AquAdvantage Salmon

Environmental Assessment

In support of supplements to NADA 141-454 to allow the production of AquAdvantage Salmon eyed-eggs and the grow out of AquAdvantage Salmon at a AquaBounty Technologies, Inc. facility near Rollo Bay, PEI, Canada

October 29, 2019

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LIST OF ACRONYMS AND CONVENTIONS EMPLOYED

AAS	AquAdvantage Salmon
AAS Broodstock	Transgenic Atlantic salmon used to produce AquAdvantage Salmon
ABRAC	Agricultural Biotechnology Research Advisory Committee
ABC	AquaBounty Canada, a wholly-owned subsidiary of AquaBounty Technologies, Inc.
ABT	AquaBounty Technologies Inc.
ABT Salmon	Any salmon genetically modified with the EO-1α construct in either the homozygous or hemizygous condition.
AFP	antifreeze protein
ANADA	Approved New Animal Drug Application
AR	Advanced rearing (area)
BW	body weight
CFIA	Canadian Food Inspection Agency
DFO	Department of Fisheries and Oceans
CVM	Center for Veterinary Medicine
DNA	deoxyribonucleic acid
DO	dissolved oxygen (concentration)
EA	environmental assessment
EO-1α	the integrated form of the AquAdvantage rDNA construct
EPA	United States (U.S.) Environmental Protection Agency
ER	Early rearing (area)
FDA	U.S. Food and Drug Administration
FL	fork length
FONSI	Finding of No Significant Impact
FWS	U.S. Fish and Wildlife Service, Department of Interior
GE	genetically engineered
GH	transgenic fish modified by the addition of an exogenous growth hormone gene
gh	growth hormone
HS	Heath Stacks
HT	Heath Trays
ISA	infectious salmon anemia
ISAV	infectious salmon anemia virus
LHO	low head oxygenator
mRNA	messenger ribonucleic acid
MT	metric tons
NADA	New Animal Drug Application
NEPA	National Environmental Policy Act
NM	nautical miles
NOAA	National Oceanic and Atmospheric Administration

NRC	National Research Council
ONADE	Office of New Animal Drug Evaluation
PEI	Prince Edward Island, Canada
PVC	polyvinyl chloride
rDNA	recombinant deoxyribonucleic acid
RAS	Recirculating Aquaculture System(s)
RFS	radial-flow settler
SOPs	Standard Operating Procedures
USDA	U.S. Department of Agriculture
UV	ultraviolet

TECHNICAL TERMS*

Allele	Any alternative form of a gene that can occupy a particular chromosomal locus.
AquAdvantage construct	The recombinant DNA construct used to generate AquAdvantage Salmon, referred to as *opAFP-GHc2*.
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 structure composed of one very long molecule of DNA and associated proteins (e.g., histones) that carries hereditary information.
°C-day [min]	Compound Unit of "time" (°C x days [min]) for relative determination of growth rate that accounts for effect of water temperature.
Conspecific	An organism of the same species as another organism.
Construct (gene 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-1	The mosaic, female founder of the AquAdvantage Salmon line created by microinjection of the *opAFP-GHc2* construct into a fertilized egg.
EO-1α	Functional, stably integrated form of *opAFP-GHc2* in the AquAdvantage Salmon genome.
Egg	Unfertilized haploid sex cells developed by females
Expression (gene)	The process by which information from a gene is used in the synthesis of a functional gene product (e.g., cell structures or proteins).
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).
Genome	The genome is 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.

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.
Molecular Cloning	Molecular cloning is a process by which scientists amplify a desired DNA sequence. The target sequence is isolated, inserted into another DNA molecule (known as a *vector*), and introduced into a suitable host cell (usually bacteria). Then, each time the host cell divides, it replicates the foreign DNA sequence along with its own DNA.
opAFP-GHc2	AquAdvantage recombinant DNA construct comprising regulatory sequences from an ocean pout AFP gene & growth hormone-coding sequences from chinook salmon.
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 a higher organism (See *haploid*, *diploid*, and *triploid*).
Promoter	A promoter is 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.
Protein-coding sequence	The DNA sequence of a gene that is transcribed into mRNA and subsequently translated into protein.
Recombinant DNA (rDNA construct)	Recombinant DNA (rDNA) is 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.
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*.
Smolt	A freshwater juvenile Atlantic salmon that has undergone the physiological changes necessary to be able to survive in salt water.
SW	Sea winter: Number of winters spent at sea (e.g., 1SW, 2SW).

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/downloads/AnimalVeterinary/SafetyHealth/AnimalCloning/UCM124756.pdf (accessed 01/29/2019); National Human Genome Research Institute, *Talking Glossary of Genetic Terms*, accessed at www.genome.gov/Glossary (accessed 01/29/2019); Human Genome Project, accessed at www.genomics.energy.gov (accessed 01/29/2019).

1 SUMMARY

AquaBounty Technologies, Inc. (ABT) has provided herein data and information in support of supplements to New Animal Drug Application (NADA) 141-454 for an intentional genetic alteration in a genetically engineered (GE) Atlantic salmon referred to as AquAdvantage Salmon, which exhibits a rapid-growth phenotype that allows it to reach a growth marker that is commonly used in the aquaculture industry faster than non-GE farm raised Atlantic salmon. The NADA (NADA 141-454) approved the production of AquAdvantage Salmon[1] at ABT's facility in Bay Fortune, PEI, Canada (Bay Fortune facility) and grow out of the fish at the ABT facility in Panama. In a 2018 supplement to the NADA, ABT received approval to grow out AquAdvantage Salmon for human food purposes at its facility in Albany, Indiana.

ABT is currently seeking approval of a supplement to NADA 141-454 to allow eyed-eggs of AquAdvantage Salmon to be produced at a land-based Hatchery Unit within ABT's Rollo Bay facility located near Rollo Bay, PEI, Canada, and for those eggs to be shipped to the United States (U.S.) for hatching and grow out at ABT's grow out facility in Albany, Indiana. In a later supplement, ABT will also be seeking approval of grow out operations for AquAdvantage Salmon in the land-based Grow Out Unit located at the Rollo Bay facility. Although Canada will be the primary market for the AquAdvantage Salmon grown for human food use in Rollo Bay, in the future supplement, ABT will seek U.S. Food and Drug Administration (FDA) approval to enable AquAdvantage Salmon grown in the Rollo Bay Grow Out Unit to be harvested and exported to the U.S. should ABT choose to do so.

Approval of such a supplemental application constitutes a major agency action and triggers environmental analysis under the National Environmental Policy Act (NEPA), unless otherwise excluded. Production and grow out of AquAdvantage Salmon at the Rollo Bay facility constitutes a major change in the conditions established in the approved NADA that requires FDA approval of a supplemental NADA and environmental analysis under NEPA. This EA constitutes part of that environmental analysis and relies extensively on the previous EA prepared by FDA for the original AquAdvantage Salmon NADA approved in November 2015. This EA describes the physical, biological, and geographical/geophysical forms of containment at the Rollo Bay facility and evaluates the potential environmental impacts of the action (approval of this specific supplemental NADA) and the no action alternative. FDA's approval of the NADA supplements would be for the specific set of conditions described in this EA and as enumerated in FDA's supplemental NADA approval letters. No other conditions of production and use of AquAdvantage Salmon would be permitted within the scope of the supplemental NADA approval, or have been evaluated in this EA.

This Environmental Assessment (EA) was prepared to support the supplements to NADA 141-454 for the Hatchery and Grow Out Units at the Rollo Bay facility. The original approval of the NADA was based on an EA prepared by FDA's Center for Veterinary Medicine (CVM), dated

[1] NADA 141-454 is for approval of the integrated α-form of the opAFP-GHc2 gene construct at the α-locus in the EO-1α line of Atlantic salmon under the conditions of use specified in the application; however, for ease of reference, this document refers to the application as being for approval of the AquAdvantage Salmon.

November 15, 2015 (2015 EA). Based on that EA, a Finding of No Significant Impact (FONSI) was issued on November 15, 2015, concluding that the action to approve the NADA for AquAdvantage Salmon, under the specific conditions described in the 2015 EA, "would not individually or cumulatively have a significant impact on the quality of the human environment in the United States."

The specific conditions in the original NADA were based on production of eyed-eggs at a single, specific facility in Bay Fortune, PEI, Canada, and shipment of eyed-eggs to a single, specific land-based grow out facility in the highlands of Panama, where they would be reared to market size and harvested for processing for food use in Panama before retail sale in the U.S. In 2018 a supplement to the NADA was approved allowing ABT to grow out AquAdvantage Salmon at its land-based grow out facility in Albany, Indiana[2]. The Panama grow out facility is currently no longer operational and FDA-registered; therefore, it will no longer receive shipments of AquAdvantage Salmon eggs for hatching and grow out.

It should be noted that, as a result of a similar request for the Rollo Bay facility made to, and accepted by, Canadian officials, production of AquAdvantage Salmon eggs is already underway at the Rollo Bay Hatchery and grow out of AquAdvantage Salmon will commence in the Rollo Bay Grow Out Unit once this unit is complete and operational. Additional information regarding the Canadian application and outcome is provided in Section 4.

Social, economic, and cultural effects of the proposed actions (approval of the supplements to the NADA) have not been analyzed and evaluated because the analysis in this EA indicates that the proposed action will not significantly affect the physical environment of the U.S. Under NEPA, social, economic, and cultural effects must be considered only once it is determined that the proposed agency action significantly affects the physical environment. 40 CFR 1508.14.

ABT's approach in this EA is one based on a characterization of hazards, an evaluation of potential exposure pathways, and a consideration of the likelihood of any resulting risk. The environmental analysis of consequences in the EA incorporates the principles described in the 2015 EA (Section 1) as well as the U.S. Environmental Protection Agency's (EPA) approach to ecological risk assessment EPA (1992). The potential hazards and harms addressed in this EA center on the likelihood and consequences of AquAdvantage Salmon escaping, surviving, and becoming established in the environment, and then dispersing or migrating such that there might be an exposure pathway causing an adverse outcome (the risk) to the environment.

These hazards were addressed in the 2015 EA and again in the EA prepared in support of the 2018 supplement to the NADA (2018 EA). In this EA, these hazards are addressed for production of AquAdvantage Salmon eyed-eggs and grow out of AquAdvantage Salmon to market size at a facility in Rollo Bay, PEI. The risk-related questions are:

[2] Approval for grow out of AquAdvantage Salmon was granted pending deactivation of Import Alert 99-40, which subsequently occurred on March 8, 2019.

What is the likelihood that AquAdvantage Salmon or the broodstock used to produce AquAdvantage Salmon eyed-eggs will escape the conditions of confinement?

What is the likelihood that AquAdvantage Salmon or the broodstock used to produce AquAdvantage Salmon eyed-eggs will survive and disperse if they escape the conditions of confinement?

What is the likelihood that AquAdvantage Salmon or the broodstock used to produce AquAdvantage Salmon eyed-eggs will reproduce and establish if they escape the conditions of confinement?

What are the likely consequences to, or effects on, the environment of the U.S. should AquAdvantage Salmon or the broodstock used to produce AquAdvantage Salmon eyed-eggs escape the conditions of confinement?

The land-based Hatchery and Grow Out Units in Rollo Bay have multiple and redundant forms of effective physical containment. As a result, the possibility that AquAdvantage Salmon or the broodstock used to produce AquAdvantage Salmon eyed-eggs could escape from containment, survive, and become established in the local environments of the Rollo Bay facility is very low. Should unintentional release from the Rollo Bay facility occur, the facility is surrounded by farmland and pasture and the only aquatic access to the local marine environment is a variable flow stream (Rollo Bay Brook) which flows through the Rollo Bay property.

Water temperatures in Rollo Bay Brook 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. The brook flows approximately 1.5 km before entering the Northumberland Strait, a tidal water body between PEI and the coast of eastern New Brunswick and northern Nova Scotia. A generally shallow depth causes strong tidal currents, water turbulence and a high concentration of suspended red silt and clay in the Strait. Water temperatures >25°C and anoxic conditions have been reported in the Northumberland Strait near Souris, PEI (Coffin et al. 2013) during the summer, and high nitrogen loads support excessive vegetative growth and algal blooms in the Strait. These conditions make the waters less than ideal for salmonids in general, particularly so during the summer months when water temperatures can exceed 25°C.

Additionally, early salmonid life-stages (i.e., egg, alevin, fry and parr)[3], which have not developed the hypo-osmoregulatory capacity necessary to survive in saltwater would not be likely to survive introduction to the saline waters of the Northumberland Strait. Only smolt, juvenile, or adult stages of AquAdvantage Salmon and AquAdvantage Broodstock would have any prospect of surviving a breach of, or escape from, the facility and any subsequent introduction into the Northumberland Strait.

[3] Atlantic salmon go through several life stages, including alevin, fry, parr, and smolt. For a description of these life stages, as well as the life history and biology of Atlantic salmon, see Appendix A.

The Rollo Bay operations will house AquAdvantage Salmon and AquAdvantage Broodstock (the fish used to produce AquAdvantage eggs). The largest number of fish that will be housed at the Rollo Bay site will be triploid, female AquAdvantage Salmon. These fish are effectively sterile and cannot reproduce with themselves or with any other salmonid.

In addition to the AquAdvantage Salmon themselves, three types of AquAdvantage Salmon broodstock will be housed at Rollo Bay: 1) diploid, wildtype (non-GE) Atlantic salmon; 2) diploid growth hormone (GH)[4] neomales (genetic females that have been converted to phenotypic males through addition of testosterone to their diets; neomales are fertile but do not have functional vas deferens and cannot transmit gametes without human intervention), and 3) diploid GH females which are fertile and reproductively competent. Approximately 300 neomales and no more than 20 GH females will be housed at Rollo Bay. As explained in detail later in the EA (Section 7.4), although reproductively competent, the GH females would not be likely to reproduce if they were to escape or be released due to biological, behavior, geographic, and geophysical factors.

The term "AquAdvantage Broodstock" is used throughout this EA when referring to GH neomales and GH females together. When appropriate, neomale(s) and GH female(s) are used in reference to the specific type of transgenic fish. Details regarding AquAdvantage Broodstock and the production of AquAdvantage eyed-eggs are provided in Section 5.5.1 and in the 2015 EA.

The addition of the Rollo Bay Hatchery and Grow Out Units theoretically add to the cumulative risk discussed in the NADA EA which was based on production only at the original facility in PEI near the Fortune River and grow out at a single facility in the highlands of Panama. As described above, the Rollo Bay facility will house additional AquAdvantage Broodstock and additional AquAdvantage Salmon will be reared and brought to market from the Rollo Bay Grow Out Unit. As a result, the cumulative risk in PEI is potentially increased. However, because it is concluded that the likelihood of escape, dispersal, survival, and establishment of AquAdvantage Salmon or AquAdvantage Broodstock from the Rollo Bay facility is small and inconsequential, the change to the cumulative risk is negligible.

