transmit the pathogen/parasite; is the pathogen or parasite capable of surviving in saltwater; etc.

However, if ABT salmon were to escape, it is unlikely that any, let alone a large number, of ABT salmon would be infected with a pathogen/parasite due to ABT's mitigation procedures that would take effect when a pathogen/parasite is detected (e.g., quarantine and culling infected fish, and disinfection of contaminated areas and equipment). These measures greatly reduce the likelihood that naïve individuals would come into close contact with any infected individuals, frequently enough to transmit a pathogen/parasite. In addition, this would reduce the pathogen load in the environment and the transmission rate. Furthermore, the only way for endangered

US Atlantic salmon to become infected by ABT Salmon would be if the pathogen/parasite was tolerant of both fresh and saltwater environments, which reduces the number of potential pathogens/parasites that are capable of surviving these pathways. However, the ABT Salmon could become infected in the natural environment after escape, and if they are more susceptible to a pathogen/parasite (see Section 9.3.2.2.e), it could increase the transmission. Based on this information, the likelihood of escaped ABT Salmon transmitting a pathogen/parasite to endangered US Atlantic salmon via horizontal or vector-born transmission is considered to be low.

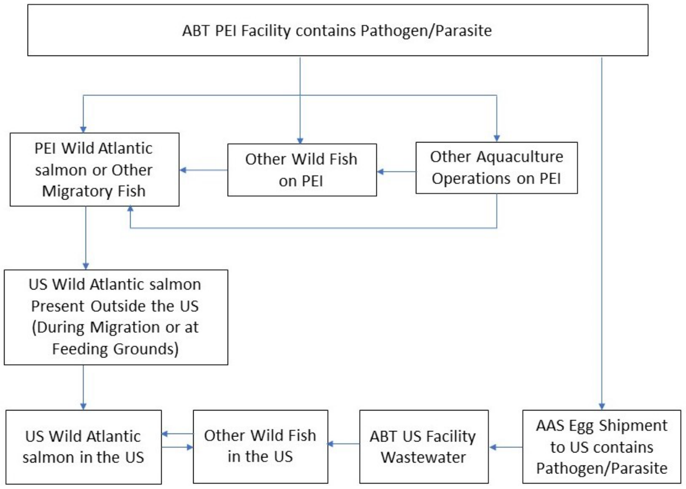
Although not discussed in the steps above, it is also possible for AAFB and diploid AAS to transmit a pathogen/parasite via vertical transmission if they are able to reproduce with endangered US Atlantic salmon in the US environment. (Only AAFB and diploid AAS are capable of reproduction.) As discussed in Section 9.2.2, the likelihood of AAFB and diploid AAS migrating to and reproducing with endangered US Atlantic salmon in the US environment is considered to be negligible to moderate. Regardless, if this were to occur, and AAFB and diploid AAS contained a pathogen/parasite that was transmitted via the mother, then the transmission to the offspring could occur. Thus, the likelihood of vertical transmission occurring is also ranked as low.

9.2.4.3. Likelihood of Transmission of Pathogens/Parasites from ABT Facilities to US Wild Atlantic Salmon

In this section, the likelihood of transmission of pathogens/parasites from the ABT facilities to US wild Atlantic salmon is evaluated. In the unlikely event that a pathogen/parasite is introduced to and spreads in an ABT facility on PEI, there are several potential pathways by which a pathogen/parasite could be transmitted to US wild Atlantic salmon. These pathways are illustrated in Figure 8-7 (see Box 11) and described in Sections 8.2.2.3 and 8.2.2.4.

In order for a pathogen/parasite to be transmitted to the natural environment from an ABT facility without the escape of any fish, it would need to be contained in the wastewater of the ABT facility. As described under Section 9.2.4.1 above, ABT has strict pathogen/parasite prevention procedures (disinfection) and mechanisms (UV and ozone treatment of water) in place at all facilities to reduce or eliminate the likelihood of introduction of a pathogen/parasite. However, if all of these procedures failed and a pathogen/parasite was introduced into the environment from the ABT facilities on PEI, then it is possible for the pathogen/parasite to enter the wastewater and it could spread via horizontal or

Box 11: Pathogen/Parasite Transmission Pathways from ABT Facilities (Figure 8-7)



vector-born transmission in the freshwater PEI environment, including to PEI wild Atlantic salmon and other wild fish and aquaculture operations on PEI.

In the unlikely event of introduction and spread in the facilities, it is expected that the pathogen/parasite load in the wastewater would likely be reduced due to the mitigation measures that ABT would put into place upon early detection of a pathogen/parasite at any of their facilities (e.g., quarantine and culling infected fish, and disinfection of contaminated areas and equipment). In addition, as discussed in Section 9.2.4.2 above, the pathogen/parasite introduced from the ABT facilities would have to be tolerant of both fresh and saltwater in order for the pathogen/parasite to be transmitted to endangered US Atlantic salmon during migration, at the feeding grounds, or in the US environment. Thus, the likelihood of this pathway occurring is also ranked as low.

Another path of transmission from the PEI facilities to the US environment would be via a shipment of AAS eggs carrying the pathogen/parasite and sent to an ABT facility in the US (see Box 10). (Currently, the only ABT facility in the US is located in Albany, Indiana.) If this occurred, the pathogen/parasite could spread within the US facility and be contained in the wastewater entering the US environment. However, as discussed in Section 9.4.1, the likelihood of any of the ABT facilities containing a pathogen/parasite is negligible and there is significant regulatory oversight by both US and Canadian authorities to ensure that eggs shipped from the PEI facilities are free of pathogens. In addition, ABT has procedures to disinfect all packages of AAS eggs upon receipt at the US facilities, including disinfection of the packaging, water, and eggs (see Section 7.5.2, above). Furthermore, at the Indiana facility, AAS are continually screened for disease prior to transferring fish from the nursery to the pre-grow out area and require a veterinary certificate to be moved. These procedures substantially reduce, if not eliminate, the likelihood of transmission of a pathogen/parasite from the ABT facilities on PEI to those in the US and would mitigate any spread within the Indiana facility if it were to occur. Thus, the likelihood of this pathway occurring is ranked as negligible.

9.2.4.4. Conclusion

The potential for transmission between ABT salmon and wild fish and the environment is expected to be largely the same as it would be for any land-based Atlantic salmon production in this region.

As discussed in Section 9.2.3, the probability of exposure [*P(E)*] is the product of the probabilities of occurrence of each sequential step required for that exposure. This logic can also be applied herein when evaluating the probability of transmission of a pathogen/parasite from ABT salmon or the ABT facilities to endangered US Atlantic salmon. Similar to the exposure pathways analyzed in Section 9.2.2 above, some of the steps of transmission pathways are limited by the occurrence of other steps. For example, the likelihood of transmission from ABT salmon to wild fish, including endangered US Atlantic salmon, is limited by the likelihood of introduction of a pathogen/parasite to ABT salmon at the PEI facilities, as well as the likelihood of ABT salmon escaping the PEI facilities. Thus, the overall likelihood of transmission to endangered US Atlantic salmon would be determined by the step with the lowest ranked likelihood, i.e., if any of the steps of the pathways are found to be negligible, then the likelihood of transmission via that pathway would also be negligible.

The likelihood of introduction of a pathogen/parasite to ABT salmon or the ABT facilities on PEI was found to be negligible due to the comprehensive prevention, surveillance, and biosecurity measures that are employed at all ABT facilities. In addition, the likelihood of spread within the PEI facilities is also considered negligible due to early detection, mitigation strategies, and treatment of water (UV and ozone). Thus, the likelihood of pathogen/parasite transmission from any of the pathways outlined in Section 8.2.2 and Figure 8-6 and Figure 8-7 is considered negligible. This is further minimized by the negligible to low likelihood of transmission from ABT salmon and the PEI facilities even if a pathogen/parasite was introduced because the pathogen/parasite would need to be tolerant of both fresh and saltwater environments in order to be transmitted to endangered US Atlantic salmon. In addition, conditions for transmission would need to be optimal.

It is also important to note that transmission of a pathogen/parasite to a naïve fish does not ensure disease will arise in naïve fish (Grant and Jones, 2010). There are many factors that influence the likelihood of disease resulting from infection, which are discussed in Section 9.3.2.2.e, below.

9.3. Harms Identification and Analysis

As previously discussed in Section 2.4 above, the Court requires that "*…on remand the FDA must complete the final step of its own risk analysis by addressing the consequences that would result from the engineered salmon successfully establishing a persistent population outside of captivity*."[95] In order to address this requirement, FDA has revised Risk-related Question 4 slightly and expanded it by adding an additional question under Question 4:

> Question 4a: What are the identified potential harms to, or effects on, the US environment if AquAdvantage Salmon or AquAdvantage Broodstock establish and/or are present?
>
> Question 4b: What is the likelihood of these potential harms occurring assuming exposure in the US environment has occurred?

In the risk assessment approach used in this assessment (see Sections 4.2 and 9.1), *risk* is described as the likelihood of harm resulting from an exposure to a hazard or $R = P(E) \times P(H|E)$. Revised Risk-related Question 4 evaluates $P(H|E)$, i.e., the conditional probability of harm assuming that exposure has occurred. First, revised Question 4a (under Section 9.3.1) identifies/characterizes the potential harms (adverse consequences, effects, or impacts) to the US environment in the unlikely event that ABT salmon escape ABT's facilities located on PEI, Canada, and establish and/or are present in the Maine environment. This is the hazard/harm identification process for this risk assessment. Second, Question 4b assesses the likelihood of these potential harms occurring assuming exposure (establishment and/or presence)

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[95] See fn 5. It is important to note that, herein, the phrase "successfully establishing a persistent population outside of captivity" is interpreted to mean both establishment and/or presence in the US environment.

of ABT salmon has occurred in the US,[96] which directly addresses one of the Court's remand requirements (see paragraph above; the other remand requirements are addressed in Sections 9.4 and Appendix I). Questions 4a and 4b are addressed below in Sections 9.3.1 and 9.3.2, respectively.

These questions are answered with consideration to the definitions of hazard and harm (see Section 4.2 above) used in this assessment:

- <u>Hazard</u> is defined as the GE organism itself and any act or phenomenon that has the potential to produce harm; herein, that is the animal containing the rDNA construct, i.e., all types of ABT salmon (AAFB, AANB, diploid and triploid AAS), and its interactions with ecosystem components.

- <u>Harm</u> is defined as an adverse effect to the environment due to the hazard (in this case, ABT salmon). Potential harms include those that could occur at the population, community, and ecosystem levels, including gene pool, species, or community perturbation resulting in negative impacts to community stability (NRC, 2002), and ecosystem displacements, disruptions, or species extinctions (Devlin *et al*., 2006).

It is important to clarify that being identified as a hazard does <u>not</u> mean something will inherently produce a harm. Likewise, something identified as harm would not necessarily be a significant risk to the environment, especially if the harm is reversible once the exposure is removed (i.e., significant impacts may not occur to the environment even if a harm exists). The likelihood of harms occurring (the risk) must be determined with consideration of the magnitude of the harm (e.g., severity, reversibility) and the context of the exposure (e.g., duration, frequency, magnitude), which is discussed further when *risk* is evaluated in Section 9.4, Risk Characterization.

9.3.1. Question 4a: What are the Identified Potential Harms to, or Effects On, the US Environment if AquAdvantage Salmon or AquAdvantage Broodstock Establish and/or are Present?

In this section, the potential harms to the US environment due to an exposure (establishment and/or presence) of ABT salmon are identified. Section 7.5 and Table 12 of the 2015 EA presented general information on five potential processes and ecological consequences (herein, the term "harms" is used) to the environment that may be associated with, or affected by, GE fish, which was based on Snow *et al*. (2005). The information in that table has been updated and presented in Figure 9-2 and is discussed further below.

As stated previously, the ABT salmon and its interactions with ecosystem components is the hazard in this assessment; however, the presence of the fish in the ecosystem does not necessarily constitute a harm, rather it is their interactions with ecosystem components (e.g., reproduction with conspecifics) that may

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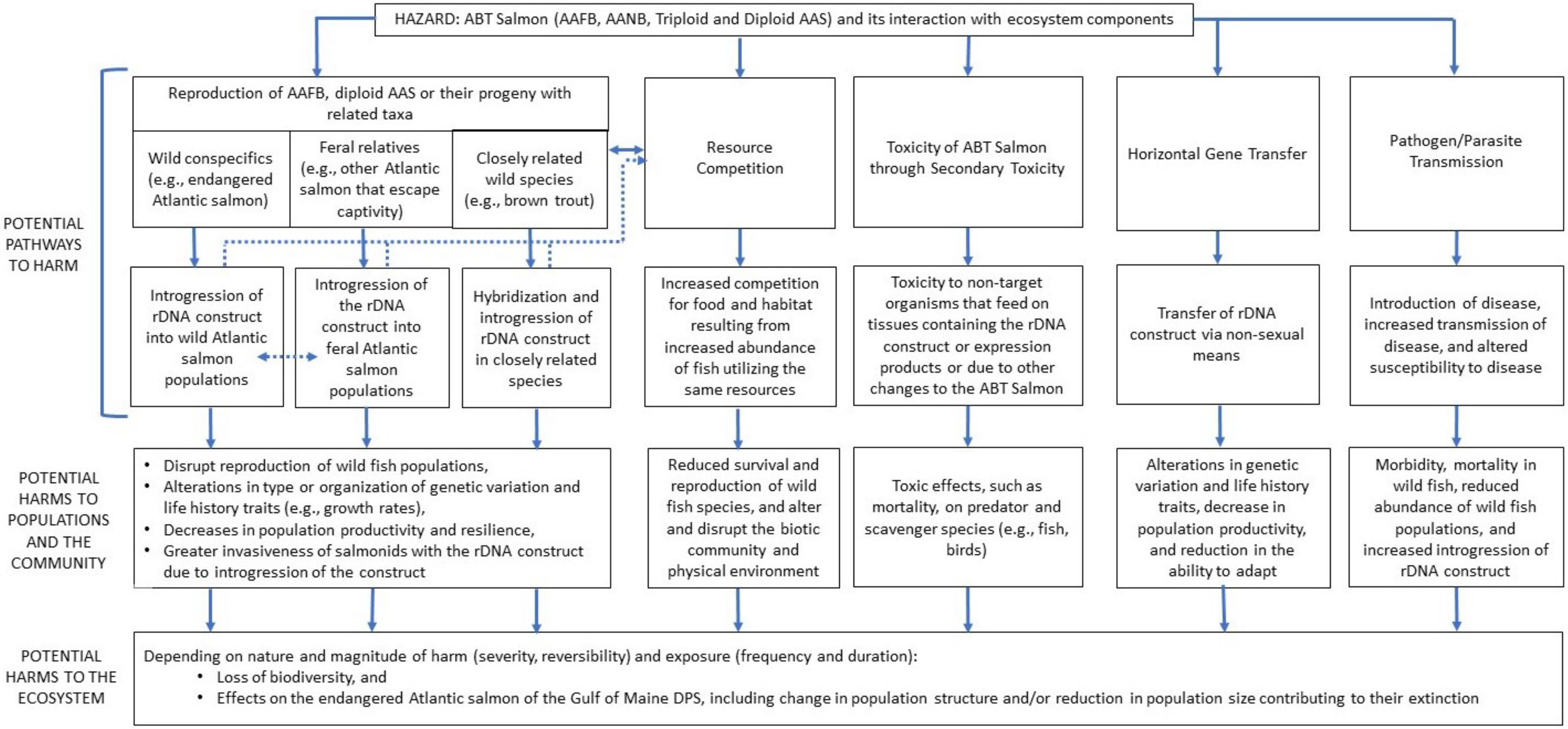
[96] For Question 4b, we ignore the outcome of our analysis in Question 3 and are making the outright assumption that exposure has occurred in order to directly address the Court's directive for remand.

potentially cause harms to the US environment (Devlin *et al.*, 2015). Herein, ABT salmon's interactions with the ecosystem components is referred to as the "pathway to harm." Thus, in addition to the potential harms due to exposure to ABT salmon, Figure 9-2 also illustrates the pathways leading to harms. Figure 9-2 was developed using information from Snow *et al*. (2005); NRC (2002); NRC (2004a); Kapuscinski *et al*. (2007b); Devlin *et al*. (2007); Devlin *et al*. (2006); Devlin *et al*. (2015); Grant and Jones (2010), as well as the 2013 and 2019 Canadian Science Advisory Secretariat Science Advisory Reports for the ABT salmon authorizations in Canada (DFO 2013; DFO, 2019). Furthermore, potential harms that could occur at the population and community, as well as ecosystem levels are presented in Figure 9-2.

Figure 9-2 only includes the potential pathways and harms applicable to this assessment, which, to date, remain largely undocumented. The potential ecological harms described in Figure 9-2 are specific to the environment of Maine because that is where both wild populations and net pen farmed Atlantic salmon are primarily located in the US. This is also the closest location of the US to where ABT salmon are produced in PEI, Canada.

Figure 9-2. Potential Hazards, Pathways, and Harms from Exposure to ABT Salmon in the US Environment



Note: ABT salmon = AquaBounty Technologies Salmon with the rDNA construct; AAFB = AquAdvantage Female Broodstock; AANB = AquAdvantage Neomale Broodstock; AAS = AquAdvantage Salmon

In addition to Figure 9-2, the potential pathways and harms are outlined below. The ultimate harms to the ecosystem are the same for all potential pathways to harm and include:

- loss of biodiversity in the Maine ecosystem, and
- effects on the endangered Atlantic salmon of the Gulf of Maine DPS, including change in population structure and/or reduction in population size contributing to their possible extinction.

9.3.1.1. Reproduction with Related Taxa

Pathway: ABT salmon that enter the Maine environment could interfere with breeding behaviors of wild fish species, including endangered Atlantic salmon, via competition for mates and reproduction with wild fish species. Under suitable environmental conditions, AAFB and diploid AAS[97] released from facilities on PEI could reproduce with:

1. Wild conspecifics (endangered Atlantic salmon of the Gulf of Maine DPS), which could result in introgression[98] of the rDNA construct into wild Atlantic salmon populations,
2. feral relatives (i.e., other Atlantic salmon without the rDNA construct that escaped from net pens or the ABT facilities), which could result in creation of a feral Atlantic salmon population that is hemizygous for the rDNA construct and that would have a potential to reproduce with wild conspecifics resulting in introgression of the rDNA construct into native populations, and/or
3. wild relatives of the same genus (e.g., brown trout), which could result in hybridization and introgression of the rDNA construct in that species.

Although AANB and triploid AAS cannot establish a reproducing population with the rDNA construct in the natural environment, they can disrupt breeding of endangered Atlantic salmon by competing for mates and displaying breeding behaviors, but ultimately, leaving the eggs unfertilized or not viable.

Harms: These interactions could possibly lead to the following harms in wild fish populations, including endangered Atlantic salmon of the Gulf of Maine DPS:

- disruption of reproduction of wild fish populations,
- alterations in type (e.g., specific alleles) or organization (e.g., co-adapted gene complexes) of genetic variation,
- alterations in life-history traits,
- decreases in population productivity,
- reductions in the ability to adapt to changes (i.e., resiliency), and

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[97] As discussed in Section 9.2, only AAFB and diploid AAS can reproduce on their own.

[98] Introgression is defined as incorporation of a new or altered gene construct into the gene pool of wild relatives by backcrossing of transgenic-wild fish hybrids to wild relatives (modified from Kapuscinski *et al.*, 2007b). This is also referred to as "genetic disturbance" or "vertical gene transfer."

- greater invasiveness of the salmonids with the rDNA construct via introgression of the rDNA construct.

9.3.1.2. Resource Competition

Pathway: ABT salmon or their progeny could be present in the Maine environment for a short period of time even if they cannot reproduce (AANB or triploid AAS), or for a long period of time (over many generations) if they establish a self-sustaining population (AAFB or diploid AAS). Presence alone, even without introgression or establishment, can potentially result in harms. ABT salmon and their progeny could compete with other organisms, including endangered Atlantic salmon populations, for resources in the Maine environment, including food (e.g., competition for prey, increased predation on available prey) and habitat (e.g., competition for spawning substrate, nursery grounds).

Food—If the presence of ABT salmon substantially increased the number of predators, then it could cause a cascade of effects due to disruption of the food chain (NRC, 2002) that could affect populations and the community. For example, it could result in a reduction of available prey species and disruption of a trophic level within the Maine ecosystem, which could ultimately result in mortality of other competing predator species, including endangered Atlantic salmon, and a disruption of the biotic community through effects on the food chain. In addition, ABT salmon and their progeny with the rDNA construct could cause harm via predation (cannibalism[99]) of endangered Atlantic salmon. If growth of ABT salmon and their progeny with the rDNA construct is also greater at an earlier age in the natural environment, then it would likely result in a greater resource need in year of young (YOY) ABT salmon and YOY progeny with the rDNA construct. Ultimately, this could reduce available food resources and also result in endangered Atlantic salmon YOY becoming a food resource for YOY progeny with the rDNA construct.

Habitat—If available spawning sites in Maine are limited, ABT salmon and their progeny could cause harms by utilizing spawning sites or superimposing their redds[100] on top of existing endangered Atlantic salmon redds. This could disrupt breeding behaviors and result in loss of eggs of endangered Atlantic salmon. In addition, ABT salmon and their progeny could cause physical destruction to critical habitat of endangered Atlantic salmon by digging spawning sites.

Harms: This could result in increased pressure on limited resources and result in the following harms to populations and the community:

- effects on survival and reproduction of wild fish species, including endangered Atlantic salmon of the Gulf of Maine DPS, and
- alteration and disruption of the biotic community (e.g., reduction in prey species) and the physical environment (e.g., disturb streambeds).

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[99] Cannibalism is defined as the eating of the flesh of an animal by another animal of the same kind (https://www.merriam-webster.com/dictionary/cannibalism; December 8, 2023)

[100] Redd superimposition is defined as the creation of a redd on top of a previously established redd (McNeil, 1964).

9.3.1.3. Toxicity of ABT Salmon

Pathway: ABT salmon or their progeny could potentially cause adverse effects on non-target predators or scavengers that feed on tissues of these fish through secondary poisoning. Insertion of the *opAFP-GHc2* construct could potentially pose hazards to non-target organisms, including direct toxicity via the construct itself and its expression product (the Chinook salmon growth hormone) or indirectly via the construct or expression product causing a change in the physiology of the fish.

Harms: This hazard could result in toxicity and/or mortality in predator and scavenger species (e.g., other fish, birds) which, if the magnitude of effect was great enough, could result in alterations to the biotic community (e.g., change in numbers and types of species).

9.3.1.4. Horizontal Gene Transfer

Pathway: Horizontal gene transfer is defined as genetic material from one organism becoming incorporated into an unrelated organism through non-sexual means (NRC, 2002). The presence of ABT salmon or other salmonids containing the rDNA construct in the US environment could result in the presence of free rDNA in the environment (e.g., in feces, carcasses of dead ABT salmon or other waste materials) and could lead to non-sexual gene transfer among organisms. The potential harms to populations and the community would depend on the particular intentionally altered trait and gene flow.

Harms: Similar to transfer of the rDNA construct via reproduction (Section 9.3.1.1, above), horizontal gene transfer could possibly lead the following harms in fish populations:

- alterations in type (e.g., specific alleles) or organization (e.g., co-adapted gene complexes) of genetic variation,
- alterations in life-history traits,
- decreases in population productivity, and
- reductions in the ability to adapt to changes.

9.3.1.5. Pathogen/Parasite Transmission

Pathway: There are several potential concerns regarding the transmission of a pathogen/parasite from ABT salmon and/or the ABT facilities to endangered US Atlantic salmon and the US environment (the transmission pathways are illustrated in Figure 8-6 and Figure 8-7 in Section 8.2.2).

The primary concern is the potential for ABT salmon or the ABT facilities on PEI to introduce a novel, exotic, or existing pathogen/parasite to the natural environment that results in widespread infection and onset of disease (i.e., the harm) in endangered US Atlantic salmon or other wild fish populations in the US. In addition, the presence of ABT salmon in the environment (in the event of an escape) could also result in an increased pathogen/parasite load (i.e., increase in the potential for fish-to-fish interaction) and potentially result in an expansion of geographical range of a disease outbreak.

Another concern is regarding whether ABT salmon and their progeny may have an altered immune system that makes them more or less susceptible[101] to pathogen and parasite infections. If ABT salmon are more susceptible to pathogens/parasites it could potentially result in greater spread of pathogens/parasites in the ecosystem, including spread to organisms that may not have been exposed, including endangered Atlantic salmon. However, an increase in susceptibility could also result in a reduced fitness (survival and reproduction) of ABT salmon in the Maine ecosystem, which could lessen other potential harms (e.g., reproduction with conspecifics or competition for resources). Alternatively, if ABT salmon are less susceptible (increased resistance) to pathogens or parasites, in theory it could provide a fitness advantage over other fish species, including endangered Atlantic salmon, and could potentially result in greater introgression of the rDNA construct into the endangered Atlantic salmon population and/or ABT salmon could outcompete wild endangered Atlantic salmon.

Harms: These harm pathways could lead to the onset of disease (i.e., morbidity[102]) and mortality (due to the disease itself or increased predation due to lethargy) in wild fish populations that could subsequently result in reduction in the abundance of affected fish populations in the US environment, including endangered Atlantic salmon. The symptoms and signs of disease (morbidity) vary from disease to disease and might include lethargy, or other behavioral or physiological changes. In addition, if ABT salmon are less susceptible to a pathogen/parasite, it could potentially result in greater introgression of the rDNA construct into the endangered Atlantic salmon population and could also result in ABT salmon and their progeny outcompeting endangered Atlantic salmon. In contrast, if ABT salmon are more susceptible to a pathogen/parasite, this would likely reduce their chance of survival and their overall harms.

9.3.2. Question 4b: What is the Likelihood of These Potential Harms Assuming Exposure in the US Environment has Occurred?

Question 4b examines the likelihood of the harms identified under Question 4a (Figure 9-2 in Section 9.3.1, above) occurring by assuming exposure (establishment and/or presence) in the Maine environment has occurred, *P(H|E)*. As discussed in Section 9.2 above, relative qualitative rankings (negligible to high) will be used to characterize the likelihood of harms occurring in the US assuming ABT salmon are present and establish. Table 9-5 below lists the definitions of each likelihood ranking, which are used in Section 9.3.2.2 below to rank the likelihood of each harm pathway to occur given what is known about ABT salmon and GH transgenic relatives.

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[101] According to Grant and Jones (2010), "Susceptibility is determined by level of resistance that is insufficient to prevent infection by a pathogen."

[102] According to Merriam-Webster dictionary, morbidity means a disease state or symptom; ill health (https://www.merriam-webster.com/dictionary/morbidity; accessed December 8, 2023).

Table 9-5. Rankings for Likelihood for Harms to Occur When Exposed to ABT Salmon in the United States Environment

Ranking	Definition
Negligible likelihood	Harm very unlikely to occur
Low likelihood	Harm unlikely to occur
Moderate likelihood	Harm likely to occur
High likelihood	Harm very likely to or will occur

These qualitative rankings will be used, along with the qualitative rankings determined for the likelihood of exposure (Section 9.2.3, above), to address Risk-related Question 5; i.e., to characterize the overall risk of potential harms occurring in the US from the production of ABT salmon on PEI in Section 9.4 (below).

9.3.2.1. Background on the Factors that Influence Potential Environmental Harms of ABT Salmon

Atlantic salmon display a high degree of phenotypic plasticity and a complex life history that enable them to adapt to variable conditions. When the phenotype of that animal is altered, such as an increase in growth rate, those phenotypic changes can influence and alter the animal's interactions with the environment (Devlin *et al*., 2006). Therefore, there are several factors that will need to be considered when ranking the likelihood of harm occurring due to the interactions of ABT salmon and their progeny with the US environment, including context of the exposure (duration, frequency, number of fish), fitness of the ABT salmon, the resiliency of the receiving ecosystem, as well as uncertainties such as genotype-by-environment (GxE) interaction. Each of these factors are discussed briefly below and considered further when ranking the likelihood of harms in Section 9.3.2.2, below.

a. Context of Exposure

Question 4b assumes exposure of ABT salmon occurs in the US environment. However, the duration (short- or long-term), frequency (number of occurrences) and magnitude (number/density) of the exposure can influence the severity and reversibility of the harms. This is similar to a chemical risk assessment, where the duration, frequency, and magnitude of exposure to a chemical determines the ultimate severity of the environmental effects of that chemical and the ability of the ecosystem to recover if the exposure is removed. The same can be said of exposure to ABT salmon. For example, if the ecosystem is exposed one time to a small number of ABT salmon for a short duration of time, then the exposure may result in no harm or only result in minor harms that are reversible, allowing the ecosystem to return to homeostasis. On the other end of the spectrum, if exposed to a large number of ABT salmon over a long, multi-generational duration or multiple occurrences, then the exposure may result in severe harms that cannot be reversed due to continual exposure to the hazard.

There are four types of ABT salmon held at the PEI facilities, AAFB, AANB, triploid and diploid AAS, and depending on the time of year, the numbers of these fish will change (e.g., triploid AAS eyed-eggs are only held for 50-125 days before being shipped to the Indiana facility for grow-out). AANB and triploid AAS cannot establish a reproducing population in the US because they are not reproductively competent (see Section 8.2 above). Therefore, they would only survive one generation in the Maine environment if they somehow reached it, and they are not expected to have a persistent presence there, unless pseudo-establishment occurred. Pseudo-establishment was found not to be a realistic possibility for the Bay Fortune and Rollo Bay facilities in Section 9.2.3 (Conclusions on Likelihood of Exposure), above, due to the redundant, multiple-level containment and security measures in place. Thus, the presence of AANB and triploid AAS, and their interactions with the components of the environment, is expected to be limited to one generation, which will reduce the likelihood of harms and would limit any overall harms to the US environment. It is important to note that triploid AAS represent the ABT salmon type with the highest production numbers (see Section 6.4.2, above).

AAFB and diploid AAS are reproductively competent and can potentially reproduce in the environment. If AAFB and diploid AAS do not reproduce in the US environment (e.g., if they cannot find a suitable mate or nesting site), then the outcome would be the same as that for AANB and triploid AAS (see paragraph above). However, if AAFB and diploid AAS do successfully reproduce with conspecifics or relatives of the same genus in the US environment, then they could hypothetically establish a reproducing population resulting in long-term exposure of the US environment to their progeny with the rDNA construct. The length of this exposure will be influenced by the fitness (survival and reproductive success) of AAFB, diploid AAS and any progeny with the rDNA construct.

In addition, the number of ABT salmon and their progeny will influence the likelihood of harms, as well as the severity of those harms (see Section 9.4.1.4, below). The magnitude of exposure is influenced by the frequency of escape and fitness of ABT salmon in the environment (Devlin *et al*., 2015). If only a small number of ABT salmon escaped and/or survived the migration to the US, then the likelihood of harm would be reduced due to a decrease in the chance of exposure. If a large number of ABT salmon escape and survive, then the likelihood of harm would increase due to a greater chance of exposure in the US environment, a higher density of salmon in the ecosystem and a greater pressure on the ecosystem resources.

Thus, for the evaluation of likelihood of harms in Section 9.3.2.2 below, the context of the exposure will also need to be considered.

b. Fitness of ABT Salmon and Their Progeny

The term “fitness” refers to all of the phenotypic attributes of an animal that affect survival and reproduction, and ultimately how the individual’s genetics contribute to future generations of the animal’s population. In general, animals are adapted to a specific niche in the ecosystem (i.e., habitat and ecological role) and exhibit maximal “fitness” for that environment. For example, the native endangered Atlantic salmon of the Gulf of Maine DPS have evolved over centuries to adapt to the conditions of Maine’s environment. In terms of population and community dynamics, if escaped ABT salmon or their progeny have a greater overall net fitness than wild Atlantic salmon occupying the same niche in the Maine environment, it is possible they may eventually replace them and become established in that ecosystem (NRC, 2002). On the other hand, if the ABT salmon or their progeny are less fit, they will either not survive in the Maine environment, or the rDNA construct will eventually be removed (by virtue of selection) from the wild Atlantic salmon population (NRC, 2002; Kapuscinski *et al*., 2007b). If the overall fitness of the ABT salmon is similar to that of wild Atlantic salmon, it is possible that both genotypes could persist (and co-occur) in the Maine environment. For purposes of assessing risk associated with animals containing an IGA, it is critical to characterize the fitness of the animals with IGAs in relation to the appropriate comparator animal(s), whether wild or domesticated, and compare the two in the context of expected environment(s) in which either population of animals can be or will be found.

A key factor affecting the fitness of a GE animal is the nature of the introduced trait, and its effects on survival, reproduction, and establishment. For example, an introduced trait could either improve or decrease the adaptability of an organism to a wider range of environmental conditions, allow it to obtain nutrition from previously indigestible sources, or limit the extent to which existing food sources provide adequate nutrition.

Fitness components and associated characteristics contributing to net fitness (e.g., oxygen requirements, swimming speed, metabolic scope, etc.) of ABT salmon have not been explicitly evaluated in the studies conducted by ABT and submitted to the agency in support of phenotypic characterization and animal safety (see Appendix F). However, peer-reviewed scientific studies investigating fitness characteristics in GH transgenic Atlantic salmon of the EO-1ɑ lineage (i.e., AquAdvantage relatives) described in Section 9.2.2.1.b (and Appendix F) generally indicate that changes in the observed phenotype of these fish result in neutral or decreased fitness characteristics. Decreased fitness would be expected to reduce the chances for establishment of ABT salmon, which would reduce the likelihood of long-term severe and irreversible harms. This reduction in fitness, however, is not expected to be compromised to such an extent that survival would be affected greatly, at least on a short-term basis.

In addition, several studies have shown that fitness of escaped farmed non-GE salmonids is reduced compared to wild conspecifics after even a single generation of rearing in captivity (Abrantes *et al*., 2011; Glover *et al*., 2012; Milot *et al*., 2013; Rodewald *et al*., 2011; Salvanes, 2017). These studies suggest that farmed ABT salmon's fitness would be further reduced compared to wild Atlantic salmon after having been raised in captivity for over 14 generations.

c. Ecosystem Resilience

Ecosystem resilience is "*the ability of an ecosystem to return to a previous state following perturbation*" (Kapuscinski *et al*., 2007a). This is an important factor in determining the severity and reversibility of harms in the Maine environment due to exposure to the ABT salmon, especially when evaluating harms to the population of endangered Atlantic salmon of the Gulf of Maine DPS. As discussed in Section 8.4.3 above, most of the native Atlantic salmon populations in the northeastern US are extirpated. The Gulf of Maine DPS is the only remaining native Atlantic salmon population in the US and is considered endangered under the ESA (NOAA and FWS, 2020). According to NOAA and FWS's most recent ESA assessment for the Gulf of Maine DPS conducted in 2020, this population is at "critically low abundance" with a 10-year average return of 1247 Atlantic salmon adults (NOAA and FWS, 2020). Of these returning adults, 84% were hatchery-reared and the remainder were wild/naturally-reared adult Atlantic salmon. Seven of the eight locally adapted populations in the DPS are "supported by conservation hatcheries to buffer the extinction risk", and the eighth population is "at very high risk of extirpation" (NOAA and FWS, 2020). NOAA and FWS (2020) found that "*These very low populations can significantly increase risk to genetic fitness, loss of adaptive traits and reduced ability* *to withstand catastrophic events.*" This information suggests that the population size of this endangered population is small enough in number that harms from ABT salmon could occur. The vulnerability of the endangered Atlantic salmon populations in the Maine environment is considered when ranking likelihood of harm in Section 9.3.2.2 below.

d. Uncertainties

There are uncertainties associated with the assessment of the likelihood of harms due to exposure to ABT salmon. The most common uncertainties identified include (1) limited or no data on the fitness of ABT salmon; (2) limited or no data on the harms produced by ABT salmon under natural conditions (especially under varying environmental conditions); (3) a limited ability to directly extrapolate data from other GH transgenic fish to ABT salmon; and (4) a lack of understanding of GxE interactions. These are discussed in more detail below.

The phenotypic and fitness characteristics of AAS and diploid ABT salmon, as well as relatives with the same rDNA construct, are discussed in Appendix F. As previously stated, many of the components contributing to the net fitness of ABT salmon have not been explicitly evaluated, and furthermore, there are no studies evaluating the potential harms of ABT salmon on the natural environment. Many studies have been published on different phenotype and fitness characteristics of GH transgenic fish (e.g., Atlantic salmon, coho salmon, medaka), as well as their potential harms on the environment. Although these studies were not conducted with ABT salmon, the data from other GH transgenic fish may be useful to suggest inferences on the fitness of ABT salmon and potentially the environmental harms they can produce. However, due to differences in life-history, physiology, and ecology of different fish species, only studies conducted on GH transgenic salmon have been used in this risk assessment to make specific inferences about ABT salmon and their fitness. Thus, studies conducted with Atlantic salmon containing the same *opAFP-GHc2* construct[103] or a different GH construct, as well as studies on GH transgenic coho salmon (*Oncorhynchus kisutch)* are considered in our analyses, but with a higher weight or inferential value given to those studies on ABT salmon and their relatives.

However, there are also uncertainties associated with this data because it was obtained using a different genus and species of salmon, a different growth hormone construct, and/or the construct is at different locations in the salmon's genome. For example, it is unclear whether fitness characteristics and other behaviors of GH transgenic salmonids are different from that of the ABT salmon. In addition, conditions that an organism is raised and studied in can affect their physiological and behavioral responses (Devlin *et al*., 2015; Leggatt *et al*., 2014), which further confounds using studies conducted on other GH transgenic salmonids to make assumptions about ABT salmon. These unknowns add additional uncertainty to extrapolating laboratory data from similar GH transgenic salmonids to ABT salmon.

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[103] These GH transgenic Atlantic salmon containing the same construct as ABT salmon are related but are not identical to ABT salmon. ABT salmon have been reared by ABT for over 14 generations, and during that time certain traits may have been chosen that differ from the GH transgenic Atlantic salmon in these studies. Therefore, ABT salmon may be genotypically different from these fish.

In addition, the studies with GH transgenic salmonids were all conducted under controlled culture conditions, laboratory conditions and semi-natural simulations (mesocosoms, naturalized streams) in a laboratory setting. No studies with GH transgenic salmonids, including ABT salmon, have been conducted in a natural environment due to the risk of escape. Therefore, it is unclear how the GxE interaction may affect these phenotypes in the natural environment, especially when certain phenotypic traits have not been studied under varying environmental conditions in the laboratory.

GxE interactions are a substantial source of uncertainty when assessing the risk of transgenic animals. The interaction of the environment with a genotype strongly affects the final phenotype of an organism (summarized in Devlin *et al*., 2015; and Vandersteen *et al*., 2019). GxE interactions refers to "*when a specific difference of the environment may have a greater effect on one genotype than on another*" (Kapuscinski *et al*., 2007a), and can result in different phenotypes under different environmental conditions. In other words, the environmental conditions can affect the genotype of ABT salmon and ultimately their fitness and harms in that environment, and these can change depending on the environmental conditions. A well-known example of GxE interaction was published by Sundström *et al*. (2007). GH transgenic coho salmon reared from fry to smolt stage in conventional hatchery conditions were almost 3x longer and had stronger predation effects on prey than non-transgenic coho salmon reared under the same conditions (Sundström *et al*., 2007). However, the magnitude of those differences was substantially reduced when contained in naturalized stream environments, i.e., GH transgenic salmon were only 20% longer than the non-transgenic coho salmon. This study demonstrates the different phenotypic responses that may occur for the same genotype under different environmental conditions. Sundström *et al*. (2007) states that "*extrapolations of ecological consequences from phenotypes developed in the unnatural laboratory conditions may lead to an overestimation or underestimation of ecological risk*." Thus, these GxE interactions make it very difficult to predict the fitness (survival and reproductive potential) of the ABT salmon in the Maine environment, and any resulting harms in the Maine environment due to exposure to ABT salmon and their progeny. In addition, phenotypic traits observed in the ABT salmon may not be the same as the phenotypes of their progeny in the natural environment, which adds additional uncertainty when evaluating the fate and harms of the progeny of ABT salmon (Devlin *et al*., 2007; Bessey *et al*. 2004; Devlin *et al*. 2004b).

In addition to GxE interactions, there are additional uncertainties associated with the use of empirical studies, including: the ability to extrapolate phenotypes to consequences in nature, pleiotropic[104] effects, and evolutionary change caused by genetic selection (Devlin *et al*., 2007). Therefore, there are many uncertainties and unknowns with regards to the fate (survival and reproduction) of ABT salmon and their progeny in the Maine environment and the harms they can produce. These uncertainties will be considered when ranking the likelihood of harms assuming

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[104] Pleiotrophy is defined as when a single gene affects more than one phenotypic trait (Kapuscinski *et al*., 2007).

exposure to ABT salmon in Section 9.3.2.2 below and are considered further in the Uncertainties Analysis of the Risk Characterization in Section 9.4.4.

9.3.2.2. Likelihood of Harms Assuming Exposure to ABT Salmon, *P(H|E)*

In this section, the likelihood of harm to the Maine environment assuming exposure to ABT salmon has occurred will be ranked based on the likelihood rankings provided in Table 9-5 above. The hypothetical harms will be ranked using information on the fitness of ABT salmon and similar GH transgenic salmonids discussed in Appendix F, peer-reviewed literature studies evaluating potential environmental harms of GH transgenic salmonids, and the factors discussed in Section 9.3.2.1 above. The final rankings are displayed in Table 9-7 in Section 9.3.3 below and are used in Section 9.4, Risk Characterization, to characterize the overall risk of ABT salmon to the US environment for the harms considered and evaluated.

In the highly unlikely event of an escape, ABT salmon are expected to occupy the same ecological niche as wild and domestic Atlantic salmon, competing for food, shelter, and other resources. Although ABT salmon would have one key increased fitness attribute (i.e., more rapid growth to smolt stage) relative to their wild and domesticated counterparts, in many other respects, their fitness would be reduced (e.g., increased need for food, increased dissolved oxygen utilization, etc.). Natural selection would act on these fitness attributes in the environment, but there is considerable uncertainty associated with predicting or quantifying any particular outcome. These potential harms, and their likelihoods, are discussed below.

a. Reproduction with Related Taxa (Introgression of the rDNA Construct)

ABT salmon could cause reproductive harms to wild relatives, including endangered Atlantic salmon of the Gulf of Maine DPS, through two interactions: (1) competing for mates and (2) reproduction with wild relatives that could result in introgression[98] of the rDNA construct into wild fish populations. These interactions could affect wild fish populations in the following ways: disrupting reproduction of wild relatives, producing alterations in the type or organization of genetic variation in fish species, causing alterations in life-history traits, decreasing population productivity, reducing resilience to adapt to changing conditions (e.g., climate change), and increasing invasiveness of the ABT salmon. However, in order for any harms to occur via this pathway, the ABT salmon must be able to display mating behaviors and reproduce with wild fish (wild or feral Atlantic salmon or relatives of the same genus). The potential for these interactions to occur for each type of ABT salmon is discussed below.

AANB cannot reproduce in the natural environment but could potentially compete for mates and display mating behaviors with wild fish in the natural environment resulting in unfertilized eggs. Triploid female AAS (the majority of ABT salmon that will be housed at the PEI facilities, see Section 6.4.2) could potentially compete and reproduce with wild fish, but cannot produce viable offspring. This could result in disruption of breeding and reduction in abundance of wild fish populations due to sterile AANB and triploid AAS “mating” with wild Atlantic salmon and wasting the wild fish’s reproductive efforts (NRC, 2002). However, because triploid AAS and AANB cannot establish in the Maine environment, any harms would not occur for longer than one generation. In addition, all of this is even more unlikely for triploid AAS because they will only be held at the PEI facilities as eggs prior to be being shipped to the US for grow-out, and to be present in Maine, would require that after escape, they hatch and survive to maturity which was found to be extremely unlikely in Section 9.2.

In contrast, reproduction and establishment (for many generations) in the local environment would be possible for AAFB and diploid AAS[89] because they are capable of reproducing with wild fish. It was determined in Section 8.2, that AAFB and diploid AAS could hypothetically encounter and potentially reproduce with: (1) wild conspecifics (i.e., native endangered Atlantic salmon), (2) feral relatives (e.g., domesticated Atlantic salmon without the rDNA construct that have escaped from net pens or ABT facilities), or (3) relatives from the same genus (e.g., brown trout). While interaction through competition can cause disruption in wild populations that are spawning, the most concerning harm that could occur from the ABT salmon entering the Maine environment is through reproduction of AAFB and diploid AAS with wild fish resulting in introgression of the rDNA construct into wild fish populations, especially introgression into the population of endangered Atlantic salmon of the Gulf of Maine DPS. Introgression is the incorporation of a new or altered gene construct into the gene pool of wild relatives by backcrossing of transgenic-wild fish hybrids to wild relatives (modified from Kapuscinski *et al*., 2007b). Introgression of the *opAFP-GHc2* construct would occur by interbreeding between all-female AAFB or diploid AAS and male wild relatives followed by backcrossing of the resulting progeny containing the rDNA construct with wild relatives (Kapuscinksi *et al*., 2007). If this were to occur, it could ultimately result in long-term establishment of a salmonid population containing the rDNA construct in Maine.