The information provided in this EA supports the approval of two supplements to the NADA to allow production of AquAdvantage eyed-eggs and grow out of AquAdvantage Salmon at ABT's facility in Rollo Bay, Prince Edward Island, Canada, because it is reasonable to conclude that these operations, conducted under the conditions proposed in this EA, will not result in significant effects on the quality of the human environment in the U.S., including populations of endangered Atlantic salmon.

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[4] GH refers to genetically engineered Atlantic salmon modified by the addition of an exogenous growth hormone gene and promoter

2 PURPOSE AND NEED

This EA was prepared as part of the regulatory considerations for approval of supplements to the AquAdvantage Salmon NADA (NADA 141-454). AquAdvantage Salmon is a GH Atlantic salmon produced by AquaBounty Technologies, Inc. (ABT). ABT is currently seeking approval of a supplement to NADA 141-454 to allow eyed-eggs of AquAdvantage Salmon to be produced at the land-based Hatchery Unit located in Rollo Bay, PEI, Canada, and for those eggs to be shipped to the U.S. for hatching and grow out at ABT's grow out facility in Albany, Indiana. In a subsequent supplement, ABT will also be seeking approval of the grow out operations that will take place in the land-based Grow Out Unit located at ABT's Rollo Bay facility. Although Canada will be the primary market for the AquAdvantage Salmon grown for human food use in the Rollo Bay Grow Out Unit, as part of the future supplement, ABT will seek FDA approval to enable AquAdvantage Salmon harvested at the Rollo Bay facility to be exported to the U.S. should ABT choose to do so.

AquAdvantage Salmon contain a recombinant DNA (rDNA) construct, opAFP-GHc2, which imparts a rapid-growth phenotype allowing populations of these animals to reach a common growth measure (smolt size, or approximately 100 g) more quickly than populations of comparator Atlantic salmon.

This EA describes the use of physical, biological, and geographical/geophysical forms of containment at the Hatchery and Grow Out Units in Rollo Bay, PEI. This is a new location where AquAdvantage Salmon eyed-eggs are being produced for use in ABT grow out facilities and where AquAdvantage Salmon will be grown to market size, harvested and minimally processed (i.e., eviscerated) before transport to off-site final processors that will prepare whole fish, fish fillets, steaks, etc. for retail sale as food.

It should be noted that, as a result of a similar request made to, and accepted by, Canadian officials, production of AquAdvantage Eggs and grow out of AquAdvantage Salmon is already underway at the Hatchery and Grow Out Units that are the subject of this Environmental Assessment. Additional information regarding the Canadian application and outcome is provided in Section 4.

3 APPROACH TO ASSESSMENT

3.1 Introduction

The approach used in this EA follows that used in both the 2015 EA and 2018 EA and centers on the likelihood and consequences of AquAdvantage Salmon or AquAdvantage Broodstock, escaping, surviving, dispersing or migrating, reproducing, and becoming established in the unconfined environment, subsequently causing an adverse outcome. These hazards were previously addressed and determined to be acceptable for the production of eggs at ABT's hatchery at Bay Fortune, PEI, Canada and for grow out to market size in Panama and Indiana. In this EA, the hazards are addressed for production of eggs and grow out to market size in Rollo Bay, PEI, Canada. The framework is that of a, conceptual risk assessment model and a series of risk-related questions (see Section 3.3). This analysis and its outcomes are discussed in the Environmental Consequences section of this EA (Section 7).

3.2 Use of Redundant Containment Measures to Mitigate Risks

The principal method of managing risks associated with the production and rearing of any fish in aquaculture is through the application of confinement or containment measures designed to minimize the likelihood of escape or release into the environment. Additional confinement measures may be implemented to reduce the subsequent likelihood of harm to the environment should escape or release occur. These confinement approaches apply to GE fish as well as to wildtype fish (Kapuscinski 2005). Three primary methods of confinement have been characterized (Mair et al. 2007):

Physical confinement: providing mechanical barriers to prevent entry into the environment;

Geographical/geophysical confinement: rearing fish in a location where they cannot survive if they enter the surrounding environment; and

Biological confinement: limiting 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.

The three primary aims of confinement as cited by Mair et al. (Mair et al. 2007) are listed below along with a brief description of the containment measures that would be used for the grow out and disposal of AAS and AAS Broodstock. Section 5 of this EA describes confinement and containment measures and how they would specifically apply to AquAdvantage Salmon and AquAdvantage Broodstock at the egg production (Hatchery) and Grow Out units in Rollo Bay. These confinement measures have been incorporated as integral components of the supplements to the NADA.

1. Limit the organism: prevent the fish from entering and surviving in the receiving environment;

The primary form of preventing AquAdvantage Salmon or AquAdvantage Broodstock from entering the environment under the conditions established in the supplements to the NADA, if approved, is the mandated use of redundant physical and physico-chemical barriers within the Grow Out and Hatchery Units described in this EA. In the unlikely event that AquAdvantage Salmon or AquAdvantage Broodstock were to escape the Rollo Bay facility, naturally occurring geographic and geophysical conditions in the receiving environment are not ideal and could limit survival of escaping fish.

2. Limit (trans)gene flow: prevent gene flow from GE fish during production or following escape;

In the unlikely event that AquAdvantage Salmon or AquAdvantage Broodstock were to escape from the Rollo Bay Grow Out Unit or Hatchery (respectively), gene flow is very unlikely because: 1) the numerically largest population of transgenic fish that will be housed at the Rollo Bay site will be triploid, female AquAdvantage Salmon which are incapable of reproduction (See Section 5.5.1.2 (below) and Section 7.4.1.2 of the 2015 EA), either among themselves or with wild fish; and 2) the transgenic AquAdvantage Broodstock that will be housed at the Rollo Bay hatchery are either diploid GH neomales, which are incapable of transmitting the transgene to other fish without human intervention (See Section 5.5.1.2 (below) and Section 5.3.1.1 of the 2015 EA) or diploid GH females that are unlikely to transmit the growth hormone gene to other salmonids due to a combination of physiological and behavioral attributes associated with being GH, i.e. transgenic fish expressing an exogenous growth hormone gene, and geographical and geophysical barriers that reduce the likelihood GH females would come in contact with spawning native Atlantic salmon. Information is presented in Section 5.4 (below) regarding fitness characteristics of GH salmon and Section 5.4.7 specifically addresses reproduction of GH salmon. Additionally, GH females will be the numerically smallest population of transgenic fish housed at Rollo Bay. As the only purpose of these fish in broodstock production is to produce neomales, fewer than 20 GH females will be present, and all will be located in the Rollo Bay Hatchery Unit.

3. Limit the genetically engineered trait's expression: it is likely that the expression of the trait, not the transgene itself, poses the hazard.

The enhanced growth rate of AquAdvantage Salmon and AquAdvantage Broodstock is readily expressed under the optimum conditions provided in a commercial environment. However, in the highly unlikely event of escape into the wild, the absence of readily available food (to which they are accustomed, and which is necessary for rapid growth) and consequent depletion of energy reserves could significantly decrease the likelihood of effective exploitation of their inherent growth capacity.

No single containment measure can be assumed to be completely effective at all times and should not be considered to exist outside the context of multiple, independent and complementary measures in series. The National Research Council (NRC 2002) has recommended the simultaneous use of multiple, redundant containment strategies for GE fish, and three to five separate measures have been recommended by a body of biotechnology risk experts (ABRAC 1995). By combining containment measures with different stringencies, attributes, and modes-of-action, the compromise of aggregate containment by the failure of a single measure becomes increasingly unlikely.

This EA describes conditions of use for the approval of the supplements to the NADA for AquAdvantage Salmon. Although each individual method has intrinsic strengths and weaknesses, by combining complementary measures based on different principles of containment, an extremely high level of effectiveness results. The reliability of these measures is further ensured by adherence to a strong management operations and emergency response plan that includes staff training, Standard Operating Procedures (SOPs), daily internal inspections of containment equipment, and routine audits, complemented by inspections by FDA, as well as Federal and Provincial Canadian authorities.

As described in Section 5, multiple and redundant forms of containment are in effect at the Rollo Bay facility to effectively prevent the escape and establishment of AquAdvantage Salmon. In addition to effective physical (mechanical) containment, effective biological containment would be present for AquAdvantage Salmon, the largest group of transgenic fish (~100,000 in number) that will be housed at Rollo Bay location, and for the small number of (~ 300) transgenic neomales that are used to produce AquAdvantage Salmon eggs.

The immediate environment surrounding the facility in Rollo Bay is farmland and pasture and the only aquatic access to the local marine environment is the Rollo Bay Brook, a small stream with variable flow. It does support a population of brook trout and it is possible that escaped or released AquAdvantage Salmon or AquAdvantage Broodstock could survive in the brook.

Rollo Bay Brook flows approximately 1.5 km to the Northumberland Strait, a tidal water body between Prince Edward Island and the coast of eastern New Brunswick and northern Nova Scotia. A generally shallow depth causes strong tidal currents, water turbulence and a high concentration of suspended red silt and clay in the strait. Water temperatures >25 °C and anoxic conditions have been reported during the summer in the Northumberland Strait near Souris, PEI (Coffin et al. 2013), and high nitrogen loads support excessive vegetative growth and algal blooms in the Strait. These conditions make the waters less than hospitable to salmonids in general, especially during the summer months when water temperatures can reach or exceed 25 °C and the concentration of dissolved oxygen (DO) can fall to levels that will not sustain salmon (Appendix A, Section A.3).

3.3 Risk-Related Questions

The critical risk-related issues are the likelihood of the GE organism surviving and becoming established in the environment (the pathway by which exposure could occur) and the outcome or consequences of this establishment on the environment. As a framework for evaluating these issues, this EA has been developed around the following cascaded risk-related questions[5]:

What is the likelihood that AquAdvantage Salmon or the AquAdvantage Broodstock used to produce AquAdvantage Salmon eyed-eggs will escape the conditions of confinement?

What is the likelihood that AquAdvantage Salmon or the AquAdvantage Broodstock used to produce AquAdvantage Salmon eyed-eggs will survive and disperse if they escape the conditions of confinement?

What is the likelihood that AquAdvantage Salmon or the AquAdvantage Broodstock used to produce AquAdvantage Salmon eyed-eggs will reproduce and establish if they escape the conditions of confinement?

What are the likely consequences to, or effects on, the environment should AquAdvantage Salmon or the AquAdvantage Broodstock used to produce AquAdvantage Salmon eyed-eggs escape the conditions of confinement?

3.3.1 Likelihood of Escape from Confinement

The likelihood of escape depends primarily on the extent and adequacy of physical containment. 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 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 (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.

Fish have life stages in which they are small, can be difficult to contain, and may be impossible to re-capture if they escape. They can be highly mobile if the aquatic environment is sufficiently hospitable. These factors generally oblige the use of redundant, multiple-level containment strategies. The U.S. Department of Agriculture's (USDA) Agricultural Biotechnology Research Advisory Committee (ABRAC) has prepared Performance Standards for safely conducting

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[5] The risk-related questions in the 2015 EA did not include the phrase "or the AquAdvantage Broodstock used to produce AquAdvantage Salmon eyed-eggs" although the analyses therein did include that group of fish. Here, this group has been explicitly included in the questions for clarity. Note this phrase was not included in the risk questions in the 2018 EA for the Indiana grow out facility, but that EA addressed a facility where no AquAdvantage Broodstock were present and no eggs were produced.

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[6] is site- and project-specific, they should generally range from three to five.

3.3.2 Likelihood of Survival, Dispersal, Reproduction, and Establishment in the Unconfined Environment

For GE animals to pose a risk to the environment, in addition to exposure, an adverse outcome must result. Exposure is thus considered a threshold phenomenon (necessary, but not sufficient) because an initial escape or release of a GE organism might not have a measurable effect on the receiving community, or the organism might be rapidly removed due to natural selection or other processes (NRC 2002). Short-term survival, and ultimately long-term establishment (which requires long-term survival and reproduction) in the environment is generally needed for escape or release to present a hazard. Therefore, for the purposes of assessing risks of GE animals in the environment, exposure has been defined as the establishment of a GE organism in the community into which it is introduced or escaped (NRC 2002). Three variables have been identified by NRC as important for determining the likelihood of establishment for a GE animal:

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);

The ability of the GE animal to escape and disperse into diverse communities; and

The stability and resiliency of the receiving community.[7]

The likelihood of establishment depends 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 establishment in the environment and thus no resulting impacts. In other words, if there is no environmental exposure, there is also no environmental risk.

The term "fitness" refers to all 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. 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

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

[7] A stable receiving community has an ecological structure and function that is able to return to the initial equilibrium following a perturbation; resiliency is a measure of how fast that equilibrium is re-attained Pimm, S.L. 1984. The complexity and stability of ecosystems. Nature **307**(5949): 321-326. doi:10.1038/307321a0..

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. To assess risk associated with GE animals, it is critical to characterize the fitness of 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 increase or decrease the adaptability of an organism to a wider range of environmental conditions, could allow it to obtain nutrition from previously indigestible sources, or could limit the extent to which existing food sources provide adequate nutrition.

In addition to the animal's "fitness," for escapees to survive and ultimately reproduce, the ecosystem in which they arrive must offer suitable food, habitat, and environmental conditions (e.g., temperature and, for fish, salinity and water quality). Often the presence of conspecifics[8] or species closely related to the GE escapee in accessible ecosystems implies that a suitable environment exists (provided that the fitness of the escapee does not differ significantly from conspecifics or closely related species in that environment) (Kapuscinski et al. 2007).

The establishment of GE fish in an accessible environment would depend on how many fish escaped and survived, the non-reproductive characteristics of their phenotypes, and their reproductive potential. The latter depends on several factors including their survival rate and fertility, the environmental conditions affecting reproduction in the accessible ecosystem, and the proximity of breeding partners (e.g., conspecifics or related species with which reproduction is possible). In many cases, highly domesticated fish may be ill equipped to mate in the wild due to the effects of captivity, such as being used to artificial diets and being raised at a high stocking density (Kapuscinski et al. 2007).

An exception to the obligatory successful reproductive component for establishment can be postulated. In this case, a type of pseudo-establishment could occur if successive waves of large numbers of reproductively incompetent fish entered the environment, with each wave replacing the former as it dies off (Kapuscinski and Brister 2001). This scenario requires successive waves of release of large numbers of fish, similar to those that might occur following continual breaches of ocean net pens in a small area.