There are many steps (interactions) that must occur for introgression of ABT salmon's rDNA construct into wild fish populations (see Table 9-6 below). These steps are outlined in detail in this Section 9.3.2.2.a.ii below. Briefly, the following needs to occur: (1) AAFB and/or diploid AAS (F0 generation) must successfully reproduce with wild male relatives in Maine resulting in viable wild fish with the rDNA construct (F1 generation); (2) F1 generation wild fish with the rDNA construct must survive and reproduce with wild fish resulting in viable F2 generation fish with the rDNA construct (this is when introgression occurs and is known as backcrossing); and (3) reproduction of subsequent generations with the rDNA construct needs to occur for the rDNA construct to remain in the wild fish population (i.e., establishment of a salmon population with the rDNA construct).

The likelihood of potential harms occurring due to the different types of ABT salmon (AAFB, AANB, diploid and triploid AAS) competing for mates, reproducing with wild fish, and introgression of the rDNA construct into the wild population is discussed further below. In order to evaluate this pathway to harm, first, the potential for the ABT salmon F0 and F1 generations to compete for mates (i.e., display mating behaviors) and reproduce with wild and feral Atlantic salmon without the rDNA construct is evaluated. Mating with wild conspecifics (i.e., native endangered Atlantic salmon) and escaped feral conspecifics (e.g., domesticated Atlantic salmon) are assessed together below because these different Atlantic salmon types were evaluated in the same studies. Second, the potential for introgression of the rDNA construct into wild and feral Atlantic salmon populations is discussed. Third, mating and potential introgression with relatives of the same genus (brown trout) is discussed separately.

i. Reproduction of ABT Salmon with Wild and Feral Atlantic Salmon

Larger male salmon generally have a mating advantage; thus, it has been hypothesized in the literature that GH transgenic salmon may have a mating advantage over wild salmon (Bessey *et al*., 2004). However, ABT salmon are not necessarily larger than wild Atlantic salmon at maturity, they are just capable of reaching adult size in less time. Nevertheless, it is important to understand whether the rDNA construct provides a reproductive advantage to ABT salmon (and specifically, AAFB and diploid AAS) over wild Atlantic salmon. In other words, is reproductive fitness increased? If AAFB and diploid AAS have a reproductive advantage, then this could increase the extent to which introgression of the rDNA construct occurs into wild populations. Herein, reproductive success and advantage in salmon consider both competition for mates and reproduction via endpoints such as: display of courtship behavior, aggressive behaviors during competition, female digging behavior, number of spawns, number of spawning partners, and number of viable progeny.

There are no studies explicitly evaluating the reproductive success of ABT salmon, and specifically, that of AAFB and diploid AAS. However, there are some studies with close relatives (GH transgenic Atlantic salmon[103]) and GH transgenic coho salmon that can be used to make inferences regarding the reproductive success of ABT salmon. Available literature studies evaluate the competition and reproductive endpoints listed above for male and female GH transgenic salmon and compare them to non-transgenic salmon either (1) reared in the wild (referred to herein as "wild-reared") or (2) reared under domestic culture/hatchery conditions (referred to herein as "culture/hatchery-reared"). In addition, the studies also evaluate how these different types of fish (wild-reared or culture/hatchery-reared) interact in competitive trials under more natural settings (e.g., mesocosms, and naturalized streams).

With regards to ABT salmon mating with wild or feral Atlantic salmon, all-female AAFB, diploid AAS, and triploid AAS would require a male wild Atlantic salmon with which to mate; therefore, the most relevant study data would be that with GH transgenic female (Atlantic or coho) salmon. However, data on reproductive fitness of male GH transgenic salmon is also discussed in the section because, in general, it indicates the influence of the GH transgene on reproductive fitness. Data on male GH transgenic salmon will also provide information on the competitive abilities of AANB, as well as the potential for introgression via the F1 generation male progeny of AAFB and diploid AAS that contain the rDNA construct.

Data on female GH transgenic Atlantic salmon and coho salmon demonstrate that these fish can display courtship and breeding behaviors, reproduce naturally, and can transmit the transgene to progeny under culture and naturalized (e.g., mesocosm) study settings (Bessey *et al*., 2004; Fitzpatrick *et al*., 2011; Leggatt *et al*., 2014). However, these studies also generally demonstrate that reproductive success of female GH transgenic fish is reduced compared to non-transgenic salmon. Bessey *et al*. (2004) found that, under experimental conditions, female GH transgenic coho salmon reached maturity a year faster and were more fecund (produced more eggs per volume) than wild-reared non-transgenic salmon, but fewer female GH transgenic salmon spawned, they displayed consistently low levels of courtship behavior, and had smaller eggs[105] compared to wild-reared non-transgenic salmon. When placed in more naturalized settings with competition, i.e., male and female GH transgenic coho salmon and wild-reared non-transgenic salmon (4 fish of each type), Fitzpatrick *et al*. (2011) found that wild-reared non-transgenic coho salmon spawned substantially more often and produced more progeny compared to GH transgenic salmon (<6% of the progeny were sired by GH transgenic salmon).

Additional studies with male GH transgenic salmon demonstrate similar findings. Male GH transgenic coho salmon displayed courtship behavior and were able to fertilize eggs of a non-transgenic coho salmon (Bessey *et al*. 2004; Fitzpatrick *et al*. 2011). However, male GH transgenic salmon routinely demonstrated reduced breeding performance when competing with wild-reared non-transgenic counterparts (Bessey *et al*., 2004; Fitzpatrick *et al*., 2011; Leggatt *et al*., 2014; Moreau *et al*., 2011a; Moreau and Fleming, 2012). Bessey *et al*. (2004) found that wild-reared male non-transgenic coho salmon were dominant (e.g., more aggressive and had primary access to females) over GH transgenic males and always spawned when competing for females. The GH transgenic males failed to spawn when competing with wild-reared male non-transgenic salmon. Fitzpatrick *et al*. (2011) observed the same impaired reproductive performance in male GH transgenic coho salmon. Male wild-reared non-transgenic coho salmon performed more courtship behaviors than male GH transgenic salmon, and had denser, faster swimming, and longer-living sperm. Leggatt *et al*. (2014) found that, when in high competition situations with wild-reared non-transgenic salmon (2 males and 1 female), females preferred to mate with non-transgenic males, and the GH transgenic males failed to spawn, did not display quivering, and were less aggressive towards competition. Moreau *et al*. (2011a) had similar observations with male GH transgenic Atlantic salmon.[103] The GH transgenic males were less competitive (reduced nest fidelity and quivering frequency) and participated in fewer spawning events than non-transgenic males.

All authors also suggested that the reproductive impairment observed in GH transgenic salmon may be due to their domestication and rearing in a culture environment (see Appendix E). Non-transgenic coho salmon reared for their full life-

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[105] Although the eggs were smaller, the authors did not determine whether that affected survival of progeny.

history in a culture environment were also found to have impaired spawning compared to wild-reared non-transgenic coho salmon (Bessey *et al*., 2004; Leggatt *et al*., 2014). However, when reared in a large mesocosm and presented with competition, domesticated culture/hatchery-reared non-transgenic coho salmon had slightly lower or similar reproduction to that of wild-reared non-transgenic coho salmon (Leggatt *et al*. 2014). Additionally, in some studies, GH transgenic salmon had similar or reduced reproductive success compared to domesticated culture/hatchery-reared non-transgenic coho salmon (Bessey *et al*., 2004; Fitzpatrick *et al*., 2011; Leggatt *et al*., 2014).

The life stage at which AAFB and diploid AAS escaped the PEI facilities could also affect their reproductive success. Many researchers postulate that the length of time under culture conditions could affect their reproductive ability in the natural environment (Bessey *et al*. 2004; Fitzpatrick *et al*. 2011). That is, if GH transgenic salmon are reared under culture conditions for most of their lives before escape, then it is theorized their reproductive success in the natural environment would be impaired. However, if the GH transgenic salmon escape into the wild at young life stages (e.g., egg or fry), and the environment shapes their phenotype, then their reproductive fitness could be similar to that of wild-reared non-transgenic salmon. Thus, life stage at time of escape could affect the reproductive success of AAFB and diploid AAS and the rate of introgression of the rDNA construct.

These studies suggest that all types of ABT salmon can display courtship behaviors and compete for mates, and AAFB and diploid AAS can reproduce with wild and feral conspecifics in the natural environment, but they may be reproductively impaired (both in competing for and reproducing with wild mates) compared to wild-reared Atlantic salmon. In addition, there was variability seen between and within studies indicating a strong GxE interaction that adds additional uncertainty to predicting the reproductive success of AAFB and diploid AAS in the Maine environment and determining the potential for introgression. The potential for introgression and establishment of the rDNA construct in wild/feral Atlantic salmon populations is further discussed in the next section.

ii. Introgression (also known as, Genetic Disturbance or Vertical Gene Transfer)

Establishment of the rDNA construct in the Maine environment requires introgression of the rDNA construct into wild fish populations, including the population of endangered Atlantic salmon of the Gulf of Maine DPS. However, many sequential steps must take place for introgression of the rDNA construct to occur in wild fish populations, and for establishment in the Maine environment to be maintained over many generations. Kapuscinski *et al*. (2007b) describes the steps required for introgression of an rDNA construct in a wild fish population, which are also summarized in Table 9-6 below.

Table 9-6. Steps Required for Introgression of the rDNA Construct in Wild Fish Populations*

Step #	Description of Step
1	AAFB and/or diploid AAS must escape or be released from ABT PEI facilities, survive, and migrate to Atlantic salmon breeding grounds in Maine.
2	Sexually mature female AAFB and/or diploid AAS must encounter sexually mature male wild or feral Atlantic salmon and successfully mate (including compete for nesting sites, display courtship behaviors, and reproduce).
3	F1 progeny of AAFB and/or diploid AAS x wild or feral Atlantic salmon must be viable, hatch, and survive to sexual maturity. Approximately 50-100% of the F1 progeny would contain the rDNA construct.
4	Sexually mature F1 progeny must encounter a sexually mature wild or feral Atlantic salmon or another F1 progeny, and successfully mate (including compete for nesting sites, display courtship behaviors, and reproduce).
5	F2 progeny of the F1 progeny with the rDNA construct x wild/feral Atlantic salmon or other F1 progeny must be viable, hatch, and survive to sexual maturity.
6	F2 progeny and subsequent progeny with the rDNA construct must continue to produce viable progeny with the rDNA construct for introgression to be maintained and establishment to occur.

*Information obtained and modified from Kapuscinski *et al*. (2007b)

According to Kapuscinski *et al*. (2007b), these steps characterize the potential for gene flow, which is defined as "*the exchange of genes between different populations of the same or closely related species*." Kapuscinski *et al*. (2007b) presented a series of equations to assess the probability of gene flow:

Probability of gene flow = probability of entry × probability of introgression

Where,

$$\text{Probability of entry} = P(\text{escape}_{\text{mature}}) + [P(\text{escape}_{\text{immature}}) \times P(\text{survival to maturity})]$$

$$\text{Probability of introgression} = P(\text{F1 hybridization}) \times P(\text{BC1 backcrossing})$$

The probability of entry is dependent upon sexually mature AAFB and/or diploid AAS escaping the ABT facilities, or immature AAFB and/or diploid AAS escaping and surviving to maturity in the natural environment. In addition, AAFB and diploid AAS must survive in the natural environment and disperse or migrate to breeding grounds in Maine (Kapuscinski *et al*., 2007b). This is represented in Step 1 in Table 9-6 above. The likelihood of these events occurring was assessed in Section 9.2 and found to be negligible (or infinitesimal, see Section 9.2.3 above). Regardless, in this section, it is conservatively assumed that AAFB and diploid AAS are present and encounter wild and feral relatives in the Maine environment in order to assess the intention of Question 4b, i.e., the likelihood of harms occurring *assuming* exposure (establishment and/or presence) occurring, $P(H|E)$.

When evaluating the probability of introgression of a transgene into a wild population, Kapuscinski *et al*. (2007b) recommends that the following probabilities be assessed:

- *P(F1 hybridization):* The probability of first-generation hybridization. For the current assessment, this would be the probability of sexually mature AAFB and diploid AAS encountering a sexually mature wild male relative in Maine and the two successfully mating. This is represented in Step 2 in Table 9-6 above, and is characterized by the following equation from Kapuscinski *et al*. (2007b):

  P(F1 hybridization) = P(encounter) × P(escapee × wild fish mating)

- *P(BC1 backcrossing):* The probability of backcrossing (BC) of the F1 progeny containing the rDNA construct. For current assessment, this would be the probability of F1 progeny with the rDNA construct surviving to maturity, the mature F1 progeny encountering a sexually mature wild relative and the two successfully mating. This is represented as Steps 3 and 4 in Table 9-6 above, and is characterized by Kapuscinski *et al*. (2007b) as:

  P(BC1 backcrossing) = P(F1 progeny survival to maturity) × P(encounter) × P(F1 x wild mating)

- Further introgression: Establishment of the rDNA construct into the wild population would require the BC1 fish to survive to sexual maturity and successfully reproduce. For establishment to be maintained, future progeny with the rDNA construct would need to continue to reproduce (represented as Steps 5 and 6 in Table 9-6 above).

Note: The terms used in these equations were obtained from Kapuscinksi *et al*. (2007). “F1 hybridization” represents the F1 progeny of AAFB or diploid AAS crossed with wild or feral Atlantic salmon. “BC1 backcrossing” represents the F2 progeny created via backcrossing of the F1 progeny with the rDNA construct and wild or feral Atlantic salmon.

P(F1 hybridization) is dependent upon sufficient numbers of reproductively competent transgenic fish escaping and entering wild populations, *P(encounter).* The scale and frequency of introduction would have a large influence on environmental risk. Any introductions would have to involve a critical mass that could offset natural mortality and be of sufficient frequency and in proper season to allow for long-term survival and establishment. If the scale and frequency of the escapes (i.e., introductions to the environment) are small, then the chances of becoming established in the natural setting are extremely low (Kapuscinski and Hallerman, 1991). *P(F1 hybridization)* is also dependent upon AAFB and/or diploid AAS mating with a wild or feral Atlantic salmon without the rDNA construct. In order for this to occur, conditions need to be sufficient for breeding, including appropriate timing of sexual maturation, morphology and coloration, and appropriate display of courtship and breeding behaviors (Kapuscinski *et al*., 2007b). In addition, AAFB and diploid AAS would need to outcompete wild female relatives for mates (discussed in Section i above) and spawning sites (see Section 9.3.2.2.b, below) in Maine. Based on the information in Section i above, AAFB and diploid AAS can display courtship behaviors and compete for mates and can reproduce with wild and feral conspecifics in the natural environment. Although, they may be reproductively impaired (both in competing for and reproducing with wild mates) and have a reduced reproductive fitness compared to wild-reared Atlantic salmon. Regardless, F1 hybridization could occur if sexually mature female AAFB and/or diploid AAS encounter a male wild or feral Atlantic salmon in the Maine environment.

P(BC1 backcrossing) assesses the probability of the F1 progeny with the rDNA construct surviving until sexual maturation, *P(F1 progeny survival to maturity)*, which is dependent upon fertilization success, gamete number and quality, parental care, successful foraging, predator avoidance, and normal developmental schedules (growth and reproduction) (Kapuscinski *et al*., 2007b). *P(BC1 backcrossing)* also assesses the probability of sexually mature F1 progeny with the rDNA construct encountering sexually mature wild relatives, *P(encounter)*, and successfully reproducing (backcrossing), *P(F1 × wild mating)*, which is dependent upon the factors outlined in the previous paragraph. Based on the information in Section i above, there is nothing to indicate that F1 progeny with the rDNA construct could not reproduce with wild/feral Atlantic salmon resulting in introgression and establishment of the rDNA construct (i.e., backcross) as long as conditions are suitable. This could ultimately result in introgression and establishment of the transgene into wild endangered Atlantic salmon in Maine.

In addition, long-term establishment of the rDNA construct in a wild population via introgression also could occur. Long-term introgression is dependent upon gene flow (exchange of the rDNA construct within the population) and natural selection against the transgenic phenotype (Kapuscinski *et al*., 2007b). However, there is no information to predict if and/or for how long the rDNA construct would persist within a wild/feral Atlantic salmon population (e.g., what frequency would the rDNA construct be transmitted; would the phenotype be selected for or against in nature). When applying Mendelian genetics, it is expected that the 50% of the F2 hybrids (F1 hybrid x wild Atlantic salmon) would be hemizygous for the rDNA construct, and so on for future generations. If an F1 hybrid reproduced with an F1 hybrid, then it is expected that 50% of the F2 hybrids would be hemizygous for the rDNA construct, 25% would be homozygous for the rDNA construct, and 25% would not contain the rDNA construct.

The potential for gene flow, that is, the ability for an rDNA construct to spread in a wild population, is determined by natural selection and has been described by a net fitness model (Muir and Howard, 1999; 2001; 2002a; 2002b). Net fitness components included in the Muir and Howard model include viability (survival) and reproductive success. Factors used to determine the potential for reproductive success include age at sexual maturity, mating success, female fecundity, and male fertility. Although specific data on these net fitness parameters for ABT salmon had not yet been published in the scientific literature, at FDA’s Veterinary Medicine Advisory Committee (VMAC) meeting for AquAdvantage Salmon on September 20, 2010, Professor William Muir (Department of Animal Sciences, Purdue University) reported that diploid ABT salmon (i.e., AAFB and diploid AAS) although potentially larger than their age-matched wild counterparts, would not have a mating advantage. They are behaviorally out competed by control males as determined by nest fidelity, quivering frequency, and spawn participation (see Section i above, and paragraphs below). Dr. Muir also concluded that male GE salmon displayed reduced reproductive performance relative to control males.[106]

In addition to pioneering the use of a net fitness model for assessing the environmental risk of GE fish (Muir and Howard, 2001 and 2002b; Muir, 2004), Dr. Muir is one of the originators of the Trojan gene hypothesis, which explored possible extinction of populations through the flow of a gene that confers a reproductive advantage while also rendering offspring less able to survive in the natural environment (Muir and Howard, 1999; Howard *et al*., 2004). This hypothesis was generated for, and addresses data derived from, mating and growth behaviors of a laboratory model fish, medaka.

In comments presented to the VMAC in September 2010, Dr. Muir addressed the Trojan gene hypothesis and his data in relation to AAS [*sic* diploid ABT salmon] as follows:

> "*I want to clearly state that this only occurs as a result of a conflict between mating success and viability fitness. And the data conclusively shows that there is no Trojan Gene effect as expected. The data in fact suggest that the transgene will be purged by natural selection. In other words, the risk of harm here is low*."

More recently, Dr. Muir has stated in a commentary by Van Eenennaam and Muir (2011) that he has reviewed actual AAS [*sic* GH transgenic Atlantic salmon] data collected by Moreau and colleagues (Moreau, 2011; Moreau *et al*., 2011a; Moreau and Fleming 2012) quantifying critical life history characteristics, such as relative viability and mating success of these fish in multiple environments (these data are discussed in the paragraph below and in Appendix F). Dr. Muir states that,

> "*Analysis of the data showed that none of the net fitness components of AquAdvantage salmon [sic GH transgenic Atlantic salmon] were enhanced by expression of the transgene. As a result, the Trojan gene effect would not be predicted to occur in the unlikely event AquAdvantage salmon [sic diploid ABT salmon] did escape from confinement. Rather, selection over time would be expected to simply purge the transgene from any established population, suggesting a low probability of harm resulting from exposure to AquAdvantage salmon [sic diploid ABT salmon]*."

In another recent publication, Dr. Muir stated that, "*[b]ased on their data, the long-term risk of GE salmon is close to zero as no fitness advantages in any component were demonstrated, resulting in a purge scenario for the transgene*" (Van Eenennaam *et al*., 2013).

There are concerns in the literature that GH transgenic Atlantic salmon, like AAFB and diploid AAS, could cause introgression of the rDNA construct into the wild population at a faster rate due to their rapid growth and maturation at a younger age

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[106] Transcript available here: https://wayback.archive-it.org/7993/20170404230911/https://www.fda.gov/AdvisoryCommittees/CommitteesMeetingMaterials/VeterinaryMedicineAdvisoryCommittee/ucm201810.htm (accessed December 8, 2023)

enabling more generations of progeny to be produced than wild Atlantic salmon (Devlin *et al*., 2015). As expected, GH transgenic salmon have been found to grow faster in laboratory naturalized stream settings and mesocosms resulting in these fish reaching maturity faster than wild-reared or hatchery reared salmon (Devlin *et al*., 2004b; Bessey *et al*., 2004; Fitzpatrick *et al*., 2011; Leggatt *et al*., 2014; Moreau *et al*., 2011a). This enhanced growth rate allows the GH transgenic fish to reproduce at a younger age (at year 1-2 rather than year 3-4), and thereby, facilitating more rapid transmission of the rDNA construct into the wild population (Devlin *et al*., 2015).

In addition, it has been hypothesized that, due to their higher energy stores and growth at a young age, GH transgenic salmon populations could have a higher proportion of precocious males in the natural environment, which could provide them a reproductive advantage by increasing the rate of transmission of the rDNA construct (Fitzpatrick *et al*. 2011; Moreau and Fleming 2012). As discussed earlier, in Atlantic salmon populations, there are two types of reproductive males: large anadromous males who court females to spawn (after migration at sea) and small, non-migratory precocious males (known as sneaker males) whose small size allows them to sneak in unknowingly to fertilize eggs during spawning events (Fitzpatrick *et al*. 2011; Moreau *et al*. 2011a; Moreau and Fleming 2012). Precocious males may have evolved due to intense competition among large anadromous males for breeding with females (OECD, 2017). Fitzpatrick *et al*. (2011) hypothesizes that *"…male progeny from transgenic salmon may be more likely to develop into sneaker males under some conditions. Therefore, introgression of a transgene into wild populations may be further accentuated by the shortened maturation age of precociously development males if transgenic salmon escaped into nature*." Precocious male parr could represent anywhere from 2-100% of the male population and could fertilize 11-65% of eggs that are released by females (OECD, 2017). Therefore, if a higher percent of progeny with the rDNA construct are precocious males, they could fertilize eggs via sneak attacks without the larger males and females knowing and shorten the generation times resulting in faster introgression (OECD, 2017).

Moreau and Fleming (2012) and Moreau *et al*. (2011a) evaluated whether precocious GH transgenic Atlantic salmon[103] could provide a reproductive advantage in transmission of the rDNA construct. In a laboratory study evaluating the rate of precocious males in GH transgenic Atlantic salmon compared to non-transgenic salmon, Moreau and Fleming (2012) found that 1.3% of the male population were precocious parr in the first year of life, but that the number of precocious males was similar between GH transgenic and non-transgenic salmon. However, in the second year of life, there were significantly more non-transgenic precocious males than GH transgenic precocious males (non-transgenic males were 1.8% more likely to mature). Moreau *et al*. (2011a) found GH transgenic precocious Atlantic salmon were able to spawn naturally in naturalized laboratory settings, but overall fertilization success was low for all precocious males (transgenic or not); anadromous males had substantially higher fertilization success compared to precocious males. When comparing only precocious male mating, non-transgenic precocious males had a greater nest fidelity and spawn participation and significantly higher fertilization success and parented more offspring than GH transgenic precocious males (Moreau *et al*. 2011a). Thus, these studies indicate that progeny with rDNA construct are not likely to have a higher number of precocious males, and it's not likely to substantially influence the rate of introgression of the rDNA construct into wild populations.

iii. Conclusion on Mating and Introgression with Wild/Feral Atlantic Salmon

In conclusion, ABT salmon and wild and domesticated Atlantic salmon are conspecifics that are generally phenotypically and behaviorally similar; thus, it is expected that ABT salmon could compete for mates, and AAFB and diploid AAS could reproduce with wild and feral Atlantic salmon if conditions are suitable (Kapuscinski *et al*., 2007b). AANB and triploid AAS have the potential to disrupt reproduction of wild Atlantic salmon. AANB can compete for mates and cause females to produce eggs that will remain unfertilized, wasting the female wild Atlantic salmon's efforts, whereas triploid AAS can mate with wild Atlantic salmon but would result in nonviable offspring. However, these potential harms from AANB and triploid AAS would only last one generation until these fish died or ceased their spawning behavior. Therefore, the likelihood of harms via AANB and triploid AAS competing for mates and producing nonviable offspring was ranked as low to moderate (harm unlikely to likely to occur).

AAFB and diploid AAS could potentially reproduce with wild and feral Atlantic salmon and could potentially produce viable F1 progeny in the Maine environment. This could result in introgression and establishment of the rDNA construct if the F1 progeny are capable of backcrossing and there is continued transmission of rDNA construct in future progeny. Thus, exposure to Atlantic salmon the rDNA construct could theoretically occur over many generations. However, the studies discussed above demonstrate that there will likely be some impairment in reproductive success for AAFB and diploid AAS in the Maine environment compared to wild Atlantic salmon. In addition, there are many steps (see Table 9-6) that need to occur in order for introgression and establishment of the rDNA construct to occur in wild fish populations in Maine, including the endangered Atlantic salmon. The initial step of entry (probability of entry) of the AAFB and diploid AAS into Maine and the likelihood of these fish encountering wild or feral Atlantic salmon in the Maine environment was found to be negligible (or infinitesimal) in Section 9.2. Thus, according to the equation above (probability of gene flow = probability of entry × probability of introgression), probability of gene flow would also be negligible. In addition, Dr. Muir[106] also concluded that the likelihood of establishment (introgression) of the rDNA construct via diploid ABT salmon (i.e., AAFB and diploid AAS) occurring is considered to be low and the data suggest that the transgene will be purged via natural selection.

Regardless, because we do not explicitly know what would occur if ABT salmon enters the natural environment, and the endangered Atlantic salmon of the Gulf of Maine DPS is a vulnerable population (see Section 9.3.2.1.c, above), thus any disruption in their breeding could result in severe and irreversible harms, we have conservatively weighted our harm ranking for reproduction higher than it likely merits based on the information above. Thus, assuming exposure occurs, the likelihood of harms due AAFB and diploid AAS competing for mates and reproducing with wild and feral Atlantic salmon is considered to be high. The risk of this harm actually occurring will be further evaluated in Question 5 (Section 9.4), below.

iv. Relatives of the Same Genus

AAFB and diploid AAS may breed with relatives of the same genus, *Salmo*, in Maine and produce hybrid progeny with the rDNA construct. For example, it is known that

Atlantic salmon (*Salmo salar*) can reproduce with brown trout (*Salmo trutta*) under laboratory conditions (Oke *et al*., 2013). Although not native and considered invasive to North America, brown trout have been widely introduced and exist in freshwater rivers in Maine.[107] Therefore, it is important to consider whether ABT salmon could compete for mates, whether AAFB and diploid AAS could reproduce with relatives of the same genus (e.g., brown trout) and produce hybrids (e.g., Atlantic salmon × brown trout) with the rDNA construct, and whether these hybrids could backcross with wild relatives and cause introgression of the rDNA construct into the wild population.

As previously discussed in Section 9.2.2.1.e, Oke *et al*. (2013) found that gametes of diploid GH transgenic Atlantic salmon, relatives of ABT salmon,[103] could produce transgenic hybrids (i.e., hybrids with the GH EO-1ɑ transgene) that were viable[85] and grew more rapidly than their GH transgenic parents. During mesocosm studies, the transgenic hybrids were competitively dominant and suppressed the growth of both GH transgenic and non-transgenic Atlantic salmon. Thus, it is possible that AAFB and diploid AAS may be able to reproduce with relatives of the same genus (e.g., brown trout) in Maine and produce viable hybrid offspring containing the rDNA construct that could dominate other fish populations (Step 1 of Table 9-6, above). However, Oke *et al*. (2013) did not evaluate whether the progeny of Atlantic salmon × brown trout hybrids are capable of producing viable offspring. There is information in the literature showing that the offspring of non-transgenic Atlantic salmon × brown trout are either non-viable or triploid (i.e., effectively sterile) (Galbreath and Thorgaard 1995). Therefore, the available information suggests that there is virtually no potential for any further introgression of the transgene into brown trout or Atlantic salmon genomes via backcrossing by the hybrids with the rDNA construct (i.e., Steps 2 and 3 of Table 9-6, above, would not be possible). In addition, fish in the genus of *Salvelinus* (such as, brook trout and artic char) also exist in Maine, but they would not be expected to have viable offspring (and Atlantic salmon and brook trout would be unlikely to mate due to size differences).

Based on the information above, it appears that AAFB and diploid AAS could cause minor harms to wild fish populations that consist of relatives of the same genus, such as brown trout, in Maine due to disruption through competing for and reproducing with mates. However, these harms would likely be reversible because any progeny would only be viable for one generation. Thus, the likelihood of harms from AAFB and diploid AAS competing for and reproducing with relatives of the same genus is considered to be low. Likewise, AANB and triploid AAS could also cause disruption to wild fish populations by competing for mates; however, eggs produced would either not be fertilized (in the case of AANB) or would not be viable. Thus, the likelihood of harms from AANB and diploid AAS due to competition for mates with relatives of the same genus was ranked as negligible to low.

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[107] https://www.maine.gov/ifw/fish-wildlife/fisheries/species-information/brown-trout.html (accessed December 8, 2023)

b. Resource Competition

As stated throughout this assessment, the physical presence of the ABT salmon (AAFB, AANB and diploid and triploid AAS) in the Maine environment could also potentially result in harms without reproduction and establishment occurring. For example, if ABT salmon were present in the Maine environment, they would occupy the same niche as endangered Atlantic salmon of the Gulf of Maine DPS and, therefore, could compete for and utilize the same resources, including food and habitat (e.g., spawning substrate, over-wintering sites). Resulting harms from this competition could potentially include a reduction(s) in the survival and reproduction of wild fish species, including endangered Atlantic salmon of the Gulf of Maine DPS, and disruption of the biotic community (e.g., reduction in prey species) and physical environment [e.g., disruption of streambeds due to redd (nest) creation]. This is especially concerning because the population of endangered Atlantic salmon of the Gulf of Maine DPS are extremely vulnerable, and any type of disruption could result in harms to that population (see Section 9.3.2.1.c above). The likelihood of harms occurring due to competition for each of these resources is discussed and ranked below.

i. Competition for and Use of Food Resources

If ABT salmon entered the Maine environment, they would increase the abundance (density) of predators, and inherently increase the pressure on available food resources, and could adversely affect growth and survival of competitors (including endangered Atlantic salmon). In addition, because ABT salmon contain a GH construct that causes them to grow at a faster rate than wild Atlantic salmon, ABT salmon may also require increased food intake compared to wild Atlantic salmon, which can add additional pressure on food resources and competitors.

There are no specific studies evaluating the foraging behaviors of ABT salmon with wild prey; however, studies conducted with other GH transgenic salmon can provide some insight into the potential foraging behavior of ABT salmon in the natural environment, as well as the potential effects that has on wild salmon. There are a large number of these types of studies in the published literature, mainly focused on GH transgenic coho salmon, that evaluate the food competition dynamic between GH transgenic salmon and non-transgenic salmon, and potential harms that can arise from this interaction. They are discussed at length in Devlin *et al*. (2007), Devlin *et al*. (2015), and Vandersteen *et al*. (2019), and some are described below.

GH transgenic coho salmon have been observed to be more competitive (Devlin *et al*., 1999), less discriminate in choosing prey (Sundström *et al*., 2004a), more likely to attack novel prey (Sundström *et al*., 2004a), and better at using lower quality food (Raven *et al*., 2006) when compared to wild relatives. Because they grow faster, there has been a suggestion that ABT salmon might be more aggressive and thus out-compete their wild counterparts for resources. During pre-smolt growth, GH transgenic Atlantic salmon consumed much larger amounts of food than size-matched controls on a daily basis when fed to satiation three times per day under hatchery conditions (Cook *et al*., 2000a). Similar responses were seen for GH transgenic coho salmon when held in laboratory settings with high food availability (Sundström *et al*., 2007). However, the availability of food, density of salmon, and specific environmental conditions (e.g., laboratory versus natural settings) also influence behavior and competition for resources.

Devlin *et al*. (2004a) found food availability effected growth and survival of GH transgenic coho salmon and non-transgenic salmon. When food availability was high, GH transgenic coho salmon did not affect the growth of non-transgenic fish, but they did grow larger than non-transgenic fish (11.6-fold greater mass). However, at very low food availability, the GH transgenic salmon reduced the growth of non-transgenic salmon, but the greatest adverse effects were observed to the GH transgenic salmon population. The population of GH transgenic salmon was reduced to near extinction due to aggression and cannibalism by dominant GH transgenic salmon. It is also notable to mention that this study included a treatment of only non-transgenic fish (i.e., GH transgenic salmon were not present). When food availability was low with non-transgenic salmon only, the population had high survival and an increase in biomass throughout the test period. This study suggests that GH transgenic salmon can become extremely aggressive in conditions with low food availability and could potentially have effects on growth and survival of wild Atlantic salmon populations.

The extreme aggression upon GH transgenic salmon and wild Atlantic salmon is important to note because it alone can result in harms to endangered Atlantic salmon due to cannibalism.[99] For example, YOY Atlantic salmon with the rDNA construct could grow faster in the natural environment resulting in increased food requirements at a young age. If food availability is very low, then YOY salmon with the rDNA construct could consume YOY wild Atlantic salmon to meet the energy requirements for their rapid growth phenotype. This cannibalism could reduce the abundance of YOY endangered Atlantic salmon.

Vandersteen *et al*. (2019) found that density had the large influence on the growth of GH transgenic coho salmon versus non-transgenic salmon. When density of salmon was low, survival of GH transgenic and non-transgenic salmon was high and GH transgenic salmon grew larger regardless of food availability (low or high). When density of fish was high, increased mortality for both GH transgenic and non-transgenic salmon was observed regardless of food availability (low or high), but there was greater overall mortality for non-transgenic salmon. Thus, increases in the density (magnitude) of fish in the Maine environment could adversely affect wild fish populations due to competition.

However, the studies discussed above were conducted under laboratory conditions in tanks without natural components (bare tanks) and may not accurately predict what could occur in a natural environment. When similar studies were conducted in conditions expected to be much more representative of those in the natural environment (e.g., naturalized stream settings with gravel bottoms, large rocks, logs, plants), the growth and survival of the GH transgenic salmon and the effects of GH transgenic salmon on survival and growth of non-transgenic salmon were highly variable (Vandersteen *et al*., 2019). For example, Moreau *et al*. (2011b) found the presence of the GH gene construct in the GH transgenic Atlantic salmon did not influence growth or survival of first-feeding fry at high or low fry densities under food-limited conditions in simulated aquatic environments (i.e., stream microcosms). In addition, GH transgenic salmon and non-transgenic salmon were also found to be equally likely to be dominant in naturalized stream settings (Moreau, 2014). Sundström and Devlin (2011) observed similar findings, when food availability was low, survival and growth of GH transgenic coho salmon and non-transgenic coho salmon was not affected in naturalized stream settings. In contrast, Sundström *et al*. (2007) found that when GH transgenic coho salmon were reared in naturalized stream environments, their growth was substantially reduced compared to when they were reared under typical culture conditions in a hatchery setting. Similarly, Leggatt *et al*. (2017a) found a substantial size advantage of GH transgenic coho salmon under hatchery conditions but little to no advantage under semi-natural stream conditions. The reason for the variability is likely due to the various factors that affect survival and growth of transgenic fish in the natural environment. Vandersteen *et al*. (2019) conducted complex experiments that altered habitat complexity, food type (artificial versus natural), amount of food, and risk of predation. All of these factors affected growth and survival of GH transgenic and non-transgenic coho salmon, but "*no predictable trend in relative survival of transgenic versus non-transgenic fry emerged*" (Vandersteen *et al*. 2019).

It is also hypothesized that the rapid growth of ABT salmon compared to wild Atlantic salmon could affect the life history of the fish (see Appendix F.2.8, Life History), which could in turn affect consumption of food sources. For example, GH transgenic coho salmon were found to hatch and emerge from redds sooner allowing them to access food earlier than non-transgenic salmon (Sundström *et al*., 2004b). If this is also the case for ABT salmon, this could result in reduction of food resources for the wild Atlantic salmon once they are ready to emerge from the redd which could affect survival of non-transgenic salmon fry. However, the early emergence from the redd also increases the likelihood of mortality for the GH transgenic salmon due to a higher chance of being preyed upon. In addition, if ABT salmon reached smolt size faster (Saunders *et al*., 1998; Devlin *et al*., 1995a), then it could hypothetically cause them to migrate to sea earlier and have access to food resources at wintering sites in Canada and Greenland earlier than wild Atlantic salmon, but that could also reduce the pressure on food resources in freshwater rivers in Maine (Devlin *et al*., 2015). However, Sundström *et al*. (2010) found that, although larger fish tend to migrate earlier, overall migration timing was not dependent on size and growth rate.

As discussed above, some researchers observed more aggressive feeding behavior by GH transgenic salmon. More aggressive foraging behavior could also affect the ABT salmon's survival in the Maine environment. Research on GH transgenic Atlantic salmon and rainbow trout in laboratory experiments indicates these fish are more likely to feed in the presence of a predator than non-transgenic controls (Abrahams and Sutterlin, 1999, Sundström *et al*., 2004b; and Crossin *et al*., 2015). Many researchers think this behavior is due to domestication and rearing in hatchery facilities rather than the presence of the transgene (Islam *et al*., 2020; Solberg *et al*., 2020). Regardless, this risky behavior could result in higher mortality rates for ABT salmon compared to wild Atlantic salmon.

Furthermore, domestication (i.e., farm rearing) of the ABT salmon can adversely affect their foraging behavior and survival in the natural environment. There is considerable research showing that a large percentage of farmed fish fail to adapt to feeding on live prey after escaping and could starve to death (Muir, 2004; Olsen and Skilbrei, 2010; Hislop and Webb, 1992; Soto *et al*., 2000). A detailed discussion on this topic is provided Section 9.2.2.1.c, above.

The studies discussed above indicate that there are many factors that would influence the actual outcome of ABT salmon competing for and utilizing food resources in the Maine environment, and whether the competition for food would cause harm, particularly to endangered Atlantic salmon. These factors include the availability of prey, the density of ABT salmon and wild Atlantic salmon competing for the same prey, the aggressiveness of ABT salmon's foraging behavior (including potential for cannibalism), the context of exposure, the life history of ABT salmon in the natural environment, the plasticity of ABT salmon and other wild organisms, the resiliency of the Maine ecosystem, the domestication of the ABT salmon, and, most importantly, GxE interactions (Devlin *et al*., 2015, and Vandersteen *et al*., 2019). In addition, there was high variability in results of these studies (Vandersteen *et al*., 2019), and uncertainties associated 1) with how results from laboratory studies (laboratory or naturalized conditions) relate to what would occur in the natural environment, and 2) how studies conducted with different fish species and different GH gene constructs relate to ABT salmon. Thus, taking all of this into consideration, it is unclear if, and under what conditions, ABT salmon would cause harms in the Maine ecosystem through competing for and utilizing food resources.

Regardless, the weight of evidence suggests that if ABT salmon were present in the Maine environment at a high enough abundance for a sufficiently long duration, or at a high frequency, they could potentially cause moderate to severe harms to the Maine environment by depleting food resources used by endangered Atlantic salmon and disrupting the biotic environment through increased consumption of prey and potentially feeding on different types of prey (including endangered Atlantic salmon). In addition, due to the low abundance of endangered Atlantic salmon of the Gulf of Maine DPS, any adverse impact to their food source could have severe and irreversible harms on this population. However, the duration of exposure will also affect the likelihood of harm. AANB and triploid AAS are only expected to be present in the Maine environment for one generation, and therefore, any potential harms would be limited. AAFB and diploid AAS could establish and potentially cause harms for multiple generations. Thus, the likelihood of harms occurring if AANB and triploid AAS are present and competing for food is ranked as moderate, whereas, for AAFB and diploid AAS, the ranking is high.

ii. Competition for and Use of Habitat

Devlin *et al*. (2007) suggest that transgenic fish could affect the breeding success of wild fish due to competition for mates and breeding resources, such as breeding sites and nest building materials. Devlin *et al*. (2007) states that "*The extent of any reproductive competition will depend on the existence of limiting resources, such as spawning habitat and mates, which are sought after jointly by transgenic and nontransgenic fish.*" If ABT salmon enter the Maine environment, it is expected that they would share the same habitat as endangered Atlantic salmon of the Gulf of Maine DPS and would have to compete with endangered Atlantic salmon for nesting sites and mates (see Section 9.3.2.2.a, above for discussion on competition for mates). If ABT salmon outcompete endangered Atlantic salmon for spawning habitat or disrupt spawning sites, it could affect breeding success of wild Atlantic salmon, and eventually, could result in a reduction of the abundance of endangered Atlantic salmon. However, the competition for habitat and the effects due to that competition are only hypothetical; there is little information available on the potential for ABT salmon or other GH transgenic salmonids to compete for nesting sites and nesting materials (Devlin *et al*., 2007). However, some concerns regarding the potential for this harm to occur in Maine are discussed below.

Spawning grounds located in freshwater river habitats in Maine are critically important to the survival of the endangered Atlantic salmon of the Gulf of Maine DPS. As discussed in Section 8.4.3 (above), one of the primary reasons for the decline of Atlantic salmon in the northeastern US is due the construction of dams that blocked the salmon’s access to their natal rivers and spawning and nursery grounds (NRC, 2004b; NOAA and FWS, 2020). Other major threats include road stream crossings that impede fish passage to critical habitat, and climate change which can result in damage to critical habitat. Therefore, the ESA designation also covers the critical habitat of the Gulf of Maine DPS, which is shown in Figure 8-11. Based on the most recent review of the endangered designation for Atlantic salmon under the ESA, NOAA and FWS (2020) stated the quantity of suitable and accessible habitat for spawning and juvenile rearing in Maine has increased due to dam removals and improvements in fish passage; however, the Gulf of Maine DPS is still at critically low abundance. Figure 8-12 shows accessibility of the critical habitat of the Gulf of Maine DPS. Based on this map, there is still limited accessibility by Atlantic salmon to much of the designated critical freshwater habitat (spawning and nursery grounds) on the coast of Maine. In fact, most of the endangered Atlantic salmon of the Gulf of Maine DPS (~85%) return to only one river in Maine, the Penobscot. This information indicates that suitable accessible habitat for Atlantic salmon to spawn and grow is limited in Maine. Thus, if present, ABT salmon could potentially compete with endangered Atlantic salmon for nesting sites and disturb existing endangered Atlantic salmon nesting sites, which could adversely affect the reproduction of endangered Atlantic salmon.

Devlin *et al*. (2007) also notes that theoretically GH transgenic fish could be a concern with regards to competition for nesting sites because they could potentially grow faster and reach maturity earlier resulting in a mating advantage over wild Atlantic salmon. Females compete for nesting sites and males compete to mate with females. Diploid and triploid AAS and AAFB are all female populations, and therefore, are expected to compete for nesting sites whether they are reproductively competent for not. If AAS and AAFB can reach sexual maturity earlier than wild Atlantic salmon, then they may obtain nesting sites earlier, potentially reducing the available nesting sites for wild Atlantic salmon. There is some information available in the literature on the effects of GH constructs on the development of transgenic salmonids compared to wild counterparts that suggest that GH transgenic salmonids could reach maturity earlier than their wild counterparts (Saunders *et al*., 1998; Devlin *et al*., 2004b; Devlin *et al*., 1995a; Devlin *et al*. 1995b; see summary in Appendix F.2.5). However, there are other studies that found that development of GH transgenic Atlantic salmon was not faster, and at times, could be delayed compared to wild counterparts (Moreau, 2014; Moreau and Fleming, 2012). In addition, hatchery rearing has been shown to result in more rapid developmental rates (see Appendix F.2.5), but also impair competitive ability compared to wild counterparts (Fleming *et al*., 2000). Further, mating of Atlantic salmon is controlled by temperature and photoperiod, not size; thus, more rapid growth is not likely to affect timing of reproduction for Atlantic salmon who reproduce during one season each year, but it could affect reproduction of Pacific coho salmon that reproduce during two seasons per year. Therefore, the information that that ABT salmon would reach maturity faster and outcompete wild Atlantic salmon for nesting sites is limited, and it is unclear how domestication and GxE interactions might affect the development of ABT salmon in the natural environment.

Although it is unclear whether ABT salmon would outcompete endangered Atlantic salmon for nesting sites in Maine, these spawning and nursery habitats are considered critically important to the preservation of endangered Atlantic salmon under the ESA. The loss of spawning and nursery habitat has been identified as one of the causes of the decline of native Atlantic salmon populations in the northeastern

US, and therefore, it is clear that any impact to those habitats is expected to affect endangered Atlantic salmon populations. In addition, ABT salmon would increase the number of female fish competing for limited nesting sites, which has the potential to affect breeding behaviors of endangered Atlantic salmon as well as the survival and successful hatching of eggs laid by endangered Atlantic salmon.

Limited breeding sites and an increase in the number of female salmon in a habitat could also result in redd (nesting site) superimposition (McNeil, 1964). Redd superimposition is defined as the creation of a redd on top of a previously established redd (McNeil, 1964). Superimposition of Atlantic salmon redds occurs when spawning is not spatially segregated (OECD, 2017) and there is a high demand for spawning sites due to a large number of female spawners (McNeil, 1964; Hendry *et al*., 2004) or the timing of spawning activities is disparate, which potentially could occur if large numbers of female ABT salmon enter the Maine environment much later in the spawning season. Redd superimposition could result in loss of endangered Atlantic salmon eggs from disturbance of the substrate (e.g., redd) due to digging to create a new redd (i.e., existing eggs could be scattered and crushed) and unsuccessful hatching of eggs in the previously created underlying redd. Hendry *et al*. (2004) found that redds of sockeye salmon (*Oncorhynchus nerka*) that are superimposed lose a substantial fraction of the eggs compared to those that are not superimposed (i.e., 19.3 and 37.6% egg survival in redds superimposed with another redd versus 73.8 and 89.0% egg survive in redds that were not superimposed). Overall, Hendry *et al*. (2004) found that superimposition could impose a substantial fitness cost.