3.3.3 Likely Consequences of Escape

The environmental risk posed by GE organisms in the environment is similar to that of any introduced species, whether the introduction is intentional or unintentional. The ecological impacts of GE animals would be related to their fitness, interactions with other organisms, role in

[8] A conspecific is an organism belonging to the same species as another. For example, farmed and wild Atlantic salmon are conspecifics because they belong to the same species (*Salmo salar*).

ecosystem processes, or potential for dispersal and persistence (Kapuscinski and Hallerman 1991). For a more complete discussion of the interactions between Atlantic salmon and other organisms, including those between wildtype domesticated (farmed) salmon and wild salmon, see Appendix A.

The scale and frequency of introductions of GE fish into the environment will have a large influence on potential ecological risks and their magnitude. Any introductions would have to involve a critical mass (sufficient number) that could offset natural mortality and be of sufficient frequency in the 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 the time since they were first developed, several groups of scientists have identified the general types of environmental concerns or possible risks associated with GE organisms in general, including GE animals (Devlin et al. 2006; Devlin et al. 2015; NRC 2002, 2004; Snow et al. 2005). Although primarily hypothetical to date, general risks identified by Snow et al. (2005) include the following:

- Creating new or more vigorous pests and pathogens;
- Exacerbating the effects of existing pests through hybridization with related transgenic organisms;
- Harm to non-target species, such as soil organisms, non-pest insects, birds, and other animals;
- Disruption of biotic communities, including agroecosystems; and
- Irreparable loss of changes in species diversity or genetic diversity within species.

The Snow et al. (2005) report goes on to present several major environmental concerns associated with GE organisms, although not all of these are applicable to GE animals or to fish in particular. For aquatic GE animals specifically, the authors cited the following possible effects in the event of an escape: heightened predation or competition, colonization of GE animals in ecosystems outside of their native range, and alteration of population or community dynamics due to activities of the GE animal. The report states that in extreme cases, these effects might endanger or eliminate wildtype conspecifics, competitors, prey, or predators. Further consideration of these effects in relation to AquAdvantage Salmon is presented in Section 7.

4 ALTERNATIVES TO THE PROPOSED ACTION

The only alternatives to the proposed action are the "no-action" alternatives, which would be the failure to approve a supplement to the NADA to allow production of AquAdvantage Salmon eyed-eggs in the Rollo Bay Hatchery Unit and for these eggs to be used for grow out of AquAdvantage Salmon at the ABT facility in Indiana, and/or failure to approve a NADA supplement to allow rearing and grow out AquAdvantage Salmon in the ABT Grow Out Unit at Rollo Bay, which would prevent ABT from exporting AquAdvantage Salmon products from Rollo Bay facility to the U.S. However, based on the analysis in this EA, ABT does not believe that significant environmental impacts will occur from the proposed action; therefore, the "no-action" alternatives were eliminated from further consideration.

It is also noted that regardless of the outcome of the proposed action, and even if FDA adopted one or both of the no action alternatives, the ABT facility at Rollo Bay would continue to operate (but with no shipment of AquAdvantage Salmon eggs or food products to the U.S.) because of prior Canadian regulatory decisions. In July 2018, ABT submitted a New Substance Notification (NSN) to Environment and Climate Change Canada (ECCC) requesting permission to produce AquAdvantage Salmon eyed-eggs and to grow AquAdvantage Salmon for sale as food at the Rollo Bay site, i.e. the same requests being made in the supplements to the NADA.

After reviewing the potential environmental risks posed by those operations and the physical and chemical containment measures in place or being put in place at the time of the review, the Canadian authorities (ECCC and Health Canada) issued a Joint Assessment Report that concluded that operations at Rollo Bay posed a low risk to the environment. Subsequent to the review, ABT was authorized to operate at the site, and as FDA inspectors observed during the June 2019 pre-approval inspection of Rollo Bay, hatchery operations are underway at Rollo Bay. The Canadian Joint Assessment Report can be accessed at this link: https://www.canada.ca/content/dam/eccc/documents/pdf/pded/new-substances-organisms/Aquadvantage-salmon-summary.pdf.

The associated risk assessment report issued by the Department of Fisheries and Oceans[9] can be accessed through this link: http://www.dfo-mpo.gc.ca/csas-sccs/Publications/SAR-AS/2019/2019_014-eng.html.

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[9] Department of Fisheries and Oceans. (2019). Environmental and Indirect Human Health Risk Assessments for the Manufacture and Grow-out of EO-1α Salmon, including the AquAdvantage® Salmon, at a Land-Based and Contained Facility near Rollo Bay, PEI. DFO Can. Sci. Advis. Sec. Sci. Advis. Rep. 2019/014.

5 DESCRIPTION OF AQUADVANTAGE SALMON, CONDITIONS OF USE, AND CONTAINMENT

This section provides details on the phenotype of AquAdvantage Salmon and the specific conditions that would apply for production and use of these animals under the proposed action, including the applicable types of physical and biological containment. Information on the rDNA construct used in the genetic engineering of AquAdvantage Salmon and the genotype of this salmon is available in Appendix E of the 2015 EA[10] and is not discussed further herein. Background information on the life history and biology of Atlantic salmon is presented in Appendix A. Appendix A also contains information on salmon farming and the interactions between domesticated (farm-raised) salmon and wild salmon. This information provides a baseline for the consequence assessment in Section 7 and for characterization of the "fitness" of AquAdvantage Salmon relative to other farmed Atlantic salmon, and where appropriate, wild Atlantic salmon.

5.1 Identification of AquAdvantage Salmon

The identification of AquAdvantage Salmon has been previously described in the 2015 EA and there are no changes to this description.

5.2 Phenotypic Characterization of AquAdvantage Salmon

This section discusses the phenotype of AquAdvantage Salmon relative to wildtype farm-raised Atlantic salmon to help characterize its fitness. Most of this information was included in the 2015 EA (Section 5.2) and was updated in the 2018 EA for the Indiana facility.

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, genotype-by-environment 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 genotype-by-environment interactions, it is not surprising that the wide spectrum of traits observed in wildtype Atlantic salmon generally encompasses those of AquAdvantage Salmon.

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[10] https://www.fda.gov/downloads/AnimalVeterinary/DevelopmentApprovalProcess/GeneticEngineering//UCM466218.pdf

5.3 Comparative Studies

Multiple studies have been conducted by ABT comparing non-genetically engineered farm-raised Atlantic salmon to AquAdvantage 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 fish including diploid, mixed-sex GE GH Atlantic salmon, and other species of salmon, most notably coho salmon.[11] The extent to which these results may be applicable to Atlantic salmon in general, and to AquAdvantage Salmon in particular, have not been demonstrated (see Veterinary Medicine Advisory Committee Meeting Briefing Packet[12]).

5.3.1 Nutritional and Hormonal Composition

As discussed in the 2015 EA, the nutritional and hormonal composition of AquAdvantage Salmon muscle and skin is similar to that of present-day farm-raised Atlantic salmon. See 2015 EA Section 5.2.1.1.

5.3.2 Gross Anatomy, Histopathology, and Clinical Chemistry

The gross anatomy, histopathology, and clinical chemistry of male and female, triploid ABT Salmon 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 AquAdvantage Salmon 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 AquAdvantage 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 AquAdvantage Salmon in commercial production.

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[11]Many of the comparisons have been made to GE GH coho salmon, which is a different species (Onchorynchus kisutch), and contains a different growth hormone construct (i.e., the sockeye salmon growth hormone under the control of the metallothionein-B promoter of the same species Mori, T., and Devlin, R.H. 1999. Transgene and host growth hormone gene expression in pituitary and nonpituitary tissues of normal and growth hormone transgenic salmon. Mol. Cell. Endocrinol. **149**(1-2): 129-139. doi:10.1016/s0303-7207(98)00248-2.

[12]https://wayback.archiveit.org/7993/20170404230823/https://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/VeterinaryMedicineAdvisoryCommittee/UCM224762.pdf)

In the same comparator-controlled study, no severe malformations were noted among the AquAdvantage Salmon and diploid EO-1α salmon enrolled. Irregularities in the fins and gill structure of triploid AquAdvantage Salmon 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. kuta* (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.

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).

Almost all of the values for hematology and serum chemistry parameters of AquAdvantage Salmon 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 AquAdvantage Salmon and comparator salmon, the effect of triploidy on red cell number and size, and unavoidable limitations in study design.

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.

5.3.3 Growth Rates

The main difference between AquAdvantage Salmon 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 F_6-generation AquAdvantage Salmon 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 the 2015 EA.

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 ($g^{1/3}$/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.

5.4 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 and 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 AquAdvantage Salmon 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 AquAdvantage Salmon 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.

5.4.1 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 AquAdvantage Salmon is rapid growth, which is an integrated composite of many physiological rates. AquAdvantage Salmon exhibit growth and behavioral traits that also appear in other fast-growing Atlantic salmon or in brown trout (*Salmo trutta*) that have been treated with time-release growth hormone (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. 2000a).

The expression of growth hormone 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. 2000b; Cook et al. 2000c). 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).

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 AquAdvantage Salmon 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.

5.4.2 Tolerance of Physical Factors

Tolerance of physical factors such as temperature, salinity, 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 AquAdvantage Salmon, studies have shown that oxygen consumption in adult GH Atlantic salmon is higher than in wildtype comparators (Abrahams and Sutterlin 1999; Cook et al. 2000a; Cook et al. 2000b; 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 et al. 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[13]) 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,[14] escape into water with a DO level less than approximately 6 mg/L would place the GH Atlantic salmon at a physiological disadvantage.

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.

Although the temperature tolerance of AquAdvantage Salmon has not been investigated, because AquAdvantage Salmon 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%

[13]The solubility of oxygen in water is inversely related to water temperature, thus, DO concentrations decrease as the water temperature increases.

[14]Growth hormone appears to have a role in osmoregulation in anadromous salmonids Down, N.E., Donaldson, E.M., Dye, H.M., Boone, T.C., Langley, K.E., and Souza, L.M. 1989. A Potent Analog of Recombinant Bovine Somatotropin Accelerates Growth in Juvenile Coho Salmon (Oncorhynchus kisutch). Can. J. Fish. Aquat. Sci. **46**(2): 178-183. doi:10.1139/f89-024, Powers, D.A. 1989. Fish as model systems. Science **246**(4928): 352-358.. 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, K. 1993. Genetically engineered fish and their possible environmental impact. Norsk Insitutt for Naturforskning (NINA), Trondheim.. Other factors (discussed in subsequent sections) tend to increase the predation risk for GE fish.

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.

5.4.3 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).

In nature, swimming performance is important in foraging and predator avoidance. GH 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). 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.

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 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. 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.

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).

The effect of triploidy on the wildtype phenotype is also important to consider as AquAdvantage Salmon are triploid. 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. 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.

Under laboratory conditions, GH coho salmon (*Oncorhynchus kisutch*) bearing the OnMTGH1 growth hormone 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. (2017b) 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 gene-environment 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. (2017a) found that, in addition to gene-environment interactions, the strain of the coho salmon influenced fitness. Moreau et al. (2014) also found that family of origin to be an important factor influencing fitness in Atlantic salmon. In fact, Moreau *et al*. 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).

5.4.4 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. 2000c) 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.

5.4.5 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 AquAdvantage Salmon have any altered resistance to disease or parasites.

An outbreak of infectious salmon anemia (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 AquAdvantage Salmon and EO-1α[15] broodstock) (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 ISA virus (ISAV), the mortality rates were almost identical for GH (both AquAdvantage Salmon and EO-1α 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 AquAdvantage Salmon exposed via injection (ABT unpublished studies). No data were generated on wildtype comparators before the studies were discontinued.

FDA examined the facility's records related to the ISA outbreak during an inspection in June 2012 (see 2015 EA Appendix F) and found extensive documentation of the outbreak and diagnosis of ISAV as the causative agent. FDA found ABT's response to the outbreak to be appropriate, and all information collected during the inspection was found to be consistent with that previously described in ABT's submissions to the Agency (see 2015 EA, Section 5.2.2.5).

Periodic inspections by the Department of Fisheries and Oceans (DFO) Fish Health Unit (2010 through 2014) and by Canadian Food Inspection Agency (2012 through January 2019) 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 and diseases encompassed by these inspections are shown in Table 5-1 and include several viruses and filterable replicating agents, such as ISAV, plus other common fish pathogens and diseases. The Canadian Food Inspection Agency (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 tests for Infectious Pancreatic Necrosis three times per year and on occasion for Infectious Salmon Anemia virus and ISAV strain HPR0. CFIA conducts annual inspections that include a review of biosecurity protocols. The US Title 50 and Provincial clearances require re-testing every six months. Further discussion about diseases and parasites is provided in the 2015 EA, section 5.4.2.

[15] EO-1α broodstock are diploid salmon, homozygous for the EO-1α insert, used for the production of AquAdvantage Salmon

Table 5-1. Pathogens and Diseases Included in Inspections by Canadian Authorities

Pathogen or Disease	US Federal Title 50	Indiana DNR	Provincial Certificate of Health	CFIA Compartment Program	DFO FHPR Program
Bacterial Kidney Disease		X	X		
Infectious Haematopoietic Necrosis	X	X	X	X	X
Viral Hemorrhagic Septicemia	X	X	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[a]	
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] *for export to Brazil*

5.4.6 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 genetically engineered coho salmon (Devlin et al. 2004b; Sundström et al. 2007) and GH Atlantic salmon (Cook et al. 2000a; Moreau et al. 2011b). This 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 genotype-by-environment 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).

These 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 were only 20% longer 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.

5.4.7 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, EO-1α broodstock 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 and Fleming 2012; Moreau et al. 2011a). 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 (*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[16] 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[17] to either Atlantic 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.