This information suggests that if there was a large escape of female ABT salmon, then this could potentially result competition for limited nesting sites and redd superimposition in the Maine environment due to an increase in female Atlantic salmon. However, the likelihood of this harm occurring is also dependent upon the duration of exposure. As stated earlier, AANB and triploid AAS cannot reproduce and would only be present to compete for habitat for one generation, whereas AAFB and diploid AAS could produce progeny that result in competition for multiple generations. Thus, based on the vulnerability of endangered Atlantic salmon and their need for the limited spawning and nursery habitats in Maine, the likelihood of harm occurring due to competition for habitat is ranked as high for AAFB and diploid AAS, but low to moderate for AANB and triploid AAS.

DFO (2019) also noted a concern for ABT salmon to cause physical alteration of the ecosystem by digging in the stream bed to create nests. Hypothetically, if there is suddenly a greater number of Atlantic salmon building nests in Maine’s rivers, it could cause physical harm to the environment. DFO (2019) concluded that the potential hazard of ABT salmon to habitat due to digging is low because domestic Atlantic salmon and GH transgenic coho salmon have been shown to have a lower digging frequency than wild or hatchery fish. Leggatt *et al*. (2014) reported that female GH transgenic coho salmon performed 30% less digs during spawning events in natural mesocosms than non-transgenic salmon. In addition, female non-transgenic coho salmon reared in nature performed 3.4-fold more digs than GH transgenic salmon when paired with a member of the same group. Reduced digging by female GH transgenic coho salmon has been reported in other studies as well (Bessey *et al*., 2004; Fitzpatrick *et al*., 2011) This information suggests that ABT salmon will not dig more than wild salmon, and in fact, may dig less than wild salmon in the natural environment. As stated in the paragraph above, AANB and triploid AAS would only cause harms for one generation, whereas AAFB and diploid AAS could produce progeny that cause harms for multiple generations. Thus, the likelihood of physical harms in the Maine environment due to AAFB and diploid AAS digging nests is considered to be low. The likelihood of physical harms caused by AANB and triploid AAS digging nests is ranked as negligible because any harms would be for less than one generation.

c. Toxicity of ABT Salmon

ABT salmon and their progeny could hypothetically cause secondary toxicity to non-target predators and scavengers in Maine through consumption of ABT salmon. Information regarding toxicity (food safety) of the ABT salmon to humans and other animals is discussed in FDA's FOI Summary for NADA 141-454 (2015)[12] and is used herein to evaluate potential harms.

As part of the Molecular Characterization of ABT salmon, FDA evaluated whether the ABT salmon contain gene sequences that are likely to pose potential hazards to the target animal, humans or animals consuming food from that animal or the environment. Based on the information and data provided, FDA found that "*there is no risk from any contaminants or other hazardous materials (with the possible exception of the GH present by design) in the EO-1α lineage.*" (NADA 141-454 FOI Summary, 2015). FDA also did not identify any sequences likely to contain hazards to animals consuming food from that animal.

In addition, FDA completed a food safety evaluation to consider whether food derived from AAS is as safe as food from farm-raised Atlantic salmon without the rDNA construct. As part of this evaluation, FDA considered whether there are risks of consumption of ABT salmon by other animals as part of animal feed. Although this evaluation is intended to evaluate the consumption of ABT salmon by domesticated animals in the US, this evaluation can also aid in identifying any potential toxicity that could occur from consumption of ABT salmon by wild non-target animals.

FDA analysis found that AAS are Atlantic salmon (NADA 141-454 FOI Summary, 2015). The evaluation found that there is no risk from consumption of the *opAFP-GHc2* construct as DNA, which is comprised of nucleic acids and is ubiquitous in cells of all living organisms, is generally recognized as safe for human consumption (57 FR 22984, 22990, May 29, 1992). The evaluation also considered the risk associated with direct effects of consumption of the Chinook salmon GH contained in ABT salmon, as well as indirect effects due to the *opAFP-GHc2* construct or its gene product changing the physiology of the fish (e.g., insulin-dependent growth factor 1, IGF1). ABT submitted data of the levels of the Chinook GH and other select hormones in ABT salmon and salmon without the rDNA construct. Published literature were considered as well. Du *et al*. (1992) found that mean plasma growth hormone concentrations were not statistically different between GH transgenic Atlantic salmon that contained the same construct as ABT salmon (i.e., ABT salmon relatives) and comparators without the rDNA construct. Based on ABT's data, mean concentrations of other select hormones (estradiol, testosterone, 11-ketotestosterone, T3 and T4) were no different between the ABT salmon and comparator salmon without the rDNA construct. However, there was a difference in IGF1, but the difference was small. There were no GH concentrations in edible tissues of ABT salmon at the level of quantification for the analytical method. In addition, FDA concluded that levels of analytes (e.g., fat content, protein, vitamins and minerals) in ABT salmon are similar to comparator salmon without the rDNA construct and any differences are within normal biological variability. A summary of FDA's findings is presented in the FOI Summary for NADA 141-454 (2015). Overall, FDA concluded that there were "*no animal feed consumption concerns*."

These evaluations indicate that the likelihood of harms from secondary toxicity in non-target animals that consume ABT salmon (AAFB, AANB and triploid and diploid AAS) in the Maine environment is negligible.

d. Horizontal Gene Transfer

Horizontal gene transfer is the nonsexual transmission of DNA between species, involving close contact between the donor's DNA and the recipient, uptake of DNA by the recipient, and stable incorporation of the DNA into the recipient's genome (NRC, 2002). Horizontal gene transfer is known to occur between prokaryotes, especially microbes; however, it is unclear how often this occurs in eukaryotes, such as plants and animals (NRC, 2002). NRC (2002) states that the risk of horizontal gene transfer is considerably lower than transfer through reproduction, but of high risk of resulting in effects if it were to occur. The harms would be similar to those that occur for gene transfer through reproductive means (see Section 9.3.2.2.a, above).

The rDNA construct (*opAFP-GHc2*) in ABT salmon does not contain any mobile genetic elements, such as viral vectors or transposons, that could increase the potential mobility or uptake of DNA through nonsexual means. The *opAFP-GHc2* construct was found to be integrated into the genome of the fish, and stably maintained at the α-locus over many generations and lineages of ABT salmon (NADA 141-454 FOI Summary, 2015). This information indicates that the *opAFP-GHc2* construct is likely incapable of being transferred through nonsexual means. Thus, the likelihood of harms occurring from horizontal gene transfer is ranked as negligible for all ABT salmon (AAFB, AANB and triploid and diploid AAS).

e. Pathogen/Parasite Transmission

ABT salmon and their production at ABT facilities on PEI could hypothetically harm the US environment by transmitting[108] a pathogen/parasite, whether novel, exotic, or existing, to endangered US Atlantic salmon or other wild fish or animals in the US environment. As described in Section 8.2.2, this could potentially occur via four transmission pathways: 1) direct transmission from escaped ABT salmon to endangered US Atlantic salmon, 2) transmission from escaped ABT salmon to PEI wild Atlantic salmon then to endangered US Atlantic salmon, 3) transmission from wastewater discharged from ABT's PEI facilities, and 4) transmission via shipment of AAS eggs that contain pathogen/parasite to a US facility. ABT salmon could also cause an increase in the spread of a pathogen/parasite in the natural environment by increasing the number of hosts available in the environment as well as by potentially having an altered susceptibility[101] to a pathogen/parasite. This could potentially lead

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[108] Potential pathogen/parasite transmission pathways for ABT salmon and the ABT facilities are presented in Section 9.2.2 above, and the likelihood of these transmission pathways occurring is evaluated in Section 9.2.4 above.

to harms to the US environment due to disease (morbidity), and possibly mortality, in wild fish populations, including endangered US Atlantic salmon.

Exposure to a pathogen/parasite does not necessarily result in infection and onset of disease. For example, a pathogen may exist in tissues of fish, but not result in onset of disease nor cause any symptoms, ultimately resulting in no harm (Grant and Jones, 2010). Grant and Jones (2010) state that the following factors affect pathogen transmission and development of disease:

1. the pathogen (virulency, infectivity, pathogenicity, concentration, and bioavailability),
2. the host (species, age, immunity, stress, density, nutrition, and health status), and
3. the environment (temperature, salinity, water quality, contaminants, currents, and other hosts and carriers).

These factors may act synergistically in the natural environment "*to exert multiple, compounding effects on a pathogen and on the physiology of the host both at the individual and population level*" (Grant and Jones, 2010). As noted above, there are many characteristics of the host (e.g., ABT salmon) that could affect the transmission of a pathogen/parasite, including health status and immunity. Based on an evaluation of general health records, tank records, fish necropsies, and study data, there is no evidence that ABT salmon have any altered resistance or susceptibility to pathogens or parasites. Appendix F.2.5 presents data on disease occurrence and mortality in ABT salmon when exposed to diseases including furunculosis (a disease caused by *Aeromonas salmonicida*) and ISAV. ABT salmon has had no difference in disease occurrence or mortality compared to Atlantic salmon without the rDNA construct. However, there are some reports in the literature of altered resistance to pathogens and impaired immune response in GH transgenic coho salmon (Jhingan *et al*. 2003, and Kim *et al*. 2013). In addition, triploidy has been shown to increase susceptibility to diseases in fish (Chalmers *et al*., 2017), including in GH transgenic fish (Jhingan *et al*., 2003). See also Benfey (2016) who states that available study results "*suggest that triploid Atlantic salmon (i) may be less resistant than diploids to pathogenic diseases and parasites, and (ii) may not react as well to vaccination*." Summaries of studies on triploids are presented in Appendix H.7.

These alterations in susceptibility have not been observed when ABT salmon have been exposed to pathogens in experimental or culture settings. For example, when ABT conducted a challenge study with furunculosis (see Appendix F.2.5), triploid AAS did not show any difference in disease occurrence or response compared to Atlantic salmon without the rDNA construct. Thus, there is uncertainty associated with whether the data from studies with GH transgenic coho salmon and triploid fish are applicable to ABT salmon. As discussed in Section 9.3.2.1.d (above), there is some uncertainty associated with extrapolating data from different fish species (e.g., coho salmon), including those with different GH constructs, to ABT salmon. In addition, the conditions the fish are raised and studied in can affect their physiological and behavior responses (Devlin *et al*., 2015). Also, it is unclear how GxE interactions would affect ABT salmon's immune response to pathogens or parasites in the natural environment especially because ABT's data on susceptibility in ABT salmon was not conducted under varying environmental conditions and with different pathogens/parasites.

However, even just the presence of ABT salmon, whether infected with a pathogen/parasite or not, could cause harm via increased transmission of pathogens/parasites. If ABT salmon infected with a pathogen/parasite were present in the natural environment, it could increase the likelihood of transmission via fish-to-fish contact due to an increased number of infected hosts, as well as increase the geographical range of a pathogen/parasite if the ABT salmon were to migrate or infect commercial aquaculture operations (see Section 8.2.2.3). Furthermore, the presence of ABT salmon alone, even if not infected with a pathogen/parasite, would increase the number of possible hosts susceptible to infection, especially if the ABT salmon are more susceptible to that particular pathogen/parasite. This could potentially increase the transmission of a pathogen/parasite to wild fish populations and increase the risk of disease occurrence.

Alternatively, transmission of a pathogen/parasite to wild fish populations on PEI could occur via the wastewater discharge of the PEI facilities, without escape of any ABT salmon. These PEI wild fish could potentially spread the pathogen/parasite in the natural environmental, eventually causing infection in endangered US Atlantic salmon or other wild fish in the US environment (see Section 8.2.2.3).

The other factors that affect pathogen/parasite transmission and development of disease include the other characteristics of the host (ABT salmon), such as age, stress, and nutrition, as well characteristics of naïve hosts (other wild fish), the pathogen/parasite, and the environment (see list above). However, all of these are unknown and would be situation specific. Without specific information on all of these factors, it is impossible to reliably predict whether ABT salmon or wastewater from the PEI facilities infected with a pathogen/parasite would cause harms, such as disease and mortality, in wild fish populations, including endangered Atlantic salmon. Due to these uncertainties, the likelihood of harms occurring due to pathogen/parasite transmission from ABT salmon (AAFB, AANB and triploid and diploid AAS) in the Maine environment is conservatively ranked as moderate.[109]

9.3.3. Conclusions for Question 4a and 4b

Risk-related Question 4 was revised slightly and expanded to address the Court's remand requirement: "*the FDA must complete the final step of its own risk analysis by addressing the consequences that would result from the engineered salmon successfully establishing a persistent population outside of captivity*."[5] Risk-related Question 4a identified the potential harms of ABT salmon (the hazard), as well as the pathways leading to those outcomes (pathways to harm). The pathways and harms are summarized in Figure 9-2 and Table 9-7 below. Risk-related Question 4b ranked the likelihood of these harms assuming that exposure has occurred, i.e., Question 4b assumes that ABT salmon establish and/or are present in the Maine environment. This directly addresses one of the Court's remand requirements (see Section 2.4 and 9.3, above). The likelihood ranking for the harms (negligible, low, moderate and

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[109] DFO (2019) rated the hazard of ABT salmon to act as a vector of a disease agent as low with a high uncertainty due to an inability to directly apply information from other GH transgenic fish models to ABT salmon, and a limited understanding of significant of altered resistance to vector capabilities. This hazard was not assessed in DFO (2013).

high) are summarized in Table 9-7 below as defined in Table 9-5 (above). This evaluation does not take into account the likelihood of exposure (establishment and/or presence, P(E)) in the US environment, which was found to be negligible in Section 9.2. The comparison of the likelihood of exposure and the likelihood of harms will be evaluated in Section 9.4, Risk Characterization, which will take into account many factors that could not be evaluated in Question 4b, including the magnitude of harm (severity and reversibility) and extent of exposure (including frequency, duration, and magnitude), above.

Most of the likelihood rankings in Table 9-7 were conservatively assigned (i.e., given a higher ranking than they would seem to merit) due to the lack of information on fitness and environmental harms of ABT salmon, and due to the factors outlined in Section 9.3.2.1, above. The two factors that influenced the rankings the most were ecosystem resiliency and uncertainties (e.g., extrapolation of data from other GH transgenic salmon and GxE interactions). Because the Atlantic salmon of the Gulf of Maine DPS and its critical habitat are endangered, any harm to that population or its habitat could result in harms that could lead to extinction. Thus, when concern for ecosystem resiliency was considered, a more conservative ranking was used. For example, the likelihood of harms due to reproduction with wild and feral Atlantic salmon and competition for food and habitat were ranked as high because these resources greatly affect the survival and reproduction of endangered Atlantic salmon; thus, any impact to these resources could affect this vulnerable population. In addition, no studies regarding the potential harms of ABT salmon to the environment were conducted; therefore, only inferences could be made from studies conducted with GH transgenic Atlantic and coho salmon, which can differ from ABT salmon due to their different genotypes and phenotypes. Additionally, all of these studies were conducted under laboratory settings, including naturalized settings, which does not allow assessment of the influence of GxE interactions.

Table 9-7. Summary of Likelihood Rankings for Potential Harms to Occur in the Maine Environment Assuming Exposure (Presence and Establishment) of ABT Salmon Occurs, *P(H|E)*[a]

ID	Potential Pathway to Harm	Likelihood Rankings[b] of AANB and Triploid AAS	Likelihood Rankings[b] of AAFB and Diploid AAS	Potential Harms
a1	Reproduction with Wild and Feral Atlantic salmon	Low to Moderate	High	• disrupt reproduction of wild fish populations, • alterations in type (e.g., specific alleles) or organization (e.g., co-adapted gene complexes) of genetic variation, • alterations in life-history traits, • decreases in population productivity, • reductions in the ability to adapt to changes (i.e., resiliency), and • greater invasiveness of the salmonids with the rDNA construct via introgression of the rDNA construct
a2	Reproduction with Relatives of the Same Genus (e.g., brown trout)	Negligible to Low	Low	• Same as a1
b1	Competition for Food	Moderate	High	• effects on the survival and reproduction of wild fish species, including endangered Atlantic salmon of the Gulf of Maine DPS, • alteration and disruption of the biotic community (e.g., reduction in prey species) and the physical environment (e.g., disturb streambeds)
b2	Competition for Habitat	Low to Moderate (Negligible for habitat destruction)	High (Low for habitat destruction)	• Same as b1

ID	Potential Pathway to Harm	Likelihood Rankings[b] of AANB and Triploid AAS	Likelihood Rankings[b] of AAFB and Diploid AAS	Potential Harms
c	Toxicity of ABT salmon	Negligible	Negligible	• toxicity and/or mortality in predator and scavenger species (e.g., other fish, birds), which if the magnitude of effect was great enough, could result in alterations to the biotic community (e.g., change in numbers and types of species)
d	Horizontal Gene Transfer	Negligible	Negligible	• alterations in type (e.g., specific alleles) or organization (e.g., co-adapted gene complexes) of genetic variation, • alterations in life-history traits, • decreases in population productivity, and • reductions in the ability to adapt to changes
e	Pathogen/Parasite Transmission	Moderate	Moderate	• increased transmission of disease, • increased introgression of rDNA construct, and • outcompete wild fish

[a] It is important to note that the evaluation under Risk-related Question 4 does not account for likelihood of exposure (evaluated in Section 9.2); rather it *assumes* exposure (establishment and/or presence) of ABT salmon occurs in Maine in order to estimate $P(H|E)$. Exposure is taken into account in the Risk Characterization when the likelihood of the potential harms occurring is evaluated: $R = P(E) \times P(H|E)$.

[b] See definition of each likelihood ranking (negligible, low, moderate and high) in Table 9-5.

9.4. Risk Characterization

In this section of the amended EA, new Risk-related Question 5 will be addressed (see Sections 4.4 and 9.1, above). This question will reevaluate under NEPA the risk of significant environmental harms (adverse consequences, effects, or impacts) occurring to the US environment, including endangered Atlantic salmon of the Gulf of Maine DPS, from the production of ABT salmon at the Bay Fortune and Rollo Bay facilities on PEI, Canada. This section also addresses part of the second requirement of the Court with regards to a revised NEPA evaluation – FDA must "*reconsider its 'no effect' determination under the ESA together with its revised NEPA evaluation.*" FDA's revised ESA determination is discussed in Appendix I below.

The November 5, 2020, Court opinion (see Section 2.4, above) addressed the 2015 EA prepared for the original NADA approval concerning AAS (NADA 141-454), which evaluated the risk of significant environmental impacts to the US environment and endangered Atlantic salmon of the Gulf of Maine DPS, from the production of AAS only at the Bay Fortune facility on PEI, Canada. The scope of this amended EA has been expanded to account for existing changes in ABT's production at all PEI facilities that have occurred since the time the 2015 EA was prepared, as well as anticipated future changes/expansion. This amended EA also addresses production at the Rollo Bay facility on PEI, including the Hatchery Unit (approved by FDA in 2019 and evaluated in the 2019 EA) and the future Broodstock Units 1 and 2. Including the Rollo Bay facility in this assessment will also indirectly account for AAS eyed-eggs that will be shipped to the Indiana facility for grow-out (evaluated in the 2018 EA[14]). This amended EA does not evaluate impacts on the US environment from grow-out of AAS at the former Panama facility, which is no longer part of FDA's approval of NADA 141-454, or the Indiana facility, which was evaluated in the 2018 EA and was not addressed in the Court opinion (see Section 3 above). Thus, Risk-related Question 5 will evaluate the risk of significant environmental impacts in the US from all current and reasonably foreseeable future production of ABT salmon on PEI only.

Information from Risk-related Questions 1-4 (Sections 9.2 and 9.3, above) will be used to answer Risk-related Question 5 in Section 9.4.1, below. The potential effects to the US environment due to escape/release during transport will be evaluated in in Section 9.4.2, cumulative impacts will be assessed under Section 9.4.3, an uncertainties analysis will be discussed in Section 9.4.4, and the conclusion on the risk of significant harms occurring is discussed under Section 9.4.5. This information will also be used to make a determination regarding the likelihood of effects to the endangered Atlantic salmon of Gulf of Maine DPS under the ESA in Appendix I, below.

9.4.1. Question 5: What is the Risk that the Potential Harms to, or Effects On, the US Environment Would Occur Given the Likelihood of Exposure Occurring in the US Environment?

Risk (R) is described as the likelihood of harm resulting from an exposure to a hazard (NRC, 2002); in this assessment the hazard is defined as all types of ABT salmon (AAFB, AANB, diploid and triploid AAS) and its interactions with ecosystem components. As presented throughout this amended EA (specifically, Sections 4.2 and 9.1), the following simple model presented in NRC (2002) was used herein as a starting point to conceptually describe risk:

$$\text{Risk (R)} = P(E) \times P(H|E)$$

Where,

$P(E)$ = the probability of exposure, and

$P(H|E)$ = the conditional probability of harm given that exposure has occurred.

Despite the use of this equation, it must be recognized that actually quantifying the environmental risks of GE organisms is a difficult and highly uncertain task because it is not possible to assign actual probability values for the variables therein. Therefore, in this assessment, simple discrete qualitative rankings from negligible to high were used to characterize the likelihood of exposure [$P(E)$] and likelihood of harm assuming exposure has occurred [$P(H|E)$]. Definitions for the rankings were developed *a priori* and are described in Table 9-1 for $P(E)$ and Table 9-5 for $P(H|E)$. The rankings for $P(E)$ and $P(H|E)$ will be used herein to evaluate the potential for risk (probability) of harm occurring. However, risk is not a simple probability, and it must be considered in the context of several other factors that are not accounted for in this risk model, such as the context of exposure (magnitude, frequency, duration, and physical location) and the severity (intensity) of harm. These factors also need to be considered when assessing the risk of significant harms (adverse consequences, effects, or impacts) to occur to the US environment and endangered Atlantic salmon. The importance of these factors was discussed in Section 9.3 above and will be discussed again below when evaluating risk.

9.4.1.1. Summary of Exposure Assessment, *P(E)*

For this assessment, exposure is defined as the establishment and/or presence of ABT salmon or its progeny with the rDNA construct in the US environment. As described in Section 8.2 and illustrated in Figure 8-4 and Figure 8-5 (above), there are 3-6 steps (e.g., escape, survival, dispersal, migration, and establishment) that must happen in order for exposure of ABT salmon to occur in the US environment. The likelihood of each of these individual steps occurring in either the Canadian[110] and/or US environment was evaluated and ranked in Section 9.2 using Risk-related Questions 1 through 3. The overall likelihood of establishment [$P(E_{establishment})$] and presence [$P(E_{presence})$] occurring in the US environment was determined in Section 9.2.3 (see summary in Table 9-4), and is the product of the probabilities of occurrence of each sequential step required for that exposure:

$$P(E_{\text{establishment}}) = P(\text{Step 1}) \times P(\text{Step 2}) \times P(\text{Step 4}) \times P(\text{Step 5}) \times P(\text{Step 6})$$

$$P(E_{\text{establishment}}) = \text{negligible to moderate} \times \text{low to moderate} \times \text{negligible to low} \times \text{low to moderate} \times \text{low to moderate}$$

and,

$$P(E_{\text{presence}}) = P(\text{Step 1}) \times P(\text{Step 2}) \times P(\text{Step 4}) \times P(\text{Step 5})$$

$$P(E_{\text{presence}}) = \text{negligible to low} \times \text{low to moderate} \times \text{negligible to low} \times \text{low to moderate}$$

$P(E_{establishment})$ is only applicable to AAFB and diploid AAS[89] because AANB and triploid AAS cannot establish a reproducing population in the natural environment.[111]

As stated in Section 9.2.3, some of the steps in the exposure pathway are limited by the occurrence of other steps (e.g., reproduction cannot occur without survival, which cannot occur without escape). Thus, theoretically, the overall likelihood of exposure would be determined by the step with the lowest ranked likelihood, i.e., if any of the steps of the pathways are found to be negligible, then the likelihood of that exposure, either presence or establishment, occurring would also be negligible. In this assessment, it was found that escape (Step 1) is a limiting step (the other

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[110] The likelihood of exposure in Canada was only evaluated in this EA as a step in the exposure pathway that may facilitate a complete exposure pathway to the US. The likelihood of harms and consequences occurring in Canada was not evaluated in this EA because NEPA does not require analysis of effects on the environment in foreign sovereign countries, see fn 25.

[111] It is important to note that *P(Step 3)*, likelihood of reproduction and establishment of ABT salmon in PEI prior to migration to the US, was conservatively omitted from the equation above. This step is not required for $P(E_{establishment})$ or $P(E_{presence})$ to occur, rather it is an alternative pathway to exposure in the US; thus, it is not considered herein.

steps cannot occur without escape). Thus, because the likelihood of escape was found to be negligible due to multiple redundant forms of physical and procedural containment and security, the overall likelihood of establishment of AAFB and diploid AAS in Maine was found to be negligible.[75] Likewise, the overall likelihood of the presence of all types of ABT salmon (AAFB, AANB, diploid and triploid AAS) is also negligible. Therefore, it was determined in Section 9.2.3 that it is extremely unlikely or not reasonably foreseeable for any ABT salmon to establish and/or be present in the US environment.

It is also important to emphasize that the rankings for all of the other steps in the exposure pathway occurring after escape were variable ranging from negligible to moderate depending upon the different possible situations the ABT salmon may encounter in the natural environment (see equations above). Regardless, the likelihood of all of these steps occurring sequentially is very small and considered to be extremely unlikely.

Likewise, the overall likelihood of pathogen/parasite transmission from ABT salmon and/or the ABT facilities on PEI is found to be negligible due to a negligible likelihood of introduction into the ABT facility (see Section 9.2.4).

9.4.1.2. Summary of Harms Analysis, *P(H|E)*

For this assessment, harm is defined as an adverse effect to the environment due to the hazard (ABT salmon). In the 2015 EA, and as referenced in the November 2020 Court opinion, the term "consequence" was used when discussing effects or impacts to the US environment. However, in this assessment, the term "harm" was chosen to be consistent with the terminology used in the 2002 NRC report. Herein, "harm" is considered synonymous with adverse "consequence", "effect", or "impact." The evaluation of harms included assessing potential harms that could occur at the population, community and ecosystem level, including gene pool, species, or community perturbation resulting in a negative impact to community stability (NRC, 2002), as well as ecosystem displacements, disruptions, or species extinctions (Devlin *et al.,* 2006). The potential harms due to exposure to ABT salmon in the US environment are identified and discussed in Section 9.3.1 and presented in Figure 9-2. The potential harms could occur through five pathways: (1) reproduction with related taxa (wild and feral Atlantic salmon and wild relatives of the same genus); (2) competition for resources (food and habitat); (3) toxicity of ABT salmon; (4) horizontal gene transfer; and (5) pathogen/parasite transmission.

The likelihood of each harm occurring assuming exposure has occurred [*P(H|E)*] was ranked for each of the five pathways in Section 9.3.2, and the rankings are summarized in Table 9-7. *P(H|E)* was ranked separately for those ABT salmon that can establish a reproducing population in the natural environment (AAFB and diploid AAS) versus those that cannot (AANB and triploid AAS) because their duration of exposure would be different (see Section 9.3.2 for additional discussion). *P(H|E)* assumes exposure has occurred (i.e., ABT salmon establish and/or are present in the US environment) but does not take into account the likelihood of exposure [*P(E)*], the context of exposure (magnitude, frequency, duration) should it occur, or the severity of any harms based on the expected exposure context. However, these factors are considered in the determination of risk in Section 9.4.1.3, below.

The rankings for *P(H|E)* range from negligible to high depending on the specific harm pathway evaluated and the ability of the ABT salmon to reproduce. These rankings were based on available information from ABT (genotype, food safety and disease resistance data) and in the published literature (reproduction and competition data). Several other factors were also considered, including fitness of the ABT salmon, resilience of the Maine ecosystem (specifically endangered Atlantic salmon and their critical habitat), and uncertainties in the available information (e.g., extrapolation of data from different GH transgenic salmon to ABT salmon, GxE interactions, and extrapolation from laboratory settings to the natural environment). There is limited fitness and phenotype data available specifically for ABT salmon; thus, this was only considered when ranking *P(H|E)* due to toxicity of ABT salmon and pathogen/parasite transmission. However, ecosystem resiliency and uncertainties played a major role in conservatively raising (adjusting upward) the likelihood rankings of *P(H|E)* due to reproduction with wild and feral Atlantic salmon and competition for food and habitat. These pathways were ranked as moderate to high for all types of ABT salmon due to the vulnerability of the endangered Atlantic salmon of the Gulf of Maine DPS and its critical habitat, as well as the uncertainty in the applicability and relevance of available data. For example, there were many uncertainties associated with the applicability of the laboratory data due to the conditions they were tested under, including the extrapolation to the natural environment, effects of domestication in hatchery/culture-raised fish, and GxE interactions. Because of this, the rankings for reproduction and resource competition were weighted conservatively as moderate (harms are likely to occur) to high (harms are very likely or will occur if establishment and/or presence of ABT salmon occurs in the US environment). Harms due to pathogen/parasite transmission were also ranked conservatively as moderate due to uncertainties. All other pathways were ranked as negligible (i.e., harm very unlikely to occur) or low (i.e., harm unlikely to occur).

9.4.1.3. Risk Analysis

The simple risk model, R = *P(E)* × *P(H|E)*, presented in NRC (2002) was used as the initial starting point for this risk assessment. Inherent in this model is the concept that both exposure and harm (i.e., adverse effects) are required components of risk, i.e., Risk = Exposure × Effects. Without either component (exposure or effect), there can be no risk. In this assessment, it was found that the likelihood of exposure [*P(E)*] is extremely negligible, i.e., approaching zero. Thus, the overall risk of significant harms (adverse consequences, effects, or impacts) occurring is also close to zero regardless of the value for *P(H|E)*.

To illustrate this visually, an example calculation is provided below. It is important to note that the values for this example were chosen arbitrarily to demonstrate this concept; a true quantification of the risk evaluated in this amended EA could not be definitively calculated. The values chosen for this example are based on a probability scale, which can only range from 0 to 1. The value for *P(E)* was chosen to be very close to zero, i.e., one in a million, to represent the extremely negligible likelihood of exposure concluded in this assessment. For *P(H|E),* the highest possible value of 1 was chosen to represent an upper worst-case conditional probability that is ranked as high, i.e., harm is very likely or will occur assuming exposure has occurred.

$$R = P(E) \times P(H|E)$$

$$R = 1\times10^{-6} \times 1$$

$$R = 1\times10^{-6}$$

Where,

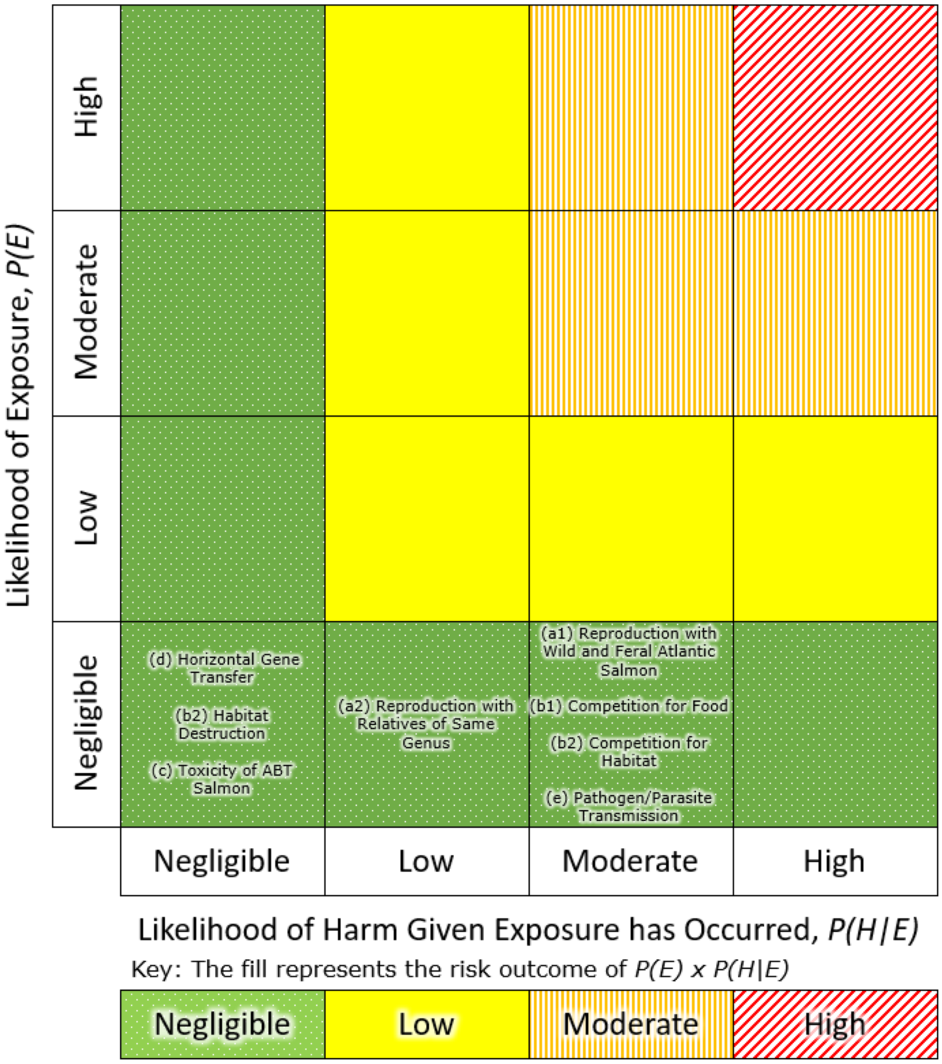
$P(E)$ = the probability of exposure $=1\times10^{-6}$

$P(H|E)$ = the conditional probability of harm given exposure has occurred = 1.0

Based on this equation, the risk (R) is still very close to zero, even when using the highest possible value for the likelihood of harm assuming exposure [*P(H|E)*]. Thus, for this assessment, the likelihood of harms occurring in the US environment due to exposure (establishment and/or presence) of ABT salmon, i.e., risk, is considered to be ***negligible*** because the likelihood of exposure is infinitesimally small or negligible (approaching zero).

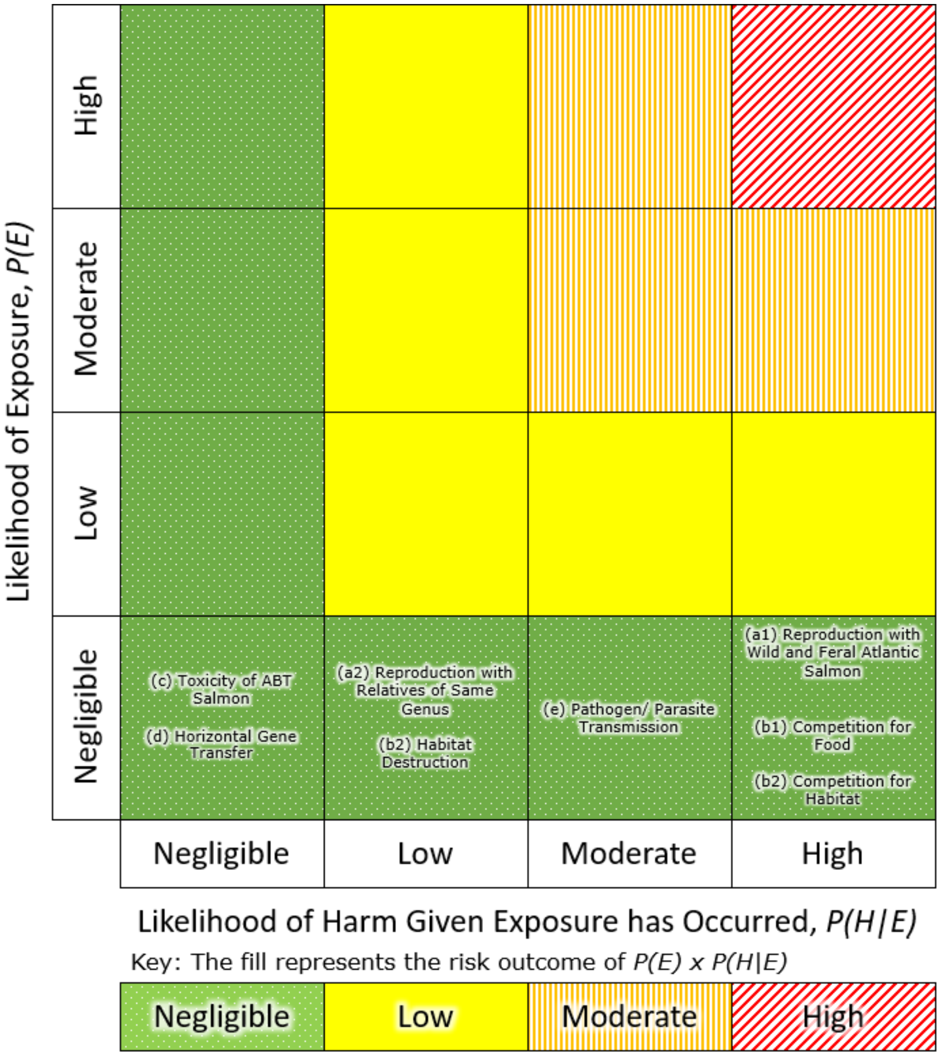
To visualize this analysis, risk matrices were also developed in Figure 9-3 (for AANB and triploid AAS) and Figure 9-4 (for AAFB and diploid AAS), below. The axes represent the two variables in the NRC (2002) risk equation: the likelihood of exposure [*P(E)*] and the likelihood of harm assuming exposure has occurred [*P(H|E)*]. Each of the harm pathways were plotted on the matrix based on their previously determined qualitative probability (likelihood) rankings of *P(E)* (summarized in Table 9-4, Section 9.2.3) and *P(H|E)* (summarized in Table 9-7, Section 9.3.3). The colors in the matrices boxes represent our evaluation of risk (i.e., the likelihood of harm resulting from an exposure to ABT salmon and its interactions with ecosystem components) based on the product of the two likelihood rankings. Green with white dots represents negligible risk, yellow represents low risk, orange with white vertical lines represents moderate risk, and red with white diagonal lines represents high risk of harms occurring. These colors were determined based on scientific knowledge and expert judgment, and considering the information presented in this amended assessment. Risk was found to be negligible for all harm pathways because, as described above, the likelihood of exposure was found to be negligible (i.e., extremely unlikely or not reasonably foreseeable).

Figure 9-3. Final Risk Ranking[a] Illustrating the Risk of Harms Due to AANB and Triploid AAS Occurring Through Different Harm Pathways in the US Environment



[a] The highest *P(H*|E) ranking from Table 9-7 was used in the risk matrix even when there was a range of rankings.

Figure 9-4. Final Risk Ranking Illustrating the Risk of Harms Due to AAFB and Diploid AAS Occurring Through Different Harm Pathways in the US Environment



However, a negligible risk does not equal zero or no risk. The negligible risk determination was based on a negligible likelihood of exposure, which is defined as an extremely unlikely or not reasonably foreseeable occurrence (Table 9-1). Thus, risk of harms occurring in the US cannot be entirely ruled out. This is especially important to consider for those harm pathways that were ranked in Section 9.3.2.2 (see Table 9-7) as having a high likelihood of occurrence in the event of an exposure to ABT salmon (i.e., reproduction and competition for resources for AAFB and diploid AAS). Thus, out of an abundance of caution, additional risk-related analyses have been conducted in Section 9.4.1.4, below, to evaluate the likely severity of impacts from these harm pathways based on a potential hypothetical worst-case exposure.

9.4.1.4. Harm Severity Analysis

a. Approach

Consideration of severity can aid in determining whether significant environmental harms (impacts) could occur due to an exposure, depending on magnitude, frequency, and likelihood of that exposure to occur. In order to evaluate the severity of harm for each of the harm pathways, rankings have been defined in Table 9-8 below using the same discrete qualitative descriptors (i.e., negligible to high) as those used previously for ranking *P(E)* and *P(H|E).* The definitions for the severity rankings take into account the severity of the harm (none, minor or severe), as well as the reversibility of the harms if the exposure were removed.

Table 9-8. Rankings for severity of harm assuming exposure (establishment and/or presence in Maine) were to occur

Ranking	Definition
Negligible	No harms, i.e., no biological response expected beyond natural fluctuations
Low	Minor harms but are expected to be reversible
Moderate	Severe harms but are expected to be reversible or minor harms that are irreversible
High	Irreversible severe harms

The potential severity of the harm is dependent upon the context and intensity of the exposure, including the magnitude (number/density of ABT salmon present), frequency and timing (e.g., season) of escape, and duration of exposure (short- or long-term) keeping in mind that exposure herein has been defined as establishment and/or presence of ABT salmon in the Maine environment. As determined in the Exposure Analysis (Section 9.2.3), due to the redundant multiple-level physical and procedural containment and security systems in place, the likelihood of an escape or unintentional release of ABT salmon is considered negligible. However, if it were to occur, it is expected to be a single, very rare event, such as a release due to a catastrophic natural disaster or malicious event (Section 9.2.3). However, even if such a rare event were to occur, ultimately, it is impossible to accurately predict the level or magnitude (number) of exposure of ABT salmon in the US environment due to the many known and unknown factors that could affect this variable. For example, how many ABT salmon would escape and when/where; how many would survive and migrate to the US; how many would migrate upstream in Maine and encounter sexually mature endangered Atlantic salmon with which to spawn; how many would survive in the US and reproduce; whether introgression of the rDNA construct would occur (see Table 9-6 above).

Because the actual extent of any exposure is unknown and cannot be reliably or accurately predicted, the severity of harm (i.e., severity of impact) for each pathway was ranked conservatively assuming a possible worst-case exposure scenario of a one-time release of a large number of ABT salmon, and with the assumption that a large population establishes and/or is present in the Maine environment. This is considered a worst-case exposure scenario. This worst-case exposure scenario is also applicable to the potential for ABT salmon or the ABT facilities to transmit a pathogen/parasite. It is assumed herein that a worst-case exposure scenario for transmission of a pathogen/parasite would be that a large number of infected ABT salmon escape the ABT facilities.

For this exercise, it is expected that if there is negligible exposure (abundance and/or duration approach zero) or transmission of a pathogen/parasite, which is the outcome determined previously for all harm pathways, then the resulting severity of harm would also be negligible. Thus, negligible severity is the lower limit for all pathways to harm. However, if an actual exposure did occur, the upper limit of severity would be dependent upon the many other factors that affect that pathway, including reproductive success of each type of ABT salmon. For this additional analysis, the upper limit of severity rankings was determined for each pathway to harm considering the possible worst-case exposure scenario described above (one-time release of a large number of ABT salmon and assuming a large population establishes and/or is present in the Maine environment). The severity of harm based on this possible worst-case exposure scenario is presented below.

b. Severity Rankings

As stated above, the severity rankings discussed below are based on a possible worst-case exposure scenario (one-time release of a large number of ABT salmon and assuming a large population establishes and/or is present in the Maine environment) and the reproductive capability of the ABT salmon. AANB and triploid AAS cannot establish a reproducing population in the natural environment; therefore, if they survived, their harms would be limited to one generation (short-term exposure). In contrast, if AAFB and diploid AAS reproduce and establish in the natural environment, their harms could occur for multiple generations (long-term exposure). The duration of the exposure influences the severity of the harms. Thus, these different types of ABT salmon are ranked separately below.

The rankings for severity of harm were determined based on scientific knowledge and expert judgment taking into consideration the information previously discussed in Question 4b (Section 9.3.2, above). The severity rankings for a worst-case exposure are summarized in Table 9-9 for all potential harm pathways.

As stated in Section 9.4.1.3 above, exposure is negligible for all types of ABT salmon (AAFB, AANB, and diploid and triploid AAS); therefore, the risk is also negligible for all potential harms. Because the risk (probability of harm) is negligible for these harms under a worst-case exposure scenario, the likely impact of harms will also be ***negligible***, regardless of their possible severity. Thus, significant harms (impacts) are not expected to occur in the US environment due to the production of ABT salmon at the facilities on PEI, Canada.

Table 9-9. Summary of Rankings for Severity of Harm for a Possible Worst-Case Exposure Scenario[a] for Each Potential Harm Pathway

Potential Harm Pathway		Severity Ranking for AANB and Triploid AAS	Severity Ranking for AAFB and Diploid AAS
a1	Reproduction with Wild and Feral Atlantic salmon	Low	High
a2	Reproduction with Relatives of the Same Genus (e.g., brown trout)	Negligible	Negligible
b1	Competition for Food	Moderate	High
b2	Competition for Habitat	Low	Moderate
c	Toxicity of ABT salmon	Negligible	Negligible

Potential Harm Pathway		Severity Ranking for AANB and Triploid AAS	Severity Ranking for AAFB and Diploid AAS
d	Horizontal Gene Transfer	Negligible	Negligible
e	Pathogen/Parasite Transmission[b]	Moderate	Moderate

[a] For the purposes of this assessment, a worst-case exposure scenario is defined as a one-time release of a large number of ABT salmon and assuming a large population establishes and/or is present in the Maine environment.

[b] It is also assumed that a large number of ABT salmon are infected with a pathogen/parasite and escape.

9.4.2. Effects on the US Environment Due to Escape/Release During Transportation to the US

As discussed above in Section 9.2.1.3, the likelihood of escape or release of AAS eyed-eggs during transportation from PEI to any US grow-out facilities is considered negligible and not reasonably foreseeable. Any release of eggs during shipment would be the result of an accidental release due to a major incident or accident during transport. Due to the fragile nature of salmonid eggs prior to hatching and the very low probability of the eggs ending up in a suitable habitat for survival (i.e., cold freshwater with sufficient dissolved oxygen), survival of the eggs through and after a significant shipping incident, such as a trucking accident or plane crash, is a highly remote scenario. As a result, no adverse effects on the US environment are anticipated due to transportation of AAS eyed-eggs.

9.4.3. Cumulative Impacts

The 1978 CEQ regulations define cumulative impact as "*the impact on the environment which results from the incremental impact of the present action when added to other past, present and reasonably foreseeable future actions...*" (40 CFR 1508.7). The scope of this amended EA accounts for all past, present and anticipated future changes/expansions at ABT's facilities on PEI, including the Bay Fortune facility and the Hatchery Unit and future Broodstock Units 1 and 2 at the Rollo Bay facility. This amended EA also indirectly accounts for AAS eyed-eggs that will be shipped from the Rollo Bay facility on PEI to the Indiana facility for grow-out. Thus, FDA has accounted for all potential reasonably foreseeable cumulative impacts in this EA.

This EA pertains to current production and use conditions for AAS (including all ABT salmon), as well as future expansions, at the Bay Fortune facility and the Hatchery and Broodstock 1 and 2 Units at the Rollo Bay facility. This EA assumes these facilities are operating at maximum capacity for all size ranges of fish that could be held at any one-time (see Section 6.4.2). Should the sponsor at a later time seek to open, or ship to, any additional egg production or grow-out facilities, or to significantly expand existing facilities, a supplemental NADA would need to be submitted, reviewed, and approved prior to using, or shipping to, such a facility, including expansion to include Broodstock Units 1 and 2 at the Rollo Bay facility. Action by FDA on such an application would be considered a major federal action under NEPA and FDA regulations, and, as such, would require the preparation of an EA and potentially an EIS, both of which would consider the cumulative impact of the addition of another facility or other proposed changes. Such a supplemental application would also require FDA to reassess any potential effects on endangered species and potentially to consult with NMFS and FWS if a "may effect" determination is made. The agency does not speculate about any future business expansion by the sponsor because any such speculation would be hypothetical, and the agency would have no particular conditions to evaluate. If such an expansion is proposed at a later time, FDA will have the obligation to consider the concrete specifics of the supplemental application at that time.

In 2021, ABT announced that it plans to build a new facility to grow-out AAS in Pioneer, Ohio, US. This assessment accounts for the eyed-eggs that will be supplied from the Rollo Bay facility to the Indiana facility and this new facility. If approval is sought for that facility through a supplemental NADA, the environmental impacts of that action will be evaluated in an EA that will also consider the cumulative impacts of the addition of that facility.

9.4.4. Uncertainties Analysis

An evaluation of the uncertainties (unknowns) is recommended when conducting a risk assessment of GE animals to ascertain limits of the assessment (NRC, 2002; NRC, 2004a; Kapuscinski, 2005; Hayes *et al*., 2007). Uncertainties in the available information and unknowns due to lack of information were discussed when applicable throughout this assessment and were taken into consideration when ranking the likelihood of exposure [*P(E)*], likelihood of harm assuming exposure occurs [*P(H|E)*], and the severity of harm. When there was a high amount of uncertainty or unknowns, the rankings for these variables were conservatively weighted higher (i.e., increased the ranking, and therefore, increased the risk outcome). The uncertainty with each of these variables is summarized below.

The uncertainty associated with the likelihood of escape/release and resulting likelihood of exposure [*P(E)*] is considered to be negligible due to multiple, redundant forms of physical, biological and procedural containment and security (see Section 9.2.1, Question 1), which are comprehensively documented in SOPs and has been verified during reviews and inspections by FDA and continues to be verified during routine inspections by Provincial and Canadian authorities. In contrast, the uncertainty associated with the likelihood of harms assuming exposure as occurred [*P(H|E)*] and severity of harm is considered to be high due to the uncertainties and unknowns outlined in Section 9.3.2.1 (e.g., laboratory to field extrapolation, GxE interactions, species and rDNA construct comparisons), as well as a lack of knowledge on what would happen if ABT salmon were introduced into the natural environment, which cannot be evaluated due to the risk of escape if such studies were undertaken.

Because the uncertainty in determining the likelihood of exposure is negligible (infinitesimally small or approaching zero), and based on this finding, the risk was also determined to be negligible, there is **high certainty** in the outcome of this assessment (i.e., negligible likelihood of significant harm).

9.4.5. Significance Factors

Under the NEPA implementing regulations published by the Council on Environmental Quality (CEQ) on November 29, 1978 (and until the revised regulations were published in September 2020), 40 CFR 1508.27 stated that two main points should be considered when determining significance: context and intensity. Context (40 CFR 1508.27(a)) relates to analyzing the significance of an action in different contexts "such as society as a whole (human, national), the affected region, the affected interests, and the locality." Context was analyzed herein under Section 9, which the identified the regions (waters of Maine) and interests in the US (endangered Atlantic salmon and their habitat) that may be affected by the production of ABT salmon on PEI, determined the potential for exposure, and evaluated potential short and long-term impacts to endangered Atlantic salmon and their critical habitat assuming that ABT salmon establish and/or are present in Maine waters. With regards to intensity, ten factors were listed in the 1978 regulations that agencies should consider. While the current implementing regulations (40 CFR 1501.3(b)) reduced the number of enumerated factors, the underlying considerations remain generally the same. Because this amended EA relates to the action taken in 2015, and the regulations in place at that time included a greater number of specific factors to consider in assessing significance, as set forth below, we determined it was appropriate to analyze the factors that were in the regulations in 2015.

1. **Impacts that may be both beneficial and adverse:** Under Section 9.4 of this assessment, we determined that the likelihood of exposure [*P(E)*] of ABT salmon in the US environment is extremely negligible (approaching zero); thus, the overall risk of significant harms (adverse consequences, effects, or impacts) occurring is also close to zero regardless of the value for *P(H|E)*. We also conducted additional analysis to evaluate the likely severity of impacts from these harm pathways based on a potential hypothetical worst-case exposure scenario and concluded that because the risk (probability of adverse harm) is negligible for the potential harms under a worst-case exposure scenario, the likely impact of harms will also be negligible, regardless of their possible severity. Among the potential benefits of this action identified by AquaBounty are help in alleviating food scarcity, use of fewer resources to grow salmon to conventional size, and lower carbon footprint associated with farming salmon closer to consumers. *See* www.AquaBounty.com.

2. **The degree to which the proposed action affects public health or safety:** There were no effects to public health or safety found during the review of these actions under the FD&C Act as discussed herein and in the 2015 FOI Summary.[12]

3. **Unique characteristics of the geographical area:** In Sections 7 and 8 of this assessment, we considered at length the unique geographic features of the locations of the facilities on PEI, as well as the US environments that could potentially be exposed. In addition, in Section 9, we considered the potential impacts that could occur in the US environment assuming exposure to ABT salmon occurred (establishment and/or presence). We determined that the likelihood of exposure [*P(E)*] of ABT salmon in the US environment is extremely negligible (approaching zero); thus, the overall risk of significant harms occurring to the US environment, including the unique characteristics of Maine, is also close to zero regardless of the value for *P(H|E)*. We also determined that there is high certainty in the outcome of this assessment (i.e., negligible likelihood of significant harm).

4. **The degree to which the effects on the quality of the human environment are likely to be highly controversial**: Controversy "sufficient to require preparation of an EIS occurs 'when substantial questions are raised as to whether a project . . . may cause significant degradation of

some human environmental factor, or there is a substantial dispute [about] the size, nature, or effect of the major Federal action.'" *Pub. Citizen v. Dep't of Transp*., 316 F.3d 1002, 1027 (9th Cir. 2003), *rev'd on other grounds*, 541 U.S. 752 (2004). While controversy relating to the uncertainty of an environmental effect may be appropriate to consider when deciding whether to prepare an EIS, public controversy relating to policy disagreements over development of GE food animals in and of itself does not weigh in favor of an EIS. *See* NEPA Implementing Regulations Provisions Phase 2 Proposal, 88 Fed. Reg. 49924, 49936 (July 31, 2023). For analysis of the uncertainty of effects on the human environment, see discussion under Factor 5.

5. **The degree to which the possible effects on the human environment are highly uncertain or involve unique or unknown risks:** This amended EA analyzes the facilities and containment measures on PEI; the potential exposure pathways; the likelihood of and nature of potential harms that could result from escape and establishment of a population; and the consequences that could result from the establishment of a persistent population outside of captivity. We determined that the likelihood of exposure is negligible (infinitesimally small or approaching zero) and, based on this finding, we also determined the risk to be negligible, with high certainty in the outcome of this assessment (*i.e*., negligible likelihood of significant harm). *See* Sections 7, 8, and 9.1-9.4.4, above.

6. **The degree to which the action may establish a precedent for future actions with significant effects:** We do not consider this action to be precedent-setting. FDA has previously approved drugs for intentional genomic alteration in animals and future approvals of similar applications would require environmental assessments specific to the action at issue, including the animal and drug involved and the location of any facilities. Additional ABT facilities in particular would require a supplemental approval with an associated NEPA review specific to its particular location. We recognize, however, that future analyses of proposed ABT facilities may rely in part on this assessment. For that reason, we have conducted this environmental assessment based on the planned maximum, rather than current, operating capacity at the PEI facilities. *See* Sections 6.4.2, 7.

7. **Whether the action is related to other actions with individually insignificant but cumulatively significant impacts:** This assessment considered all current operations at all PEI facilities (Bay Fortune and Rollo Bay facilities), as well as planned future expansions and changes at the facilities on PEI (see Section 7 above).

8. **The degree to which the action may adversely affect resources listed in or eligible for listing in the National Register of Historic Places:** These actions are not expected to adversely affect resources listed in or eligible for listing in the National Register of Historic Places.

9. **The degree to which the action may adversely affect an endangered or threatened species or its habitat**: The potential for this action to affect an ESA-listed species or its habitat was evaluated under Appendix I below, and FDA concluded that these actions are not likely to adversely affect a listed

species or its critical habitat because the effects are discountable, i.e., extremely unlikely to occur.

10. **Whether the action threatens a violation of Federal, State, or local law or requirements imposed for the protection of the environment:** These actions do not violate or threaten a violation of any laws imposed for the protection of the US environment.

9.4.6. Conclusions for Risk-related Question 5

The risk or probability of significant harms (adverse consequences, effects, or impacts) occurring in the US environment due to the production of ABT salmon at the Bay Fortune facility and the Hatchery Unit and Broodstock Units 1 and 2 at the Rollo Bay facility is found to be negligible due to a negligible likelihood of exposure (Figure 9-3 and Figure 9-4). However, negligible risk does not mean zero risk. Because there is a high likelihood of harm assuming exposure to ABT salmon for some of the harm pathways (reproduction and competition), additional risk analyses were conducted to consider severity of harms. It was concluded that the likely impact of harms will also be negligible, regardless of their possible severity, because the risk (probability of harm) is negligible for these harms. Therefore, significant environmental impacts are not expected in the US environment from maximum current and planned production of ABT salmon at the Bay Fortune or Rollo Bay (Hatchery Unit and Broodstock Units 1 and 2) facilities. Furthermore, based on an evaluation of the uncertainties associated with the exposure and harms analysis, it was found that there is high certainty in the outcome of this assessment. Because this amended EA relates to an action taken in 2015, we made our NEPA determination based on the NEPA implementing regulations in place in 2015.

The final part of the Court's November 2020 decision required FDA to "reconsider its 'no effect' determination under the ESA together with its revised NEPA evaluation." FDA's reconsideration and "not likely to affect" determination, using the information from Risk-related Questions 1-5 in Section 9 above, is set forth in Appendix I.

10. PERSONS AND AGENCIES CONSULTED

This amended EA is the culmination of many individual steps that have either been generated, prepared, or peer-reviewed under the direction or request of the Center for Veterinary Medicine at FDA.

The information and analyses in this amended EA also reflect comments and input received from the NMFS and the FWS during an ESA technical assistance review initiated in June 2022 with initial discussions beginning in March 2021.

In addition, this amended EA takes into account oral comments received from the public during an FDA public meeting held on December 15, 2022, and written comments submitted to a public docket during a public comment period from November 16, 2022, to January 17, 2023 (see Appendix A for history). Materials presented at the December 15, 2022, public meeting, a recording of the meeting, as well as a transcript of the meeting are available on FDA's website.[112] Public comments can be viewed on regulations.gov under Docket No. FDA-2022-N-2672.[113]

11. PREPARATION OF AMENDED EA

This amended EA has been prepared by the Center for Veterinary Medicine at FDA, and includes changes made in response to technical assistance from the Services.

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[112] https://www.fda.gov/animal-veterinary/workshops-conferences-meetings/virtual-public-meeting-aquadvantage-salmon-draft-amended-environmental-assessment-12152022#Transcript (accessed December 8, 2023)

[113] https://www.regulations.gov/docket/FDA-2022-N-2672 (accessed December 8, 2023)

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Appendix A. Significant Steps During the Preparation of the EAs for the 2015 NADA Approval, 2018 and 2019 NADA Approvals, and Current Court Remanded Revisions. This list was first published in Section 8 (page 121) of the 2015 EA.

2015 EA: Bay Fortune and Panama Facilities

- In 1995, the sponsor requests an investigational exemption for AAS under 21 CFR Part 511.
- FDA issues an EA and FONSI for the investigational phase of the AAS NADA in 2001.
- Pivotal studies in support of an eventual NADA, including studies that support this EA, begin in 2001 once the sponsor establishes genetic stability of AAS over four generations.
- FDA conducts an inspection of the PEI, Canada, broodstock facility in October 2008. Participants include subject matter experts from CVM as well as an inspector from FDA’s Office of Regulatory Affairs.
- CVM issues Draft Guidance for Industry 187 for public comment in 2008. The guidance clarifies FDA’s continuing authority to regulate GE animals and details the overall process for review of data submitted in support of an eventual NADA with CVM; the Guidance is issued in final form in early 2009.
- CVM experts in aquaculture, biotechnology, and environmental risk assessment conduct a site visit to the Panamanian grow-out facility in November 2009, accompanied by a fisheries expert from the NMFS to provide additional expertise and consultation.
- On September 19-20, 2010, FDA’s Veterinary Medicine Advisory Committee (VMAC) held a meeting to address science-based issues associated with the material submitted by the sponsor in support of the NADA for AAS. CVM presented information on animal health, food safety, environmental concerns, and data supporting the safety and effectiveness of AAS. Both days of the VMAC meeting were open to the public. Interested members of the public were invited to present data, information, or views to the committee, orally or in writing. Materials presented at the meeting as well as the VMAC Chair’s final report are available on FDA’s website.[106] It is important to note that the final 2015 EA differed from that released for the VMAC meeting because it took into account numerous comments submitted by the public.
- In October 2010, FDA sends FWS and NMFS letters stating that FDA has made a “no effect” determination under the ESA. FDA clarifies the proposed conditions of use (PEI and Panama) and reaffirms that any additional facilities would require a supplemental application, a new environmental analysis, and a new ESA determination.
- In December 2010, FWS issues a concurrence letter to FDA regarding FDA’s “no effects” determination with regard to AAS and populations of endangered Atlantic salmon. A copy of the FWS letter is provided in Appendix D of the 2015 EA.
- In April 2011, FDA hosts an Intergovernmental Workshop on FDA’s review of AAS with authorities from the United States, Canada and Panama in attendance. In addition to staff from FDA, representatives of several other US Federal agencies, including the NMFS, FWS, and the USDA were present at this workshop.
- In July 2011, NMFS issued a letter to FDA responding to FDA’s “no effects” determination with regard to AAS and populations of endangered Atlantic salmon. A copy of the NMFS letter is provided in Appendix D of the 2015 EA.

- On December 26, 2012, FDA published notice of the release of a draft EA and the accompanying preliminary FONSI in the Federal Register. The comment period closed on April 26, 2013. In accordance with 21 CFR 25.51, after reviewing and considering the public comments, FDA revised the draft EA and responded to the public comments.[114]
- In November 2013, the Canadian government issued a Significant New Activity (SNAc) for AAS based on risk assessments (including a qualitative Failure Mode Analysis) conducted by DFO that concluded that these salmon were not "CEPA Toxic" (see Section 2.3 of this EA).
- On November 19, 2015, FDA approved NADA 141-454 allowing for the commercial production of eyed-eggs for AAS at the sponsor's facility located near Bay Fortune on PEI (known as the Bay Fortune facility), and the grow-out of AAS at the sponsor's facility in Panama. At the time of the NADA approval, FDA issued the final EA and final FONSI.

2018 EA: Indiana Facility

- In June 2017, ABT publicly announces its plans to acquire and renovate a land-based aquaculture facility in Albany, Indiana.
- FDA conducted an inspection of the Albany, Indiana facility in February 2018. Participants included subject matter experts from CVM as well as an inspector from FDA's Office of Regulatory Affairs.
- On April 25, 2018, FDA approved a supplement to NADA 141-454 allowing for grow-out of AAS at ABT's land-based, freshwater aquaculture facility near Albany, Indiana, US. FDA issued the final EA and final FONSI at that time.

2019 EA: Hatchery Unit at Rollo Bay Facility

- In November 2017, ABT publicly announces its plans to build a new production facility in Rollo Bay, PEI.
- In March 2019, the Canadian government issued a New Substance Notification (NSN) to allow ABT to produce AAS at a new facility near Rollo Bay, PEI. The review of an NSN includes environmental and indirect human health risk assessments. Human and environmental exposure is expected to be low, and therefore, AAS was deemed "not toxic" under CEPA.[115]
- FDA conducted an inspection of the new Hatchery Unit at the Rollo Bay facility on PEI in June 2019. While there, FDA also conducted a follow-up inspection on the Bay Fortune facility to ensure no unapproved changes had been made to the containment and procedures at the facility. Participants included subject matter experts from CVM as well as an inspector from FDA's Office of Regulatory Affairs. We verified the facilities were in compliance with the NADA application and applicable FDA regulations, and no Form FDA 483 was issued at the conclusion of these inspections.

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[114] FDA's response to public comments: https://www.fda.gov/animal-veterinary/animals-intentional-genomic-alterations/aquadvantage-salmon-response-public-comments-environmental-assessment (accessed December 8, 2023).

[115] https://www.canada.ca/content/dam/eccc/documents/pdf/pded/new-substances-organisms/Aquadvantage-salmon-summary.pdf (accessed December 8, 2023).

- On November 5, 2019, FDA approved a supplement to NADA 141-454 to allow production of AAS in a Hatchery Unit at ABT's second land-based, freshwater aquaculture facility located near Rollo Bay, PEI, Canada (known as the Rollo Bay facility). At that time, FDA issued the final EA and final FONSI. In addition, under this approval action, ABT closed the Panama facility.

Current Amended EA:

- In March 2016, a number of organizations filed suit in the US District Court, Northern District of California, challenging FDA's jurisdiction to regulate AAS under the FD&C Act, as well as FDA's NEPA and ESA determinations.
- On December 19, 2019, the US District Court issued the first of two decisions in the suit, stating: "*…the FDA's assertion of authority is valid. Under the plain language of the Food, Drug, and Cosmetic Act, the FDA has the authority to require companies to seek its approval before creating and breeding genetically engineered animals. Perhaps the genetic material used to modify an animal does not seem like a "drug" in the colloquial sense, but it is the statutory definition that matters. The statutory definition of "drug" is far broader than the ordinary meaning of that word, and the modification of an animal's genetic makeup falls squarely within the statutory definition*." *Inst. for Fisheries Res. v. United States Food and Drug Adm'n*, 499 F. Supp. 3d 657, 660 (N.D. Cal. 2020).
- On November 5, 2020, the US District Court issued another decision in the suit stating: "*The FDA did not…meaningfully analyze what might happen to normal salmon in the event the engineered salmon did survive and establish themselves in the wild. Even if this scenario was unlikely, the FDA was still required to assess the consequences of it coming to pass. This is especially true because the FDA knew that the company's salmon operations would likely grow, with additional facilities being used for farming. Obviously, as the company's operations grow, so too does the risk of engineered salmon escaping. Thus, it was particularly important at the outset for the agency to conduct a complete assessment of the risks posed by the company's genetic engineering project, including an assessment of the consequences for normal salmon if the engineered salmon established themselves in the wild." The Court ordered FDA "to go back and complete the analysis*", and to reconsider its "no effect" determination under the ESA together with a revised NEPA evaluation. *Inst. for Fisheries Res. v. United States Food and Drug Adm'n*, 499 F. Supp. 3d 657, 660 (N.D. Cal. 2020). However, the Court declined plantiff's motion to vacate the approval.
- FWS and NMFS (collaboratively referred to as the Services) Technical Assistance: FDA initiated discussions with the Services in March 2021. Between June and October 2022, FWS and NMFS reviewed and commented on the draft amended EA as part of the technical assistance process under Section 7 of ESA. Those comments were incorporated by FDA into the draft EA released for public comment.
- A draft of the Amended EA, titled "Draft Amended Environmental Assessment for Production of AquAdvantage Salmon at the Bay Fortune and Rollo Bay Facilities on Prince Edward Island, Canada," was released for public comment following publication on November 17, 2022, of a Notice of Availability in the Federal

Register that also established a docket for public comment.[116] The public had an opportunity to submit comments on the Draft Amended EA to this docket. The Draft Amended EA was made available on FDA's website for viewing.[117] The comment period closed on January 17, 2023, and comments can be viewed on regulations.gov under FDA-2022-N-2672.[113]

- A virtual public meeting, titled "Public Meeting on Draft Amended Environmental Assessment for Production of AquAdvantage Salmon at the Bay Fortune and Rollo Bay Facilities on Prince Edward Island, Canada" was held on December 15, 2022, and was announced in the Federal Register on November 17, 2022.[118] Interested members of the public were invited to present data, information, or views orally. Materials presented at the meeting, a recording of the meeting, as well as a transcript of the meeting are available on FDA's website.[112]
- FDA conducted follow-up inspections of the Bay Fortune and Rollo Bay Hatchery facilities in June 2023 to evaluate the physical and biological containment, procedures, and physical- and bio-security at the facilities. Participants included subject matter experts from CVM as well as an inspector from FDA's Office of Regulatory Affairs. We verified the facilities were in compliance with the NADA application and applicable FDA regulations, and no Form FDA 483 was issued at the conclusion of these inspections.
- In June 2023, a representative of NMFS conducted a site visit with FDA to observe the PEI operations, including physical containment, security, and biosecurity, at the Bay Fortune facility and the Rollo Bay facility (both the Hatchery Unit and Broodstock Unit 1).
- FDA conducted an inspection of the Albany, Indiana facility in August 2023 to evaluate the physical containment, procedures, and physical- and bio-security at the facility. Participants included subject matter experts from CVM as well as an inspector from FDA's Office of Regulatory Affairs.
- FDA revised the draft amended EA based on public comments and the June and August 2023 inspections. On January 5, 2024, FDA concluded that the production of AAS and AquAdvantage broodstock at the Bay Fortune facility and the Rollo Bay facility, including planned changes to the Bay Fortune facility and expansion at the Rollo Bay facility, "may affect, but is not likely to adversely affect endangered Gulf of Maine Atlantic salmon or their critical habitat." On January 8, 2024, FDA submitted a request to NMFS to initiate an informal consultation under Section 7 of the ESA. FDA also initiated informal consultation with the FWS due to joint jurisdiction over endangered Atlantic salmon of the Gulf of Maine DPS; however, FWS chose to defer the decision to NMFS. FDA provided the revised amended EA and supporting references to NMFS. NMFS initiated the informal consultation on January 21, 2024. On April 22, 2024, NMFS provided an ESA Letter of Concurrence (LOC) with FDA's assessment that the action may affect but is not likely to adversely affect endangered Gulf of Maine Atlantic salmon or their critical habitat. Following receipt of the LOC, FDA notified NMFS of several errors in the LOC and requested a corrected letter. NMFS provided a corrected

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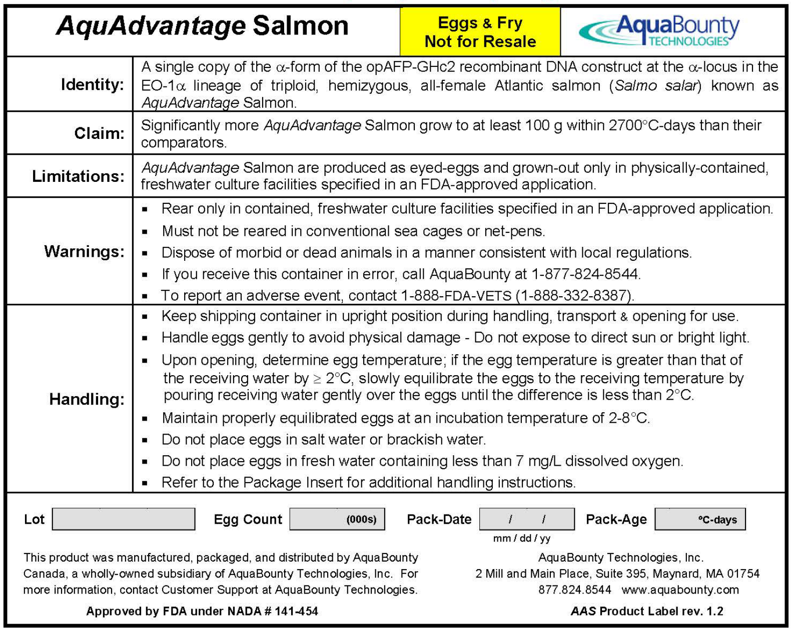
[116] https://www.federalregister.gov/documents/2022/11/17/2022-25001/draft-amended-environmental-assessment-for-production-of-aquadvantage-salmon-at-the-bay-fortune-and; accessed June 21, 2024
[117] https://www.fda.gov/media/163153/download?attachment; accessed June 21, 2024
[118] https://www.federalregister.gov/documents/2022/11/17/2022-25002/draft-amended-environmental-assessment-for-production-of-aquadvantage-salmon-at-the-bay-fortune-and; accessed June 21, 2024

LOC dated April 24, 2024. FDA notified NMFS of new errors in the corrected LOC. In response, NMFS requested additional information regarding the water usage and discharge volumes from the PEI facilities and ABT’s procedures for responding to an infectious disease emergency at the PEI facilities. On June 3, 2024, NMFS provided FDA the following response: “*In reviewing the responses to questions and additional information provided by the FDA regarding pathogens and water discharge, NMFS has concluded that additional revisions to the letter of concurrence are not needed. The additional water discharge load information does not yield any changes to our analysis of the anticipated effects of the action on ESA-listed species or designated critical habitat, or the conclusions of the letter of concurrence.*” Thus, the April 24, 2024, corrected LOC is the final concurrence from the Services. The corrected LOC is included in Appendix I.

Appendix B. AAS Label



Please read label completely before opening container

***AquAdvantage* Salmon**	**Eggs & Fry Not for Resale**
Identity:	A single copy of the α-form of the opAFP-GHc2 recombinant DNA construct at the α-locus in the EO-1α lineage of triploid, hemizygous, all-female Atlantic salmon (*Salmo salar*) known as *AquAdvantage* Salmon.
Claim:	Significantly more *AquAdvantage* Salmon grow to at least 100 g within 2700°C-days than their comparators.
Limitations:	*AquAdvantage* Salmon are produced as eyed-eggs and grown-out only in physically-contained, freshwater culture facilities specified in an FDA-approved application.
Warnings:	▪ Rear only in contained, freshwater culture facilities specified in an FDA-approved application. ▪ Must not be reared in conventional sea cages or net-pens. ▪ Dispose of morbid or dead animals in a manner consistent with local regulations. ▪ If you receive this container in error, call AquaBounty at 1-877-824-8544. ▪ To report an adverse event, contact 1-888-FDA-VETS (1-888-332-8387).
Handling:	▪ Keep shipping container in upright position during handling, transport & opening for use. ▪ Handle eggs gently to avoid physical damage - Do not expose to direct sun or bright light. ▪ Upon opening, determine egg temperature; if the egg temperature is greater than that of the receiving water by ≥ 2°C, slowly equilibrate the eggs to the receiving temperature by pouring receiving water gently over the eggs until the difference is less than 2°C. ▪ Maintain properly equilibrated eggs at an incubation temperature of 2-8°C. ▪ Do not place eggs in salt water or brackish water. ▪ Do not place eggs in fresh water containing less than 7 mg/L dissolved oxygen. ▪ Refer to the Package Insert for additional handling instructions.

Lot ☐ **Egg Count** (000s) **Pack-Date** / / (mm / dd / yy) **Pack-Age** °C-days

This product was manufactured, packaged, and distributed by AquaBounty Canada, a wholly-owned subsidiary of AquaBounty Technologies, Inc. For more information, contact Customer Support at AquaBounty Technologies.

AquaBounty Technologies, Inc.
2 Mill and Main Place, Suite 395, Maynard, MA 01754
877.824.8544 www.aquabounty.com

Approved by FDA under NADA # 141-454

***AAS* Product Label rev. 1.2**

Display a copy of this label on each egg-incubation unit & rearing tank used

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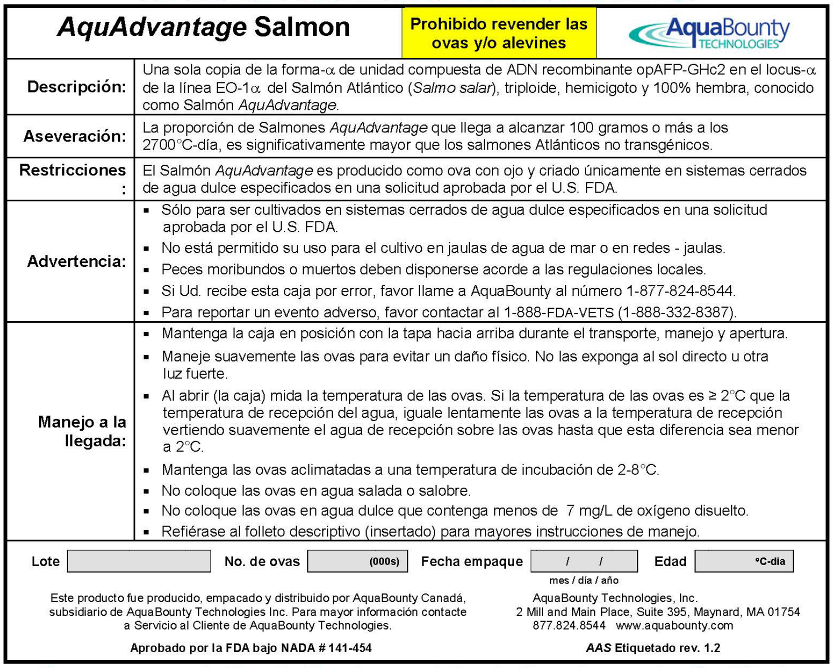


Favor lea esta etiqueta completamente antes de abrir la caja

***AquAdvantage* Salmon**	**Prohibido revender las ovas y/o alevines**
Descripción:	Una sola copia de la forma-α de unidad compuesta de ADN recombinante opAFP-GHc2 en el locus-α de la línea EO-1α del Salmón Atlántico (*Salmo salar*), triploide, hemicigoto y 100% hembra, conocido como Salmón *AquAdvantage*.
Aseveración:	La proporción de Salmones *AquAdvantage* que llega a alcanzar 100 gramos o más a los 2700°C-día, es significativamente mayor que los salmones Atlánticos no transgénicos.
Restricciones:	El Salmón *AquAdvantage* es producido como ova con ojo y criado únicamente en sistemas cerrados de agua dulce especificados en una solicitud aprobada por el U.S. FDA.
Advertencia:	▪ Sólo para ser cultivados en sistemas cerrados de agua dulce especificados en una solicitud aprobada por el U.S. FDA. ▪ No está permitido su uso para el cultivo en jaulas de agua de mar o en redes - jaulas. ▪ Peces moribundos o muertos deben disponerse acorde a las regulaciones locales. ▪ Si Ud. recibe esta caja por error, favor llame a AquaBounty al número 1-877-824-8544. ▪ Para reportar un evento adverso, favor contactar al 1-888-FDA-VETS (1-888-332-8387).
Manejo a la llegada:	▪ Mantenga la caja en posición con la tapa hacia arriba durante el transporte, manejo y apertura. ▪ Maneje suavemente las ovas para evitar un daño físico. No las exponga al sol directo u otra luz fuerte. ▪ Al abrir (la caja) mida la temperatura de las ovas. Si la temperatura de las ovas es ≥ 2°C que la temperatura de recepción del agua, iguale lentamente las ovas a la temperatura de recepción vertiendo suavemente el agua de recepción sobre las ovas hasta que esta diferencia sea menor a 2°C. ▪ Mantenga las ovas aclimatadas a una temperatura de incubación de 2-8°C. ▪ No coloque las ovas en agua salada o salobre. ▪ No coloque las ovas en agua dulce que contenga menos de 7 mg/L de oxígeno disuelto. ▪ Refiérase al folleto descriptivo (insertado) para mayores instrucciones de manejo.

Lote ☐ **No. de ovas** (000s) **Fecha empaque** / / (mes / día / año) **Edad** °C-día

Este producto fue producido, empacado y distribuido por AquaBounty Canadá, subsidiario de AquaBounty Technologies Inc. Para mayor información contacte a Servicio al Cliente de AquaBounty Technologies.

AquaBounty Technologies, Inc.
2 Mill and Main Place, Suite 395, Maynard, MA 01754
877.824.8544 www.aquabounty.com

Aprobado por la FDA bajo NADA # 141-454

***AAS* Etiquetado rev. 1.2**

Disponga una copia de esta etiqueta en cada incubadora y tanque de cría utilizado

Appendix C. Physical Containment and Site Plans for the Bay Fortune Facility

Figure C-1. Schematic of Site Effluent and Waste Flow with Key Containment Locations at the Bay Fortune Facility as of January 2024

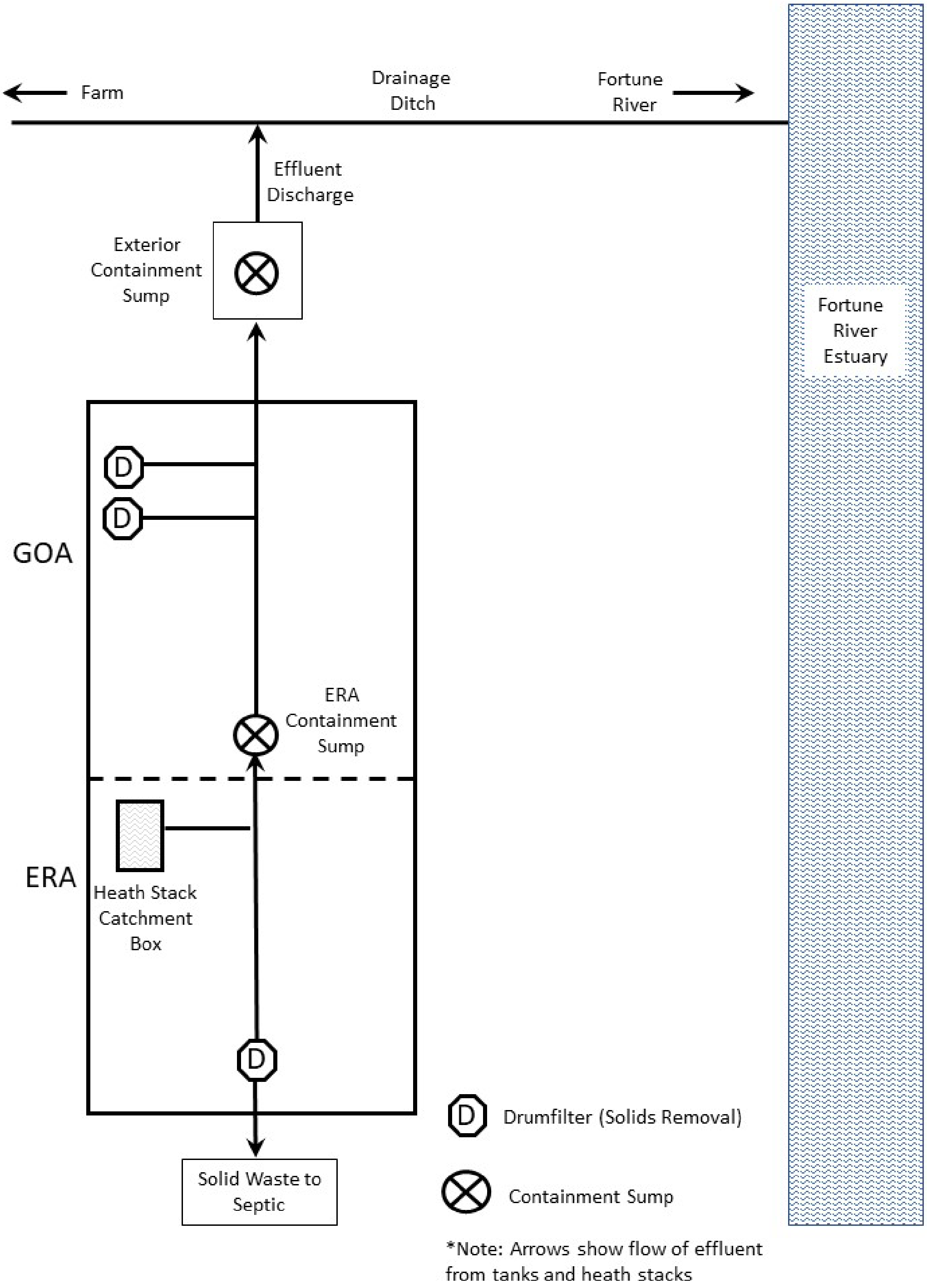
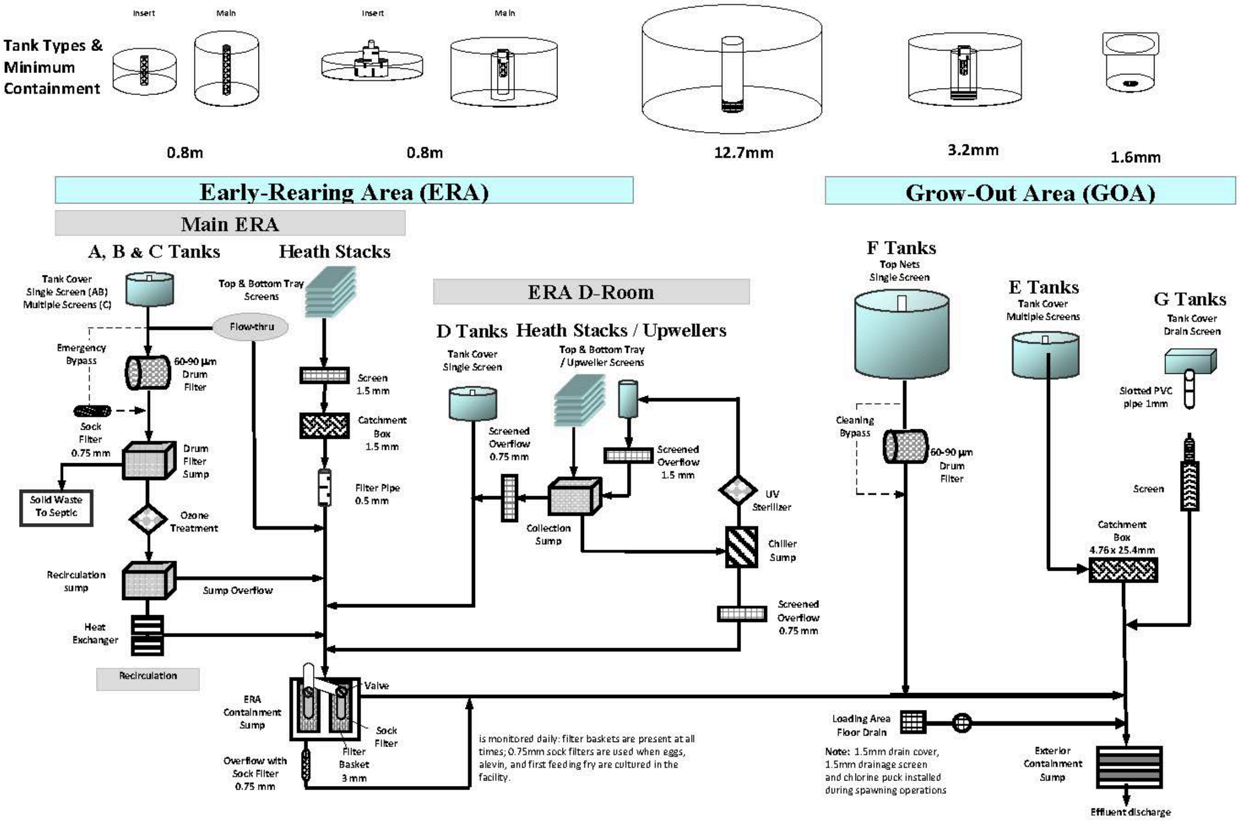


Figure C-2. Schematic of Physical Containment Components at the Bay Fortune Facility as of January 2024



is monitored daily: filter baskets are present at all times; 0.75mm sock filters are used when eggs, alevin, and first feeding fry are cultured in the facility.

Note: 1.5mm drain cover, 1.5mm drainage screen and chlorine puck installed during spawning operations

Table C-1. Containment Components and Level of Containment for the Bay Fortune Facility as of January 2024 (this table is identical to that presented in Table 3 of the 2015 EA, pages 57-59). (Note: ERA = early rearing area; GOA = grow-out area.)

Area	Tank Type	Containment level	Component	Details
ERA	Heath Stacks	1	Screened trays	Each drawer is a 1.5 mm screened compartment (top and bottom). The water must pass through both of these screens at each level as it descends towards the drain.
ERA	Heath Stacks	2	Vertical screen	Effluent is screened through a vertical 1.5 mm perforated PVC screen.
ERA	Heath Stacks	3	Catchment box	Effluent is filtered through 1.5 mm perforated catchment box filter with a horizontal screen.
ERA	Heath Stacks	4	Filter pipe	Effluent is filtered through a 0.5 mm slotted PVC capped pipe.
ERA	Heath Stacks	5	ERA containment sump sock filters	When smaller hatchlings and first feeding fry are present, sock filters are attached to the drain pipe outlets. The socks used have 1.5 and 0.75 mm mesh as appropriate for the fish size.
ERA	Heath Stacks	6	ERA containment sump	Three drain lines feed water into two stainless steel perforated baskets with 3 mm punched holes and 30 cm high sides. When cleaning a containment basket and/or sock filter, water flow is restricted while clean socks are put in place. The sump overflow is fitted with a 0.75 mm sock filter.

Area	Tank Type	Containment level	Component	Details
ERA	Upwelling Units	1	Screened inflow and overflow	Each unit is equipped with a 1.5 mm screened disk on both top and bottom. The water passes through the bottom screen when entering the upwelling unit and exists through the top sceen.
ERA	Upwelling Units	2	Effluent screen	Effluent is screened through a 0.75 mm sock filter.
ERA	Upwelling Units	3	Overflow screen	Overflow from collection sumps are filtered through a 0.75 mm sock filter.
ERA	Upwelling Units	4	ERA containment sump sock filters	When similar hatchlings and first feeding fry are present, sock filters are attached to the drain pipe outlets. The socks used to have 1.5 and 0.75 mm mesh, as appropriate for the fish size.
ERA	Upwelling Units	5	ERA containment sump	Three drain lines feed water into two stainless steel perforated baskets with 3 mm punched holes and 30 cm high sides. When cleaning a containment basket and/or sock filter, water flow is restricted while clean socks are put in place. The sump overflow is fitted with a 0.75 mm sock filter.
ERA	Early Rearing Tanks	1	Slotted tank stand pipe	Covered slotted standpipe with progressively wider slots as fish size increases; deep C tanks have an additional barrier
ERA	Early Rearing Tanks	2	Septic tank for solids collection	The solids (plus some water) from ERA drains are separated out by the drum filter and then pass into the drum filter solid waste septic tank.

Area	Tank Type	Containment level	Component	Details
ERA	Early Rearing Tanks	3	ERA containment sump sock filters	When smaller hatchlings and first feeding fry are present, sock filters are attached to the drain pipe outlets. The socks used have 1.5 and 0.75 mm mesh as appropriate for the fisk size.
ERA	Early Rearing Tanks	4	ERA containment sump	Three drain lines feed water into two stainless steel perforated baskets with 3 mm punched holes and 30 cm high sides. When cleaning a containment basket and/or sock filter, water flow is restricted while clean socks are put in place. The sump overflow is fitted with a 0.75 mm sock filter.
GOA	Large Circulars (F Tanks)	1	External stand pipe screens and standpipe cover	Screened ports or drilled holes depended upon fish size. The top of the standpipe is fitted with a solid PVC cap.
GOA	Large Circulars (F Tanks)	2	Facility containment screen #1	All effluent passes through a 6.2 mm punched stainless steel basked screen.
GOA	Large Circulars (F Tanks)	3	Facility containment screen #2	All effluent passes through a 10 mm punched stainless steel basket screen.
GOA	Large Circulars (F Tanks)	4	Facility containment screen #3	All effluent passes through a 13 mm flat punched stainless steel basket screen.
GOA	Small Circulars (E Tanks)	1	Internal stand pipe screens and stand pipe cover	Screened ports or drilled holes dependent upon fish size. The top of the standpipe is fitted with a solid PVC cap.
GOA	Small Circulars (E Tanks)	2	External stand pipe screens	Baskets with appropriate screening are located within exterior standpipe. Appropriate size screen is fitted to top of the external standpipe drain above the water level.