5.4.8 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. 1995a) and smoltification in Atlantic salmon (Saunders et al. 1998) and in four species of Pacific salmon (Devlin et al. 1995b). 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 (19ºC) or constant light. Moreau et al. (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

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[16] 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, R.H., and Price, D.J. 1991. Natural hybrids between Atlantic salmon, Salmo salar L., and trout, Salmo trutta L., in juvenile salmonid populations in south-west England. J. Fish Biol. **39**(Suppl.A): 335-341. doi:10.1111/j.1095-8649.1991.tb05095.x, Jansson, H., Holmgren, I., Wedin, K., and Anderson, T. Ibid.High frequency of natural hybrids between Atlantic salmon, Salmo salar L., and brown trout, S. trutta L., in a Swedish river. (sA): 343-348. doi:doi:10.1111/j.1095-8649.1991.tb05096.x, McGowan, C., and Davidson, W.S. 1992. Unidirectional Natural Hybridization between Brown Trout (Salmo trutta) and Atlantic Salmon (S. salar) in Newfoundland. Can. J. Fish. Aquat. Sci. **49**(9): 1953-1958. doi:10.1139/f92-216, Verspoor, E. 1988. Widespread hybridization between native Atlantic salmon, Salmo salar, and introduced brown trout, S. trutta, in eastern Newfoundland. J. Fish Biol. **32**(3): 327-334. doi:doi:10.1111/j.1095-8649.1988.tb05370.x. and in the laboratory through artificial fertilization Gray, A.K., Evans, M.A., and Thorgaard, G.H. 1993. Viability and development of diploid and triploid salmonid hybrids. Aquaculture **112**(2): 125-142. doi:https://doi.org/10.1016/0044-8486(93)90439-6, Refstie, T., and Gjedrem, T. 1975. Hybrids between Salmonidae species. Hatchability and growth rate in the freshwater period. Ibid. **6**(4): 333-342. doi:https://doi.org/10.1016/0044-8486(75)90112-X.. 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.

[17] 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.

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.

5.4.9 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.

5.5 Conditions of Production and Use

Under the conditions of the NADA, the commercial production of eyed-eggs of AquAdvantage Salmon may occur only at the Bay Fortune facility on PEI (Bay Fortune). A detailed description of Bay Fortune, including containment and security measures employed there, is included in Sections 5.3 and 5.4 of the 2015 EA. Under the conditions of the approved NADA, commercial rearing and grow out of eyed-eggs of AquAdvantage Salmon was allowed only at ABT’s land-based, freshwater aquaculture facility in the highlands of Panama. However, the Panama grow out facility is no longer operational and FDA-registered; therefore, it will no longer receive shipments of AquAdvantage Salmon eggs for hatching and grow out.

In April 2018, FDA approved a supplement to the NADA, that allows the commercial rearing and grow out of eyed-eggs of AquAdvantage Salmon at ABT’s land-based, freshwater aquaculture facility in Indiana. The description, containment and security measures for the Indiana facility can be found in the publicly-available 2018 EA prepared to evaluate the environmental impacts of that supplement[18].

This EA was prepared to support new supplements to the AquAdvantage Salmon NADA, which propose to also allow production of eyed-eggs of AquAdvantage Salmon and commercial rearing and grow out of AquAdvantage Salmon at ABT’s land-based, freshwater aquaculture facility in

[18] This EA is available through the following FDA website: https://www.fda.gov/animal-veterinary/animals-intentional-genomic-alterations/aquadvantage-salmon

Rollo Bay, PEI (Rollo Bay facility). Section 5.6 describes the Rollo Bay site and aquaculture units (Hatchery and Grow Out) for which approval is being sought.

5.5.1 Production and Quality Control of AquAdvantage Eyed-Eggs and Eggs of EO-1α Broodstock and Wildtype Broodstock

5.5.1.1 *Production of AquAdvantage Salmon Eyed-Eggs*

The production of eyed-eggs to be sold into commerce by ABT for the land-based culture and retail sale of AquAdvantage Salmon was described in detail in Section 5.3.1 of the 2015 EA and is summarized below. Egg production will occur in the Hatchery Unit at the Rollo Bay facility and the procedures used will not differ substantively from those currently used at the Bay Fortune facility.

Gonads from masculinized genetic females (neomales) homozygous for the EO-1α construct are collected and milt recovered. The milt is used to fertilize eggs collected from wildtype Atlantic salmon females. Fertilized eggs are pressure shocked to induce triploidy, making the fish produced from these eggs functionally sterile (See Section 5.3.2.4 of the 2015 EA). Because the sperm is collected from genetic females, all AquAdvantage Salmon are female.

After fertilization and post-fertilization rinsing, eggs will be held in cool water for a defined time before being pressure shocked and disinfected. Disinfected eggs will be placed into either Upwelling Jars or Heath trays for incubation. The eggs will be removed from the incubators before they hatch (i.e., when “eyed”) and shipped to the ABT grow out facility in Indiana or transferred via closed containers to the on-site Rollo Bay Grow Out Unit.

Egg production and shipping protocols were defined in multiple SOPs previously reviewed and approved by FDA pursuant to the approval of the NADA and in use at the Bay Fortune facility. SOPs for the Rollo Bay operation are based on those in use at Bay Fortune and are described in Section 5.6.6.

5.5.1.2 *Production of EO-1α Broodstock and Wildtype Eggs*

Two lines of fish are maintained and used as broodstock for AquAdvantage Salmon: 1) homozygous EO-1α neomales, and 2) wildtype females from the conventional strain of salmon used to develop the EO-1α line of salmon. Neomales are genetic females fed a diet beginning at first feeding that contains either 17α-methyl-testosterone or 17α-methyl-dihydrotestosterone. Introduction of testosterone at first-feeding stage causes the fish to produce male gonads and viable sperm, however, due to the absence of a functional vas deferens (sperm duct), neomales cannot release milt which can only be collected by sacrificing the fish and manually extracting it from the gonads. See also the related discussion in Section 7.5.1.1.1 of the 2015 EA.

The wildtype line of Atlantic salmon used in AquAdvantage Salmon production has been maintained and improved by ABT through line breeding for multiple generations. Both male and female wildtype fish will be housed in the Rollo Bay Hatchery Unit.

Eggs of AquAdvantage Broodstock are produced in the same manner as described above except they are not pressure shocked to induce triploidy. Broodstock eggs, both transgenic and wildtype, will be incubated in the Hatchery Early Rearing area (ER). After hatching, alevin will be transferred to the ER A-tanks where they will be reared to approximately 10 g before being transferred to the Hatchery Advanced Rearing area (AR). They will be reared to adults and housed for their lifetime in the AR area.

The predictable biology and genetics of this production strategy, and the in-process confirmation of successful triploid induction that is a routine part of the operation, has ensured the commercial product are sterile, female Atlantic salmon hemizygous for EO-1α. The AquAdvantage Salmon NADA and 2015 EA described a second approach to sex modification in transgenic fish, gynogenesis, as an alternative to the use of neomales in the production of AquAdvantage Salmon broodstock. The sponsor has abandoned the use of gynogenesis and now relies exclusively on the use of neomales for production and maintenance of AquAdvantage Salmon broodstock.

5.5.2 Packaging and labeling of AquAdvantage Salmon eyed-eggs

The 2015 EA included descriptions and illustrations of the methods used by ABC to package and ship AquAdvantage Salmon eyed-eggs from the Bay Fortune facility to the grow out facility in Panama. The methods described in the 2015 EA for packaging and labeling AquAdvantage Salmon eyed-eggs were identified as draft methods with final versions pending approval of AquAdvantage Salmon by the FDA. Since receiving FDA approval for AquAdvantage Salmon in 2015, ABC has established SOPs for packaging and shipping eyed-eggs and finalized the details of the labels and package inserts that are required by the FDA. The SOP defining the procedure for packaging and shipping eggs is in place at Rollo Bay.

The FDA-approved product label is bilingual (English and Spanish), printed on tear- and water-resistant paper, and 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.

A bilingual (English and Spanish) Package Insert comprising detailed handling recommendations and important information regarding performance, animal safety, and environmental considerations is also included. Shipments are identified as “Eggs & Fry” that is “Not for Resale.” The following additional warnings (or facsimile thereof) 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.

5.5.3 Shipping and handling of AquAdvantage Salmon eyed-eggs

Details of shipping AquAdvantage Salmon eyed-eggs from the Bay Fortune facility to Panama were provided in the 2015 EA and will be followed when eyed-eggs are shipped from Rollo Bay to the Indiana facility in the U.S. Shipping procedures are defined in an SOP. 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 U.S. That will also be the case for AquAdvantage Salmon eyed-eggs produced at the Rollo Bay facility.

Eggs produced at Rollo Bay Hatchery Unit for use in the Rollo Bay Grow Out Unit will be moved between buildings in closed containers in accordance with facility SOPs but will not require the use of the same containment or shipping methods that are used for transport of eyed-eggs to Indiana.

5.5.4 Shipping and handling of AquAdvantage Salmon

ABT intends to produce AquAdvantage Salmon for commercial sale of fillets and whole fish in Canada or export to countries where AquAdvantage Salmon is approved. ABT will harvest AquAdvantage Salmon and deliver whole, killed fish to a processor for final product preparation.

5.5.5 Quality control of AquAdvantage Salmon product integrity and durability

As explained in detail in the 2015 EA and summarized in Section 5.5.1.1 of this EA, the AquAdvantage Salmon that will be grown for commercial sale and consumption are sterile females carrying a single copy of the EO-1α construct. All AquAdvantage Salmon are female because the neomales used to manufacture AquAdvantage Salmon eyed-eggs are genetically female and the egg donor is a female domesticated Atlantic salmon. AquAdvantage Salmon are sterilized by treating fertilized eggs with hydrostatic pressure which causes the eggs to become triploid and triploid salmon are effectively or functionally sterile (See Sections 5.3.2.4 and 7.4.1.3 of the 2015 EA). As a result, in the very unlikely event that AquAdvantage Salmon were introduced to the environment, they would not be able to mate with any other fish and could neither transmit the transgene nor become established in the environment.

The sponsor has an established SOP that governs the methods used to induce triploidy in hemizygous EO-1α females and qualification of triploid induction is conducted in accordance with another SOP. Six commercial batches of AquAdvantage Salmon eggs were shipped to Panama for grow out prior to closure of that facility. In the six shipments made since 2015, the percentage of triploid eggs has never dropped below 98.5% and was ≥ 99% in five shipments (Table 5-2). Additionally, only a small fraction of the non-triploid eggs were diploid (0.08%, i.e. 1 diploid egg of 1200 tested, Table 5-2, column 4).

At full capacity, the Rollo Bay Grow Out unit will house approximately 100,000 AquAdvantage Salmon and, assuming an average diploid rate of 0.08% among the non-triploids (the averages achieved thus far, see Table 5-2), fewer than 100 diploid EO-1α females would be present in the Grow Out Unit at any given time.

Table 5-2. Quality control data collected on triploid conversion of AquAdvantage Salmon eggs

Commercial Batch	Date of Analysis	Flow Cytometry Record #	Estimated % Diploid	Estimated % Non-viable	Estimated % Inconclusive	Estimated % Triploid
AAS-120815-005	25 Jan 2016	904-907	0.5%	0.0%	0.0%	99.5%
AAS-121615-008	02 Feb 2016	920-923	0.0%	0%**	1%**	99%
AAS-111016-001	27 Dec 2016	993-1000	0.0%	0.0%	1.5%**	98.5%
AAS-112216-003	10 Jan 2017	1012-1014	0.0%	0.0%	0.0%	100%
AAS-112817-001	24 Jan 2018	1142-1146	0.0%	0.0%	0.0%	100%
AAS-011111-010	23 Feb 2018	1174-1179	0.0%	0.0%	0.0%	100%
		Average % Diploid	0.08%		Average % Triploid	99.5%

** Scored inconclusive prior to the implementation of aneuploid/non-viable scoring method implemented in November 2017 and documented in a SOP.

5.6 Rollo Bay Facility: Facility Descriptions, Containment, and Security

If approved, under the conditions that would be established in the approval of these supplements to NADA 141-454, production of AquAdvantage eyed-eggs and commercial rearing and grow out of eyed-eggs of AquAdvantage Salmon will also take place at a land-based, freshwater aquaculture facility near Rollo Bay, Prince Edward Island, Canada. This facility will be operated by AquaBounty Canada (ABC), a wholly-owned subsidiary of ABT.

In July 2018, ABT submitted a New Substance Notification (NSN) to Environment and Climate Change Canada (ECCC) proposing production of eyed-eggs and grow out and rearing of AquAdvantage Salmon at the Rollo Bay site. In March 2019, after reviewing the NSN and physically inspecting the Rollo Bay site, including the Hatchery and Grow Out Units, ECCC concluded that the physical and chemical containment measures that will be used at Rollo Bay resulted in a low potential for exposure to the environment and authorized ABT operations at the site. The Canadian report can be accessed at this link: https://www.canada.ca/content/dam/eccc/documents/pdf/pded/new-substances-organisms/Aquadvantage-salmon-summary.pdf.

5.6.1 Locations and Operations of the Rollo Bay Facility

The Rollo Bay site, formerly known as Atlantic Sea Smolt, was purchased in 2016 from Snow Island Salmon Inc. The previous owners used the site to produce salmon smolts for sale to the local salmon aquaculture industry. At the time of purchase, the site had one permanent building used as a hatchery and a set of outdoor tanks for rearing smolts. ABT acquired the location in order to expand production of AquAdvantage Salmon eggs (needed to support ABT's growing operations) and as a site for grow out and commercial rearing of AquAdvantage Salmon. Fish harvested at the site will be minimally processed, i.e. killed and gutted, and transported to licensed processors for further processing and packaging. The primary market for AquAdvantage Salmon harvested from Rollo Bay is Canada. However, ABT is seeking approval of the Rollo Bay Grow Out Unit to enable export of products to the U.S. should that prove to be of interest.

The Rollo Bay site is located at 46 36 27N, 62 34 30W and lies in a predominantly agricultural area on approximately 70 acres bound by Route 307 (Bear River Road), a north-south highway that connects Highway 2 (Veteran's Memorial Highway) and Highway 16 (Northside Highway) (Figure 5-1). The site is in eastern PEI (Kings County) ~1 km north of the closest coastal waters and ~7 km northwest of Souris, PEI (pop. 1,232). Souris is ~78 km northeast of the provincial capital of Charlottetown (pop. ~38,174). The Rollo Bay site is ~12 km (by road) from the Sponsor's Bay Fortune site which was approved for production of eyed-eggs under the NADA. The local economy is primarily dependent on farming, fishing-aquaculture, and tourism.

The Rollo Bay location will house all activities required to produce AquAdvantage Salmon eggs on a year-round schedule and to rear AquAdvantage Salmon for sale to seafood processors and wholesalers. When the location is fully operational, aquaculture activities will take place in three units: Hatchery, Grow Out (Grow Out), and a future Broodstock Unit. When the site is fully operational, ABT will be able to spawn salmon year-round and supply eyed-eggs for grow out at the ABT Grow Out facility in Indiana and in the Rollo Bay Grow Out Unit that is described and addressed in this EA. At the time this EA was prepared, the Broodstock Unit is under construction and therefore this EA addresses only the Hatchery and Grow Out Units. An additional supplement to the NADA will be submitted for approval of the Broodstock Unit when construction on it is complete, and the Unit is operational.