Area	Tank Type	Containment level	Component	Details
GOA	Small Circulars (E Tanks)	3	E Tank quarantine containment screen	Slotted (4.76 x 25.4 mm) stainless steel basket or 13 mm perforated stainless steel basket in place prior to water entering the floor drain.
GOA	Small Circulars (E Tanks)	4	Facility containment screen #1	All effluent passes through a 6.2 mm punched stainless steel basket screen.
GOA	Small Circulars (E Tanks)	5	Facility containment screen #2	All effluent passes through a 10 mm punched stainless steel basket screen.
GOA	Small Circulars (E Tanks)	6	Facility containment screen #3	All effluent passes through a 13 mm flat punched stainless steel basket screen.
GOA	Deep Swede (G Tanks)	1	Tank drain screen	Perforated metal or plastic screens with progressively larger perforations or a covered slotted standpipe with progressively wider slots as fish size increases.
GOA	Deep Swede (G Tanks)	2	PVC filter pipe	A length of 2” slotted PVC pipe fitted with an end cap. Minimum slot size is 1 mm.
GOA	Deep Swede (G Tanks)	3	Sock filter on shared drain outlet	A 1.5 mm mesh sock filter is fitted to the outlet of the shared drain pipe of the G tanks.
GOA	Deep Swede (G Tanks)	4	Slotted PVC screened sieve box	1.5 mm slotted PVC screen.
GOA	Deep Swede (G Tanks)	5	Facility containment screen #1	All effluent passes through a 6.2 mm punched stainless steel basket screen.
GOA	Deep Swede (G Tanks)	6	Facility containment screen #2	All effluent passes through a 10 mm punched stainless steel basket screen.
GOA	Deep Swede (G Tanks)	7	Facility containment screen #3	All effluent passes through a 13 mm flat punched stainless steel basket screen.

Area	Tank Type	Containment level	Component	Details
GOA	Loading Area Floor Drain	1	Floor drain covers	Normal drain covers are perforated steel plates with 7.0 mm openings. During spawning or packaging eggs for shipment, a 1.5 mm drain cover screen is installed.
GOA	Loading Area Floor Drain	2	Floor drain screen	An additional drainage screen is installed consisting of 1.5 mm perforations.
GOA	Loading Area Floor Drain	3	Chlorine puck	Installed under the floor drain screen while spawning.
GOA	Loading Area Floor Drain	4	Facility containment screen #1	All effluent passes through a 6.2 mm punched stainless steel basket screen.
GOA	Loading Area Floor Drain	5	Facility containment screen #2	All effluent passes through a 10 mm punched stainless steel basket screen.
GOA	Loading Area Floor Drain	6	Facility containment screen #3	All effluent passes through a 13 mm flat punched stainless steel basket screen.

Figure C-3. Schematic of Planned Layout of the Bay Fortune Facility with Future Incubation Room (Changes to Occur in 2024). The new incubation room will replace ERA D-Room and the Heath Stacks in the Main ERA. This will consolidate egg incubation into one location; no expansion of egg production is expected from this change.

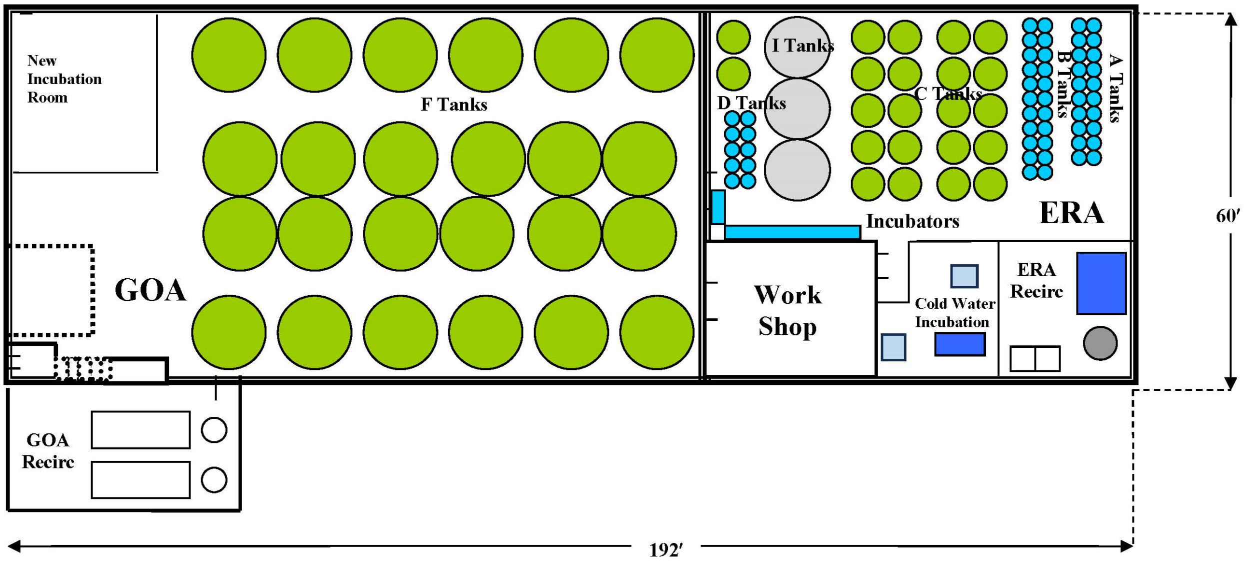
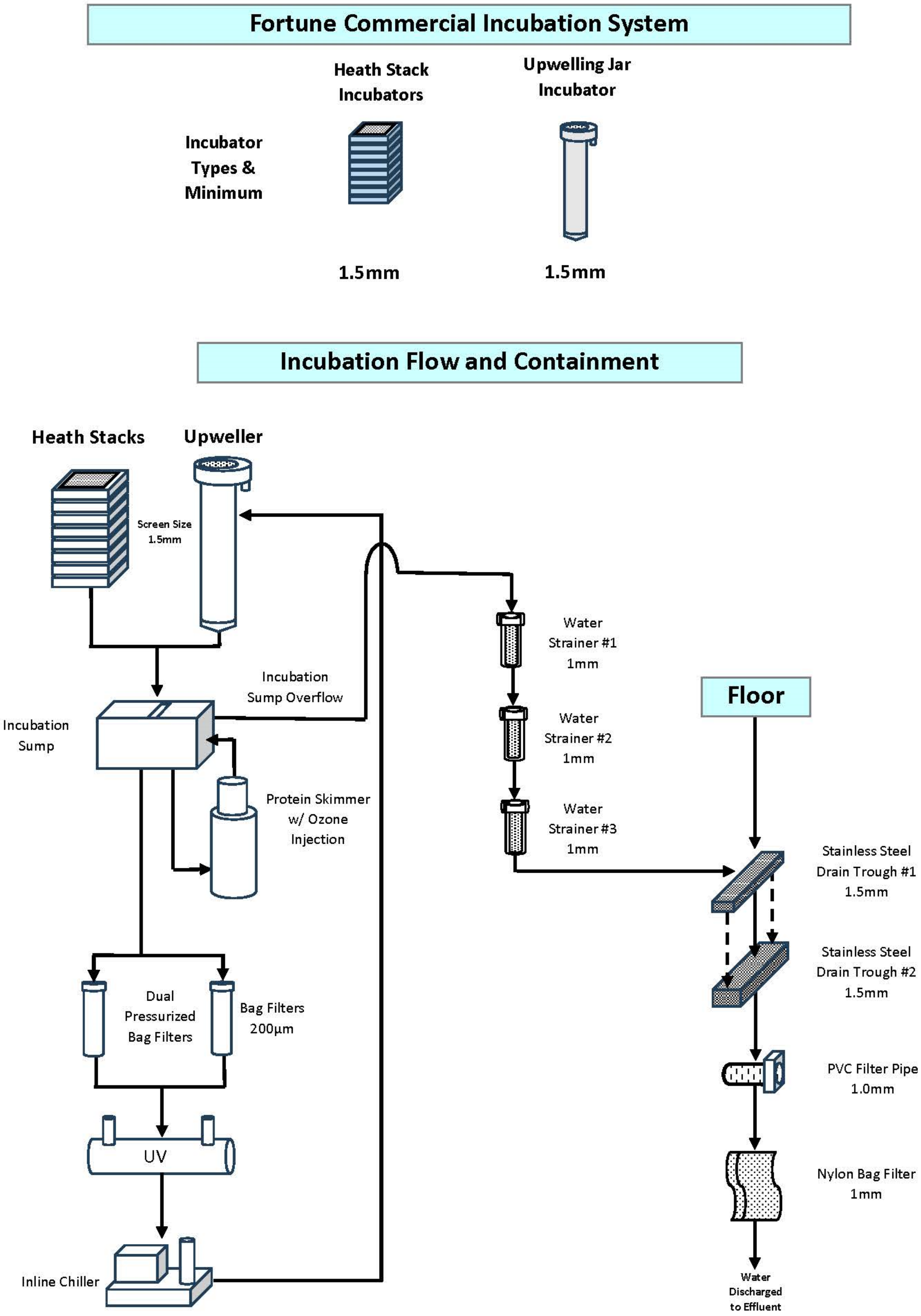


Figure C-4. Schematic of the Planned Physical Containment Components in the Future Incubation Room at the Bay Fortune Facility (Changes to Occur in 2024).



Appendix D. Physical Containment and Site Plans for the Rollo Bay Facility

Figure D-1. Schematic of Site Effluent and Waste Flow with Key Containment Locations at the Rollo Bay Facility

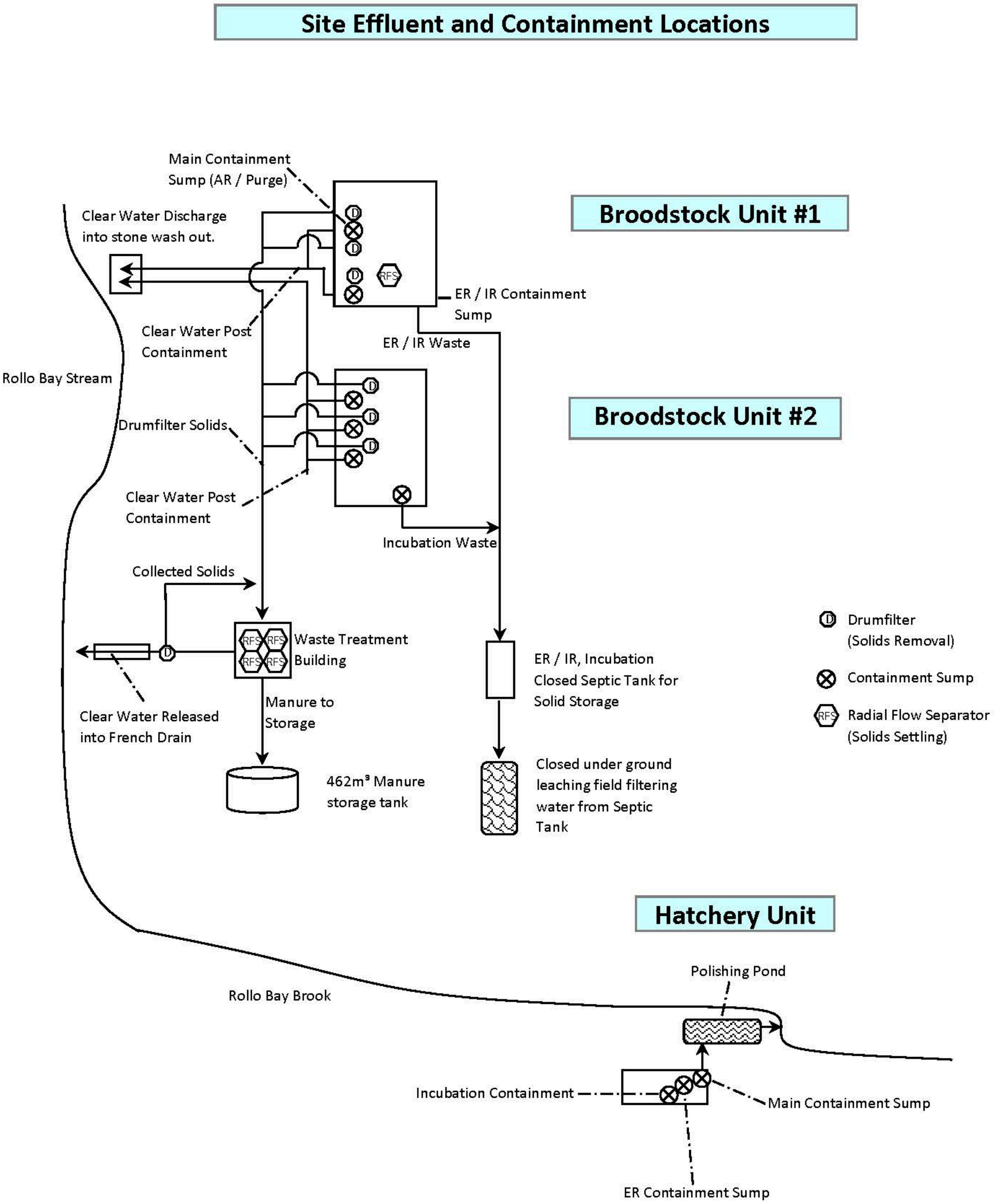


Figure D-2. Schematic of Physical Containment Components at the Rollo Bay Hatchery Unit

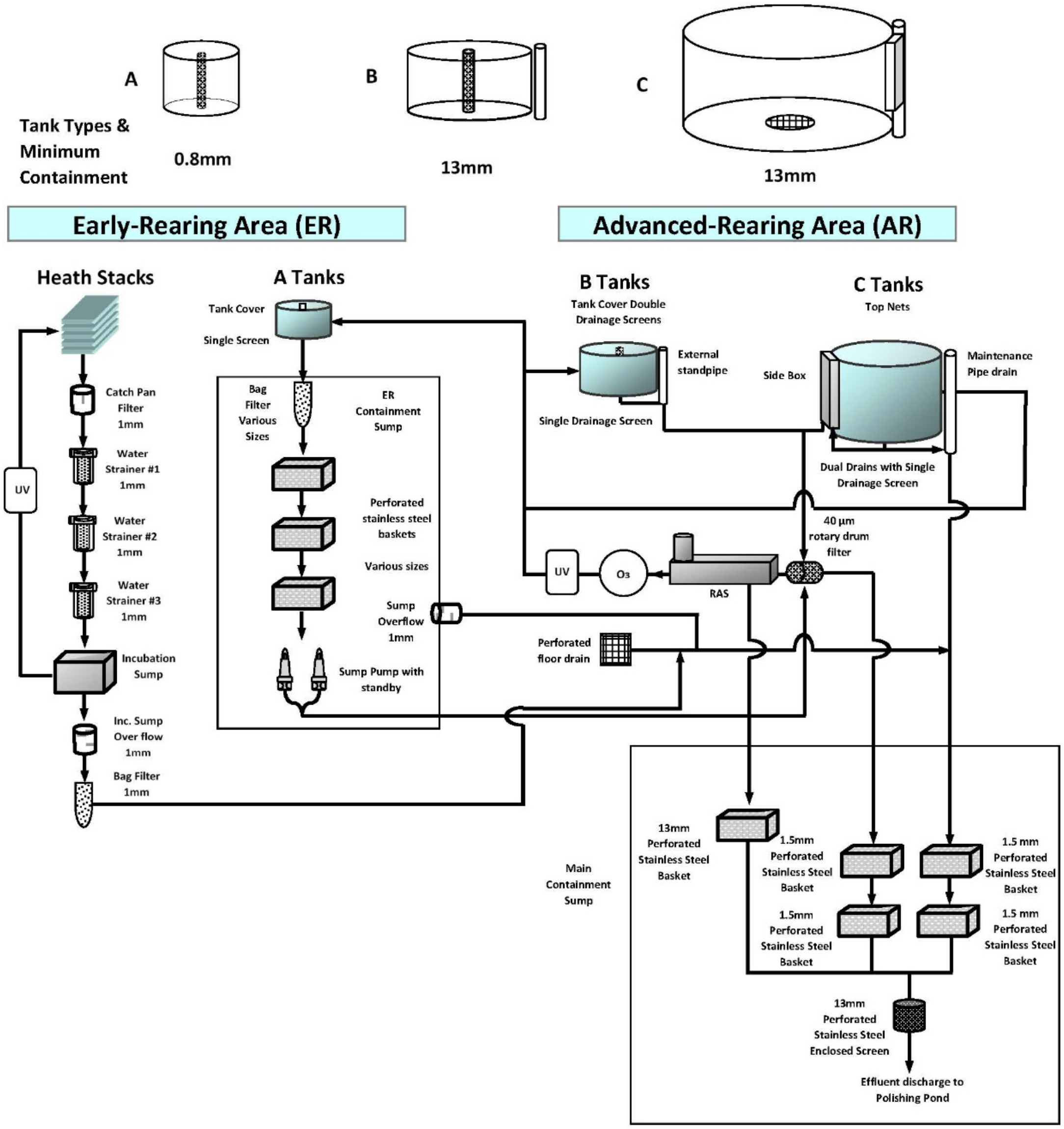


Table D-1. Containment Components and Level of Containment for the Rollo Bay Hatchery Unit. These tables are identical to those presented in Tables 5-6, 5-7 and 5-8 of the 2019 EA. (Note: ER=early rearing area; AR=advanced rearing area.)

Area	Tank Type	Containment Point	Location	Barrier Type	Barrier Materials	Perforation Size (mm)	Fish Size (g)
ER	Hatchery Incubator	1	Heath Stack Egg Trays	Top and bottom screens	Molded plastic inserts with Polyester screen	1.50	0.1
ER	Hatchery Incubator	1	Upweller Jars (Incubator Jars)	Top and bottom screens	PVC	1.50	0.1
ER	Hatchery Incubator	2	Heath stack catchment pan	Perforated pipe	PVC	1.00	0.1
ER	Hatchery Incubator	2	Upweller Jar filter pipe	Perforated pipe	PVC	1.00	0.1
ER	Hatchery Incubator	3	Recirculation line	Strainer	Nylon	1.00	0.1
ER	Hatchery Incubator	4	Recirculation line	Strainer	Nylon	1.00	0.1
ER	Hatchery Incubator	5	Recirculation line	Strainer	Nylon	1.00	0.1
ER	Hatchery Incubator	6	Incubation Sump Overflow	Perforated pipe	PVC	1.00	0.1
ER	Hatchery Incubator	7	Incubation Sump Overflow	Bag filter	Nylon	1.00	0.1
ER	Hatchery Incubator	8	Floor drain	Perforated pipe	PVC	1.00	0.1
AR	Hatchery Incubator	9	Main containment sump	Containment basket	Stainless steel	1.50	0.1
AR	Hatchery Incubator	10	Main containment sump	Containment basket	Stainless steel	1.5	0.1
AR	Hatchery Incubator	11	Main containment sump	Containment basket	Stainless steel	13.00	0.1
ER	A-tanks	1	A-tank cover	Tank cover nets	Polyethylene	9.00	0.1 – 10.0
ER	A-tanks	2	A-tank drain	PVC standpipe	Perforated PVC	0.8 – 3.5	0.1 – 10.0
ER	A-tanks	3	ER containment sump inlet	Sock filter	Polyester	0.75 – 3.5	0.1 – 10.0
ER	A-tanks	4	ER containment sump	Containment basket	Stainless steel (SS)	1.5 – 6.3	0.1 – 10.0
ER	A-tanks	5	ER containment sump	Containment basket	SS	1.5 – 6.3	0.1 – 10.0
ER	A-tanks	6	ER containment sump	Containment basket	SS	1.5 – 6.3	0.1 – 10.0
ER	A-tanks	7	ER containment sump	Overflow screen	Perforated PVC	1.5	0.1 – 10.0

Area	**Tank Type**	**Containment Point**	**Location**	**Barrier Type**	**Barrier Materials**	**Perforation Size (mm)**	**Fish Size (g)**
ER	A-tanks	8	AR drum filter	Drum filter screen	SS frame with polyester micromesh	0.04	0.1 – 10.0
ER	A-tanks	9	Floor drains	Perforated pipe	SS	1.0	0.1 – 10.0
ER	A-tanks	10	Main containment sump	Perforated box	SS	13.00	0.1 – 10.0
ER	A-tanks	11	Main containment sump	Containment box/screen	SS	1.50	0.1 – 10.0
ER	A-tanks	12	Main containment sump	Containment box/screen	SS	1.50	0.1 – 10.0
ER	A-tanks	13	Main containment sump	Containment box	SS	13.00	0.1 – 10.0
AR	Tanks B & C	1	B- and C-tanks	Tank cover nets	Polyethylene	13.0 – 25.4	10 - 5000
AR	Tanks B & C	2	B-tank drains	PVC standpipe and cover	PVC and perforated plastic screen	5.0 – 13.0	10 - 5000
AR	Tanks B & C	3	C-tank side boxes	Screen	Polyethylene	5.0 - 13.0	10 - 5000
AR	Tanks B & C	4	C-tank bottom drains	Drain cover	SS	5.0 – 25.0	10 - 5000
AR	Tanks B & C	5	AR drum filter	Drum filter screen	SS frame with polyester micromesh	0.04	10 - 5000
AR	Tanks B & C	6	Floor drain	Perforated covers	Bronze	8.00	10 - 5000
AR	Tanks B & C	7	Main containment sump	Clear water overflow baskets	SS	13.00	10 - 5000
AR	Tanks B & C	8	Main containment sump	Effluent baskets	SS	1.50	10 - 5000
AR	Tanks B & C	9	Main containment sump	Screen	SS	13.00	10 - 5000

Figure D-3. Schematic of Rollo Bay Broodstock Unit 1 Layout

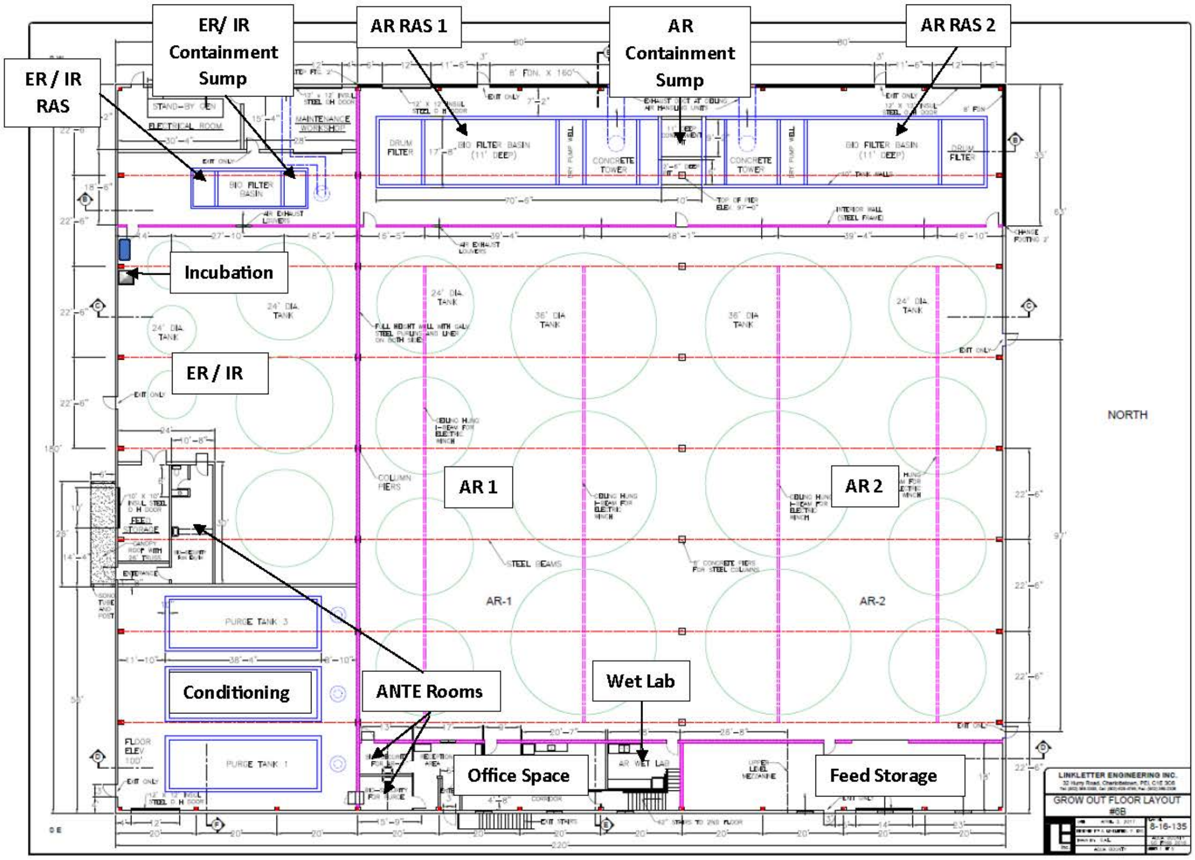


Figure D-4. Schematic of Physical Containment Components at the Rollo Bay Broodstock Unit 1 Early Rearing Area

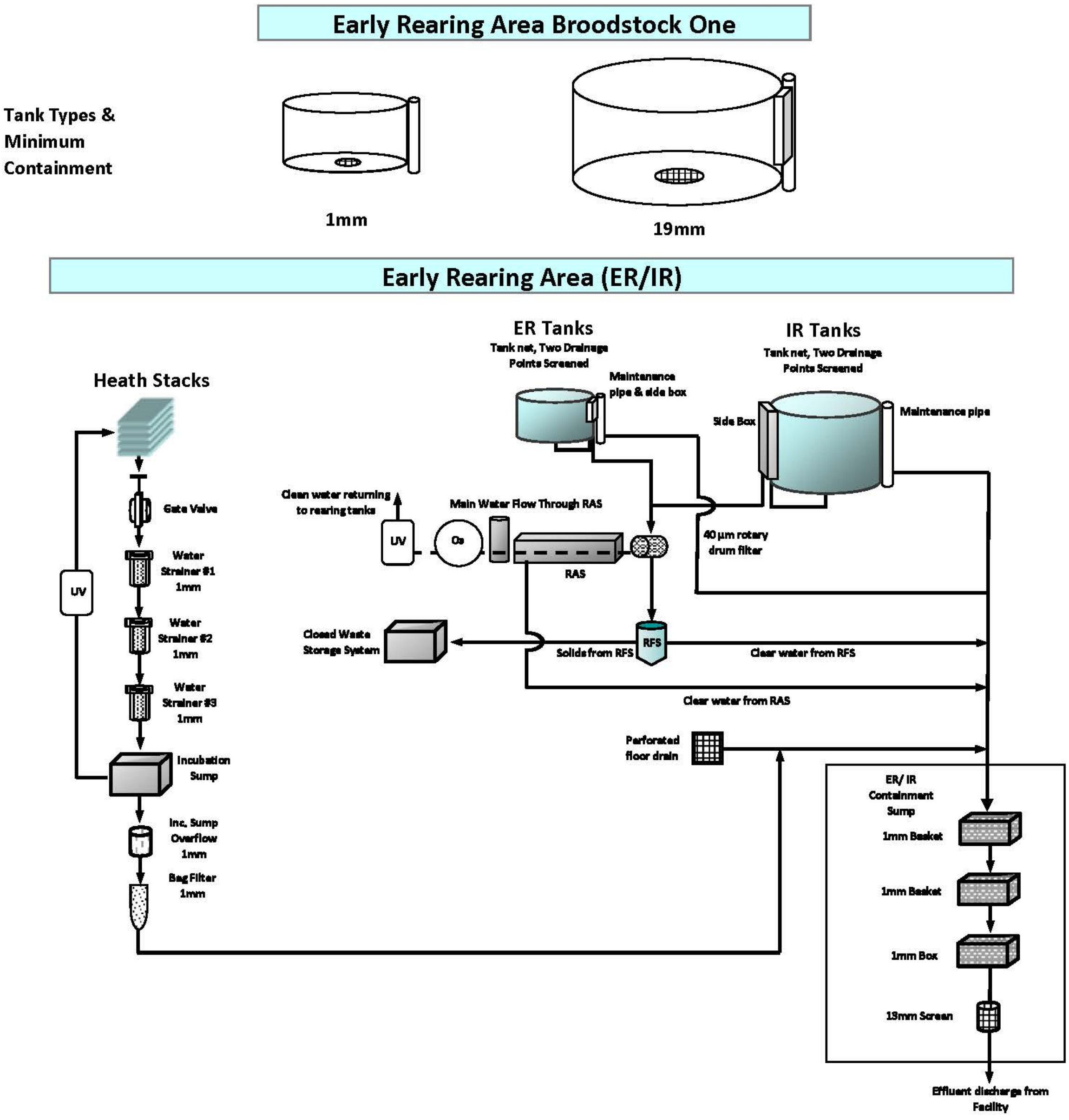
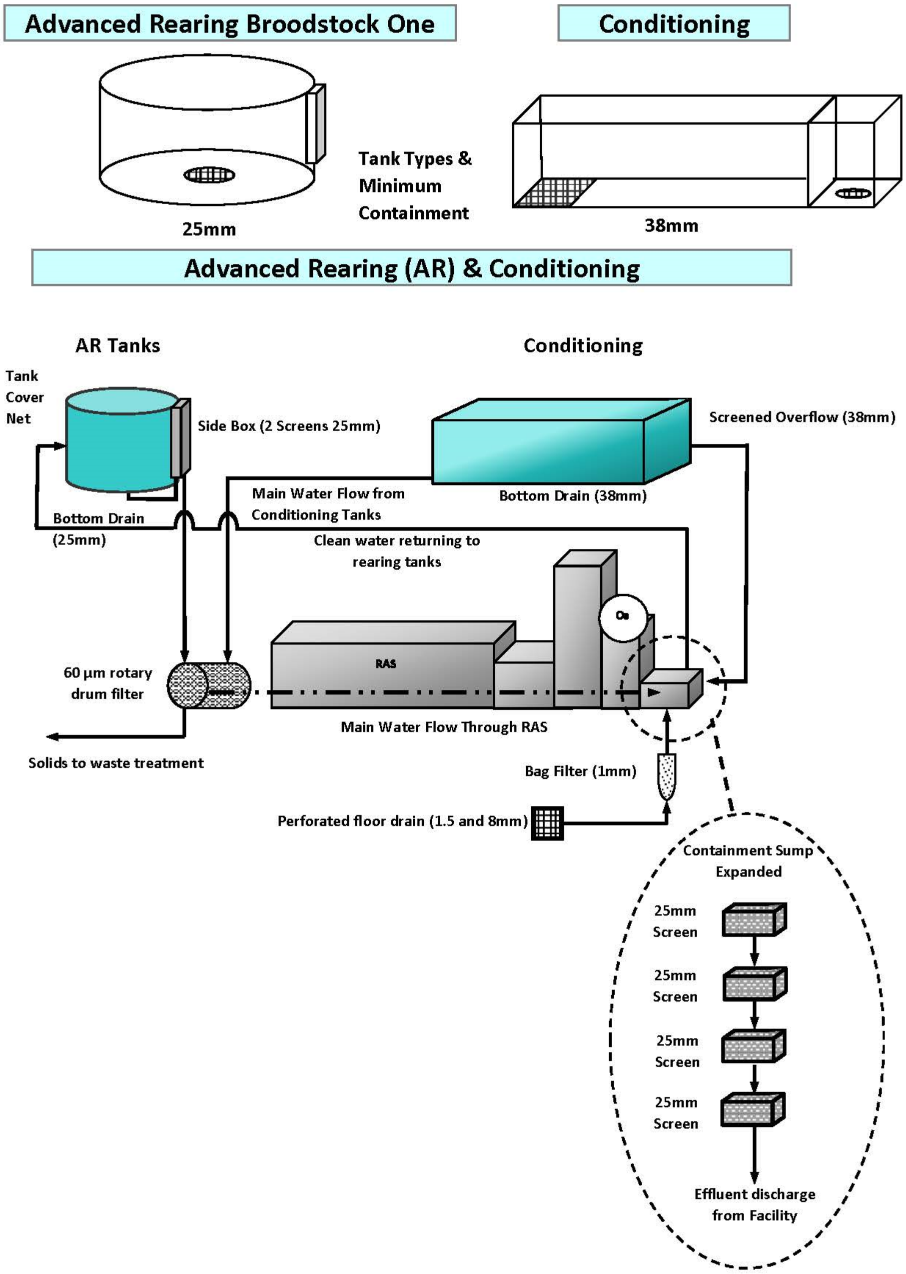


Figure D-5. Schematic of Physical Containment Components at the Rollo Bay Broodstock Unit 1 Advanced Rearing and Conditioning Areas



Appendix E. Background on the Biology of the Atlantic Salmon

This section characterizes the biology, ecology, life history, and distribution/status of Atlantic salmon, factors important in describing the fitness of Atlantic salmon without an IGA, including farmed Atlantic salmon. It also includes background information on Atlantic salmon farming and relevant information on common interactions between domesticated and wild salmon in the areas where salmon farming occurs. These characteristics form the baseline of information against which the potential environmental impacts of AAS can be evaluated. For additional information, consult the Consensus Document on the Biology of Atlantic Salmon (*Salmo salar*) (OECD, 2017).

E.1. Wild Atlantic Salmon

E.1.1. Geographic Range: Historical and Current

Atlantic salmon have historically inhabited the North Atlantic Ocean and associated coastal drainages. In North America, the species was distributed in river systems and marine waters from the Hudson River in New York state northward. In Canada, Atlantic salmon were found in the Bay of Fundy, throughout the Gulf of St. Lawrence and along the whole coast of Newfoundland and Labrador to the Fraser River. Self-sustaining populations no longer exist in many historical rivers at the southern distributional limits in the eastern United States and the adjacent Maritime Provinces of Canada (Webb *et al*., 2007). Native populations have also become extinct in the upper St. Lawrence River, including Lake Ontario. Where stocks of Atlantic salmon remain, populations are generally depressed and frequently supported by supplemental stocking programs. Populations of Atlantic salmon in the Eastern Atlantic historically ranged from northern Portugal at the southern end to the tributaries of the Barents Sea and White Sea (Russia) in the northeast, including most rivers draining into the Baltic and North Seas. Native, wild stocks are no longer found in the Elbe and Rhine Rivers or in many of the rivers draining into the Baltic Sea (Webb *et al*., 2007). The species is also severely depressed or extinct in the rivers of France, Spain, and Portugal at the species’ southern limit.

E.1.2. Life History

Atlantic salmon populations exhibit diverse physiological, anatomical, and behavioral characteristics that derive in part from local genetic adaptation. In populations for which seaward migration is not prevented by physical barriers, females are usually anadromous (i.e., living in salt water and spawning in fresh water); however, males often reproduce after living 1-4 years in fresh water, after which they may or may not migrate to sea. Anadromous populations also exhibit considerable variation in the type of freshwater habitat chosen for rearing (estuarine or lacustrine), the total duration of their seawater habitation (20-50% of lifetime), and the timing of spawning migration (spring or fall). Some Atlantic salmon complete their entire life cycle in fresh water, such populations being common throughout the North American range, but more limited to large lakes in the European distribution.

The developmental phases of Atlantic salmon include the following:

- Alevin: A newly-hatched fish in the larval stage that has not yet emerged from the nesting area and is dependent upon a yolk sac for its nutritional requirements;

- Fry: An alevin that has fully absorbed its yolk sac and must hunt for, and consume, live food;
- Parr: A young salmon in fresh water that has developed a characteristic skin coloration known as "parr marks;"
- Smolt: A young salmon that has undergone the physiologic adaptation necessary for transition to salt water;
- Grilse: A salmon returning to fresh water one year after migrating to the sea;
- Kelt: A salmon after spawning.

The Atlantic salmon is iteroparous, meaning it may spawn repeatedly. Typically, Atlantic salmon spawn during October to February, with the peak of spawning usually occurring in late October and November. The nesting site, or redd, is chosen by the female, and is usually a gravel-bottom riffle upstream from a pool (Bigelow, 1963; Scott and Crossman, 1973). The ecomorphological demands of the spawning grounds are stringent and include the following: water descent of 0.2-3%; water depth of 50 to 90 cm; running speed of 0.3 to 0.7 m/s; gravel size of 3 to 5 cm; and, nest size of 1 to 2 m (MUNLV, 2001).

The eggs are buried in gravel at a depth of about 12-25 cm (Bigelow, 1963; Scott and Crossman, 1973). The female rests after spawning and then repeats the operation, creating a new redd, depositing more eggs, and resting again until spawning is complete. The male continues to guard the female, and to drive away competitors aggressively until she has completed making redds and depositing her eggs. This may take as long as a week and require the building of up to seven redds to deposit her nearly 7,500 eggs. Thereafter, the post-spawn adult fish, or kelt, may return to the ocean without delay, move to a pool downriver for a period of rest, or over-winter in the nursery river and return to sea in the spring. Many kelt do not survive the first mating; some survive to mate twice, but very few mature males or females salmon survive to spawn three or more times.

Only about 9-20% of the fertilized eggs in the redds survive to develop over the winter, and depending on temperature and water conditions will usually hatch in April. The hatchlings, often referred to as "alevin," are mostly transparent, and have large yolk sacs. These alevin remain in the gravel feeding on their yolk sacs until they are absorbed, after which the young fish emerge from the redd and begin foraging for food in the water column. This typically occurs in May or June. Once "swim up" has occurred, these small fish are referred to as fry (as in "small fry") or swim-up fry. Hungry, they swim freely, and begin to eat— insect larvae, other small organisms called zooplankton, and fish eggs, including those of their own species.

As the fry mature, and become more fish-like in appearance, they develop a series of spots along their sides, from which dark vertical stripes descend. These markings, referred to as parr marks, aid in camouflaging the young fish, which are preyed on by other fish, mammals, and birds that live along rivers and streams. At this stage, the juveniles are referred to as "parr." They remain in their natal (birth) streams, feeding on the larvae of insects, worms, and shellfish, and sometimes each other or related species (such as trout).

If there is plenty of food, and other environmental conditions are good (the water is clean and there is enough oxygen), those parr not consumed by other fish, birds, or other animals, grow rapidly during their first summer. Parr can be very territorial, and aggressively protect their space from other parr. As the parr become larger, their territories expand, probably to ensure a reliable source of food.

Parr may spend between one and six years (usually two to three years) in their natal streams; at some point, if they are not in land-locked lakes, they begin their downstream migration and prepare for life in the sea. They are usually about 15 cm (six inches) long at this point in their development (as depicted here.). The seaward migration involves a change in physiology which allows the young salmon to adapt to salt water conditions. This transformation in physiology is referred to as “smoltification” and the young fish that migrate to the sea are called “smolts”. In general, smolts tend to live for a while in brackish (part salt) water, such as bays and estuaries while they complete their adaptation to salt water. It is thought that the “imprinting” of the natal river occurs during smoltification (NOAA, 2022). At this stage, the fish lose their parr marks and take on silver color. They also become more elongated than they were as parr and have darker fins.

At the end of the spring during which they have adapted to living in salt water, the smolt generally swim to sea. For example, Atlantic salmon leave Maine rivers some time in April or May and can be found in the waters off Labrador and Newfoundland by mid-summer. They then migrate to take advantage of available food supplies and generally spend their first winter at sea off the coast of Greenland. While at sea, salmon are sometimes referred to as “opportunistic pelagic feeders.” That means they eat whatever is edible in the open sea: other finfish, shellfish (including shrimp, krill, and other crustaceans), and zooplankton. In fact, it is the pigments in these organisms (crustaceans and zooplankton) that are in large part responsible for the orange-pink hue of most salmon. Salmon that do not eat crustaceans with pigment, especially those salmon that tend to spend their lives in freshwater lakes, tend to have a whiter flesh.

As they mature, Atlantic salmon feed on finfish such as Atlantic herring, alewives, rainbow smelt, young cod, sand lances, flatfish, and small Atlantic mackerel. Atlantic salmon must also avoid being eaten themselves, as they are preyed on by marine birds, seals, and larger fish. After two years at sea, an adult salmon can weigh about 8-15 pounds, and be up to 30 inches long.

During their time in the open sea, which can last from one to several winters, the fish become sexually mature. Upon first entering the sea, the salmon keep the silver hue and darker fins of the smolts, and gain some black spots on their backs. Their bodies become even more elongated, and they become strong and elegant swimmers.

Post-smolt salmon age is counted in units of “winters at sea.” In general, a salmon that spends one winter at sea prior to becoming sexually mature and returning to its natal stream to spawn is called a “grilse.” A salmon that spends two years at sea is referred to as a “2SW” (sea winter) fish. In general, the longer a salmon spends at sea feeding, the larger it becomes, although Atlantic salmon rarely get bigger than about 25 pounds.

Salmon typically form schools after they enter the sea and may travel with or be mistaken for herring, mackerel or other pelagic fish, since post-smolts occur as by-catch in these fisheries according to the North Atlantic Salmon Conservation Organization (NASCO, 2007). Post-smolts follow ocean currents, feeding as they migrate, and adding fish to their diet of marine invertebrates at a size of about 27 cm (fork length) after a few months at sea. Survival in fresh water from egg to smolt varies from 0.3-2.6%. Survival in the sea from smolt to return as grilse varies from 1.3-17.4% (Hutchings and Jones, 1998). Most Atlantic salmon (70-80%) survive spawning and migrate to sea a second time as kelt; only about 10% of them return to spawn a second time (Fleming, 1998).

Regardless of their age, as Atlantic salmon migrate back to their natal rivers and streams, the fish become sexually mature, and their shape and coloration begin to change, with pigment changes more prominent in the males. In general, males become redder on their bellies, or red with purple spots; females tend to be blue-black in color. They become less elongated and thicker in the body; the females, in particular, become swollen with eggs. The males also develop teeth and an exaggerated hooked lower jaw referred to as a "kype." These are useful in fending off the unwanted attentions of other males to their selected females during spawning.

A few salmon never make the transition to saltwater environments because they spend their entire lives in landlocked lakes. In addition, a small percentage of the males become sexually mature in fresh water as parr and are referred to as "precocious males." Rather than migrating to sea, these small, young males establish residence in the still water in which mature salmon spawn. When the females release their eggs, the precocious males dart in and deposit their milt before the sexually mature large males can. Because they are small, the precocious males are not recognized as threats by the larger mature males and are generally not the object of their aggression. Precocious parr make up approximately 1% of the male population but may end up fertilizing up to 20% of the total eggs that are released by females.

The size of the adult fish is more dependent on time spent feeding at sea than on age. Sea-run Atlantic salmon usually attain a larger size than do landlocked salmon (i.e., those living entirely in fresh water). Sea-run salmon range from 2.3 to 9.1 kg and commercially-raised fish average 4.5 to 5.4 kg. (Teufel *et al*., 2002). Many aspects of Atlantic salmon behavior are affected by size. Investigations of growth in parr have shown that they may segregate into two or more groups at the end of the first growth season. Parr in the upper modal group may smoltify at 1+ years versus the lower modal groups, which may smoltify later (Metcalfe *et al*., 1988). Within populations, therefore, the onset of the parr-smolt transition is dependent on growth rate. Smolt size can also vary widely among populations (Klemetsen *et al*., 2003). 1-SW salmon spawn usually every year, while older sea-age salmon are primarily biennial spawners; within populations, the proportion of biennial spawners increases with the size of fish at first maturity. The proportion of repeat spawners decreases with size of fish. This may be related to energy expenditure due to spawning: 1SW salmon may allocate 50% of their energy (Jonsson *et al*., 1991) for spawning compared to 70% for older salmon (Jonsson *et al*., 1997). Fecundity, or potential reproductive capacity, is another trait that varies considerably both within and among salmon stocks.

Fecundity is typically expressed in terms of numbers of eggs (gametes). Egg number and egg size increase with body size (Thorpe *et al*., 1984; Jonsson *et al*., 1996). Although absolute fecundity varies greatly among individuals, as expected owing to high variability in adult body size, relative fecundity (eggs/kg total egg mass) as a measure of reproductive effort varies much less. The faster that parr grow in fresh water before smoltification, the smaller their relative egg size becomes when they attain maturity. This phenotypic response has been explained as an adaptation to the potential growth opportunities in their nursery river. Usually, both egg size and fecundity increase with size of fish (Klemetsen *et al*., 2003).

Atlantic salmon compete for food and space in fresh water (Chapman, 1966) where they may be “keystone species” like Pacific salmon (steelhead, *Oncorhynchus mykiss*), which along with California roach (*Hesperoleucas symmetricus*) were found to influence the entire food web in a Northern California river (Power, 1990). In marine waters, however, even at their highest levels of historical abundance, Atlantic salmon are rare relative to the available space and few in proportion to total biomass of fish populations, and are thus, expected to play a more minor role in the food web (Hindar, 2001).

E.1.3. Habitat Requirements

The physical habitat requirements of the Atlantic salmon vary depending upon the life stage. The preferred spawning habitat is a transitional area between pool and riffle with coarse gravel. Shelter (e.g., undercut banks or overhanging vegetation) is also important. Juvenile freshwater habitat includes rivers, lakes and estuarine (i.e., brackish) environments. Highest population densities are typically found in rivers with riffle, run and pool sections, with moderate-size cobble substrates. As parr grow, they prefer deeper and swifter parts of riffles. In general, juvenile salmon occupy shallow fast-flowing water with a moderately coarse substrate and overhead cover provided by surface turbulence. Once in the sea, the distribution of adult salmon appears to reflect environmental factors such as surface temperature, currents, and food availability.