Figure 5-1. Rollo Bay Site (white box) and Surrounding Area, including AquaBounty Bay Fortune Facility

The Hatchery Unit is housed in a refurbished building of sound construction built by professional contractors. Exterior walls of the building consist of 2 by 6 in. studs, insulation, and steel siding inside and out. The walls of the concrete foundation measure approximately 1.2 m high. Load bearing trusses were designed for the upper floor to support the required equipment for water treatment that was used by previous owners. The roof trusses are covered in steel, are appropriate for the climate, and will withstand high winds and the weight of snow in winter months.

The Grow Out Unit is housed in a pre-engineered steel building complete with insulation and steel siding inside and out. The walls of the concrete foundations measure from 1.5 to 10 m high, depending on the area of the building. The roof beams are covered in steel and appropriate to the local climate, which can include high winds and heavy snow loads.

During the winter months, snow loads on the roofs will be monitored and removed as needed. No trees will be located adjacent to the buildings to prevent damage from falling limbs or trees in the event of a tropical storm or hurricane.

Multiple systems will be in place to monitor site security, prevent unapproved intrusion, avoid inadvertent escape of fish, and prevent loss of operational capacity. The aquaculture buildings will be equipped with independent back-up generators that will meet power requirements in the event of an electrical outage. Culture tanks in all buildings will be monitored continuously for water level, dissolved oxygen levels, pH, temperature, carbon dioxide, and ozone.

All ABT operations, including Bay Fortune and Rollo Bay, are exclusively land-based aquaculture systems. Except for the conditioning operations that will be housed in the Grow Out Unit, 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 more detailed description of the RAS systems is provided below.

Eyed-eggs will be produced in the Hatchery Unit and market-sized fish will be produced in the Grow Out Unit. The Hatchery and Grow-out Units are self-contained, and only eyed-eggs produced in the Hatchery will be moved between those two buildings. Details of the buildings and operations within each building are provided in the following sections.

Table 5-3 describes the buildings/units and key units at the Rollo Bay Facility and a schematic diagram of the site is provided in Figure 5-2.

Table 5-3. Buildings and key Units at Rollo Bay Facility

Building or Unit	**Purpose and Description**
Hatchery	Produce eyed-eggs, house broodstock, conduct research. The building includes offices, a lunchroom, and an apartment for overnight workers.
Grow Out	Grow market-size fish (5 kg) from eyed-eggs through conditioning-harvest; capacity of 250 MT per year. The facility also houses a wet lab, mechanical room, office spaces, conference room, lunchroom, and overnight apartment for staff.
Broodstock[19]	Under construction. To house broodstock used for production of AquAdvantage Salmon
Waste Treatment/Manure Storage	Permanent, concrete storage tanks for solid waste storage
Production Wells	Four wells provide water for aquaculture operations
Polishing Pond	Located adjacent to Hatchery; first discharge point for hatchery effluent

[19] The Broodstock building and equipment therein is under construction and not addressed in this EA. A new supplement to the NADA and a supporting EA will be submitted when the Broodstock building is completed and functioning.

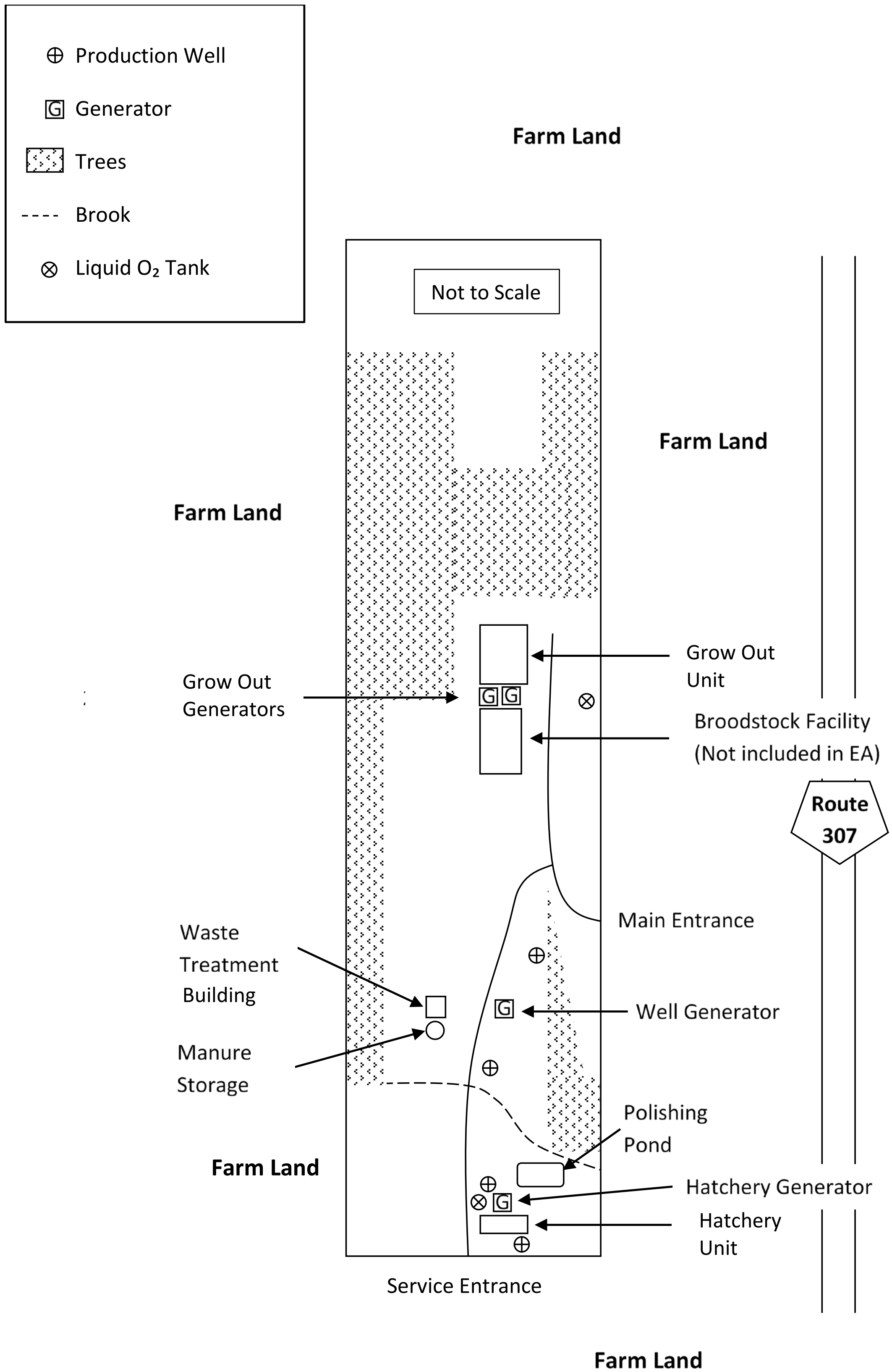


Figure 5-2. Site diagram of the Rollo Bay Location

5.6.2 Facility Descriptions

5.6.2.1 *Rollo Bay Hatchery Unit*

The Hatchery Unit is used to produce AquAdvantage Salmon eyed-eggs and to produce and house AquAdvantage Salmon Broodstock. It is housed in a recently renovated 8800 ft^2 building that includes approximately 5340 ft^2 of aquaculture space in two discrete areas, the Early-Rearing area (ER); and Advanced Rearing area (AR). Additional detail about the tanks and the fish that are housed in the Hatchery is provided in Table 5-4.

5.6.2.1.1 *Hatchery ER*

Production of transgenic and non-transgenic (wildtype) eggs takes place in a designated area within the ER. Incubation of eggs and rearing of alevin will take place in the ER. Fertilized eggs are incubated in Heath Stacks and Upweller Jars can be added to the incubation area if desired/need. Both types of incubators are described in more detail in Section 5.6.3.1.1.1.

Eyed-eggs of the AquAdvantage Salmon produced in the Hatchery will be transported to the ABT facility in Indiana or transferred to the Rollo Bay Grow Out Unit. Eyed-eggs may also be held under cold temperatures to delay development and coordinate with the grow out facilities' production cycles.

Broodstock eggs are incubated, hatched and the alevin reared to approximately 10 g in the ER. They are then transferred to the Hatchery AR for rearing to adulthood and long-term housing. Additional details about manufacturing and processing eyed-eggs are provided in Section 5.5.1.1 (above).

There are 30 tanks of 0.22 m^3 capacity (A-Tanks) in the ER. Newly hatched alevin are transferred from the incubators to A-tanks where they are reared to approximately10 g in size.

Table 5-4. Tank and Fish Information, Rollo Bay Hatchery Unit

Culture Tanks		**Fish Size**[1]			**Tank Containment Screens**[2]			
Group	Max Volume (L)	~BW (g) Min	~BW (g) Max	~FL (mm)	Number	mm	inch	
A (alevin)	220	0.1	10	20 - 200	1	0.8	0.03	1/32
B (fry)	1,500	≥ 10	100	≥ 100	1	6.4	0.25	1/4
C (smolt)	14,790	≥ 100	≤12,000	≥ 200	2	12.7	0.50	1/2
Heath Stacks (eggs)	10,000/tray[3]	na		5[4]	Top & Bottom[5]	1.5	0.06	1/16

1 Size-range of fish in body weight (BW) and fork length (FL) typically housed in each tank (0.1 g, alevin; 10 g, fry; 100 g, smolt)

2 Minimum number of internal tank screens and Minimum size of the opening in screening used (Note: screen size is increased as the fish grow to facilitate wash-out of feces and unconsumed feed).

3 Number of eggs per tray

4 Diameter of eggs (mm)

5 Location of screens on trays

5.6.2.1.2 *Hatchery AR*

When alevin reach approximately 10 g they are transferred from the Hatchery ER to the Hatchery AR. The AR is used for culture of fry and smolt, to grow juvenile broodstock to adult size, and to house adult broodstock.

The Hatchery AR houses 15 tanks of 1.5 m^3 capacity (B-tanks) and 12 14.8 m^3 tanks (C-tanks). The B-tanks are used for intermediate rearing of fish from approximately10 g in size to approximately100 g and the C-tanks are used to rear and hold broodstock from approximately100 g to adults of up to 12,000 g.

5.6.2.2 *Rollo Bay Grow Out Unit*

The Grow Out Unit occupies a newly constructed building of approximately 42,500 ft^2 of total space. Aquaculture operations will occupy just over 38,000 ft^2 and will be conducted in three distinct areas: Early Rearing /Intermediate Rearing area (ER/IR), Advanced Rearing area (AR), and the Conditioning area. At full capacity, approximately 250 MT of fish will be produced each year for processing and sale. Information on the tanks and fish that will be reared in each type of tank are provided in Table 5-5.

Table 5-5. Tank and Fish Information, Rollo Bay Grow Out Unit

Culture Tank		**Fish Size**[1]		**Internal Screening**[2]	
Group	~Max Volume (L)	~BW (g)	~FL (mm)	Number	mm
A	12,840	0.1 - 30	20 - 100	2	1.5
B	70,700	30 - 200	≥ 100	2	5
C	122,000	200 - 1500	≥ 200	2	12.7
D	274,100	1500 - ~5000	≥ 400	2	25
E (Conditioning)	83,920	~5000	≥ 800	2	25
Heath Stacks	10,000/tray[3]	n/a	5[4]	Top & Bottom[5]	1.5

[1] Size-range of fish in body weight (BW) and fork length (FL) typically housed (0.1 g, alevin; 10 g, fry; 100 g, smolt; >100 g post-smolt; approximately 5000 g market size)
[2] Minimum number of internal tank screens and minimum size of the opening in screening used (Note: screen size is increased as the fish grow to facilitate wash-out of feces and unconsumed feed)
[3] Number of eggs per tray
[4] Egg diameter (mm)
[5] Location of screens on each tray

5.6.2.2.1 *Grow Out ER/IR*

The Grow-out ER/IR will be used to incubate and hatch eyed-eggs and rear fish from alevin to juveniles of approximately 200 g. It will house Heath Stacks for incubation of eyed-eggs, three tanks of 12.84 m^3 capacity (A-tanks) and three tanks of 70.7 m^3 capacity (B-tanks). Eyed-eggs of AquAdvantage Salmon will be transferred in closed containers (see Section 5.5.3) from the Hatchery to the Grow-out ER/IR for incubation and hatching. After eggs have hatched and fry have consumed most of their yolk sac, alevin of < 1.0 g will be transferred from the incubators to the A-tanks where they will be reared to approximately 30 g. After reaching 30 g the fish will be transferred to the B-tanks where they will be reared to approximately 200 g.

5.6.2.2.2 *Grow Out AR*

The Grow-out AR will be used to rear fish from approximately 200 g to market weight of 5 kg. The area will be organized into two identical sets of seven tanks: four of 122.0 m^3 capacity (C-tanks) and three of 274.1 m^3 capacity (D-tanks). Each set of tanks will have its own RAS. Juveniles will be transferred from the ER/IR B-tanks into the AR C-tanks where they will be reared to a size of ~ 1.5 kg and then transferred to the D-tanks where they will be reared to market size of ~ 5 kg.

5.6.2.2.3 *Grow Out Conditioning Area*

The Conditioning area will be used to prepare fish for harvest and is comprised of three rectangular concrete tanks, each measuring 11.58 m × 3.66 m × 1.98 m (L×W×H) with a capacity of 83.92 m^3. Market-sized fish (approximately 5000 g) will be moved from the AR D-tanks into the conditioning tanks approximately one week prior to harvest.

Water for conditioning operations enters the system directly from the wells (after degassing and ultraviolet [UV] treatment, see Section 5.6.3) and flows through the conditioning tanks. Water leaving the conditioning tanks is routed to the AR RAS units for cleaning and recirculation through the AR.

5.6.3 Water and Waste Management

The Rollo Bay facility is a ground water-based aquaculture operation. Water for aquaculture operations is provided through four wells (Main, North, South, and Lower) located within the site boundaries (Figure 5-3). The four wells have more capacity than is needed to operate the facilities and water for operations is typically pumped from one or more wells at any given time. There will be domestic wells on the property located adjacent to the three main buildings that supply water only for domestic needs to each building. The domestic wells supplying the Hatchery and Grow Out Units are in place and the third well will be added to supply domestic needs of the future Broodstock building.