Temperature plays a major role in influencing salmon behavior. Fish move to sea earlier in southern than in northern rivers; and, in Europe, sea temperature is close to 8 °C when smolt enter the ocean whether the river is southern or northern (Klemetsen *et al*., 2003). An optimal surface-seawater temperature range for Atlantic salmon is estimated to be 4-10 °C (Reddin, 2006). The upper incipient lethal temperature (i.e., the temperature at which all salmon would exit a habitat if the opportunity were available) is estimated to be approximately 28 °C (Garside, 1973); the lower lethal temperature is below 0 °C (Reddin, 2006). Stead and Laird (2002) have cited the upper lethal temperature for salmon as being 23 °C. In a study examining the tolerance and resistance to thermal stress in juvenile Atlantic salmon, Elliot (1991) acclimated the fish for two weeks to various temperatures (5, 10, 15, 20, 25 and 27 °C) then raised or lowered the temperature by 1 °C per hour. The incipient lethal levels defined the tolerance zone within which salmon lived for a considerable time (i.e., survival over seven days). Salmon acclimated to 27 °C initially demonstrated the highest incipient lethal level at 27.8 ± 2 °C; for these fish, the lower mean incipient lethal level was 2.2 ± 4 °C. Temperature limits for feeding increased slightly with acclimation temperature to upper- and lower-mean values of 22.5 ± 0.3 °C and 7.0 ± 0.3 °C, respectively. The fish acclimated to 25 °C and 27 °C did not feed, while fish acclimated to the lower temperatures fed normally at 21.6-22 °C (Elliot, 1991).

This research collectively indicates that although fish acclimated to relatively high temperatures may be able to survive more than seven days at these high temperatures, they do not feed at temperatures above ~23 °C and would eventually starve. Willoughby (1999) presents the feeding and activity range for smaller Atlantic salmon (i.e., < 100 g) in fresh water as favorable up to ~23 °C, with mortality occurring at ~26 °C. For larger Atlantic salmon, the available data for sea water show the feeding and activity range as favorable up to ~20 °C, with mortality occurring at ~22 °C. Elliott (1991) noted that little is known about the upper temperature limits for survival of Atlantic salmon in the field and reported studies showing tolerances similar to those observed in his laboratory. Other experimental studies summarized by Elliott (1981, 1991) indicate that the optimum temperatures for growth of young Atlantic salmon are in the range 16-19 °C.

The minimum **pH tolerance** is between pH 5.0-5.4 depending on other river variables (e.g., aluminum levels), with eggs being the developmental stage least sensitive to acidity, followed by parr, and then smolt and fry, which are the most sensitive (Amiro, 2006).

Salmonids are known for requiring more **dissolved oxygen** than "warm-water fish." Shepherd and Bromage (1995) state that the DO content of water in a salmonid farm should never drop below 6 mg/L and that carbon dioxide (which influences the pH of the water) starts to be a problem for salmonids above 15 mg/L. Similarly, Stead and Laird (2002) suggest that DO levels should never fall below 5 mg/L; for good growth, a minimum of 7 mg/L is essential.

Other challenges to survival come from **obstructions and siltation**. Passage of salmon upstream can be blocked by natural and man-made obstructions (e.g., dams), as most vertical obstructions in excess of 3.4 m will block the upstream passage of salmon. In addition, high concentrations of fine sediments in the spawning gravel may decrease embryo survival and fry emergence through a reduction in the intragravel flow necessary for adequate water oxygenation. For example, the presence of as little as 0.02% silt (<0.063 mm) during incubation has been shown to decrease embryo survival (Julien and Bergeron, 2006). Atlantic salmon have the capacity to cope with a wide variety of flow conditions, and juvenile salmon have been known to prefer pools at lower discharges and move from pool to riffle habitats at higher discharges. Their ability to adapt to changes in flow and tolerance of relatively high water temperatures enables juvenile salmon to occupy extensive sections of streams that experience variations in flow outside the range of useful habitat of some competitive sympatric species (Amiro, 2006).

E.1.4. Status of Wild Atlantic Salmon Populations in the United States

The historical range of the North American Atlantic salmon (fish found in Canadian and U.S. waters) ranged from northern Quebec to Newfoundland, and southwest to Long Island Sound. In colonial times, they could be found in almost every river north of the Hudson. Beginning in the 19th century, these populations began to decline precipitously. In the 1800s, Atlantic salmon became extinct in the Connecticut (CT), Merrimack (MA), and Androscoggin (NH, ME), rivers mostly likely due to the results of dam building to harness the energy of the water. These dams blocked access of the fish to their natal streams (and thus their spawning areas). Industrial pollution, from paper mills and textile factories, also contributed to the decrease in populations, as did commercial overfishing and climate changes that affect the temperature of the water in the ocean at the depths at which Atlantic salmon are found (2-10 meters below the surface). (Atlantic salmon need clear, sediment-free water and cold temperatures to survive). As an example, "weirs" (structures in rivers or estuaries that let water through while either directing fish to nets to be caught, or directly trapping fish) in Maine were reported as catching 90 metric tons of Atlantic salmon in the late 1800s and half that in the early 1900s.

Today, very few rivers in Maine support wild Atlantic salmon. In fact, Atlantic salmon are extinct in 84 percent of the rivers in New England that historically supported salmon. They are in “critical condition” in the remaining 16 percent. In 2004, only 60-113 individual fish were counted in the eight rivers in Maine that support Atlantic salmon. In 2000, the NOAA Fisheries Services and FWS listed the Gulf of Maine Distinct Population Segment of Atlantic salmon as “endangered” under the ESA. That designation was extended in 2009 to include fish in several rivers in Maine. Populations in Canada have also declined. In the 1970s, approximately 1.5 million salmon returned to their natal rivers in Eastern Canada; by 2004, that number had dropped to approximately 350,000 (http://www.traffic.org; accessed October 25, 2022).

The Northeast Fishery Management Council developed a Fishery Management Plan for Atlantic Salmon in 1988. This authority extends over all Atlantic salmon of United States origin, and prohibits “possession” of Atlantic salmon, either as the intended catch of commercial fishing, or as the indirect (by catch) result of fishing for other fish. Commercial fishing of wild Atlantic salmon is now prohibited in US federal waters, although recreational fishing is allowed. (Commercial fishing of wild Atlantic salmon still occurs off the coast of Greenland, where adult Atlantic salmon feed).

There is now a Recovery Plan for the Gulf of Maine Population Segment of Atlantic salmon, which identifies steps that need to be taken to stop the decline of the population.[119] In addition, as previously mentioned, the US is a member of the North Atlantic Salmon Conservation Organization (www.nasco.int; accessed October 25, 2022), a group dedicated to the conservation, restoration and management of Atlantic salmon.

E.1.5. Interactions with Other Organisms

In fresh water, Atlantic salmon compete with other conspecifics, grayling, brown trout, and brook trout. Carps, minnows, darters, perches, and similar fishes compete with Atlantic salmon in pools. It is difficult to characterize the extent of competitive interactions in marine waters due to the vast scale of the habitat that is used. Predators of smolt and juvenile salmon in fresh water include birds, reptiles, mammals, and other fish (including salmon and trout); predators in estuaries, coastal waters, and the sea include birds, fish, and mammals. In fresh water, juvenile salmon are opportunistic predators of invertebrates, especially those drifting at the surface (including mayflies, stoneflies, caddisflies, midges, and beetles). Larger parr eat fish (including smaller trout and salmon) and their eggs. In marine waters, post-smolts feed primarily on small fish and crustaceans such as euphausiids

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[119] Available at https://www.fisheries.noaa.gov/resource/document/recovery-plan-2019-gulf-maine-distinct-population-segment-atlantic-salmon-salmo; accessed December 8, 2023.

(krill), amphipods (scud), copepods, and crab larvae. Large juveniles prey mostly upon fish.

E.1.6. Domesticated Salmon

General practices used in salmon aquaculture are presented in this section; specific production and grow-out practices for AAS are described in Section 6.4 of this EA. This section of the appendix discusses information about the interaction of domestic salmon with their wild counterparts to provide context for predicting how ABT salmon might fare in the unlikely event that they would be released into the wild (Section 9).

E.2. Domesticated Atlantic salmon

E.2.1. Salmon Farming

Atlantic salmon farming can occur at locations throughout the world where there is access to clean, cold water. The greatest production currently occurs in Norway, Chile, Scotland, and Canada where smolts are typically grown to market size (generally 2 - 5 kg) in ocean net pens or cages. Other countries with significant production of Atlantic salmon include Australia, China, New Zealand, the Faroe Islands, and the US. Salmon farming industries rely on domesticated breeding lines selected for commercially important phenotypic traits, most importantly, faster growth and delayed sexual maturation (Gjedrem *et al.*, 1991). The oldest of these lines, developed in Norway and incorporated into virtually all commercial breeding programs (except those in eastern Canada which are based on a local line), achieved a growth rate improvement of about 10% per generation over the first seven generations of development (Gjøen and Bentsen, 1997).

Although Atlantic salmon can complete their entire life cycle in fresh water, most commercial Atlantic salmon farming involves both fresh and saltwater phases. In the freshwater phase, eggs are provided with a continuous flow of oxygenated water until they hatch. Typically, the alevin are transferred to small fiberglass tanks while they absorb the yolk sac prior to first-feeding. Once established on feed, the fry are transferred to larger tanks and grown to the parr stage, when they are sorted by size, segregated by growth rate, and transferred to separate tanks. In some locations, the parr may be transferred to lakes for the final phase of freshwater rearing. When the parr reach 60-120 g and begin to take on the silver coloration of smolt, they are typically transferred to saltwater production units called net pens or sea cages.

Under ambient light and temperature conditions, the freshwater phase typically takes 14-16 months, but is often shortened to eight months by increasing the early-rearing temperature and introducing a short period of darkness after the summer solstice to trigger smoltification at the next equinox (fall rather than spring) (McCormick *et al.*, 1987). Virtually all commercial smolt are vaccinated against pathogens of local concern to reduce the risk of disease, pathogen amplification, and the need for antibiotic treatment before transfer to sea water. The saltwater grow-out phase begins when the smolt are transferred to sea water and lasts for 12-26 months, depending on ambient sea temperature and the contingencies of harvest-to-order marketing. Feeding usually occurs twice a day, with feed generally moved by compressed air through tubes from a central hopper to each individual sea cage. The fish are fed until uneaten feed is detected by an underwater sensor.

E.2.2. Farmed versus Wild Atlantic salmon

1. Anatomy and Malformations

Morphologic irregularities occur in wildtype salmonids, most commonly affecting cartilaginous and boney structures (Brown and Núñez, 2010), and are often associated with the development of new commercial lines or husbandry techniques and culture conditions. Developmental malformations of cartilage and bone have been observed quite commonly in association with intensive commercial farming of salmon (*Salmo*) and trout (*Oncorhynchus*) species, including *S. salar* (Baeverfjord *et al*., 1996; Fjelldal *et al*., 2012; Silverstone and Hammell, 2002; Vågsholm and Djupvik, 1998), *S. trutta*, (Poynton, 1987), *O. mykiss* (Madsen and Dalsgaard, 1999; Mbuthia, 1994), and *O. keta* (Akiyama *et al*., 1986). They are also observed in salmonids in the wild (DeVore and Eaton, 1983). These malformations include irregularities of the head, jaw, and operculum, and twisting or compression of the spine. In farmed wildtype Atlantic salmon, vertebral deformities are now categorized into 20 different types, with those associated with fusions and compressions as the most common in harvest sized fish (Fjelldal *et al*., 2012). Although the incidence of these malformations has not been studied systematically, a background incidence of 3–5% is not uncommon in experimental control animals (Ørnsrud *et al*., 2004). Veterinary field studies have identified the periodic occurrence of spinal compression (humpback) in 70% of salmon in Norwegian farming operations (Kvellestad *et al*., 2000) and jaw malformation in 80% of salmon at commercial sites in Chile (Roberts *et al*., 2001). Nonetheless, aggregate data for the industry have not been reported, and the experience of individual commercial operations remains closely held. Such irregularities are not limited to salmonids and have been reported in the culture of other fish species.

2. Growth Rates

Growth rate is the most consistently observed trait difference between farmed and wild strains of Atlantic salmon (Glover *et al.,* 2017). Non-transgenic, farmed Atlantic salmon also grow at greater rates than wild Atlantic salmon, attributed to generations of breeding for this trait (Glover *et al.,* 2017). Bicskei *et al.* (2016) analyzed Atlantic salmon embryo transcriptomes and found that farmed salmon showed upregulation of mRNA translation, that were likely associated with this enhanced growth.

When farmed, wild, and hybrid Atlantic salmon were held under the same conditions, farmed salmon generally displayed the greatest growth (Glover *et al.,* 2017). Under hatchery conditions with unrestricted access to feed, Solberg *et al.* (2016) showed that at a typical hatchery temperature (12 °C) farmed salmon had the highest growth rate, followed by hybrid salmon and then wild salmon. Decreasing the temperature to “low” and “extra low” (5.6 °C and 3.9 °C, respectively) resulted in some growth overlap between strains, but wild salmon were consistently associated with the slowest growth (Solberg *et al.,* 2016). Hamoutene *et al.* (2017) observed wild, farmed, and hybrid Atlantic salmon under hatchery conditions and found that wild-type salmon had greater total lengths at hatch, but that this difference diminished over time and was no longer significant by 70 days post hatch. Farmed Atlantic salmon were found to be moderately larger (5-20%) than wild Atlantic salmon across all life stages in both hatchery and wild environments (Reed *et al.,* 2015). Skaala *et al.* (2019) observed a marginal growth advantage of domesticated Atlantic salmon in freshwater that became significant in the marine environment.

Generally, the observed growth difference between farmed and wild Atlantic salmon has been greater under hatchery conditions than in the natural environment (Glover *et al.,* 2017; Harvey *et al.,* 2016). For example, along the Norwegian coastline Bolstad *et al.* (2017) did not find an association between size at maturity in escaped domesticated or wild Atlantic salmon populations. Harvey *et al.* (2016) tested whether the diminished growth difference observed under wild conditions may be due to farmed fish adaptation to commercial fish food by analyzing the growth and survival of wild, farmed, and F1 hybrids fed one of three diets: salmon pellet diet, commercial carp pellet diet, or mixed natural diet. Farmed fish grew larger than wild salmon under all dietary conditions and while the growth difference was lowest (1:1.6) for salmon fed the mixed natural diet, dietary effects were not statistically significant. The authors concluded that farmed fish have not adapted to commercial diet but rather have increased appetite which drives domesticated Atlantic salmon to have higher growth rates compared to wild populations under hatchery conditions (Harvey *et al.,* 2016).

3. Metabolism

Several studies have also shown upregulation of metabolic processes in non-transgenic farmed Atlantic salmon (Bicskei *et al.,* 2014; Glover *et al.,* 2017). Robertsen *et al.* (2017) hypothesized that the lower survival rates observed for farmed salmon compared to wild salmon in natural conditions was related to higher standard metabolic rates (SMR). However, when the embryo SMRs of farmed, wild, and hybrid Atlantic salmon were compared in the laboratory, the results were inconclusive (Robertsen *et al.,* 2017).

4. Tolerance to Physical Factors

Solberg *et al*. (2016) hypothesized that farmed fish, bred at elevated temperatures, would have reduced tolerance to cold temperatures during early development. The results did not support this theory, as 35 families of farmed, wild, and farmed-wild hybrid Atlantic salmon were shown to successfully transition from alevin to fry under different temperature regimes (3.9 °C, 5.6 °C, and 12 °C), irrespective of origin (Solberg *et al.,* 2016).

5. Smoltification and Osmoregulatory Capacity

Most salmonid species spend one-half to three years in freshwater before migrating to a saltwater environment (Wong *et al*., 2019). The parr-smolt transformation, or smoltification, is the development of physiological, morphological, and behavioral characteristics that allows salmon to transition from a freshwater to saltwater environment. During smoltification, morphological and physiological changes occur, such as a change in Na^+, K^+- ATPase (NKA) activity, that allow salmon to tolerate an increase in salinity (McCormick *et al*., 2003; Urke *et al*., 2014b, Franklin *et al*., 1992; McCormick and MacKinlay, 1998; McCormick *et al*., 1999). A change in ion activity is indicative of saltwater tolerance and has been found in wild salmon and hatchery reared salmon (Grant *et al*., 2009; McCormick *et al*., 1999; McCormick *et al*., 2003; Urke *et al*., 2014b; Franklin *et al*., 1992; McCormick and MacKinlay, 1998) and coincides with downstream migration in hatchery-released and wild salmon populations (Urke *et al*., 2014a; Urke *et al*., 2014b).

Hatchery and wild pink salmon (*Oncorhynchus gorbuscha*) smolts exposed to saltwater challenges (i.e., fish previously only exposed to freshwater are rapidly introduced to saltwater) have both demonstrated hypo-osmoregulatory capacity (Grant *et al*., 2009; Grant *et al*., 2010). Pink salmon smolts subjected to a saltwater mesocosm experiment for 22 weeks showed that hatchery and wild fish had similar salinity tolerance by their Na^+ and Cl^- whole body concentrations and NKA levels (Grant *et al*., 2009).

Post-smolt hatchery Atlantic salmon maintained in salt water that were subjected to a freshwater challenge (i.e., fish that were maintained in salt water for a year and then introduced to fresh water) showed low mortality and significantly lower but normal plasma Na^+ and Cl^- concentrations (Bakke *et al*., 1991). Although hatchery fish demonstrate salinity or freshwater tolerance in mesocosm challenges, these results do not directly translate to the survival of hatchery fish in the wild (Heard *et al*., 2013; Jensen *et al*., 2016; Morera *et al*., 2021). Hatchery-reared smolts showed acceptable plasma chloride levels (mean ± S.D. = 123.4 ± 10.0 mM) and limited mortalities in a 24-hour, 34 ppt seawater challenges (Jensen *et al*., 2016). However, Jensen *et al*. (2016) found that stocked Atlantic salmon had reduced growth, age of maturity and survival compared to wild Atlantic salmon when released into the natural environment. For example, the mean return rate of post-smolt Atlantic salmon from marine to freshwater was 2.35% (range = 0.77% - 5.40%) for wild Atlantic salmon and 0.98% for hatchery-reared Atlantic salmon (range = 0.44% - 2.69%) (Jensen *et al*., 2016). Additionally, Morera *et al*. (2021) discussed deficient smoltification as a primary cause of mortality in Chilean Atlantic salmon aquaculture, with 11.2% of deaths attributed to fish experiencing problems with osmoregulation.

In a direct comparison of the smoltification of farmed (AquaGen) and wild-type Atlantic salmon (Namsen and Alta), farmed salmon were shown to undergo smoltification a month earlier than wild-type salmon in the same maintained, freshwater environment, indicating that the smoltification process of wild salmon has adapted to the local environment (Strand *et al*., 2007). The change in smoltification and salinity tolerance associated with genetic differences arising from generations of breeding decreases the likelihood that farmed salmon will survive seawater migration (Handeland *et al*., 2003; McCormick 1994). Hybrids of farmed and wild type Atlantic salmon are as likely to survive seawater migration in very cold temperatures as their wild counterparts (Hamoutene *et al*., 2015).

Although wild and hatchery-reared Atlantic salmon smolts may differ in their survival in the natural environment, the loss of salinity tolerance for both types are highly influenced by temperature (Bernard *et al*., 2019; McCormick, 1994; McCormick *et al*., 1999; McCormick *et al*., 2003). If significant increases of both temperature and salinity occur simultaneously, this combination can be fatal (Vargas-Chacoff *et al*., 2018). Exposure to higher water temperatures, largely above 16 °C, reduces salinity tolerance in both hatchery-reared and wild smolts (Bernard *et al*., 2019; McCormick and Bjornsson, 1994; Vargas-Chacoff *et al*., 2018).

The timing of transportation, or the movement of fish from one hatchery to another, can also influence salinity tolerance in salmon (Stewart *et al*., 2017). Chinook salmon (*Oncorhynchus tshawytscha*) that were transported to the experimental facility only 3 weeks prior to a salinity challenge had significantly higher mortality rates in a 24-hour, 34 ppt saltwater challenge compared to fish that were transported to the facility >2 months prior to the experiment (Stewart *et al*., 2017).

6. Behavior

Behaviors associated with swimming, feeding, reproduction, territorial defense, migration, or other developmental events could be affected by genetic engineering. The ecological impacts of these changes in behaviors could affect life history patterns, population dynamics, and species interactions (ABRAC, 1995).

In experiments with artificial conditions, farmed salmon have displayed decreased predator awareness compared to wild salmon, attributed to the effects of breeding in an environment without predator selection pressure (Glover *et al.,* 2017). Under controlled hatchery conditions, farmed fish spend less time in refuges than their wild-type counterparts and demonstrate a smaller reduction in growth than wild salmon in the presence of a predator, potentially leaving farmed salmon more vulnerable to predation (Glover *et al.,* 2017).

Studies conducted under seminatural conditions with artificial predators, however, have not shown a clear relationship between domestication and predator risk (Glover *et al.,* 2017; Solberg *et al..* 2015). A study by Solberg *et al.* (2015) investigated the reaction of farmed, wild, and hybrid Atlantic salmon to an artificial predator (a net) under “seminatural” conditions intended to mimic Atlantic salmon spawning areas. No association between salmon type and predator susceptibility was observed, indicating either anti-predator behavior was consistent between salmon types or that the experimental setup was not appropriately representative of natural or predator conditions (Solberg *et al.,* 2015). While there is evidence that predation is a factor in the decreased survival associated with farmed fish released to the environment, the causal relationship has not been established (Glover *et al.,* 2017).

Hybrid, farmed, and wild-type juvenile Atlantic salmon have been shown to successfully compete for food resources under seminatural conditions, indicated by similar growth rates. Robertsen *et al.* (2017) did not observe differences in the growth of juvenile wild and domesticated-wild hybrid Atlantic salmon under conditions of either high or low food availability. Comparable growth was also observed for wild and farmed Atlantic salmon while they were competing for food resources in the presence of an artificial predator (Solberg *et al.,* 2015).

Domestication affects risk behaviors in Atlantic salmon. Farmed (non-transgenic) Atlantic salmon strains of both North American and European origins were more explorative, bold, and aggressive than the wild North American (Saint John River) strain and related hybrids (Islam *et al.,* 2020). Domesticated strains of Atlantic salmon in Norway exhibited behaviors which increased susceptibility to predation and lowered survivorship in semi-natural conditions, but their hybrids showed similar survival to wildtypes (Solberg *et al.,* 2020).

In addition, hatchery-rearing alone has been shown to affect the behavior of Atlantic salmon, including migration behavior in the wild. Non-transgenic hatchery-reared Atlantic salmon are associated with weakened imprinting compared to wild Atlantic salmon (Moe *et al.,* 2016; Skilbrei *et al.,* 2015). Following a large-scale release of Atlantic salmon during a simulated Norwegian hatchery escape conducted by Skilbrei *et al.* (2015), post-smolts were recaptured over a large geographic area, indicating a weaker homing mechanism. In another study conducted in Norway, the movements of wild and escaped Atlantic salmon were tracked during spawning in the Namsen river system (Moe *et al.,* 2016). While wild salmon were distributed throughout the river, escaped salmon were primarily in the upper section, indicating that farmed salmon have not imprinted to a specific area and lack a "stop signal" during migration (Moe *et al.,* 2016). Another small study of wild and domesticated Atlantic salmon crosses in Norway showed decreased homing accuracy in hatchery releases and hybrids (Jonsson and Jonsson, 2017).

7. Impact of Disease and Parasites

Analysis of the transcriptomes of Atlantic salmon embryos and fry provide some evidence that farmed salmon have weaker immune responses than wild salmon, as domestication was associated with down-regulation of pathways related to immune function (Bicskei *et al.,* 2016; Bicskei *et al.,* 2020). Previous studies under controlled hatchery conditions have shown that farmed salmon, compared to wild-type salmon, are more susceptible to salmon lice, less susceptible to vibriosis, and equally susceptible to furunculosis and infectious salmon anemia virus, indicating susceptibility is situational and disease-specific (Glover *et al*., 2004; Glover and Skaala, 2006; Bergh *et al*., 2006; Skar *et al*., 2006; Lawlor *et al*., 2009; as reviewed in Glover *et al.,* 2017).

The exposure of wild Pacific salmon to farmed Atlantic salmon may result in the transmission of diseases to wild populations. A study conducted in British Columbia showed that the piscine orthoreovirus (PRV) infection rate of wild-type pacific salmon increased with the density of fish farms in the area (Morton *et al.,* 2017). PRV can result in lethargy and erratic swimming behavior, diminishing the ability of salmon to migrate. Taranger *et al.* (2015) conducted a risk assessment of Norwegian Atlantic salmon farming that included determining the likelihood of virus transmission to wild salmon populations. The probability of transmission was virus- specific, with the likelihood characterized as low for piscine myocarditis virus, low to moderate for salmonid alphavirus, moderate for infectious pancreatic necrosis, and likely for PRV (Taranger *et al.,* 2015).

8. Life History

Independent of GH, hatchery-rearing has been shown to influence the developmental stages of Atlantic salmon. Under hatchery conditions with river water, Hamoutene *et al.* (2017) observed salmon with wild-type maternal parentage (both wild-type and wild-farmed hybrid) hatching a week earlier than salmon with farmed maternal parentage (both farmed and farmed-wild hybrid). Yates *et al.* (2015) compared the male parr maturation of domesticated-wild hybrid salmon with wild-type salmon and found that while the maturation timing of the Atlantic salmon strains was independent of fish origin, the proportion of salmon that underwent maturation was lower for hybrid than for wild salmon. Weigel *et al.* (2019) observed the migration timing of wild and hybrid steelhead populations in the Willamette Basin, Oregon to be origin-dependent. Skaala *et al.* (2019) also observed differences in migration timing, with domesticated and hybrid Atlantic salmon migrating from freshwater (the River Guddalselva in Western Norway) to seawater about 12 days earlier than wild smolts.

9. Acute Stress Response

Non-transgenic hatchery-reared fish are believed to have developed a greater stress tolerance than wild Atlantic salmon. Solberg *et al.* (2015) conducted an experiment where both wild and farmed Atlantic salmon were held in large tanks intended to mimic natural spawning areas. Only wild salmon died of undefined causes, indicating wild salmon may be experiencing higher stress levels under the experimental conditions. Bicskei *et al.* (2020) conducted a study to investigate the relative stress response of wild and farmed Atlantic salmon by determining transcriptomic differences between farmed and domesticated offspring under hatchery conditions with a crowding stressor. Functional analysis showed origin-dependent differences in stress response pathways and found that wild salmon were associated with a higher number of pathways that were responsive to stress (Bicskei *et al.,* 2020).

E.2.3. Interactions Between Non-GE Domesticated and Wild Salmon

This section will discuss the interactions between domesticated Atlantic salmon without an IGA and wild salmon. Four general areas of potential interaction between natural salmonid populations and escaped, farm-reared, non-genetically engineered fish that could conceivably lead to environmental impacts:

- Transfer of exotic pathogens or amplification of endemic pathogen loads (Saunders, 1991; McVicar, 1997);
- Genetic disturbance caused by transmission of fitness-reducing alleles (Ryman and Utter, 1987; Frankham, 1995), disruption of locally-evolved allelic combinations (Templeton, 1986; Ryman *et al*., 1995; McGinnity *et al*., 2003), or "swamping" of the native gene pool (Sægrov *et al*., 1997);
- Direct competition for environmental resources, such as habitat, food, or mating opportunities (McGinnity *et al*., 1997; Fleming *et al*., 2000); and
- Ecological disturbance through interference competition or disruption of local equilibria in complex systems, such as food webs, predator-prey relationships, or migration patterns (Lacroix and Fleming, 1998).

To provide additional context for potential application to AAS, each of these potential interactions is discussed in more detail below.

1. Pathogen Transfer

Documented examples of pathogen transmission between artificially-propagated and wild fish are not common, but have been known to occur through stock enhancement programs involving transfer of live fish and eggs (Brackett, 1991). For example, several incidents in the late 1980s suggest circumstantial involvement of farmed salmon in the movement of an endemic bacterium, *Aeromonas salmonicida*, which causes furunculosis, from Scotland to Norway (Johnsen and Jensen, 1994; Inglis *et al*., 1991). There is little direct evidence of bacterial disease transmission from commercial to wild salmon. None of the reviews that have evaluated the available scientific literature on the potential for disease interchange between wild and farmed salmon has found irrevocable evidence that fish farming has contributed to detectable adverse changes in wild fish populations (McVicar *et al*., 2006).

When wild fish are exposed to pathogens shed from farmed fish, it is not inevitable that infection or disease will occur in the wild fish population (Olivier, 2002). Critical factors affecting the spread of disease include:

- The occurrence and persistence of the infection in the source population;
- The availability of susceptible potential new hosts;
- The viability and concentration of the infectious organism in the environment; and

- The ability of the infection to affect the recipient population from individual fish infections.

The initial risk level of infection in wild fish associated with escaped farmed fish depends on the length of survival, behavior of the escaped fish after leaving the farm, and the reduced disease transmission opportunity in the lower fish densities outside of the farm (McVicar *et al*., 2006). In general, farmed fish are considered less fit or maladapted for survival in the wild (Fleming *et al*., 2002). In the event of escape, the presence of disease, if it occurs, would be expected to lead to the early disappearance of the most seriously affected fish, thus rapidly limiting the spread of disease transmission.

In contrast to disease transfer, the transmission of parasites by cultured fish is less subject to debate (McVicar *et al*., 2006). The introduction of *Gyrodactylus salaris* (the salmon fluke) to Norwegian waters in 1975 has been clearly linked to resource management activities (Johnsen and Jensen, 1991), but the role of farmed salmon in the subsequent epidemiology remains under investigation (Bakke and Harris, 1998). Salmon lice, *Lepeophtheirus salmonis*, are endemic throughout the native range of Atlantic salmon, making a direct link to salmon aquaculture difficult to establish. White (1940) associated the occurrence of “white spot” and salmon mortalities with sea lice infections in wild Atlantic salmon populations in eastern Canada as early as 1940, well before the advent of commercial salmon farming. Natural populations of parasites may be amplified in areas associated with salmon farming (Bakke and Harris, 1998), but sea lice abundance may be associated with rising marine temperatures as much as with the availability of hosts.

2. Genetic Disturbance

Atlantic salmon have been subject to significant selection pressure, both intentional and inadvertent, as a result of human activity for more than a century. The former include, but are not limited to, size-selective harvesting, stock-enhancement efforts, transplantation across drainages and ecosystems, and increasing importance of commercial and recreational objectives; the latter derive (in part) from hydro-electric dams, acid rain, agricultural (and other) run-off, increased sedimentation and water temperature due to deforestation, and stocking of native (striped bass) and non-native (rainbow & brown trout) salmonid predators. Despite these challenges, evidence of genetically-differentiated population structuring is still evident for salmon at local, regional, and continental scale based on allozyme, mitochondrial, and nuclear DNA analyses (Ståhl, 1987; Bourke *et al*., 1997; Bermingham *et al*., 1991; McConnell *et al*., 1995; Taggart *et al*., 1995; King *et al*., 2001). The temporal stability of this structure has been traced over decades through the analysis of genetic material contained in archived scales (Nielsen *et al*., 1997; Tessier and Bernatchez, 1999).

Farmed salmonid strains are typically genetically distinct from local wild populations because of breeding and selection practices that have been designed primarily to optimize growth rates and other commercially desirable traits. As a result, many farmed strains used in Ireland and Scotland are of Norwegian origin. Escaped farmed salmon can interbreed with local populations, intermixing their genomes with the locally adapted populations (Teufel *et al*., 2002). The persistence of genetic population structuring, even in the extreme circumstance of low population abundance and significant management intervention, indicates a degree of genetic resilience in locally-adapted wild populations (NRC, 2004a). Evidence of such persistence in nearly-extirpated Atlantic salmon populations raises doubt about the capacity of cultured salmon (ranched, farmed, or genetically-engineered) to undermine even small populations of wild salmon over time through genetic introgression or parallel colonization.

In agricultural breeding programs, including aquaculture, breeders must strike a balance between inbreeding within population that appear to be well-suited to an environment, or that may possess certain traits of interest, and "outbreeding" or the introduction of new traits by introducing distinct parental lineage. "Inbreeding" refers to mating between individuals more closely related than those drawn by chance from the general population, which can often result in a decrease in fitness. "Outbreeding" refers to mating between individuals from different populations, which can either increase (enhance) or decrease (depress) fitness relative to both parental genotypes. Outbreeding depression can be the result of poor adaptation of the hybrid to the environment (e.g., the hybrid inherits a combination of traits that make it less suitable for that environment than either parent) or of the combination of alleles in the hybrid to each other. Outbreeding depression has been observed in an Irish experiment with first- and second-generation offspring of wild and farmed Atlantic salmon (McGinnity *et al*., 2003) and in hybrid offspring produced by the crossing of anadromous and landlocked Atlantic salmon (Sutterlin *et al*., 1987).

The degree of introgression of domesticated Atlantic salmon genotypes into wild populations appears highly dependent on the number of aquaculture escapes and the number of escapes relative to the size of the wild population (Glover *et al.,* 2019; Karlsson *et al.,* 2016; Keyser *et al.,* 2018; Wringe *et al.,* 2018). Modeling studies indicate that escapes need to exceed 10% of the local population to impact wild populations (Bradbury *et al.,* 2020). Experience from escapes of Atlantic salmon and subsequent hybridization with wild populations further demonstrate that without continued supply of escapes into the wild population, the presence of hybrids decreases over time (Wringe *et al.,* 2018). Keyser *et al.* (2018) used data from aquaculture production sites, reports of escaped salmon, and estimates of introgression to model the distribution of escaped salmon in the Northwest Atlantic region and associated genetic effects. The model found that the abundance of escaped salmon and predicted introgression levels increased with proximity to areas with more intense aquaculture production sites (Keyser *et al.,* 2018).

3. Direct Competition for Resources

Although domesticated Atlantic salmon have been known to survive and breed successfully in the local environment after escaping from confinement (Lura and Sægrov, 1991; Webb *et al*., 1991), only a small proportion of the number that escape from farms actually breed (Webb *et al*., 1993; Clifford *et al*., 1998), and then at a fraction of the spawning rate of wild Atlantic salmon (Fleming *et al*., 1996; Clifford *et al*., 1998). There are two primary reasons for this:

- **Although socially dominant in culture environments, farmed Atlantic salmon are subordinate in nature:** salmon form dominance hierarchies around foraging opportunities; farmed salmon establish their social status in confinement where foraging opportunities differ significantly from those in the wild. In nature, despite the imposition of dominance by large fish, there is a residual "resident advantage" held by the wild fish that deters even the largest fish from evicting territory holders from home ground; and

- **Farmed salmon compete poorly for mates and spawning locations:** males are particularly disadvantaged in both access to mating opportunities and breeding success (Fleming *et al*., 2000); farmed females enter rivers out-of-phase with wild salmon, make fewer, poorly-covered nests, breed for a shorter period of time, and retain more eggs that remain unfertilized (Jonsson *et al*., 1997; Webb *et al*., 1991).

Consequently, even when they are within their "home range", the reproductive success of escaped, domesticated Atlantic salmon from spawning to F1-adult return ranges only from 2-19% (Clifford *et al*., 1998; Fleming *et al*., 2000; McGinnity *et al*., 2003) of that achieved by wild Atlantic salmon; the additional loss of 68% of eggs in the F2-generation is a further barrier to successful introgression or establishment of escaped farmed salmon within or coexistent with natural populations (McGinnity *et al*., 2003).

4. Ecological Disturbance

Ecological disturbance includes community disturbances such as interference competition or disruption of local equilibria in complex systems, such as food webs, predator-prey relationships, or migration patterns (Lacroix and Fleming, 1998).

Although farmed salmon have been known to enter marine systems in large numbers by escape from containment nets, they can only become established by reproducing in adjacent freshwater ecosystems. Consequently, the fitness and behavior of feral Atlantic salmon is of continuing interest as a matter of risk management in Atlantic salmon aquaculture, specifically with respect to the extent to which any homing migration imprinting may have occurred, the extent to which feral Atlantic salmon succeed in spawning, and the relative survival of their offspring. Escaped farmed salmon feed poorly in fresh and salt water and may not begin feeding on wild prey for a considerable period after escape owing to their acclimation to pelleted feed. For example, only 5-15% of escaped Atlantic salmon recovered from British Columbian and Alaskan waters had fed after their release (Alverson and Ruggerone, 1997).

One key risk parameter, the number of animals escaping containment, is difficult to establish with certainty due to inconsistencies in reporting, lack of long time-series, decomposition of small fish that die in sea cages, and limited data collection on escapees at sea. One generally accepted estimate of escapees from sea cages in the North Atlantic is approximately 2,000,000 Atlantic salmon (McGinnity *et al*., 2003). This number represents an escape rate of about one percent. Less than two percent of wild Atlantic salmon currently return to spawn at their natal streams. Escaped farmed salmon survive marine conditions and migration at one-third to one-half of the rate for wild Atlantic salmon and return to fresh water at about 1% of the numbers that are estimated to escape (Butler *et al*., 2005).

E.3. References

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Appendix F. Phenotypic Characterization of AquAdvantage Salmon

This section discusses the phenotype of AAS relative to wildtype hatchery- or farm-raised Atlantic salmon to help characterize its fitness. Most of this information was previously presented in Section 5.2 of the 2015 EA and was updated in the 2018 EA for the Indiana facility and the 2019 EA for the Rollo Bay facility. It is presented again here, supplemented with newly published information as well as relevant information not previously included. In general, the phenotypic characteristics of AAS are similar to those of farm-raised Atlantic salmon without an IGA (referred to as non-GE herein), the major exception being the enhanced growth rate of AAS, particularly in the early pre-smolt life stage.

Any consideration of the fitness of Atlantic salmon, regardless of its status with respect to genetic engineering, requires understanding that in general, Atlantic salmon display a high degree of phenotypic plasticity and have a complex life history that enable them to adapt to variable conditions and rigorous environments. In addition, GxE interactions will produce different phenotypes when animals with the same genetic background are exposed to different environmental conditions. Given the high degree of phenotypic plasticity of Atlantic salmon, and the impact of GxE interactions, it is not surprising that the wide spectrum of traits observed in wildtype Atlantic salmon generally encompasses those of AAS.

F.1. Comparative Studies

Multiple studies have been conducted by ABT comparing AAS to farm-raised Atlantic salmon. Data and information published in peer-reviewed journals, which may include comparisons to wild Atlantic salmon, are also considered. In a few instances, when potentially relevant, results have also been included from studies that have been conducted in other GE Atlantic salmon, including diploid, mixed-sex GE GH Atlantic salmon that contain the same rDNA construct as in AAS and are derived from the same lineage. These salmon are considered to be relatives of AAS, and thus, these studies are considered to be highly relevant for the analysis in this EA.

A discussion of additional studies on other species of salmon that have been genetically engineered with a growth hormone construct, most notably coho salmon (*Oncorhynchus kisutch*), a species of salmon found in the Pacific Ocean, are contained in Appendix G. These studies are considered less relevant for the analyses in this EA because not only is the coho salmon a different species (and of a different genus), but this GE fish contains a different GH construct than the one in AAS (i.e., the sockeye salmon growth hormone under the control of the metallothionein-B promoter of the same species (Mori and Devlin, 1999), and this construct is at a different location in the salmon's genome.

F.1.1. Nutritional and Hormonal Composition

As discussed in the 2015 EA, the nutritional and hormonal composition of AAS and skin is similar to that of present-day farm-raised Atlantic salmon. See 2015 EA, Section 5.2.1.1.

F.1.2. Gross Anatomy, Histopathology, and Clinical Chemistry

The gross anatomy, histopathology, and clinical chemistry of male and female, triploid AAS and size-matched, wildtype comparator salmon were evaluated in an identity-masked, controlled study. Normal behavior was observed in all groups of fish. Eight physical features were evaluated; the incidence of abnormalities was similar for triploid AAS and the wildtype comparators, with the number of abnormal findings being greater for triploid fish (both GE and wildtype) than for diploid fish, especially with regard to irregularities in gill structure. An examination of nine internal organs or structures, as well as relative organ weights, revealed no differences between GE and wildtype salmon or between diploid and triploid salmon. The pathology findings associated with the rDNA construct were limited to an increased presence of minimal-to-mild focal inflammation of unknown cause in some tissues, especially among diploid fish, and a low occurrence of jaw erosions among both male and female diploids. Most of the other findings, which included gill and fin abnormalities, soft tissue mineralization, hepatic vacuolization, and cardiac shape abnormalities, affected the triploids of both groups. In aggregate, these findings were generally of low magnitude, limited distribution, and non-debilitating nature; they were deemed unlikely to compromise the overall health of AAS in commercial production.

In this study, almost all of the values for hematology and serum chemistry parameters of AAS were consistent with published values that represent the normal range for Atlantic salmon. The statistically significant differences that were observed are believed to be related to the inherent difference in metabolic rates between AAS and comparator salmon, the effect of triploidy on red cell number and size, and unavoidable limitations in study design.

In the same comparator-controlled study, no severe malformations were noted among the AAS and diploid EO-1α salmon enrolled. Irregularities in the fins and gill structure of triploid AAS as well as triploid wildtype salmon were noted, while diploids in both groups had a low incidence of jaw erosion. The observed abnormalities are within the range of frequency and severity commonly noted in cultured salmonids, as described in the following paragraphs.

Morphologic irregularities occur in wildtype salmonids, most commonly affecting cartilaginous and boney structures (Brown and Nunez, 2010), and are often associated with the development of new commercial lines or husbandry techniques and culture conditions. Developmental malformations of cartilage and bone have been observed quite commonly in association with intensive commercial farming of salmon (*Salmo*) and trout (*Oncorhynchus*) species, including *S. salar* (Baeverfjord *et al.*, 1996; Fjelldal *et al.*, 2012; Silverstone and Hammell, 2002; Vågsholm and Djupvik, 1998), *S. trutta*, (Poynton, 1987), *O. mykiss* (Madsen and Dalsgaard, 1999; Mbuthia, 1994), and *O. keta* (Akiyama *et al.*, 1986). They are also observed in salmonids in the wild (DeVore and Eaton, 1983). These malformations include irregularities of the head, jaw, and operculum, and twisting or compression of the spine. In farmed wildtype Atlantic salmon, vertebral deformities are now categorized into 20 different types, with those associated with fusions and compressions as the most common in harvest sized fish (Fjelldal *et al.*, 2012). Although the incidence of these malformations has not been studied systematically, a background incidence of 3–5% is not uncommon in experimental control animals (Ørnsrud *et al.*, 2004). Veterinary field studies have identified the periodic occurrence of spinal compression (humpback) in 70% of salmon in Norwegian farming operations (Kvellestad *et al.*, 2000) and jaw malformation in 80% of salmon at commercial sites in Chile (Roberts *et al.*, 2001). Nonetheless, aggregate data for the industry have not been reported, and the experience of individual commercial operations remains closely held. Such irregularities are not limited to salmonids and have been reported in the culture of other fish species.

Neither intensive selection for growth nor inbreeding depression are deemed responsible for these morphologic irregularities (Baeverfjord *et al.*, 1996), which have been linked more commonly to suboptimal culture conditions (e.g., nutrition, water quality, and environmental stressors). In general, mild-to-moderate malformations of the head, jaw, operculum, or spine have limited impact on morbidity or mortality when other rearing conditions are optimized. Rearing conditions that are otherwise deficient and present significant environmental stressors can lead to the increased mortality of these fish.

1. AquAdvantage Relatives

Tibbetts *et al.* (2013) reported on the growth and nutrient utilization of GH Atlantic salmon (both diploid and triploid) fed a practical grower diet (see following section for a description of results related to growth). The study included a skeletal bone analysis, as well as an appearance assessment conducted using a ranking system (1= no obvious skeletal disorder, marketable; 2 = minor skeletal disorder, marketable; and 3 = major marketable disorder, unmarketable). The overall occurrence of major skeletal disorders (rank = 3) was low (<4%) in all salmon regardless of ploidy or whether or not the fish contained the GH transgene. Triploid salmon had a slightly higher prevalence of major skeletal disorders (2.9% for wildtype; 3.7% for GH) than diploids (0.3% for wildtype; 0.9% for GH). These results are very similar to those presented by Fjelldal and Hansen (2010) for vertebral deformities in diploid and triploid wildtype Atlantic salmon underyearling smolts (triploids 1–3%; diploids 0–1%) and suggest that triploidization has a greater effect than transgenesis on the malformation rate, although neither had a substantial effect on producing skeletal disorders that would make the salmon unmarketable.

F.1.3. Growth Rates

The main difference between AAS and wildtype Atlantic salmon, and the basis for the value of the product, is the significant increase in growth rate of the former. Studies of early-generation GH salmon conducted in academic settings deriving from the program that led eventually to identification and development of the EO-1α line provided estimates of growth rate that were two- to six-fold greater than wildtype comparators during the first year of life (Du *et al.*, 1992). A comparator-controlled study of growth performance in F6-generation AAS confirmed their significant growth advantage over a period of approximately 2,700 °C-day in both average size (261.0 g versus 72.6 g for diploid controls) and proportion of animals larger than 100 g (98.6% versus 4.9% for diploid controls). Data from this study are summarized in Figure 3 in the 2015 EA.