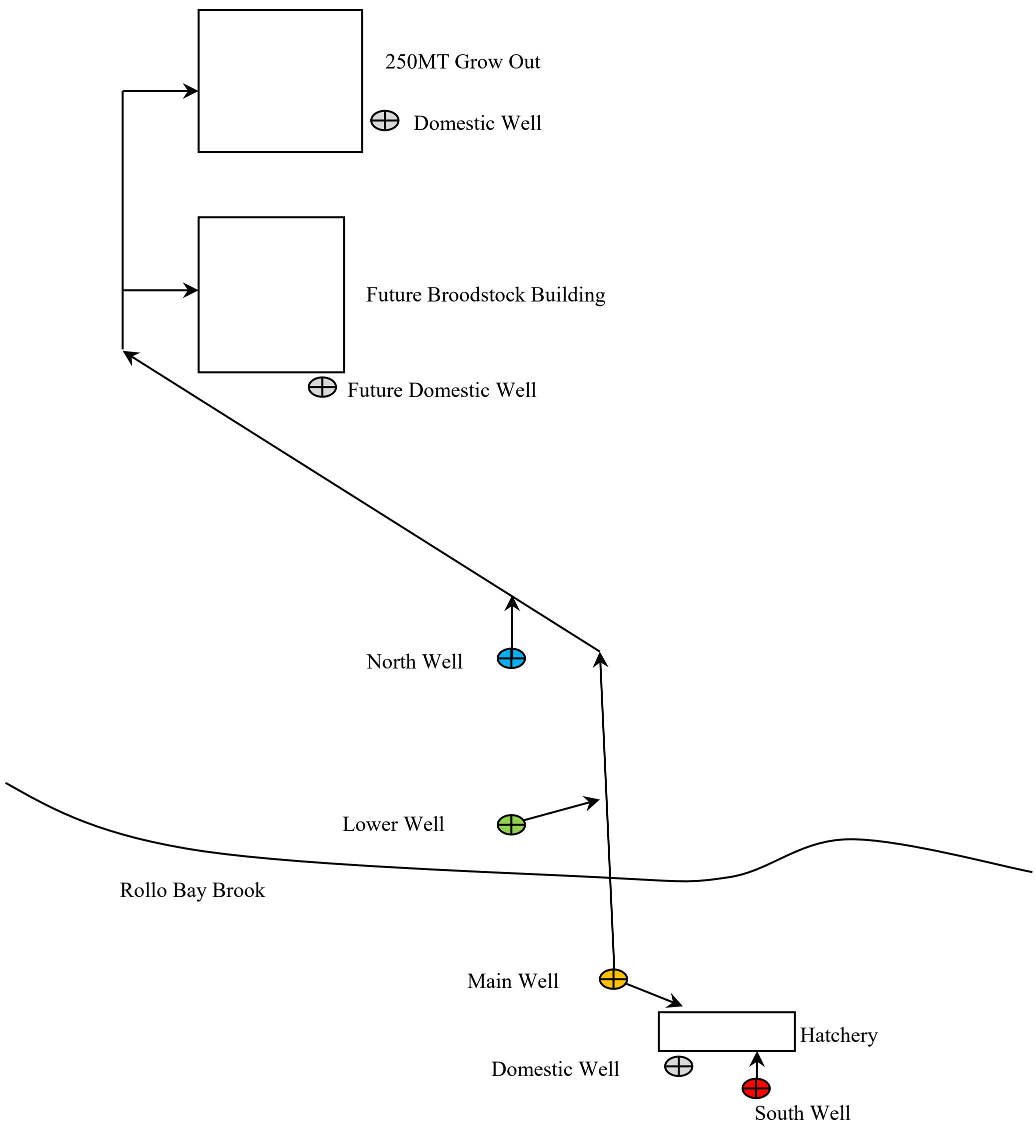


Figure 5-3. Location of Wells and Flow of Well Water at the Rollo Bay Site

5.6.3.1 *Water and Waste Flow*

Figure 5-4 provides a site-level view of water and waste flow and the location of key containment points that will be in place at the Rollo Bay facility. Detailed descriptions of the containment barriers, including diagrams and images, are provided in Section 5.6.4.

Clear water

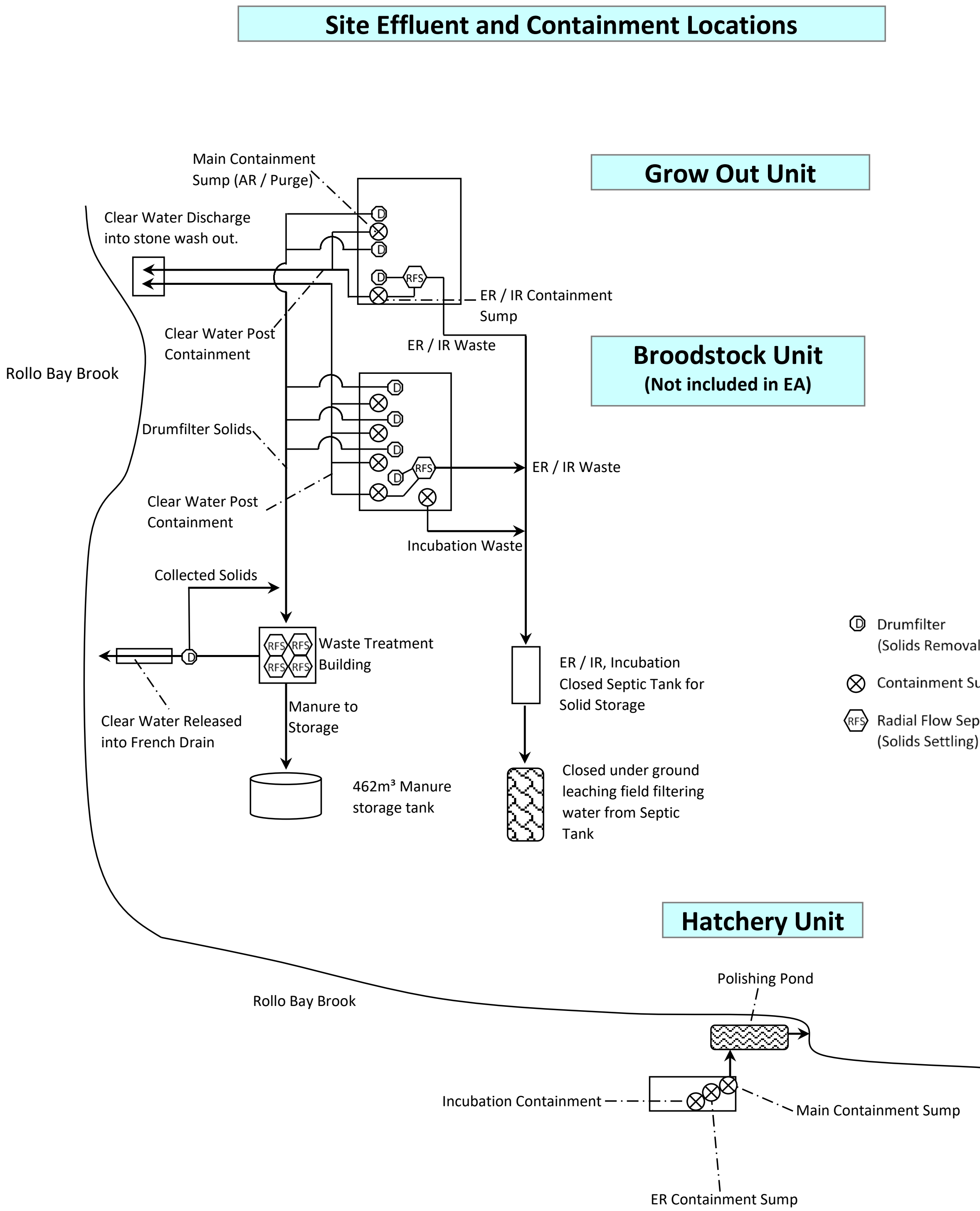


Figure 5-4. Site Effluent and Waste Flow with Key Containment Locations

5.6.3.1.1 *Incoming Water Flow*

All well water used for aquaculture will pass through UV sterilizers and have nitrogen removed and oxygen added. After sterilization, nitrogen degassing and oxygenation, incoming water enters the RAS systems or is fed directly to the Conditioning tanks located in the Grow-out facility.

5.6.3.1.2 *Hatchery Effluent and Clear Water Discharge*

Discharge water leaving the Hatchery Unit will consist of clear water overflow from the RAS and water from the floor drains. As shown in Figure 5-4 (above), effluent from the Hatchery building is discharged into an adjacent polishing pond and then into the Rollo Bay Brook which flows through the property. A detailed description of containment and water flow in the hatchery is provided in Section 5.6.4.1 and detailed diagram of water flow through the Hatchery is provided in Figure 5-6.

5.6.3.1.3 *Grow Out Effluent and Clear Water Discharge*

Discharge water leaving the Grow Out Unit will consist of clear water overflow from the RAS, water collected in floor drains, and waste effluent water from the drum filter.

Discharged water flows through a through a 20” PVC pipe into a stone out-wash located approximately 140 m west of the building housing the Grow Out Unit. The water is filtered through the stone and must flow approximately 40 m across a natural area populated with trees and undergrowth before eventually entering the Rollo Bay Brook. As shown in Figure 5-4 (above), the brook flows through the property and past the Hatchery before exiting the property (see Section 6.1.1). 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 to 364 L/m from July through September and 546 L/m the rest of the year. There are no limits on maximum discharge volumes.

5.6.3.1.4 *Grow Out ER/IR Solid Waste*

Solid waste (sludge) collected in the drum filters of the Grow Out ER/IR will flow into a Radial Flow Separator (RFS) where solids are collected. The solids will then be pumped into an underground concrete septic tank located adjacent to the Grow Out Unit. Waste from the tank will be removed as required and transported to an offsite waste treatment facility and water from the septic tank will pass into an underground leaching field.

5.6.3.1.5 *Grow Out AR Solid Waste*

Solid waste (sludge) collected in the Grow Out AR will flow to an on-site Waste Treatment Building where the sludge will be directed to four radial flow settlers (RFS) (See Figure 5-4 (above) and 5-10) where further de-watering takes place. Solids collected from the RFS will be stored in an underground, concrete storage tank. The waste will be emptied as needed and the solid waste moved to an offsite waste treatment facility or used for agricultural purposes (land application) according to provincial and federal regulations and guidelines. Clear water from the RFS will pass through a drum filter where any remaining sediments ≥0.04 mm are captured and returned to the RFS. Clear (filtered) water from the drum filter will be directed to an underground French drain located adjacent to the Waste Treatment Building which allows water to dissipate back into the ground. Additional details of water and waste flow in the Grow Out Unit are provided in Section 5.6.3.2.

5.6.3.1.6 *Recirculating Aquaculture Systems (RAS)*

Except for the Conditioning tanks located in the Grow-out facility, all aquaculture activities at the Rollo Bay site will operate as Recirculating Aquaculture Systems (RAS). The RAS units that will provide water for ER and AR operations in the Hatchery and Grow Out Units are designed to operate at a 99.7% recirculation rate, i.e., with 0.3% make up water being added continuously. RAS units will be fitted with drum filters, CO_2 strippers, biofilters, ozone generators, and low head oxygenators (LHOs). Within each RAS, water will return from the tanks and pass through a drum filter where solids will be removed. Drum filters will remove solid particles ≥ 0.04 mm in the Hatchery RAS units and the Grow-out ER/IR RAS. The Grow-out AR RAS drum filter will screen out particles ≥ 0.06 mm.

The path of water through the RAS units is shown in Figure 5-5. In the RAS, water will flow from the drum filters to the media bed where approximately 0.3% make-up water will be added from the fresh water supply. The media bed will contain bio-media which will be aerated and kept in constant motion to maintain favorable microbes in the recirculated water. Water will then be pumped up to the CO_2 strippers and gravity fed through the LHOs where it will be treated with oxygen and ozone. The oxygenated water will then be collected in a head tank to create a constant flow rate for the water returning to the rearing tanks. Water exiting the head tanks in all RAS except the Grow-out AR will be UV sterilized.

As described in Sections 5.6.2.1.2 and 5.6.2.1.3 (above), clear water from the RAS overflows is the primary source of effluent from the Hatchery and Grow Out units. The amount of water exiting the facility is equivalent to the amount of water being added as make-up water (i.e., approximately 0.3%) as described in the previous paragraph.

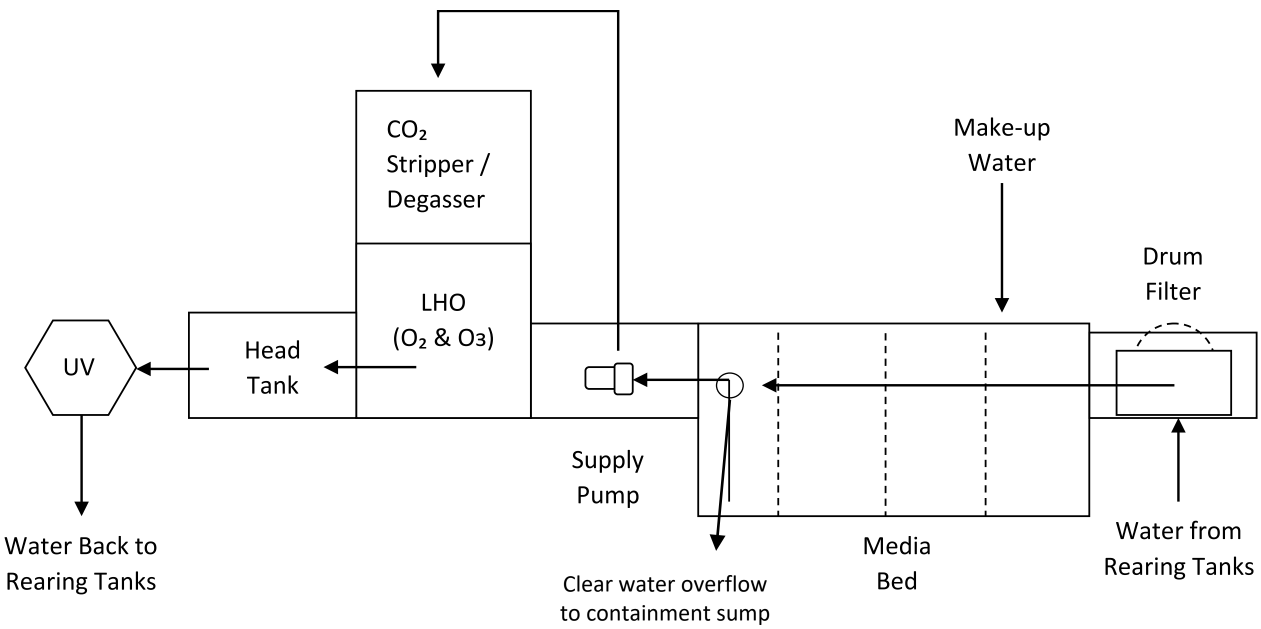


Figure 5-5. Generalized Diagram of Water Flow through the RAS Units

The Hatchery operates on two RAS that provide water for operations in the ER and AR and both are physically located in the Hatchery AR. Three RAS units will supply water for operations in the Grow Out Unit: the ER/IR A- and B-tanks will be supplied by a RAS located in a separate room inside the ER/IR, and the AR will utilize two RAS, one for each set of C- and D-tanks. The Grow Out AR RAS are in a separate room located at one end of the Grow-out AR.

The RAS used for incubators in the Hatchery and Grow-out ERs will be less complex than the other RAS. Incubator RAS consist of a recirculating pump, temperature and pH control, and UV sterilizer. More information about the incubator RAS system is provided in Section 5.6.4.1.1.

Conditioning operations (i.e. preparing market weight fish for harvest) will take place only in the Grow Out Unit. As described above, incoming well water will be sterilized and have nitrogen removed and oxygen added before entering the Conditioning tanks. Water will exit the Conditioning tanks and travel to the Grow Out AR drum filters where it will be incorporated into the AR RAS supply.

5.6.4 Containment

Multiple, redundant containment measures will be employed in the Hatchery and Grow Out Units to prevent the escape of all life stages of fish. Containment measures will include mechanical (e.g., screens, filters, and nets) and chemical (e.g., chlorine) barriers. The containment methods and locations have been designed specifically for the operations and life stages that will be present in each area of each building. The combination of appropriately sized barriers placed in key locations makes the risk of escape extremely low.

5.6.4.1 *Hatchery Unit Containment*

Figure 5-6 provides a schematic diagram of the water flow and containment scheme that will be used in the Hatchery Unit. The containment points in place in the three Hatchery operational areas are described in detail in following sections.

5.6.4.1.1 *Hatchery Containment: Egg Handling, Incubation, and Early Rearing*

Production of fertilized eggs, both transgenic and wildtype, and housing of broodstock, both transgenic and wildtype, will take place in the Hatchery Unit. Eyed-eggs of the AquAdvantage Salmon produced in the Hatchery will be transported to the ABT facility in Indiana or transferred to the Rollo Bay Grow Out Unit. Eyed-eggs may also be held under cold temperatures to delay development and coordinate with the grow out facilities' production cycles.

Spawning and egg production activities will take place in the loading bay adjacent to the Hatchery ER. Chlorine pucks will be placed in all drains and all precautions and procedures taken at Bay Fortune and described in the 2015 EA have been implemented at the Rollo Bay Hatchery. Egg incubation and rearing of alevin to approximately 10 g takes place in the Hatchery ER where fertilized eggs will be incubated in Heath Stacks or potentially in Upweller Jars. Eggs to be used for grow out of AquAdvantage Salmon will be incubated to the eyed-stage and then shipped to Indiana or transferred to the Rollo Bay Grow Out Unit. Broodstock eggs will be incubated, hatched and transferred to the Hatchery ER A-tanks as first-feeding alevin.

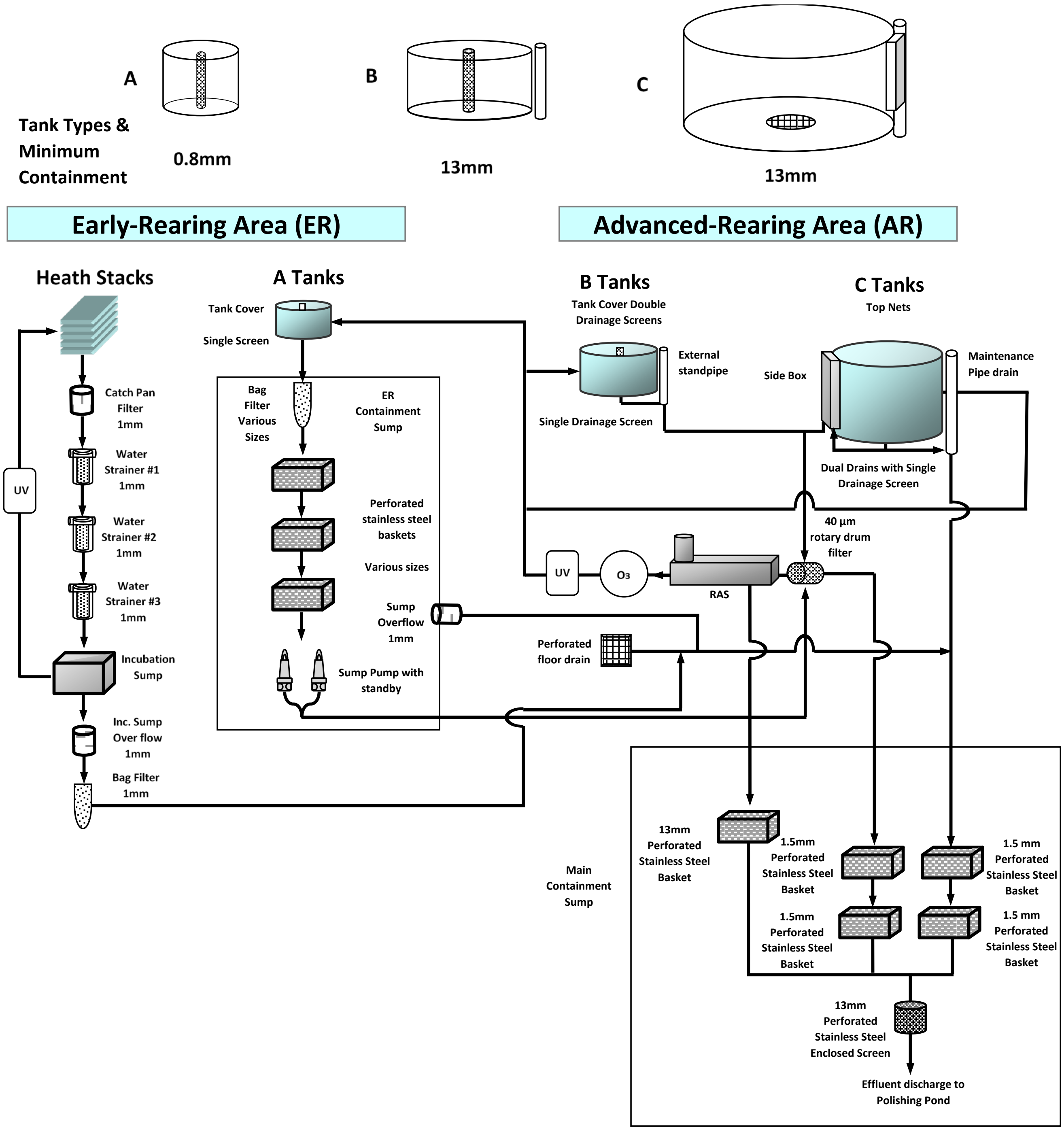


Figure 5-6 Water Flow and Containment Barriers in the Rollo Bay Hatchery

5.6.4.1.1.1 Hatchery Incubator Containment

There are 11 points of containment in the Hatchery incubator system (Table 5-6).

Table 5-6. Hatchery Incubator Containment Points

Facility	Area	Containment Point	Location	Barrier Type	Barrier Material(s)	Perforation Size (mm)	Fish Sizes (g) in Unit
Hatchery Incubators							
Hatchery	ER	1	Heath Stack Egg Trays	Top and Bottom Screens	Molded plastic inserts with Polyester screen	1.50	0.1
Hatchery	ER	1	Upweller Jars (Incubator Jars)	Top and Bottom Screens	PVC	1.50	0.1
Hatchery	ER	2	Heath stack catchment pan	Perforated Pipe	PVC	1.00	0.1
Hatchery	ER	2	Upweller Jar filter pipe	Perforated Pipe	PVC	1.00	0.1
Hatchery	ER	3	Recirculation line	Strainer	Nylon	1.00	0.1
Hatchery	ER	4	Recirculation line	Strainer	Nylon	1.00	0.1
Hatchery	ER	5	Recirculation line	Strainer	Nylon	1.00	0.1
Hatchery	ER	6	Incubation Sump Overflow	Perforated Pipe	PVC	1.00	0.1
Hatchery	ER	7	Incubation Sump Overflow	Bag Filter	Nylon	1.00	0.1
Hatchery	ER	8	Floor drain	Perforated Pipe	PVC	1.00	0.1
Hatchery	AR[20]	9	Main Containment Sump	Containment Basket	Stainless Steel	1.50	0.1
Hatchery	AR	10	Main Containment Sump	Containment Basket	Stainless Steel	1.50	0.1
Hatchery	AR	11	Main Containment Sump	Containment Basket	Stainless Steel	13.00	0.1

The recirculation systems and containment measures that are in place for the Heath Stack systems to incubate eyed-eggs in the Hatchery and Grow Out Units are generally the same in all relevant aspects. Water flow and containment details of the Heath Stack system are described here and will be referenced in the Grow-out containment description. In the Hatchery, in addition

[20] The hatchery main containment sump is physically located in the Hatchery AR.

to Heath Stacks, fertilized eggs may be incubated in Upweller Jars (also referred to as Incubator Jars). If installed, they will utilize the same downstream containment points described here for the Heath Stacks.

Figure 5-7 provides a schematic diagram of the water recirculation system and containment points used for the incubators incubator systems (Heath Stacks and/or Upweller Jars) in the Hatchery and Grow-out ER.

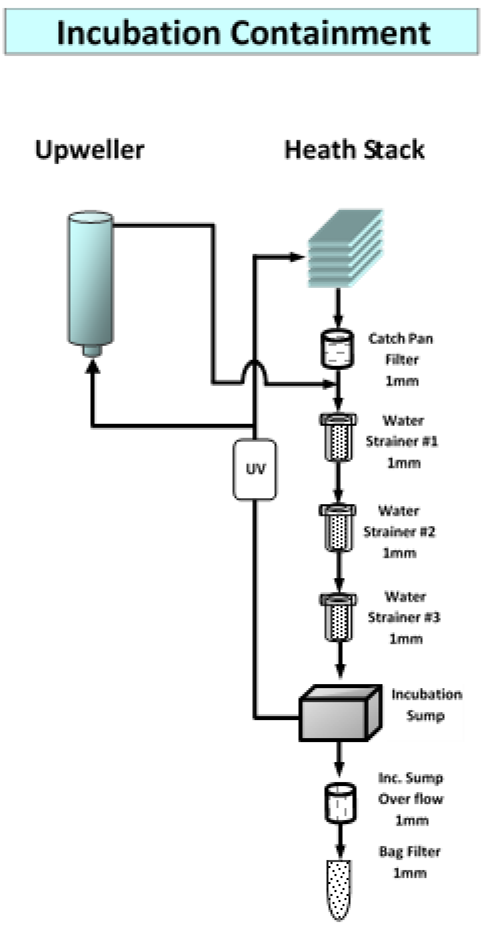


Figure 5-7 . Egg Incubation Recirculation System and Containment Points.

Eyed-eggs will be incubated in either Heath Stacks (HS, "Incubator Stacks", or "Stacks"), vertical cabinets which hold multiple Heath Trays (HT; also referred to as "incubator trays", "egg trays", or simply "trays") in each stack (Image 5-1) or Upweller Jars (Image 5-2). Eyed-eggs are approximately 5 mm in size and will be incubated until they have hatched and absorbed most of their yolk sac.

Image 5-1. Heath Stacks used to incubate eyed-eggs in the Hatchery and Grow Out Units.

Image 5-2. An example of the Upweller/Incubator Jars that may be used to incubate eyed-eggs in the Hatchery.

As shown in Table 5-6 (above), 11 containment points are utilized in the Hatchery incubator system to prevent eyed-eggs and newly hatched fry from entering the environment. The 11 containment points used in the incubation and hatchery areas include:

Containment Point 1 - Heath Stack Tray Screens: 1.5 mm polyester screens, smaller in size than salmon eggs or newly-hatched fry, sit on top and bottom of removable egg trays (Image 5-3). After loading with eggs, the removable tray will be locked into a HT by holding the front of the egg tray up and pushing it backwards until two notches located on the back corner of the tray slide under corresponding pins located on the HT (Image 5-4).

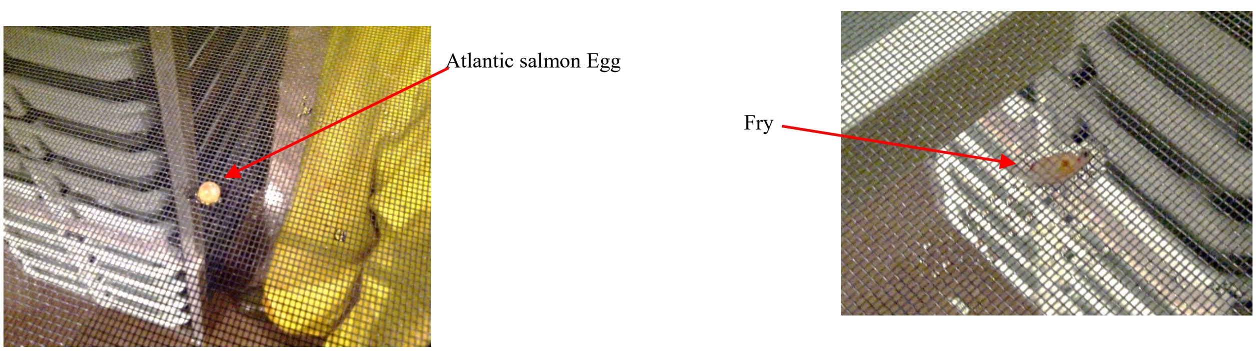


Image 5-3 1.5 mm mesh screens cover the top and bottom of each removable egg tray

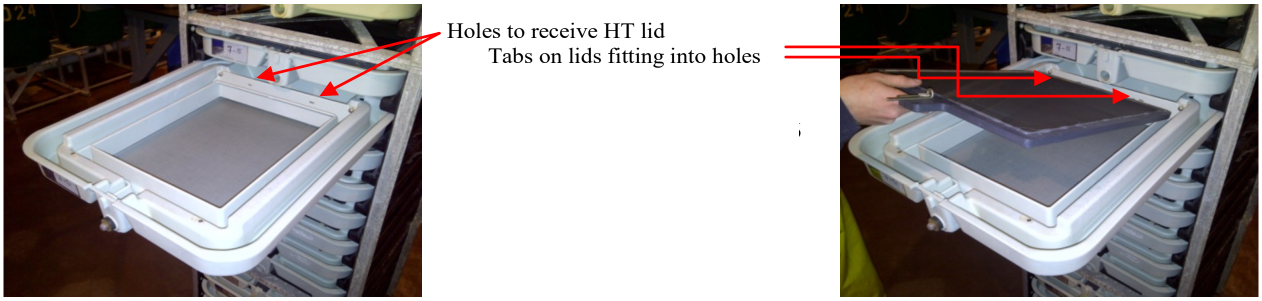


Image 5-4. Placing the removable egg tray in the Heath Tray

Water will enter at the rear of the top tray in the HS and flow through the stack from top to bottom. As the water fills the tray, it will upwell through the eggs before passing out of the front of the tray into a trough which diverts water towards the back of the rack. As the water flows to the back of the tray it passes out of two ports, one on either side of the tray, to the tray below it. This process continues until the water exits the last tray in the stack. The components of the HT and removable egg tray are shown in Image 5-5.