1. AquAdvantage Relatives

Tibbetts *et al.* (2013) also reported on the growth and nutrient utilization of GH Atlantic salmon (with a single copy of the EO-1α gene construct), both diploid and triploid, compared to full- sibling, size-matched wildtype Atlantic salmon, both diploid and triploid. GH salmon consumed a significantly higher amount of feed daily, resulting in a three-fold increase in target weight gain in less time than wildtype fish. GH Atlantic salmon also had enhanced specific growth rates (%/day), higher thermal growth coefficients (⅓/degree day), better feed conversion ratios, and higher nitrogen retention efficiencies. As a result, the overall total amount of feed required to produce the same fish biomass was reduced by 25% in GH fish. Feed intake was lower in triploid GH salmon compared to diploid GH salmon, but feed efficiency, digestibility and nutrient retention efficiencies were equal to those of GH diploids. In addition, without exception, GH triploids out-performed their related wildtype counterparts regardless of ploidy.

2. Other Factors

The effect of ploidy alone on growth rates has also been examined in salmonids, see Appendix H for additional information. The effects of ploidy (i.e., triploid vs diploid) depend on the age and species evaluated, but triploid salmon generally exhibit equal or reduced growth relative to diploids. For example, non-GE all-female triploid populations of Atlantic salmon reared under optimal conditions in freshwater were significantly lower in weight at the late alevin and fry stages but were similar in weight to diploids at the parr stage (Nuez-Ortín *et al.* 2017). In addition, growth rates in Atlantic salmon may vary between farmed (hatchery-reared) salmon and wild salmon, see Appendix E.

F.2. Other Phenotype and Fitness Characteristics

Rapid-growth phenotypes, including those produced in domesticated Atlantic salmon through selective breeding, appear to share several key physiological and behavioral attributes regardless of breeding methodology, including: the use of a common endocrine pathway to accelerate growth; elevated metabolism, feeding motivation, and efficiency; increased aggression and foraging activity; and reduced anti-predator response (in farm-raised Atlantic salmon (Fleming *et al.*, 2002); in early-generation, GH Atlantic salmon (Abrahams and Sutterlin, 1999; Cook *et al.*, 2000b), and in multiple species of growth-accelerated GE fishes (Devlin *et al.*, 2015)). Differences appear to occur in the scale of trait expression rather than in the scope or character of the trait expressed.

The extent to which the “fitness” of AAS has been altered relative to comparator Atlantic salmon can be estimated by the evaluation of the following phenotypic changes, as suggested by Kapuscinski and Hallerman (1991):

1. Metabolic rate;
2. Range of tolerance values for physical factors;
3. Behavior;
4. Resource or substrate use; and,
5. Resistance to disease, parasites, or predation.

If AAS were to escape into an uncontained environment, these factors could affect the fitness of the escaped AAS, their potential for survival and establishment, and their interactions with other organisms and the ecosystem. Other potential factors include morphology and limits to growth, reproduction, life history, and acute stress response. Information about each of these factors is presented in the following sections.

F.2.1. Metabolic Rates

Metabolic rates influence the components of the overall energy budget for an individual; the components of the energy budget in turn influence an individual's impact on nutrient and energy flows, and other organisms. The distinguishing feature of AAS is rapid growth, which is an integrated composite of many physiological rates. AAS exhibit growth and behavioral traits that also appear in other fast-growing Atlantic salmon or in brown trout that have been treated with time-release GH implants (Johnsson and Björnsson, 2001). Selection for faster growth in domesticated Atlantic salmon is generally associated with increases in pituitary and plasma GH levels (Fleming *et al.*, 2002). However, such increases are also observed in wild salmon during winter famine, smoltification, and sexual maturation (Björnsson, 1997). The only unique attributes of GH fish appear to be an increase in the magnitude of trait expression associated with the increase in growth rate when food is available, and the allocation of energy to growth that occurs at the expense of stored reserves (Cook *et al.*, 2000c).

Polymeropoulos *et al.* (2014) studied the effects of both GH transgenesis and polyploidy in Atlantic salmon on metabolic, heart, and ventilation rates and heat shock protein response. The experiments were conducted on alevins of AAS reared at the Bay Fortune, PEI facility. Mass-specific metabolic rates were increased under both normal and hypoxic conditions as compared to diploid wildtype alevins. However, this was not reflected in improved oxygen uptake through heart or ventilation rate or in altered heat shock protein responses under normal oxygen conditions. Under severe hypoxic conditions, ventilation rate decreased in both diploid wildtypes and triploid transgenics. The findings of this study show that cardiorespiratory functions under oxygen-limiting conditions are altered in early development of Atlantic salmon by the combination of GH transgenesis and induced triploidy. Hypoxia did not induce a cellular stress response, which may have a negative effect on the ability of the fish to deal with harsh environments.

1. AquAdvantage Relatives

The expression of GH alters aggregate metabolic activity in several ways: lipid breakdown and mobilization are increased, and energy is deployed more readily for maintenance or growth; protein synthesis is increased, providing the raw material for additional body mass; mineral uptake is increased, promoting skeletal development and a longer, leaner morphology; and, feeding efficiency (i.e., feed conversion ratio) is improved (Björnsson, 1997). The cost to the animal is higher oxygen utilization due to increased digestive demand and protein synthesis. In comparison to wildtype comparators, GH Atlantic salmon had lower initial energy reserves, 2.1- to 2.6-fold greater feed consumption, and a propensity to deplete body protein, dry matter, lipids, and energy more quickly during starvation (Cook *et al.*, 2000a; Cook *et al.*, 2000b). Routine oxygen uptake in GH Atlantic salmon was 1.7 times that of controls (Stevens *et al.*, 1998) and oxygen consumption during activity was 1.6-fold greater, further increasing with effort (Stevens and Sutterlin, 1999).

Although these GH Atlantic salmon have demonstrated an ability to reduce their metabolic rate in response to starvation, their enhanced metabolic profile and lower initial energy reserves would greatly reduce the likelihood of their growing rapidly, or even surviving, outside of the highly supportive conditions provided by commercial farming (Hallerman *et al.*, 2007).

F.2.2. Tolerance of Physical Factors

Tolerance of physical factors such as temperature, salinity, oxygen, and pH, can potentially be altered in GE organisms. If an increased tolerance of these factors is sufficiently large, changes in lethal limits or optimum values could possibly shift or change preferred habitats, seasonal patterns, and/or the organism's geographic range.

Although specific information addressing these potential changes is limited for AAS, studies in relatives have shown that oxygen consumption in adult GH Atlantic salmon is higher than in wildtype comparators (Abrahams and Sutterlin, 1999; Cook *et al.*, 2000b; Cook *et al.*, 2000c; Deitch *et al.*, 2006). In contrast, oxygen consumption of eyed embryos, newly hatched larvae (alevin), and first-feeding juveniles (fry) is similar to that of wildtype salmon (Moreau, 2011; Moreau, 2014). The increased requirement for oxygen in adult GH Atlantic salmon would engender a reduced tolerance for diminished oxygen content in general, and a reduced capacity for survival when the dissolved oxygen (DO) concentration is critically low (which is more likely to occur when water temperatures are elevated[120]) compared to their wildtype counterparts. In experiments with GH Atlantic salmon, oxygen uptake was independent of oxygen concentration above 10 mg/L but started to decrease at approximately 6 mg/L DO in GH fish versus 4 mg/L DO in control fish (Stevens *et al.*, 1998). Although under conditions of high DO, GH salmon are not at a disadvantage compared to controls, as oxygen demand is readily satisfied,[121] escape into water with a DO level less than approximately 6 mg/L would place the GH Atlantic salmon at a physiological disadvantage.

Exposure to higher water temperatures, largely above 16 °C, reduces salinity tolerance in both hatchery-reared and wild smolts (Bernard *et al.*, 2019; McCormick and Bjornsson, 1994; Vargas-Chacoff *et al.*, 2018). However, growth hormone-transgenic smolts have only demonstrated slightly lower NKA activity in 19 °C water (Hallerman *et al.* 2007).

Additional information on the effects of triploidy in relation to temperature tolerance and other physical factors is contained in Appendix H.

F.2.3. Behavior

Behaviors associated with swimming, feeding, reproduction, territorial defense, migration, or other developmental events could be affected by genetic engineering. The ecological impacts of these changes in behaviors could affect life history patterns, population dynamics, and species interactions (ABRAC, 1995).

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[120] The solubility of oxygen in water is inversely related to water temperature, thus, DO concentrations decrease as the water temperature increases.

[121] Growth hormone appears to have a role in osmoregulation in anadromous salmonids (Down *et al.*, 1989; Powers, 1989). During migration from fresh water to sea water, levels of GH are elevated, leading to an increase in sodium exclusion at the gills. Migrating GE smolt would therefore be likely to avoid predation better than wild smolt upon entering sea water because they would adjust faster to the saline environment and thereby escape estuarine and coastal predation (Hindar, 1993). Other factors (discussed elsewhere) tend to increase the predation risk for GE fish.

In nature, swimming performance is important in foraging and predator avoidance. GH Atlantic salmon did not differ from wild counterparts in critical swimming speed (Stevens *et al.*, 1998); however, they did demonstrate twice the movement rate of wildtype fish (Abrahams and Sutterlin, 1999). GH also increases appetite in various species of salmonids (Abrahams and Sutterlin, 1999; Devlin *et al.*, 1999; Raven *et al.*, 2006), which influences behavioral traits associated with feeding, foraging, and social competition. The availability of food also influences behavior. Abrahams and Sutterlin (1999) have demonstrated that GH Atlantic salmon would spend significantly more time feeding in the presence of a predator than wildtype salmon, indicating that they possess a higher tolerance for predation risk.

The differences between GH and other fast-growing Atlantic salmon are less quantifiable for behavioral traits and further confounded by the effects of hatchery culture, particularly in acclimation to high rates of social interaction. Salmon form dominance hierarchies around foraging opportunities, and hatchery fish have more opportunities to reinforce their social status in confinement. In nature, social dominance is dampened by a resident advantage that generally deters other fish from evicting territory holders from home ground. Experimental studies have shown that a 25% difference in size is necessary to overcome the resident advantage in Atlantic salmon (Metcalfe *et al.*, 2003).

Moreau (2014) also found that family of origin to be an important factor influencing fitness in GH Atlantic salmon. In fact, Moreau found no differences in the competitive ability or survival of first-feeding GH or wildtype Atlantic salmon fry reared in low-feed, near natural stream conditions (Moreau *et al.*, 2011b).

Observed risk behaviors in GH transgenic salmon are consistent with those seen in domesticated salmon in general, which indicate that farmed (non-transgenic Atlantic salmon are bolder and more aggressive than wild salmon of the same strain and related hybrids (Islam *et al.* 2020), see Appendix E. Hatchery-rearing alone has been shown to affect the behavior of Atlantic salmon, including migration behavior in the wild. Non-transgenic hatchery-reared Atlantic salmon are associated with weakened imprinting compared to wild Atlantic salmon (Moe *et al.* 2016; Skilbrei *et al.* 2015), see Appendix E.

F.2.4. Resource or Substrate Use

Changes in resource or substrate use might occur through direct or indirect impact of transferred genes, either via interbreeding or genetic engineering. An example of an indirect impact is the potential for fast growing fish, including fish bearing a GH gene construct, to alter food webs as their increased size at a given age can lead to increases in size of their selected prey (Kapuscinski and Hallerman, 1991). As previously mentioned, GH increases appetite; however, (Cook *et al.*, 2000a) and Tibbetts *et al.* (2013) have also found that feed conversion efficiency was improved by 10% in GH Atlantic salmon, suggesting some potential offset in the need for food.

F.2.5. Impact of Disease and Parasites

If a GE organism were to have improved resistance to disease or parasites, in theory it could out-compete its wildtype counterparts. Based on an evaluation of general health records, tank records, fish necropsies, and study data, no evidence has been found that AAS have any altered resistance to disease or parasites.

An outbreak of ISA occurred in the Bay Fortune, PEI facility during the third quarter of 2009 (see 2015 EA, Section 5.4.2 for additional details). During this outbreak, no consistent difference in disease occurrence was noted between GH and wildtype Atlantic salmon for different year classes of fish. For the 2007 year class, the incidence of mortality during the ISA outbreak was much higher for wildtype salmon (21.7%) than for GH salmon (both AAS and EO-1ɑ broodstock[122]) (6.3%), while for the 2006 year class the rates were very similar (6.9% versus 6.1%). For the 2008 year class, in which the highest numbers of fish were potentially exposed to the ISAV (the mortality rates were almost identical for GH (both AAS and EO-1ɑ broodstock) and comparator fish (0.88% versus 0.83%) for animals that were held in the same area of the Bay Fortune, PEI facility. Pilot challenge studies conducted with ISAV strain HPR4 in 2009 indicated similar survival profiles for diploid and triploid AAS exposed via injection (ABT unpublished studies). No data were generated on wildtype comparators before the studies were discontinued.

F.2.6. Morphology and Limits to Growth Maximization

Changes in the morphology of the organism (e.g., size, shape, and color) could alter species interactions (ABRAC, 1995). However, it should be noted that accelerated growth, or increased body size, is not an assured outcome for GE salmon in nature. The rapid-growth phenotype is expressed only if supported by sufficient food, as has been shown in both GH Atlantic salmon (Cook *et al.*, 2000c; Moreau *et al.*, 2011b) and GE coho salmon (Devlin *et al.*, 2004b; Sundström *et al.*, 2007). Outcomes in more complex naturalized environments where food is less prevalent may be much less dramatic. See Appendix G for additional discussion on this issue in relation to GH coho salmon.

The rapid-growth phenotype is a function of both the productivity of the habitat and the density and behavior of competitors for the resource. In the experiments of Moreau *et al.* (2011b) on GH Atlantic salmon in food-limited stream microcosms, the GH transgene did not influence the growth in mass or survival of fry at either high or low fry densities. In addition, in this study transgenic and wildtype individual were equally likely to be dominant in competitions for foraging territory. In the previous investigations of Abrahams and Sutterlin (1999), it was found that GH-transgenesis influences the GxE interaction via powerful stimulation of appetite in the presence of food and a larger capacity for food consumption given the opportunity. GH Atlantic salmon consumed approximately five times more food than same-age controls that were also size-matched by delaying hatch time of the genetically engineered salmon. This consumption differential appears to derive from the increased feeding motivation of the GE salmon, which were 60% more likely than controls to be observed at both safe and risky foraging sites, and the increased willingness of the transgenic salmon to feed in the presence of a predator (Abrahams and Sutterlin, 1999).

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[122] EO-1ɑ broodstock are diploid salmon, homozygous for the EO-1ɑ insert, used for the production of AAS.

F.2.7. Reproduction

Changes in the age at maturation, fecundity, and sterility could alter population and community dynamics and interfere with the reproduction of related organisms (ABRAC, 1995). Due to their enhanced growth rate, diploid EO-1α broodstock and AAS could be expected to achieve reproductive maturity sooner than their wildtype siblings. Because many animals, including Atlantic salmon, select mates based upon male body size, diploid GE males exhibiting larger-than-average body size might be perceived as having an advantage over their wild counterparts.

Research conducted to date on GH Atlantic salmon, particularly under simulated natural conditions, generally does not indicate that these fish have a reproductive advantage compared to their wildtype counterparts. In fact, studies with two alternative male reproductive phenotypes of Atlantic salmon (i.e., large anadromous adults that have migrated to the sea and returned to their natal streams and small precocial parr that have matured in freshwater, having never been to sea) indicate that GH salmon display reduced breeding performance relative to wildtype (Moreau *et al.*, 2011a; Moreau and Fleming, 2012). In pair-wise competitive trials with a naturalized stream mesocosm, wild anadromous (i.e., large, migratory) males outperformed captively reared GH counterparts in terms of nest fidelity, quivering frequency, and spawn participation (Moreau *et al.*, 2011a). In addition, captively reared wildtype mature parr were superior competitors to their GH counterparts with respect to nest fidelity and spawn participation. The wildtype parr also had higher overall fertilization success than GH parr and their offspring were represented in more spawning trials. Similarly, for precocial males with an alternative (small, non-migratory) phenotype, GH-transgenesis did not influence male maturation in the first year of life, despite facilitating growth to sizes typical of mature wildtype parr, and in the second year, the number of maturing transgenic parr was only half that of the wildtype individuals (Moreau and Fleming, 2012).

Oke *et al.* (2013) reported on the hybridization of diploid GH Atlantic salmon with closely related wild diploid brown trout. Experimental crosses produced in the laboratory using gametes from diploid fish resulted in transgenic hybrids (i.e., hybrids with the GH EO-1α transgene) that were viable[123] and grew more rapidly than GH salmon and other wildtype crosses in hatchery-like conditions. In stream mesocosms designed to emulate natural conditions, transgenic hybrids appeared to express competitive dominance and suppressed growth of transgenic and wildtype salmon. The researchers did not investigate the fertility of the transgenic hybrids or the viability of any progeny resulting from hybrid backcrosses[124] to either Atlantic

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[123] This is not the first time that viable offspring (hybrids) have been produced by crossing diploid Atlantic salmon with diploid brown trout; these species are closely related and others have demonstrated hybridization both in wild populations through natural hybridization (Hurrell and Price, 1991; Jansson *et al.*, 1991; McGowan and Davidson, 1992; Verspoor, 1988) and in the laboratory through artificial fertilization (Gray *et al.*, 1993; Refstie and Gjedrem, 1975). This study differs from the others, as it appears to be the first report of production of viable hybrids from a cross of *transgenic* diploid Atlantic salmon with diploid brown trout. One clear implication is that transgenic Atlantic salmon are no different from non-transgenics with respect to this characteristic.

[124] Backcrosses are the result of a crossing of a hybrid with one of its parents or an individual genetically similar to its parent, in order to achieve offspring with a genetic identity which is closer to that of the parent.

salmon or brown trout. However, they did identify and discuss several lines of evidence from the literature that combine to suggest introgression of the transgene into the brown trout genome via backcrossing is unlikely. The implications of these observations (i.e., viable hybrids) for risk of establishment and further introgression are mitigated, however, as it has long been observed that progeny resulting from backcrosses of Atlantic salmon X brown trout hybrids are either non-viable, or triploid and therefore effectively sterile (Galbreath and Thorgaard, 1995). Thus, there is virtually no potential for any further introgression of the transgene into brown trout or Atlantic salmon genomes via backcrossing.

F.2.8. Life History

Changes in embryonic and larval development, metamorphosis, and life span could alter life-history patterns as well as population and community dynamics (ABRAC, 1995). GH constructs in salmonids have been shown to influence larval developmental rate in coho salmon (Devlin *et al.*, 2004a; Devlin *et al.*, 1995b) and smoltification in Atlantic salmon (Saunders *et al.*, 1998) and in four species of Pacific salmon (Devlin *et al.*, 1995a). Saunders *et al.* (1998) found that diploid GH Atlantic salmon reached smolt size sooner than normal and the smoltification process was not inhibited by high temperatures (19 °C) or constant light. Moreau (2014) reported that GH Atlantic salmon hatched less than one day earlier than their wildtype counterparts but were somewhat developmentally delayed, having more unused yolk and being slightly smaller; however, differences in family of origin were more significant than transgenesis. Somewhat unexpectedly, Moreau and Fleming (2012) found that enhanced growth through GH-transgenesis actually reduces precocial male maturation in Atlantic salmon. The authors concluded that the evidence suggests that the physiological mechanisms promoting growth do not play a causative role in precocial male maturation in fishes.

F.2.9. Acute Stress Response

Physiological responses to stress could be altered by GH transgene expression potentially resulting in changes in fitness and phenotype. Cnaani *et al.* (2013) investigated the effects of stress on diploid GH Atlantic salmon, wildtype triploid Atlantic salmon, and what the authors refer to as wildtype Atlantic salmon. Groups of fish were subjected to either no stress (control), one-week of fasting, or low DO (1.5–2.0 ppm). Nine markers of primary and secondary stress response were quantified from blood samples taken from these fish. In general, the GH salmon showed greater responses to stress than the two other genotypes, with the triploid fish producing intermediate responses. Wildtype fish are better able to maintain homeostasis than transgenic or triploid fish, exhibiting smaller changes in all measured stress-response parameters. The researchers concluded that poor stress response may reduce the fitness of GH and wildtype triploid Atlantic salmon in the wild.

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Appendix G. Phenotype of Other GE Salmonids

Aside from Atlantic salmon, many studies have been conducted in other GE salmonids including diploid, mixed-sex GE GH Atlantic salmon, and other species of salmon, most notably coho salmon (*Oncorhynchus kisutch*) a species of Pacific salmon. The extent to which the results of these studies may be applicable to Atlantic salmon in general, and to AAS in particular, have not been demonstrated and they are considered to be less relevant than those conducted on AAS and their relatives (see Appendix F). This is because not only are these salmon of a different genus and species, but these GE fish contain a different GH construct than the one in AAS (i.e., the sockeye salmon GH under the control of the metallothionein-B promoter of the same species (Mori and Devlin, 1999), and this construct is at a different location in the salmon's genome.

G.1. Growth Rates

GH transgenic coho salmon were found to grow faster and to a larger size with no difference in survival when compared to domesticated coho salmon, whether reared post smolt in fresh water or sea water, or reared together or separately (Gaffney *et al*., 2020). However, triploid post-smolt GH transgenic coho salmon grew slower than both the diploid GH transgenic and diploid coho salmon, in both fresh water and salt water conditions. No difference in growth rates were observed between 12-month-old triploid and diploid rainbow trout over a four-year experiment using different cohorts and breeding conditions (Scott *et al*., 2015).

G.2. Tolerance of Physical Factors

Lõhmus *et al*. (2010) found growth and survival of transgenic coho salmon expressing a growth hormone (GH) gene to be different over a range of temperatures than wildtype comparators at different life stages. Maximum hatching and survival of alevin was highest for both genotypes at 12 °C and the body weight of growth-hormone alevin was lower than wildtype alevin as temperatures increased. The growth of juvenile GH-modified fish was stimulated to a greater extent by increasing temperatures than the non-transgenic comparators and resulted in a significantly different growth curve of the transgenic salmon.

A study comparing upper thermal tolerances of size-matched wild-type, domesticated, and GH transgenic coho salmon found no difference in critical thermal maxima (c. 26.9 °C) (Chen *et al*. 2015). GH transgenic coho salmon fed to satiation had significantly higher optimum temperature, i.e., maximum heart rate and Arrhenius break point temperature (mean ± s.e. = 17.3 ± 0·1 °C). However, when GH transgenic coho salmon were ration restricted, this difference largely disappeared.

G.3. Behavior

Behaviors associated with swimming, feeding, reproduction, territorial defense, migration, or other developmental events could be affected by genetic engineering. The ecological impacts of these changes in behaviors could affect life history patterns, population dynamics, and species interactions (ABRAC, 1995).

Crossin and Devlin (2017) reported that GH rainbow trout displayed a greater capacity for burst- swimming than did their wildtype siblings, both in predator and predator-free semi-natural stream mesocosms. They also found that the rearing environment is important, as all fish reared in a static hatchery environment, free from predators and with abundant food, had much lower capacity for burst-swimming.

Crossin *et al.* (2015) found that GH rainbow trout fry reared in a naturalized stream mesocosm environment were more susceptible to predation than wildtype rainbow trout fry and suffered higher mortality even in the absence of predators, likely reflecting their inability to satiate their greater metabolic needs when reared in a food- limited environment.

Under laboratory conditions, GH coho salmon bearing the OnMTGH1 GH construct have been observed to be more competitive (Devlin *et al*., 1999), less discriminate in choosing prey (Sundström *et al*., 2004a), more likely to attack novel prey (Sundström *et al*., 2004b), and better at using lower quality food (Raven *et al*., 2006) when compared to wild relatives. Leggatt *et al*. (2017a) found that GH coho salmon had decreased swimming performance and efficiency, in contrast to GH Atlantic salmon, which had similar performance but decreased efficiency relative to wildtype counterparts (Stevens *et al*., 1998). Although these effects would have the potential to influence wild relatives both directly and indirectly, such observations were demonstrably muted when the GE fish were reared under simulated natural conditions (Sundström *et al*., 2007), indicating the complexity of GxE interactions. Sundstrom *et al.* further noted that the feeding and risk-taking behavior of GH coho salmon was strongly affected by rearing conditions which, to a large extent, had a greater effect than transgenesis. Leggatt *et al.* (2017b) found that, in addition to GxE interactions, the strain of the coho salmon influenced fitness.

Behavioral differences between GH transgenic and wild-type coho salmon have also been evaluated in a simulated ocean (marine) environment using underwater acoustic tag telemetry (Hollo *et al*., 2017). Swimming speeds were consistently greater for GH transgenic coho salmon and the proportion of time spent in feeding zones was longer during the 1-1.5 h interval post-feeding. While the data suggest that some behavior differences between GH transgenic and wild-type coho salmon in freshwater conditions may be preserved in marine conditions, the study was performed under a single set of marine conditions. It remains unclear how variation in marine conditions and/or behavior within and among strains would alter this outcome.

G.4. Impact of Disease and Parasites

There are some reports in the literature of altered resistance to pathogens and impaired immune response in GH transgenic coho salmon (Jhingan *et al*. 2003; and Kim *et al*. 2013). Kim *et al.* (2019) also found that GH transgenic coho salmon raised under ideal feeding conditions exhibited attenuated immune responses to bacterial and viral pathogens relative to wildtype. Interestingly, under ration-restricted conditions GH transgenic coho salmon had potentially enhanced immune capabilities. However, these conclusions were based on patterns of transcriptomic response to immune stimulation and it remains to be determined whether these differences in gene expression translate into differences in susceptibility, particularly in the wild.

G.5. Morphology and Limits to Growth Maximization

Changes in the morphology of the organism (e.g., size, shape, and color) could alter species interactions (ABRAC, 1995). However, it should be noted that accelerated growth, or increased body size, is not an assured outcome for GE salmon in nature. The rapid-growth phenotype is expressed only if supported by sufficient food, as has been shown in both GH Atlantic salmon (Cook *et al.*, 2000c; Moreau *et al.*, 2011b) and GE coho salmon (Devlin *et al.*, 2004b; Sundström *et al.*, 2007). For example, when GH transgenic and non-transgenic coho salmon fry were reared under a mix of natural and artificial conditions, only in the most artificial conditions (artificial food and fed to satiation) were GH transgenic fry able to achieve a size advantage over non-transgenic fry (Vandersteen *et al.,* 2019). Leggatt *et al.* (2017) also found substantial size advantage of GE coho salmon under hatchery conditions but little to no advantage under semi-natural stream conditions. The authors conclude that GE *"…fry would not gain size advantage over wild fish in most natural conditions, and potentially be at a size disadvantage in severely limiting conditions, despite intrinsic potential for accelerated growth*."

Considerable differences in growth and feeding behavior between wildtype salmon, whether wildtype or domesticated, and GE salmon have been observed in simplified hatchery environments; outcomes in more complex naturalized environments where food is less prevalent may be much less dramatic. By way of example, hatchery-reared, GH coho salmon exhibited greater predation and ~3-fold greater fork-length than age-matched wildtype conspecifics. However, when reared under naturalized stream conditions, they exhibited more modest predation activity and had only 20% greater fork-length than controls (Sundström *et al.,* 2007). In a subsequent paper, Sundström *et al*. (2016) suggested that ecological impacts of GH coho salmon in natural environments may be weaker than those observed using hatchery-reared animals.

G.6. References

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Appendix H. Effects of Triploidy on Phenotype and Biology of Salmon

The effect of triploidy on the wildtype phenotype is also important to consider as AAS are triploid. According to Benfey (2016), "*Aside from sterility, there are no population-wide phenotypic effects of triploidy, although triploids do tend to perform less well than diploids with respect to commercial culture characteristics and are also less likely than escaped diploids to outcompete or displace native salmon*." Effects of triploidy on the phenotype and biology of salmon are discussed further below.

H.1. Anatomy, Histopathology and Clinical Chemistry

Triploidization induced by hydrostatic pressure has been shown to induce vertebral deformities in Atlantic salmon (Fjelldal and Hansen, 2010; Leclercq *et al.*, 2011). The prevalence of deformities in young triploid Atlantic salmon as determined by palpation or visual observation has been reported to range from 1-3% (Fjelldal and Hansen, 2010) and 1.2–2.5% (Taylor *et al.*, 2011), but were not always higher than in diploids. Using sensitive radiography, more triploids were found to have one or more deformed vertebrae than diploids (mean 22.0 versus 42.7% and 24.4 versus 48.9% in diploid and triploid, parr and post-smolts, respectively (Fraser *et al.*, 2013). Increasing the level of dietary phosphorus in freshwater can counteract the problem (Fjelldal *et al.*, 2012).

Triploid Atlantic salmon post-smolts are also more prone to cataracts than diploids (Benfey, 2016; Sambraus *et al.,* 2018). A correlation between N-acetyl-histidine (NAH) levels and cataract formation has been documented in both diploid and triploid Atlantic salmon (Waagbø *et al.,* 2010, Taylor *et al.,* 2015; as cited in Sambraus *et al.,* 2018) and may contribute to the increased prevalence of cataract formation in triploids.

Triploid Atlantic salmon reared under higher temperatures (15-18 °C) in seawater have been found to have generally less muscle energy stores and produced lower hemoglobin and hematocrit levels than diploids (Sambraus et al., 2018). In the same study, protein expression and whole-body lipid class profiles did not differ between triploids and diploids for any life stage but whole-body lipid content was higher for triploids at the parr stage.

H.2. Growth Rates

The effect of ploidy alone has also been examined in salmonids. The effects of ploidy (i.e., triploid vs diploid) depend on the age and species evaluated, but triploid salmon generally exhibit equal or reduced growth relative to diploids.

Non-GE all-female triploid populations of Atlantic salmon reared under optimal conditions in freshwater were significantly lower in weight at the late alevin and fry stages but were similar in weight to diploids at the parr stage (Nuez-Ortín *et al.*, 2017).

H.3. Metabolism

Studies looking at the effects of triploidy alone found negligible differences in metabolism between juvenile triploid and diploid Atlantic salmon reared in normoxic conditions (Bowden *et al*., 2018).

H.4. Tolerance to Physical Factors

Triploid and diploid rainbow trout were evaluated for hypoxia tolerance each year for four years using a new cohort each year and it was found that hypoxia tolerance of 12-month-old triploids was consistently lower than diploids—i.e., shorter time to loss of equilibrium (Scott *et al.,* 2015). However, this difference was not observed in adult, lake-reared trout.

Although the temperature tolerance of AAS has not been investigated, because AAS are triploid fish, triploidy itself, and not just the presence or expression of the rDNA construct, may also affect the tolerance limits of these fish. Data exist for a variety of species of fish to indicate that triploidy could be responsible for reduced survival of early-life stages and reduced survival and growth of later-life stages, particularly when environmental conditions are not optimal (Piferrer *et al.*, 2009). Atkins and Benfey (2008) have shown that compared to diploid siblings, triploid salmonid fishes such as brown, brook, and rainbow trout, exhibit reduced tolerance to chronically elevated rearing temperatures, resulting in high mortality of the triploids at temperatures that are sub-lethal for sibling diploids. In addition, triploid Atlantic salmon also were observed to have higher metabolic rates than diploids at lower temperatures, and lower metabolic rates than diploids at higher temperatures, suggesting that triploids have lower thermal optima than diploids. The authors postulate that given a lower optimum temperature for metabolic processes, triploids may not be able to sustain a high metabolic demand, resulting in increased cardiac output and, ultimately, cardiac failure, at high temperatures that are not lethal to diploids. Hansen *et al.* (2015) found that triploid Atlantic salmon had reduced feed intake, condition factor, and growth, compared to diploids, at high seawater temperatures (19 °C) and this was further exacerbated by reductions in DO from 100% to 70% of saturation. The authors suggest this indicates triploid Atlantic salmon have a lower aerobic scope at 19 °C and were approaching their upper thermal tolerance limit. Sambraus *et al.* (2017) monitored triploid and diploid Atlantic salmon post-smolts at different temperatures and oxygen saturation and found that triploids progressively reduced feed intake with increasing temperature after peak feeding at 15 to 18 °C. They also found triploids were more sensitive to hypoxia (60% oxygen saturation), exhibited lower feed intake than diploids at 6 °C and higher mortality at 18 °C. Benfey (2016) concluded that triploid Atlantic salmon were less likely to survive in habitats that are relatively warm or low in DO than their diploid counterparts.

H.5. Feeding Rates and Performance

In a study of large (2.5.kg) post-smolt Atlantic salmon reared in saltwater conditions, differences in feeding rates between triploid vs diploid Atlantic salmon were dependent on temperature, with triploids having higher feeding rates at lower temperatures (3 and 9 °C), including under hypoxic conditions, but rates that were similar at 12 °C, and dropped pronouncedly more than diploids at high temperatures (15 °C) (Sambraus *et al*., 2018). In the same study triploids had generally less muscle energy stores and produced less hemoglobin and hematocrit levels with increasing temperatures.

Multiple, but not all, studies report better performance of triploids at colder temperatures. A study with Atlantic salmon reared in freshwater found negligible differences in thermal tolerance (across 10-18 °C) between diploid and triploid individuals (Bowden *et al*., 2018). However, the study used juveniles and previous studies have shown that smaller individuals can have greater thermal tolerance than adult conspecifics (Clark *et al*., 2012, Messmer *et al*., 2016, and Clark *et al*., 2017 as cited in Bowden *et al*., 2018). The study was also done under normoxic conditions, whereas salmon in wild or cultured environments may be subjected to periods of hypoxia.

H.6. Behavior

Ocean migration studies in Ireland revealed that male triploids returned to their natal area in nearly the same proportions as diploids, whereas female triploids mostly did not (Wilkins *et al*., 2001). In another Irish study, the return rates of female triploid Atlantic salmon, both to the coast and to fresh water, were substantially reduced (four- to six-fold lower) compared to those for their diploid counterparts (Cotter *et al*., 2000), inferring that triploidy could be used as a means both for eliminating genetic interactions between cultured and wild populations and for reducing the ecological impact of escaped farmed fish. Genetic analyses on escaped of triploid Atlantic salmon in Norway demonstrated that triploids have significantly reduced motivation to migrate into freshwater following escape (Glover *et al*., 2016).

Triploid Atlantic salmon demonstrated ram ventilation behavior under both normal and hypoxic conditions, which was not seen in diploid Atlantic salmon in experiments conducted by Hansen *et al*. (2015). However, Benfey (2016) concluded that results from laboratory studies on behavior and cognitive ability and from field trials suggest that triploid Atlantic salmon, if free from obvious deformities, would not differ from diploids in their abilities to forage, escape predation, and disperse in the wild in freshwater environments.

H.7. Impact of Disease and Parasites

According to Benfey (2016), "*Some uncertainties exist with respect to the disease resistance of triploids and their potential to become reservoirs for the spread of pathogens to wild populations*." There are indications of detrimental effects on complement activity in triploid Atlantic salmon compared to diploids in terms of their innate immune response to bacterial pathogens (Langston *et al*., 2001). Triploid Atlantic salmon also have a lower relative abundance of B-cell lymphocytes than diploids (Fraser *et al*., 2012b).

A study comparing the immune response of triploid and diploid Atlantic salmon to an experimental challenge with *Neoparamoeba perurans*, the causative agent of amoebic gill disease, (AGD) found no difference in the severity of AGD pathology or the serum innate immune response (Chalmers *et al*., 2017). However, triploids did have significantly lower lysozyme activity after 21 days post-infection but within the natural range previously reported for Atlantic salmon and other salmonids.

Although controlled exposure (e.g., disease challenge) studies are lacking, in his review of available study data, Benfey (2016) states the study results "*suggest that triploid Atlantic salmon (i) may be less resistant than diploids to pathogenic diseases and parasites, and (ii) may not react as well to vaccination*."

H.8. Survival and Return Rates

Genetic analyses of populations containing hundreds of thousands of escaped farmed triploid Atlantic salmon from facilities across 17 rivers in western Norway found only 0.18% (seven individuals out of 3794) were triploid and only 0.08% (three individuals) were trisomic (Glover et al., 2016). They concluded that "triploids should not only be seen as an effective way of stopping genetic introgression, it will also significantly reduce the numbers of escapees entering rivers, which in turn limits ecological interactions and potential disease transmission." A re-examination of genetic data of 5,994 Atlantic salmon from 56 Norwegian and 24 Russian wild populations found that naturally occurring triploid populations to be lower than the frequency of spontaneous triploids observed in aquaculture, lending further support that triploid survival is substantially lower in natural conditions than aquaculture rearing conditions (Jørgensen et al., 2018).

There is also growing evidence that triploid salmon performance and survivorship may be reduced in marine conditions. A study comparing diploid and triploid steelhead trout found inferior growth and survivorship of triploids transferred from fresh to salt water conditions;after a year of freshwater rearing and transfer to a saltwater tank for 15 months, triploid survivorship (35%) was roughly half that of diploids (72%) (Johnson et al. 2019). Triploid GH transgenic coho salmon had lower survival than both the diploid GH transgenic and diploid coho salmon, when reared post smolt in seawater, whether reared together or separately (Gaffney et al. 2020). As cited in Gaffney et al. (2020), it is hypothesized that the very low survival of triploid GH transgenic coho salmon in marine conditions is due to poor smolt success, as observed in other triploid coho salmon.

H.9. Reproduction

Triploidy is believed to effectively sterilize Atlantic salmon (and other fish) because it interferes with normal gametogenesis (the formation of cells that become eggs or sperm) when cells enter meiosis. This is believed to be due to mechanical problems associated with the pairing of homologous chromosomes in the presence of a third set of homologues (Benfey, 1999). Published literature indicates that it is highly likely that triploid Atlantic salmon, particularly female salmon, will be effectively sterile due to failure of the gametes to mature normally. Most germ cells do not progress through the first meiotic prophase (an early stage in the formation of the sex cells) in triploids of either sex. Triploid females rarely produce eggs, but, if they do, the eggs usually are very few, undeveloped and unfertilizable (Piferrer *et al*., 2009). Most triploid oögonia fail to proceed to the oöcyte stage and, as a result, there are very few (if any) ovarian follicles that develop to a stage of functional steroid biosysnthesis (Benfey, 2016). Triploid females do not produce sufficient vitellogenin for oöcytes to develop to a stage necessary for the production of viable eggs, and any oöcytes that do complete vitellogenesis will not be released due to the lack of endocrine signaling for final maturation and ovulation (Benfey, 2016).

Although there have been isolated reports of limited gonadal development in triploid fish of several different species, mostly in males (Benfey, 1999; Mair *et al.,* 2007), relevant research on triploids of Atlantic salmon and related species indicates functional sterility in females. In a study on triploid landlocked Atlantic salmon, Benfey and Sutterlin (1984) found the ovaries of triploid females had the external appearance of undeveloped gonads, but still produced a small number of oocytes (from 1 to 12, versus several hundred in each diploid female). The viability of these oocytes was never determined. Subsequently, Johnstone and colleagues (Johnstone *et al*., 1991; Johnstone, 1992) showed that approximately 0.1% of triploid Atlantic salmon females underwent sexual maturation after two years. When fertilized with normal sperm, eggs stripped from triploid females were markedly variable in size, and most underwent little obvious development (Johnstone, 1992). Approximately 10% of the fertilized eggs developed to the eyed-egg stage, but the embryos were clearly malformed and none survived beyond hatching, confirming that triploid females are functionally sterile. Similar results have been reported from a study on Arctic char (*Salvelinus alpinus*), a salmonid species related to Atlantic salmon, in which although a few of the triploid females developed ovaries, fecundity was low, and the fertilized eggs from the triploid females did not hatch (Gillet *et al*., 2001), also demonstrating that successful reproduction was functionally and effectively precluded through triploidization. Therefore, based on the available evidence, FDA has concluded that triploidy would ensure functional sterility and reproductive incompetence in the all-female populations of AAS.

These techniques are not new and have been under study for many years for aquaculture purposes and have been used by fisheries biologists to reproductively isolate stocked game fish from their wild counterparts and protect species that may be threatened or endangered (Thorgaard, 1983; Benfey and Sutterlin, 1984; Benfey, 2001). As of 2005, officials in 10 different states were sterilizing (i.e., triploiding) hatchery salmonids as part of their stocking programs for hatchery-reared salmonids (Kozfkay *et al*., 2006). In addition, going back to the 1980s and 1990s, the use of sterile Atlantic salmon triploids has been proposed as a possible strategy to reduce interactions and interbreeding between escaped farmed and wild salmon (Heggeberget *et al*., 1993; McGeachy *et al*., 1995; McGinnity *et al*., 1997; Benfey, 2001; Benfey, 2016). The use of sterile triploids has also been proposed for biological containment of GE fish going as far back to the early 1990s (Devlin and Donaldson, 1992; Thorgaard *et al*., 1992). The usefulness of triploidy as a means of eliminating genetic interactions and reducing the general impact of escaped farmed fish on wild populations has been demonstrated in a large-scale field study (Cotter *et al*., 2000a).

Because AAS, as defined and specified in the NADA would only be produced as all-female triploids, it is important to consider the interactive effects of triploidy and sex on Atlantic salmon in their natural environment and how this might influence interactions between farm-raised fish that have escaped, including AAS, and wild salmon. Ocean migration studies in Ireland with tagged Atlantic salmon showed that male triploids return to their natal area in nearly the same proportions as diploids, whereas female triploids mostly do not (Wilkins *et al*., 2001). In another Irish study, the return rates of female triploid Atlantic salmon, both to the coast and to freshwater environments, were substantially reduced (four- to six-fold lower) compared to those for their diploid counterparts (Cotter *et al*., 2000a). Of direct relevance to triploid AAS females, the triploid females in this study had severely immature ovarian development (Murphy *et al*., 2000) and abnormal gonadal steroid and gonadotropin hormone profiles (Cotter *et al*., 2000b). From the reduced rate-of-return and inability to produce viable offspring demonstrated in these studies and others (e.g., Johnstone *et al*., 1991; Johnstone, 1992), FDA can infer that triploidy combined with all-female populations can be effectively used as a means of eliminating reproduction and genetic interactions between cultured and wild populations.

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Appendix I. Endangered Species Act Analysis

This section addresses the final part of the Court's November 2020 opinion by reassessing the ESA determination from the 2015 EA (FDA must "*reconsider its 'no effect' determination under the ESA together with its revised NEPA evaluation*"[5]) using the information from Risk-related Questions 1-5 in Section 9 above.

NEPA and ESA are different statutes that are implemented under different regulations, frameworks, and agencies. Thus, this section of the EA will discuss FDA's ESA determination following the requirements discussed in Section 7(a) of ESA, 50 CFR Part 402, and the ESA Section 7 Consultation Handbook (FWS and NMFS, 1998). Section 7(a)(2) of the ESA states that each Federal agency shall, in consultation with the Secretary of the Interior or Commerce, as appropriate, insure that any action they authorize, fund, or carry out is not likely to jeopardize the continued existence of a listed species or result in the destruction or adverse modification of designated critical habitat.

For the 2015 approval of the Bay Fortune facility, and the 2019 approval of the Hatchery Unit at the Rollo Bay facility, FDA made a "no effect" determination under ESA, 16 USC § 1531 et seq., concluding that AAS, when produced and reared under the conditions in the application, and as described in the EA, would not affect US populations of threatened or endangered Atlantic salmon or their critical habitat. For the 2015 EA, the two federal agencies responsible for administering the ESA, the National Marine Fisheries Service (NMFS) of the National Oceanic and Atmospheric Administration (Department of Commerce) and the US Fish and Wildlife Service (FWS) of the Department of Interior, were provided with a "no effect" determination and the underlying information in support of it by FDA. Based on their statutory authorities and regulations, the agencies either concurred with, or indicated no disagreement with, FDA's "no effect" determination (see Appendix D of the 2015 EA).

This amended EA was prepared to address FDA's NEPA obligations, but was also prepared to assist in FDA's re-evaluation of its ESA decision per the 2020 Court order, i.e., to re-evaluate whether the production of AAS and AquAdvantage broodstock at the PEI facilities, including planned changes to the Bay Fortune facility and expansion at the Rollo Bay facility (see Section 7 of this EA), would affect US populations of threatened or endangered Atlantic salmon or their critical habitat. FDA followed the requirements outlined in Section 7(a) of ESA, 50 CFR Part 402, and the ESA Section 7 Consultation Handbook (FWS and NMFS, 1998), and received input and comments on a draft of the amended EA from NMFS and FWS during ESA technical assistance reviews that occurred between June and October 2022 with initial discussions beginning in March 2021. Changes were made to this EA in response to those comments. In addition, a representative of NMFS conducted a site visit with FDA in June 2023 to observe the PEI operations, including containment, at the Bay Fortune facility and the Rollo Bay facility, including both the Hatchery Unit and Broodstock Unit 1. These collaborations are discussed in Appendix A.

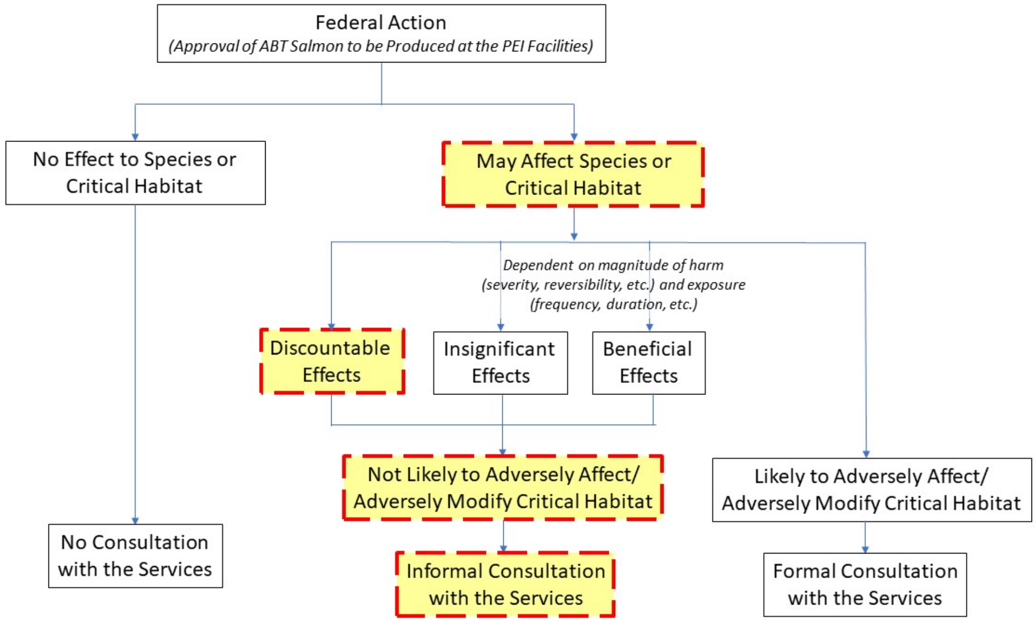
A conceptual model in Figure I-1 below reflects the ESA process and FDA's conclusions under the ESA are emphasized with red bold, dashed boxes highlighted in the yellow. The first step in the ESA process is to determine whether the action may have an effect on a listed species or designated critical habitat (see Figure I-1 below). In order to apply the assessment conducted in this EA to the evaluation under ESA, the terminology used under ESA needs to be considered. According to the ESA Section 7 Consultation Handbook, "affect" is defined as "*to bring about a change*" and "effect" is "*the result*." There are two possible outcomes of the first step in the ESA process: the action will have "no effect" on or "may affect" a listed species or designated critical habitat. "No effect" is defined in the ESA Section 7 Consultation Handbook (FWS and NMFS, 1998) as "*the appropriate conclusion when the action agency determines its proposed action will not affect a listed species or designated critical habitat*." "May affect" is defined as "*the appropriate conclusion when a proposed action may pose* ***any*** *effects on listed species or designated critical habitat.*" Based on the comprehensive physical and procedural containment and security at the PEI facilities, FDA concluded that a "no effect" determination was appropriate for the 2015 and 2019 NADA approvals. However, when re-considering these definitions in light of the court's decision and the expanded assessment conducted herein, FDA now concludes that it is possible that, in the highly unlikely event ABT salmon escape the PEI facilities, they *may affect* endangered Atlantic salmon of the Gulf of Maine DPS and their critical habitat, as described in Section 9.3 and Table 9-7 of this EA. Thus, FDA is changing the 2015 and 2019 ESA determinations from "no effect" to "may affect."

The second step in the ESA process is then to consider whether the effects may be discountable, insignificant, or beneficial per the definitions in the ESA Section 7 Handbook. Based on the assessment herein, FDA has determined that the effects from the proposed action are discountable. Discountable effects "*are those extremely unlikely to occur. Based on best judgment, a person would not: … expect discountable effects to occur.*" Based on the analyses in Section 9 of this EA, it is determined that the risk of significant harms occurring in the US environment, including to endangered Atlantic salmon and their critical habitat, due to the action is negligible. This is based on the finding that the likelihood of exposure (establishment and/or presence) of ABT salmon in the US environment is negligible (extremely unlikely or not reasonably foreseeable occurrence) due to a negligible likelihood of escape from the facilities on PEI. Both PEI facilities have multiple redundant forms of physical and procedural containment, as well as constant security and monitoring (see Section 9.2, above). In addition, biological containment (triploidy and all-female populations) provides additional containment for AAS in the highly unlikely event of their escape/release. This negligible likelihood of exposure is further reduced when considering the negligible to moderate likelihood of ABT salmon completing the additional steps required for exposure in the US to actually occur (i.e., survival, dispersal, migration, reproduction), see the discussion in Section 9.4.1.1, above. Thus, it is determined in this amended EA that there is a negligible likelihood of a complete exposure pathway to the US (see Section 9.2.3, above). Additional analyses were also performed to evaluate the severity of harms in the highly unlikely circumstance of escape and exposure. The likelihood of significant harms is also found to be negligible for all harm pathways, including reproduction of ABT salmon with endangered Atlantic salmon (see Section 9.4.1). Thus, significant harms to US endangered Atlantic salmon and their critical habitat[125] are not expected to occur.

Based on the assessment in this amended EA, FDA concludes that the action, involving production of ABT salmon at the Bay Fortune and Rollo Bay (Hatchery Unit and Broodstock Units 1 and 2) facilities, will result in **discountable effects**, i.e., those that are extremely unlikely to occur (FWS and NMFS, 1998). Based on this outcome, it is further concluded that the action is ***not likely to adversely affect*** endangered Atlantic salmon of the Gulf of Maine DPS or their critical habitat (see the red bold, dashed boxes highlighted in the yellow in Figure I-1 below). "Not likely to adversely affect" is defined in the ESA Section 7 Consultation Handbook as "*the appropriate conclusion when effects on listed species are expected to be discountable, insignificant, or completely beneficial.*"

Thus, FDA concludes that, when produced and reared under the conditions in the application, and as described in this EA, the production of AAS and AquAdvantage broodstock at the PEI facilities (including future planned changes and expansions described in Section 7 of this EA) may affect, but is not likely to adversely affect US populations of threatened or endangered Atlantic salmon or their critical habitat.

Figure I-1. Possible Outcomes of an Analysis under ESA. The highlighted boxes represent the outcome of the current analysis. This figure takes into account Figure 3-3 of the ESA Section 7 Consultation Handbook (FWS and NMFS, 1998).



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[125] See Section 9.3.2.2.b of this EA (Competition for and Use of Habitat) discussing the potential physical harms to the critical habitat of the Atlantic salmon of the Gulf of Maine DPS that could occur if it is assumed that ABT salmon establish and/or are present in the Maine environment, and Section 9.4 of the amended EA regarding FDA's finding of negligible risk of habitat destruction due to the action.

FDA submitted this assessment and supporting references to NMFS on January 8, 2024, and requested initiation of an informal consultation under Section 7 of the ESA. Due to joint jurisdiction over endangered Atlantic salmon of the Gulf of Maine DPS, FDA also initiated an informal consultation with FWS; however, FWS chose to defer the final concurrence to NMFS. NMFS initiated the informal consultation on January 21, 2024.

On April 22, 2024, NMFS provided an ESA Letter of Concurrence (LOC) with FDA's assessment that the action may affect, but is not likely to adversely affect endangered Gulf of Maine Atlantic salmon or their critical habitat. Following receipt of the LOC, FDA notified NMFS of several factual errors in the LOC and requested a corrected letter. The errors included incorrect statements regarding AAS growing to larger sizes than Atlantic salmon without the rDNA construct and that there is no wastewater discharge from the Rollo Bay facility, as well as minor typographical errors. NMFS sent FDA a corrected LOC dated April 24, 2024, which is included as Figure I-2 below.

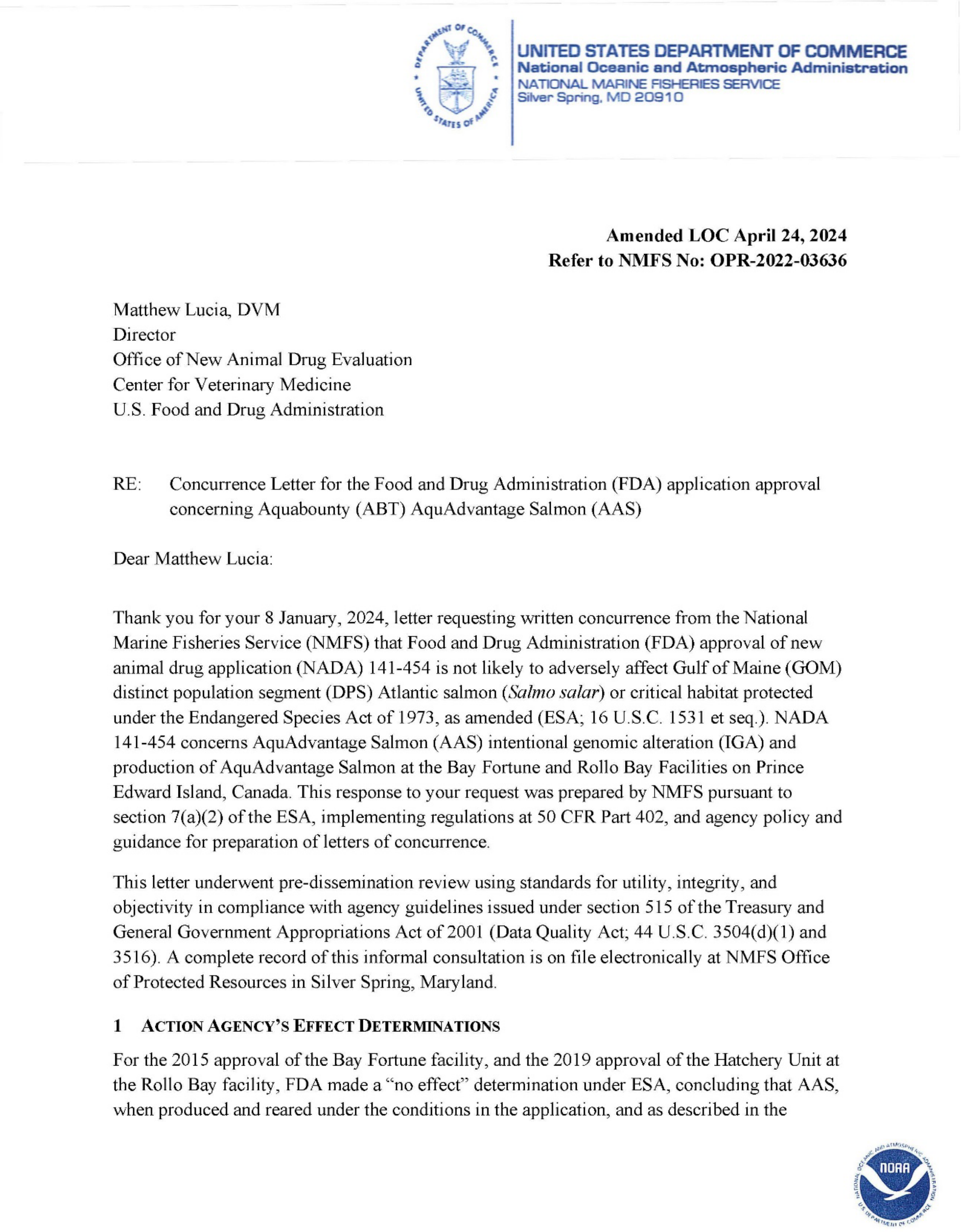
FDA found and notified NMFS of new factual errors in the corrected LOC. NMFS requested additional information on the amount of water entering and exiting each unit at the Rollo Bay and Bay Fortune facilities, and ABT's procedures for responding to an infectious disease emergency. FDA provided the requested information and referenced Sections 7.5, 8.2.2, 9.2.4, 9.3.1.5, and 9.3.2.2.e of this assessment, which discuss the potential environmental exposure of pathogens/parasites from the ABT facilities, as well as ABT's extensive and comprehensive measures to prevent the introduction of pathogens or parasites at these facilities.

On June 3, 2024, NMFS provided FDA the following response: "*In reviewing the responses to questions and additional information provided by the FDA regarding pathogens and water discharge, NMFS has concluded that additional revisions to the letter of concurrence are not needed. The additional water discharge load information does not yield any changes to our analysis of the anticipated effects of the action on ESA-listed species or designated critical habitat, or the conclusions of the letter of concurrence.*" Thus, the April 24, 2024, corrected LOC provided in Figure I-2 below is the final LOC from the Services. The errors in the final LOC are listed below:

- <u>Section 4.1 of the corrected LOC (Figure I-2):</u> NMFS states "*Recirculating aquaculture facilities do not release any of their contents to the outside environment.*" However, ABT does release some water from the PEI facilities. ABT uses recirculating aquaculture practices that reuse the water in the system by cleaning the water through the use of a biofilter, UV and ozone and capturing waste (e.g., fecal matter, uneaten food). The majority of the cleaned water is recirculated back to the fish tanks; approximately 97% and 99.7% in the Bay Fortune and Rollo Bay facilities, respectively. The rest of the water is discharged to the drainage ditch adjacent to the Bay Fortune facility or Rollo Bay brook at the Rollo Bay facility (see Section 7.2 for a more detailed description of the facilities). In addition, at the Rollo Bay facility, ABT is required by the province to discharge to Rollo Bay Brook to recharge the well from which they are obtaining water for the facility. NMFS reviewed information regarding the water discharge from ABT and found that it does not alter their decision to issue the LOC.

- Section 4.2 of the corrected LOC (Figure I-2): NMFS states "*…based on the limited releases from the Rollo Bay facility and the redundant measures of physical and procedural containment protocols, the risk of releasing pathogens is extremely unlikely and therefore, discountable*." However, the physical containment measures at the PEI facilities (presented in Appendix C and Appendix D) are designed to contain eggs and fish from being released; they are not in place to contain pathogens and parasites. The screen perforation sizes used at these facilities range from 40 µm to 25 mm and would be too large to contain pathogens and parasites in the unlikely event they were introduced. At this time, there are no physical or mechanical methods to contain pathogens or parasites in an aquaculture facility; therefore, ABT has established extensive and comprehensive biosecurity protocols to prevent the introduction of pathogens and parasites into these facilities (described in Sections 7.5 and 9.2.4 of this assessment). In addition, in the event of an infectious disease emergency, ABT also has procedures that would be implemented to decrease the probability of pathogens or parasites spreading within the premises and from the premises, which includes working with Canadian provincial veterinarians and CFIA on mitigation measures. NMFS reviewed additional information regarding ABT's planned response to a disease emergency and found that it does not alter their decision to issue the LOC.

Figure I-2. Letter of Concurrence (dated April 24, 2024) under the Endangered Species Act from the National Marine Fisheries Service



UNITED STATES DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
NATIONAL MARINE FISHERIES SERVICE
Silver Spring, MD 20910

Amended LOC April 24, 2024
Refer to NMFS No: OPR-2022-03636

Matthew Lucia, DVM
Director
Office of New Animal Drug Evaluation
Center for Veterinary Medicine
U.S. Food and Drug Administration

RE: Concurrence Letter for the Food and Drug Administration (FDA) application approval concerning Aquabounty (ABT) AquAdvantage Salmon (AAS)

Dear Matthew Lucia:

Thank you for your 8 January, 2024, letter requesting written concurrence from the National Marine Fisheries Service (NMFS) that Food and Drug Administration (FDA) approval of new animal drug application (NADA) 141-454 is not likely to adversely affect Gulf of Maine (GOM) distinct population segment (DPS) Atlantic salmon (*Salmo salar*) or critical habitat protected under the Endangered Species Act of 1973, as amended (ESA; 16 U.S.C. 1531 et seq.). NADA 141-454 concerns AquAdvantage Salmon (AAS) intentional genomic alteration (IGA) and production of AquAdvantage Salmon at the Bay Fortune and Rollo Bay Facilities on Prince Edward Island, Canada. This response to your request was prepared by NMFS pursuant to section 7(a)(2) of the ESA, implementing regulations at 50 CFR Part 402, and agency policy and guidance for preparation of letters of concurrence.

This letter underwent pre-dissemination review using standards for utility, integrity, and objectivity in compliance with agency guidelines issued under section 515 of the Treasury and General Government Appropriations Act of 2001 (Data Quality Act; 44 U.S.C. 3504(d)(1) and 3516). A complete record of this informal consultation is on file electronically at NMFS Office of Protected Resources in Silver Spring, Maryland.

1 ACTION AGENCY'S EFFECT DETERMINATIONS

For the 2015 approval of the Bay Fortune facility, and the 2019 approval of the Hatchery Unit at the Rollo Bay facility, FDA made a "no effect" determination under ESA, concluding that AAS, when produced and reared under the conditions in the application, and as described in the

Environmental Assessment (EA) (FDA 2024), would not affect US populations of threatened or endangered Atlantic salmon or their critical habitat.

Upon reconsideration of various effects pathways, FDA now concludes AAS production may affect endangered Atlantic salmon of the GOM DPS and their critical habitat, as described in Section 9.3 and Table 9-7 of the EA (FDA 2024). Thus, FDA is now requesting concurrence that production of AAS at the Bay Fortune and Rollo Bay (Hatchery Unit and Broodstock Units 1 and 2) facilities when produced and reared under the conditions in the application, may effect, but is not likely to adversely affect GOM Atlantic salmon and their critical habitat.

2 PROPOSED ACTION AND ACTION AREA

2.1 Proposed Action

The FDA expanded its assessment beyond the 2015 EA (FDA 2015) in order to consider the risk of AAS Salmon in the natural environment related to AquAdvantage Salmon production at the Bay Fortune and Rollo Bay Facilities on Prince Edward Island, Canada associated with NADA 141-454. The AAS are modified to betriploid, hemizygous, all-female Atlantic salmon bearing a single copy of the α-form of the *opAFP-GHc2* recombinant DNA (rDNA) construct at the α-locus in the EO-1α lineage. This will allow AAS to grow to market size more quickly than native GOM Atlantic salmon.

The PEI facilities produce eyed-eggs, which are then shipped to grow out facilities. The FDA made no effect determinations for risks to listed species in the vicinity of the grow out facilities. The two PEI facilities maintain broodstock to produce eggs and store the eggs before they can be shipped to grow out facilities.

AquaBounty provided FDA with a projection of the maximum number of each type of fish that could be held at any one time throughout the year in all PEI facilities based on weight ranges (eggs, <100 grams (g) or ≥100 g). The maximum values below include current production capabilities, as well as projections due to the planned future expansion of new broodstock units at Rollo Bay (known as Broodstock Units 1 and 2). ABT is planning to construct a new incubation room within the current Bay Fortune facility to consolidate egg production at a centralized location.
This change will not expand or increase the production of AAS eyed-eggs at the Bay Fortune facility. These reconfigurations will consolidate and streamline operations within the existing facilities.

Table 1 Types, size, and approximate maximum number of type of fish potentially housed at one time at all ABT facilities currently located on PEI, Canada.

Type		Approximate Maximum Number (eggs and fish)
AAFB (diploid, all-female)	homozygous	~ 200,000 eggs; 60,000 <100g fish; and 1200 ≥100g fish
	hemizygous	~ 50,000 eggs; 28,000 <100g fish; and 350 ≥100g fish
AANB (diploid, neomale)	homozygous	~ 70,000 <10 g fish; and 8,000 ≥100g fish
AAS (at least 95% triploid: up to 5% diploid, all-female)	hemizygous	~30,000,000 eggs*; and 4,000 <100g fish
Atlantic salmon without the rDNA construct	Female	~ 30,000,000 eggs; 200,000 <100g fish; and 30,000 ≥100g fish
	Male	~ 20,000 eggs; 20,000 <100g fish; and 3,000 ≥100g fish
	Neomale	~65,000 <100g fish; and 7,500 ≥100g fish

** Most AAS eggs will be held at the facility for a short period of time (~50-125 days) before being shipped to the US for grow out. However, some AAS eggs may remain in PEI for grow out for the Canadian market (although this is not routinely done at this time, it may occur in the future), as well as for quality assurance evaluations and use in research studies.*

The size delineations in Table 1 were chosen based on the approved claim for AAS: "Significantly more AquAdvantage Salmon grow to at least 100g within 2,700 °C-days than their comparators.[1]" In addition, as defined by FDA (2015), smolt are defined by a weight of 100g. These fish are assumed to be ready to begin the smolting process, which is the physiological change that allows young salmon to adapt to saltwater conditions. Therefore, pre-smolt will encompass fish <100g and post-smolt are those fish weighing ≥100g.

The numbers include anticipated expansion, including the planned Broodstock Units 1 and 2 at the Rollo Bay facility. However, any new facilities under NADA 141-454 will require a supplemental approval (NADA), which will include a NEPA evaluation and ESA consultation, as necessary.

The EA (FDA 2024) outlines the pathways necessary for AAS to escape confinement from the PEI facilities, migrate to adjacent rivers, and establish a persistent population in the US. The EA also evaluates the potential pathways for pathogen/parasite transmission from AAS and the ABT

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[1] Degree-days (DD or °C·days) are a method of quantifying the thermal experience of an organism over the linear range and are an effective method for describing growth and development in fish. The amount of ambient thermal energy that a fish has experienced can be quantified using a degree-day approach. The degree-day for a single day (DD; °C·days) is calculated as $\boldsymbol{DD = \left(\frac{T_{max}+T_{min}}{2}\right) - T_0}$ where T_{Max} and T_{Min} are the maximum and minimum daily ambient temperatures, respectively, and T_0 is the temperature below which growth or development is nonlinear and effectively zero.

facilities on PEI to wild fish populations, including endangered US Atlantic salmon. Then, the EA identifies and evaluates the potential breeding, competition, and predation possibly affecting the endangered Atlantic salmon population, if these scenarios were to occur.

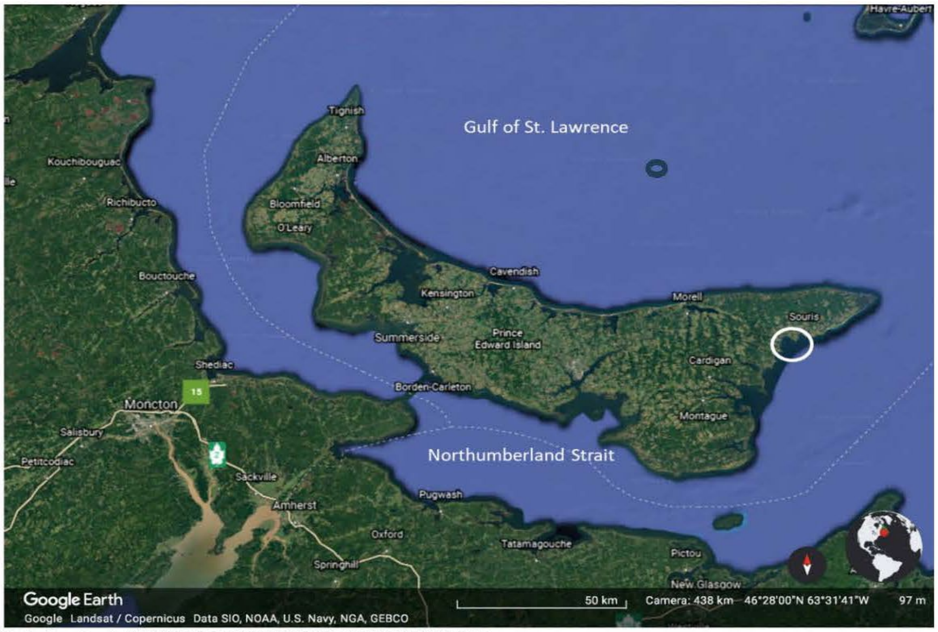
2.2 Action Area

The action area is all areas to be affected directly or indirectly by the Federal action and not merely the immediate area involved in the action (50 CFR §402.02). The facilities are located in PEI, adjacent to the foreign waters of Canada; however, due to the nature of the proposed action, the stressors potentially caused by the proposed action could extend into the Gulf of Maine where the ESA-listed GOM DPS of Atlantic Salmon and its critical habitat may be affected.

The Gulf of Maine is a large gulf of the Atlantic Ocean on the east coast of North America. The boundaries extend from Cape Cod, at the eastern tip of Massachusetts, in the southwest, to Cape Sable Island, at the southern tip of Nova Scotia, in the northeast. The Gulf includes the entire coastline of the U.S. states of New Hampshire and Maine, as well as a portion of Massachusetts north of Cape Cod, and the southern and western coastlines of the Canadian provinces of New Brunswick and Nova Scotia, respectively. PEI is located in the Gulf of St. Lawrence, west of Cape Breton Island, north of the Nova Scotia peninsula, and east of New Brunswick (Figure 2).

ABT currently produces AAS at two facilities in PEI, Canada: Bay Fortune and Rollo Bay. Figure 1 illustrates the location of PEI in relation to Canada and the US. Figure 2 shows the location of the ABT facilities on PEI and in relation to the Northumberland Strait and the Gulf of St. Lawrence, and Figure 3 illustrates the locations of the facilities in relation to each other and the Bay Fortune Estuary and Rollo Bay.

Figure 1 Location of Prince Edward Island, Canada



https://earth.google.com/web/@46.46686655,-63.52817583,97.46370584a,437931.56824864d,35y,-0h,0t,0r

Figure 2 Locations of Bay Fortune and Rollo Bay, PEI, Canada. The white circle indicates the area where the two facilities are located.

Figure 3 Locations of Bay Fortune and Rollo Bay, PEI, Canada. The white circles indicate the areas where the two facilities are located.

2.2.1 **Bay Fortune**

The Bay Fortune facility is a land-based aquaculture facility situated on the northeast side of PEI, near a tidal (estuarine) portion of the Fortune River, i.e., Bay Fortune (Figure 3). The Bay Fortune facility is located approximately one mile inland from the river's confluence at a southern portion of Rollo Bay. This bay in turn connects with the Northumberland Strait, which ultimately connects to the Gulf of St. Lawrence and the Atlantic Ocean.

The Bay Fortune facility consists of one building containing aquaculture operations, laboratory, office, and living space, a storage facility and several ancillary structures. Aquaculture operations are conducted in two principal areas:

1. Early-Rearing Area (ERA) for production of AAS eggs, and rearing of alevin and fry (size ranges of 0.1 up to 100 g); and
2. Grow-Out Area (GOA) for rearing of alevin, fry and smolt (see definitions of these life stages in EA Section 9.1), as well as longer-term cultivation of juveniles and broodstock (size ranges of 0.1 to greater than 100 g).

The incubator trays in the ERA operate with 100% recirculation of water during early egg incubation that transitions to continuous flow-through operation once the eggs hatch. The rest of the aquaculture system operates with approximately 97% recirculation of water.

A schematic of these areas, including the containment and water flow, is provided in FDA (2024) Appendix C. Planned modifications to the Bay Fortune facility, discussed above, are illustrated in Figure C-3 of Appendix C, and the planned physical containment components for the new incubation room are depicted in Figure C-4 of Appendix C. The physical containment is to be equal to systems currently in place but will be verified in a supplemental approval under the NADA. The incubation room will operate under Recirculating Aquaculture Systems (RAS).

All effluent streams are combined and pass through a single containment sump before being discharged to a nearby drainage ditch (FDA 2024) that ultimately empties into the Fortune River Estuary then discharges to Rollo Bay, which is connected to the Northumberland Strait, the Gulf of St. Lawrence, and the Atlantic Ocean. Fish wastes (biosolids) from the facility are subject to extensive treatment prior to discharge to the estuary.

2.2.2 **Rollo Bay**

The Rollo Bay facility is also a land-based aquaculture facility located in eastern PEI on 70 acres in a predominantly agricultural area. It is about 1kilometer (km) north of Rollo Bay, and about 12 km north from the Bay Fortune facility. A small stream with variable flow, Rollo Bay Brook, runs through the property and travels approximately 1.5 km from the property before entering Rollo Bay.

In the 2019 EA for the approval of production of AAS eyed-eggs at the Rollo Bay facility, three Units at the Rollo Bay facility were named: Hatchery, Grow-Out, and Broodstock. Since that time, ABT decided to change the Grow Out Unit into another unit that will hold broodstock, now known as Broodstock Units 1 and 2. The Rollo Bay facility consists of three buildings each containing one aquaculture Unit: Hatchery, Broodstock Unit 1, and Broodstock Unit 2 (see site plan in Figure D-1 of Appendix D of the EA) (FDA 2024).

Currently, only the Hatchery Unit is approved under NADA 141-454. Construction of Broodstock Unit 1 is completed and is currently approved by Canada for grow-out of AAS (DFO 2019). While production of AAS in Broodstock Unit 1 is not currently approved by FDA or included as part of the NADA, ABT has notified FDA that they plan to submit this Unit for approval under a supplemental NADA in the future. With reference to Broodstock Unit 2, a building shell has been constructed and a floor plan created, but the containment plans have not been finalized and an operation date has not been set. ABT also plans to submit Broodstock Unit 2 for approval under a supplemental NADA in the future. While there are some aspects of the Rollo Bay facility that are not fully constructed, the preliminary plans submitted have been reviewed by FDA. In discussions with FDA during the course of this ESA consultation, NMFS agreed to assess the impacts to ESA-listed GOM Atlantic salmon and critical habitat to include the anticipated reconfigurations of the Rollo Bay and Bay Fortune facilities based on designs and containment measures outlined in the EA (FDA 2024).

The Hatchery Unit is used to produce AAS eyed-eggs; to produce and house AAFB and AANB, for breeding of improved AAS lines, and for other research activities. The Hatchery Unit may also produce eggs of Atlantic salmon without the rDNA construct. AAS eggs and eggs of Atlantic salmon without the rDNA construct will not be produced (i.e., fertilized) in the Hatchery Unit on the same day or held in the same incubators (i.e., within the same set of Heath stack trays or the same upwelling chamber) to eliminate the possibility of comingling. However, eggs of AAS and Atlantic salmon without the rDNA construct may be reared in the same Unit at the same time. ABT ensures that comingling will not occur, through proper labeling and training of staff. All batches of eggs and/or fry of Atlantic salmon without the rDNA construct are tested with a defined and validated testing protocol to confirm absence of the rDNA construct before sale and shipment to conventional Atlantic salmon producers. Likewise, all batches of AAS eggs are tested to confirm presence of the rDNA construct before shipment to ABT grow out facilities. This testing is conducted to ensure that the proper eggs are shipped to the correct operators.

The Hatchery Unit contains two Areas defined above: the ERA for production of eggs and rearing of alevin (size ranges up to 30 g), and GOA for rearing of fry, smolt and broodstock (size ranges from 10 to greater than 100 g). All aquaculture activities at the Rollo Bay site operate on Recirculating Aquaculture Systems (RAS) designed to operate at a 99.7% recirculation rate (i.e., with 0.3% make-up water being added continuously). A schematic of the Hatchery Unit and

Rollo Bay Site Plan, including the containment and water flow, are provided in Figure D-1 and Figure D-2 of Appendix D of the EA (FDA 2024).

Broodstock Unit 1 consists of three areas: ERA, ARA or GOA, and Conditioning, which are depicted in a schematic of the floor plan in Figure D-3 of Appendix D of the EA. Similar to the Hatchery Unit, production of eggs and rearing of alevin (size up to 30 g) will occur in the ERA, while rearing of fry, smolt and broodstock (size ranges from 10 to greater than 100 g) will occur in the ARA. The Conditioning Area is used for purging of adult fish prior to harvest; however, that area will likely only be used to hold immature Atlantic salmon without the rDNA construct now that the Unit has been converted from a grow out facility to a broodstock production facility. The ERA and ARA in Broodstock Unit 1 will operate at a 99.7% recirculating rate, while the Conditioning Area will operate in flow-through mode only. The containment schematics for the ERA, ARA and Conditioning for Broodstock Unit 1 are provided in Figure D-4 and Figure D-5 of Appendix D of the EA (FDA 2024).

ABT also plans to construct and operate a second broodstock unit called Broodstock Unit 2. This unit is illustrated on the Rollo Bay site plan in Figure D-1 of Appendix D of the EA (FDA 2024). At this time, a building shell has been constructed; however, the building plans, including containment schematics, have not been finalized. Broodstock Unit 2 will have containment similar to the Hatchery and Broodstock Unit 1.

All effluent from the Hatchery Unit passes through a polishing pond before entering Rollo Bay Brook. Effluent from both broodstock units will be discharged to a stone wash-out and vegetative strip before entering Rollo Bay Brook. All solids from Broodstock Unit 1 are collected in either 1) radial flow separators located within Early and Advanced Rearing Areas and sent to a closed septic tank for solid storage, or 2) in the waste treatment building where solids from the Advanced Rearing/Purge Area are dewatered and stored in a tank. Collected solids will either be transported to offsite waste treatment or used for agricultural purposes (land application), and water removed during solid waste processing will either be discharged to Rollo Bay Brook or pass into an underground leach field. The Rollo Bay Brook flows downstream to Rollo Bay.

3 CONSULTATION HISTORY

- 15 Mar 2021 – FDA notified NMFS that the FDA was preparing an EA and planned to initiate consultation on the effects of AAS.
- 2 Jun 2022 – FDA provided a draft EA to the Services for review and comment.
- 22 Aug 2022 – NMFS met with FDA to discuss comments on the amended EA for AquAdvantage Salmon.
- 17 Oct 2022 – NMFS received a second draft of an EA that addressed comments provided by NMFS.

- 28 Oct 2022 – NMFS reviewed revised EA and provided comments to FDA.
- 26-30 Jun 2023 – David Bean, NMFS/NOAA/Protected Resources Division GARFO conducted a site visit with FDA to observe the PEI operations, including containment, at the Bay Fortune facility and the Rollo Bay facility, including both the Hatchery Unit and Broodstock Unit 1.
- 18 Sep 2023 – FDA held a meeting to update the Services on the amended EA for AAS.
- 13 Dec 2023 – NMFS met with FDA to discuss amended EA for AAS and steps needed for submission of an ESA section 7 consultation request.
- 8 Jan 2024 – NMFS received request to initiate informal consultation under Section 7(a)(2) of the ESA from FDA.
- 21 Jan 2024 – After reviewing the submitted ESA section 7 consultation initiation package, the package was deemed sufficient to initiate consultation.

4 EFFECTS ANALYSIS

An action warrants an NLAA finding when its effects are wholly beneficial, discountable, or insignificant. *Wholly beneficial* effects have an immediate positive effect without any adverse effects to the species or habitat. Wholly beneficial effects are usually discussed when the project has a clear link to the ESA-listed species or its specific habitat needs and consultation is required because the species may be affected, albeit positively. *Discountable* effects are those that could occur while an ESA-listed species is in the action area but, because of the intensity, magnitude, frequency, duration, or timing of the stressor, exposure to the stressor is extremely unlikely to occur. *Insignificant* effects relate to the response of exposed individuals where the response in terms of an individual's growth, survival, or reproduction would be immeasurable or undetectable, or an impact to the conservation value of a physical or biological feature of critical habitat would be immeasurable or undetectable. For stressors that meet these criteria for wholly beneficial, discountable, or insignificant effects, the appropriate conclusion is NLAA.

The stressors possibly produced by the proposed action should salmon escape the facilities include:

- Breeding with native/natural fish thus passing on the modification to native species
- Predation on native species and outcompeting for resources
- Pathogens

The species that may be affected is GOM DPS of Atlantic salmon and its critical habitat. The GOM DPS of Atlantic salmon was listed as an endangered species on November 17, 2000 (65 FR 69459). A subsequent rule, 74 FR 29344, June 19, 2009, expanded the geographic range for the GOM DPS to include the Penobscot, Kennebec, and Androscoggin Rivers. A separate rule, 74 FR 29300, June 19, 2009, identified and established the Gulf of Maine DPS critical habitat (revised by 74 FR 39903, Aug. 10, 2009), which includes the following relevant physical and biological features essential to the conservation of the species:

- Deep, oxygenated pools and cover (boulders, woody debris, vegetation, etc.), near freshwater spawning sites, necessary to support adult migrants during the summer while they await spawning in the fall.
- Freshwater spawning sites that contain clean, permeable gravel and cobble substrate with oxygenated water and cool water temperatures to support spawning activity, egg incubation, and larval development, as well as freshwater spawning and freshwater rearing sites with the same characteristics to support emergence, territorial development and feeding activities of Atlantic salmon fry.
- Freshwater rearing sites with space to accommodate growth and survival of Atlantic salmon parr.
- Freshwater rearing sites with a combination of river, stream, and lake habitats that accommodate parr's ability to occupy many niches and maximize parr production.
- Freshwater rearing sites with cool, oxygenated (6 mg/L) water and diverse food resources (mayflies, stoneflies, chironomids, caddisflies, blackflies, aquatic annelids, and mollusks, as well as numerous terrestrial invertebrates, alewives, dace, or minnows) to support growth and survival of Atlantic salmon parr.
- Freshwater and estuary migratory sites free from physical and biological barriers that delay or prevent access of adult salmon seeking spawning grounds needed to support recovered populations or prevent emigration of smolts to the marine environment.
- Freshwater and estuary migration sites with pool, lake, and instream habitat that provide cool, oxygenated water and cover items (e.g., boulders, woody debris, and vegetation) to serve as temporary holding and resting areas during upstream migration of adult salmon.
- Freshwater and estuary migration sites with abundant, diverse native fish communities to serve as a protective buffer against predation.
- Freshwater and estuary migration sites with sufficiently cool water temperatures and water flows that coincide with diurnal cues to stimulate smolt migration.
- Freshwater migration sites with water chemistry (particularly pH) needed to support seawater adaptation of smolts.

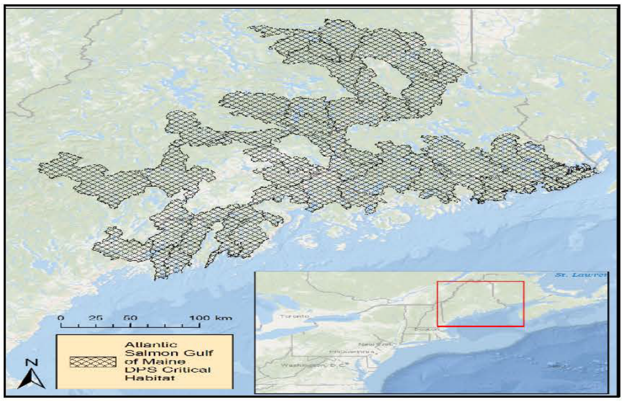


Figure 4 Map of designated critical habitat for the endangered Gulf of Maine distinct population segment of Atlantic salmon.

4.1 Escape leading to Breeding, Predation, and Competition with GOM DPS Atlantic Salmon

The stressors identified, with the exception of pathogens, are predicated on the escape of the genetically modified AAS fish or their broodstock. The FDA (2024) EA provides extensive detail of the redundant, multi-level physical containment strategies and procedural measures for containment within the Rollo Bay and Bay Fortune facilities that are purposely designed to prevent escape of AAS or their broodstock.

Additional information on the Bay Fortune and Rollo Bay facilities and their containment strategies and procedures is available in the following portions of the FDA EA (FDA 2024):

- Sections 7.1 and 7.2 contain information on the location and descriptions of the Bay Fortune and Rollo Bay facilities
- Figure C-1 and Figure D-1 in Appendix C and Appendix D contain the site plans for the facilities
- Table 7-1 in Section 7.3 contains a summary of the containment measures implemented at the Bay Fortune and Rollo Bay facilities
- Figure C-2, Figure C-3, Figure D-2, Figure D-3, and Figure D-4 in Appendix C and Appendix D, respectively, illustrate the physical containment measures at the Bay Fortune facility (including the planned incubation room) and in the Hatchery and Broodstock 1 and 2 Units at Rollo Bay
- Additional in-depth details on containment are provided in Section 5.4 of FDA (2015) for egg production in the Bay Fortune facility and Section 5.6 of FDA (2019) for egg production in the Rollo Bay facility.

The Rollo Bay facility is 99.7% recirculating with .3% being make up water continuously added to system and the Bay Fortune facility is 97% recirculating. Recirculating aquaculture facilities do not release any of their contents to the outside environment. Therefore, the Rollo Bay facility poses discountable risk of escape and only 3% of the operational extent of the Bay Fortune facility poses any possible risk for escape of AAS. Based on the limited water releases from the Bay Fortune facility and the redundant measures of physical and procedural containment protocols, the risk of escape is extremely unlikely and therefore, discountable. Because the stressors of breeding, predation, and competition with GOM DPS Atlantic salmon depend on the escape of AAS from these facilities, and further, the survival of AAS to the point of being able to interact with GOM DPS Atlantic salmon, the risk of those stressors is also discountable. Therefore, we concur with FDA that the risk of escape of AquAdvantage Salmon or AquAdvantage Broodstock, and the risk of AquAdvantage Salmon or AquAdvantage Broodstock breeding, predation, or competition with GOM DPS Atlantic salmon may affect, but are not likely to adversely affect endangered GOM DPS Atlantic salmon, due to myriad preventative measures.

Gulf of Maine DPS Atlantic salmon critical habitat is designated throughout Maine (see Figure 4). The ABT facilities are located in PEI, which requires movement through the ocean to reach the coast of Maine. Because the escape of AAS is discountable, the presence of AAS in GOM DPS Atlantic salmon critical habitat is not expected to affect the physical and biological features essential to their conservation that rely on water quality, water quantity, or passage/accessibility. It is possible that escaped AAS would adversely affect the two features that prioritize juvenile survival and growth and avoidance of predation. However, because escape of AAS is discountable and, for AAS to reach GOM DPS Atlantic salmon freshwater habitat at a size to pose a predatory risk, they would need to survive and grow in the wild, the likelihood of such an interaction is even less likely. Therefore, the operation of the ABT facilities on PEI may affect, but is not likely to adversely affect GOM DPS Atlantic salmon critical habitat.

4.2 Pathogens

In order for pathogens to be transmitted by AAS or ABT’s PEI facilities to the natural environment, they first need to be introduced into the ABT facility or to AAS in the facility and be spread within the facility. As described in Section 8.2.2 of the EA (FDA 2024), a pathogen could be introduced via 1) eggs/milt carrying a pathogen being brought into an ABT facility, 2) ABT personnel carrying a pathogen into the facility, or 3) contamination of the water source for the facility. It could then spread within the facility via water, movement of fish within the facility, equipment, and ABT personnel. The pathogen could then be transmitted to the environment via wastewater discharged from the PEI facility or via infected AAS escaping from the facility. As stated above, the Rollo Bay facility operates as a 99.7% recirculating aquaculture system with multiple outflow containment filters ranging from 13mm to 40µm screen perforation sizes. After being screened, effluent from the Hatchery Unit passes through a polishing pond before entering Rollo Bay Brook. Effluent from both broodstock units will also pass through screens prior to being discharged to a stone washout and vegetative stip before entering Rollo Bay Brook. Therefore, based on the limited water releases from the Rollo Bay facility and the redundant measures of physical and procedural containment protocols, the risk of releasing pathogens is extremely unlikely and therefore, discountable. Thus, the stressor of pathogens from Broodstock and Hatchery Units the Rollo Bay facility may affect, but are not likely to adversely affect GOM DPS Atlantic salmon.

While only 3% of the water used in the Bay Fortune facility is discharged, we must evaluate the risk of that discharge containing pathogens. One important consideration here is that pathogens in the facility's water pose a risk to AAS and their eggs. Therefore, there are preventative measures in place to prevent pathogen entry into the facility (as described in Section 8.2.2 of the EA). In the event of an introduction of a pathogen, ABT's comprehensive surveillance program at all facilities will identify the pathogen quickly so they can implement mitigation strategies to slow or eliminate spread in the facility (including reducing the load in wastewater discharge). Then, in order for the pathogens to reach GOM DPS Atlantic salmon, they would need to escape through wastewater discharge, thrive in the freshwater stream it discharges into, and then continue to survive in a host as it moves through saltwater into the Gulf of Maine.

Given the extensive procedures and mechanisms in place to prevent pathogens from entering the closed system of egg production at ABT facilities, the measures in place to quickly prevent the spread and propagation of a pathogen in their AAS tanks, and, finally, the improbability of a discharged pathogen surviving in both freshwater and marine environments to reach the Gulf of Maine, effects from pathogens are extremely unlikely to occur and therefore discountable. Thus, the stressor of pathogens may affect, but is not likely to adversely affect GOM DPS Atlantic salmon.

With respect to GOM DPS Atlantic salmon critical habitat, pathogens will not affect the physical and biological features essential to the species' conservation that rely on water quantity, passage/ accessibility, or predation, because none of these features will be impacted by the presence of pathogens. The physical and biological features essential to the conservation of the species that address water quality, growth, and survival could be affected by a pathogen. However, because the risk of pathogens is discountable to individual GOM DPS Atlantic salmon, the pathway to their critical habitat is just as unlikely and also discountable. Therefore, pathogens may affect, but are not likely to adversely affect GOM DPS Atlantic salmon critical habitat.

5 CONCLUSION

Based on the assessment conducted and information provided in the 2024 Amended EA, NMFS concurs with the FDA, Office of New Animal Drug Evaluation Center for Veterinary Medicine's assessment that the action involving production of AAS at the Bay Fortune and Rollo Bay (Hatchery Unit and Broodstock Units 1 and 2) facilities, and all stressors it may produce, including future planned changes and expansions as described in Section 7 of the EA, may affect, but is not likely to adversely affect endangered GOM Atlantic salmon or their critical habitat.

6 REINITIATION OF CONSULTATION

Reinitiation of consultation is required and shall be requested by the federal agency, or by NMFS, where discretionary federal involvement or control over the action has been retained or is authorized by law and (1) new information reveals effects of the action that may affect an ESA-listed species or designated critical habitat in a manner or to an extent not previously considered; (2) the identified action is subsequently modified in a manner that causes an effect to the ESA-listed species or designated critical habitat that was not considered in this concurrence letter; or (3) a new species is listed or critical habitat designated that may be affected by the identified action (50 CFR §402.16).

Please direct questions regarding this letter to Rory Driskell, Consultation Biologist, at (301) 427-8477, or by e-mail at Rory.Driskell@noaa.gov, or me at (240) 723-6321, or by e-mail at tanya.dobrzynski@noaa.gov.

Sincerely,

DOBRZYNSKI.TANYA.JANINE.1365846517
Digitally signed by DOBRZYNSKI.TANYA.JANINE.1365846517
Date: 2024.04.25 08:43:18 -04'00'

Tanya Dobrzynski
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Office of Protected Resources

cc:

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REFERENCES

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FDA. 2015. AquAdvantage® Salmon Environmental Assessment. Center for Veterinary Medicine.

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