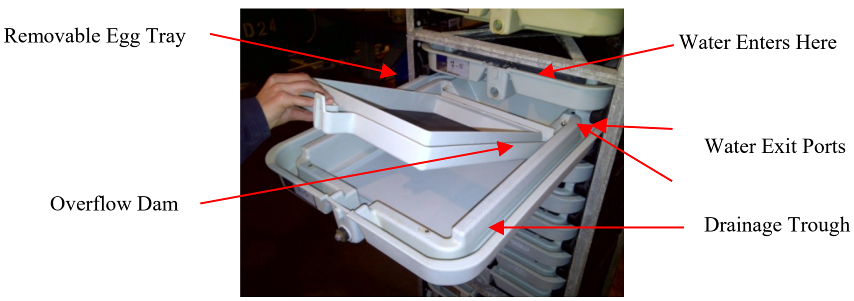


Image 5-5. Removable Egg Tray

The screen is secured to the tray by a sliding lock located on the front of the lid (Image 5-6); sliding the lock farther forward into a hole on the HT will secure the egg tray to the HT (Image 5-7).

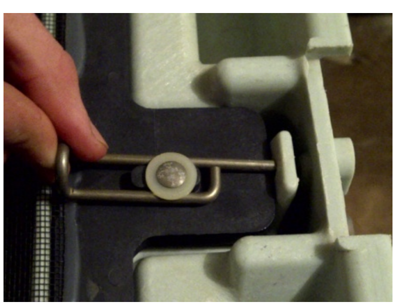


Image 5-6. Securing screen to egg tray

Image 5-7. Locking tray to Heath Stack

Containment Point 1 - Upweller Jars Screens: Upwelling jars can hold ~ 100,000 eggs in a single jar. Eggs are suspended in the water column, gently rolling, and can be held until just prior to hatch.

Each upweller is screened with 1.5 mm mesh screens permanently fixed on both the top and the bottom of the jar. Two additional 3 mm screens are present in each jar; one is fixed inside the jar at the bottom, and the second is integrated with the lid of the jar (Image 5-8).

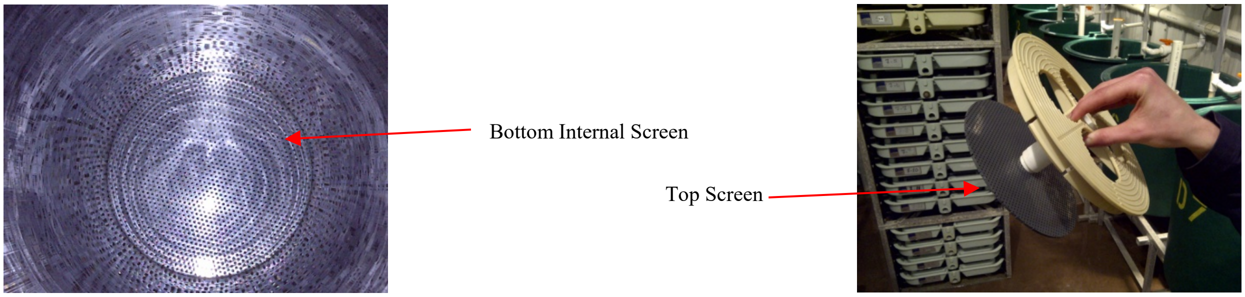


Image 5-8. Top and Bottom Screens Used in the Upweller Jars

The lid is placed into the jar and pressed down until it seats into place and tabs on the lid hold it securely into the top of the jar (Images 5-9 and 5-10).

Image 5-9. Placing Lid in Upweller Jar

Image 5-10. Securing Lid

Containment Point 2 – Heath Stack Catchment Pan: After flowing through the HS, water will flow into and through a PVC pipe perforated with 1.0 mm perforations.

Containment Point 2 – Upweller Jar Filter Pipe: After passing through the top screen and lid of the Upwellers, water will pass through a 1.0 mm pipe filter (which replaces the catchment basket used on Heath Stacks).

Containment Points 3,4,5 – Recirculation Line Strainers: After passing through the catchment pan (or the pipe filter in the case of the Upweller Jar), water will flow through three, independent water strainers, each fitted with 1.0 mm mesh screens.

Containment Points 6, 7 – Incubation Sump Overflow: After exiting the third in-line water strainer, water from the incubators will flow into the Incubation Sump located in the ER. Although most (> 99%) water will be recirculated through the UV sterilizer and back into the incubators, a small amount of overflow water will pass through a 1.0 mm perforated polyvinyl chloride (PVC) pipe (containment point 6) fitted with a 1.0 mm bag filter (containment point 7) before being discharged into the AR floor drains.

Containment Point 8 – Floor Drain: Floor drains in the ER area are capped with 1.0 mm perforated PVC pipe (Image 5-11) to allow water to drain from the floor but prevent downstream passage of eggs or fry if any inadvertently were to find their way onto the floor. The PVC pipe is covered with a solid PVC cap. All water entering the floor drains will flow to the Hatchery Main Containment Sump.

Image 5-11. Hatchery ER Floor Drain Covers (1.0 mm)

Containment Points 9 & 10 – Main Containment Sump Containment Baskets: Overflow water from the Incubation sump will be discharged into the AR Containment Sump where it will mix with water from the Hatchery floor drains. Water will pass through two 1.5 mm stainless steel screens.

Containment Point 11 – Main Containment Sump Containment Screen: After passage through the two 1.5 mm screens (Containment Points 11& 12), effluent will be mixed with other water (primarily overflow from the AR RAS, described in detail in Section 5.6.3.1.3) and pass through a perforated stainless steel containment screen (perforations are 13 mm) before being discharged into the polishing pond located adjacent to the Hatchery (refer to Figure 5-4, above, for a diagram of water flow through the property).

5.6.4.1.1.2 *Additional Hatchery Incubator Containment*

Chlorine pucks will be introduced into the floor drains during spawning, egg operations, and when newly hatched fry are transferred to the ER. The chlorine will kill eggs, milt, or fry if any were to enter the drains (Image 5-12). Staff will monitor the chlorine pucks during spawning, egg handling, and fry transfer operations to ensure an adequate supply.

During spawning operations, excess fertilized and green eggs are killed by freezing or treating them with a chlorine solution (10 mL NaClO per L of water) before disposal.

Image 5-12. Chlorine pucks in floor drains during spawning, egg handling, and transfer of fry

5.6.4.1.2 *Hatchery Containment: Early Rearing*

After hatching, the juvenile fish (first-feeder fry or alevin) will be about 3.5 by 15 mm in size and weigh approximately 0.1 g and are transferred to the A-tanks in the Hatchery ER. After reaching approximately10 g in size they will be transferred to the Hatchery AR.

5.6.4.1.2.1 *Hatchery ER A-Tank Containment*

Thirteen containment points will be in place to prevent fry (which range from 0.1 to approximately 10 g in size) housed in the ER A-tanks from entering the environment (Table 5-7.). Descriptions and images of the containment points follow the table.

Table 5-7. Hatchery Containment Points – ER A-Tanks

Facility	Area	Containment Point	Location	Barrier Type	Barrier Material(s)	Perforation Size (mm)	Fish Sizes (g) in Unit
Hatchery	ER	1	A-tank cover	Tank cover nets	Polyethylene	9.00	0.1 - 10.0
Hatchery	ER	2	A-tank drain	PVC standpipe	Perforated PVC	0.8 - 3.5	0.1 - 10.0
Hatchery	ER	3	ER Containment sump Inlet	Sock Filter	Polyester	0.75 - 3.5	0.1 - 10.0
Hatchery	ER	4	ER Containment sump	Containment Basket	Stainless Steel (SS)	1.5 – 6.3	0.1 - 10.0
Hatchery	ER	5	ER Containment sump	Containment Basket	SS	1.5 – 6.3	0.1 - 10.0
Hatchery	ER	6	ER Containment sump	Containment Basket	SS	1.5 -6.3	0.1 - 10.0
Hatchery	ER	7	ER Containment sump	Overflow screen	Perforated PVC	1.50	0.1 - 10.0
Hatchery	ER	8	AR drum filter	Drum filter screen	SS frame with polyester micromesh	0.04	0.1 - 10.0
Hatchery	ER	9	Floor drains	Perforated Pipe	SS	1.00	0.1 - 10.0
Hatchery	ER	10	Main Containment Sump	Perforated box	SS	13.00	0.1 - 10.0
Hatchery	ER	11	Main Containment Sump	Containment Box/Screen	SS	1.50	0.1 - 10.0
Hatchery	ER	12	Main Containment Sump	Containment Box/Screen	SS	1.50	0.1 - 10.0
Hatchery	ER	13	Main Containment Sump	Containment Box	SS	13.00	0.1 - 10.0

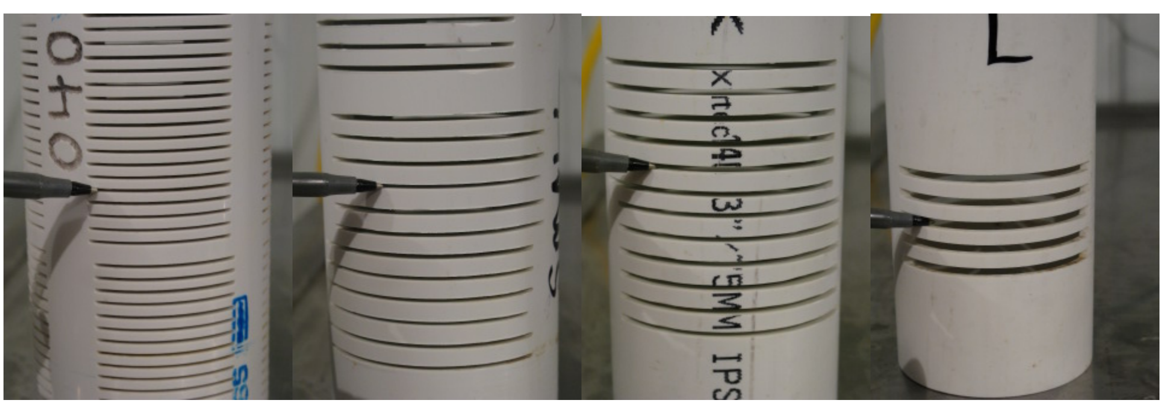
Containment Point 1 – Tank nets (Image 5-13): Tanks are covered with a 9 mm net to prevent fish from jumping out of the tank.

Image 5-13. Hatchery ER A-Tanks with 9.0 mm Nets

Containment Point 2 – Tank Floor Drain (Image 5-14): Water drains from the tank through a floor drain enclosed by two standpipes: an internal solid pipe which regulates water depth, and an external pipe which acts as a barrier to prevent release of fish but allows feces and excess feed to exit the tank.

Image 5-14. Hatchery ER A-Tank Standpipes

Four perforation sizes will be used for the external standpipe on the A-tanks: 0.76 mm, 2.0 mm, 2.5 mm, and 3.5 mm (Image 5-15). Perforation sizes will be chosen to facilitate removal of excess feed and feces and prevent the smallest fish in the tank from entering the drain system.



1mm 2mm 2.5mm 3.5mm

Image 5-15. Outside Standpipe of Hatchery ER A-Tanks; Size Correlates to Size of Fish

As shown in Image 5-16, a coupling is placed on the top of the selected standpipe and the internal standpipe is placed into the center drain. Three holes are used to set the desired water level. When a tank is used to house first-feeding fry, the depth is set at the lowest point and increased as the fish grow.

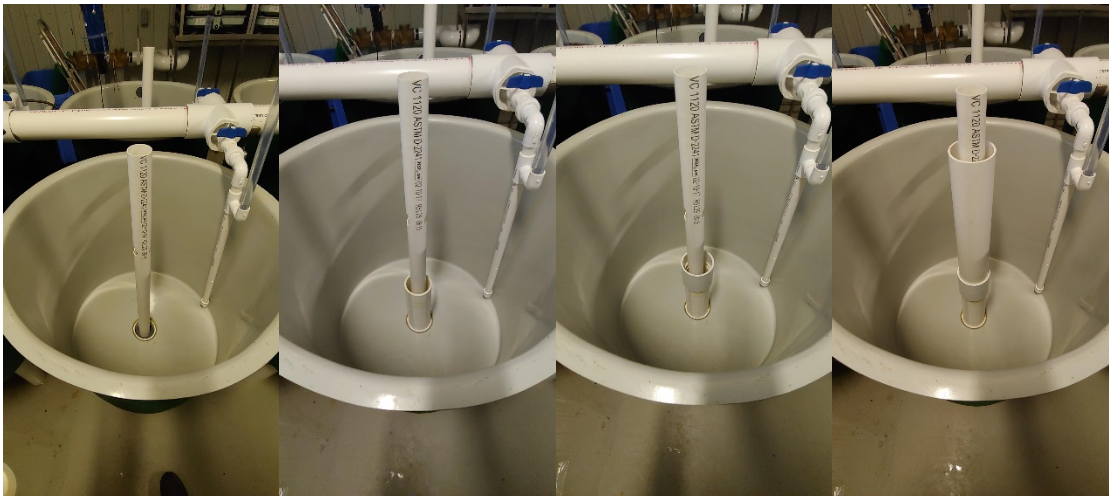


Image 5-16. Coupling and Outside Standpipes on Hatchery A-Tanks

Containment Point 3 – ER Containment Sump Inlet: Water from A-tank bottom drains is co-mingled in a single 4 in. line and passes through a sock filter as it enters the ER Containment sump. Sock filter sizes will change (0.75 mm, 1.5 mm, and 3.5 mm) as the size of the fish housed in the A-tanks change.

Containment Points 4, 5 & 6 – ER Containment Sump Baskets (Image 5-17): Inside the containment sump, water will pass through a series of three perforated stainless steel containment baskets, one of which is completely enclosed with a cover. The perforations will increase from 1.5 to 6.3 mm as the fish being held in the A-tanks grow.

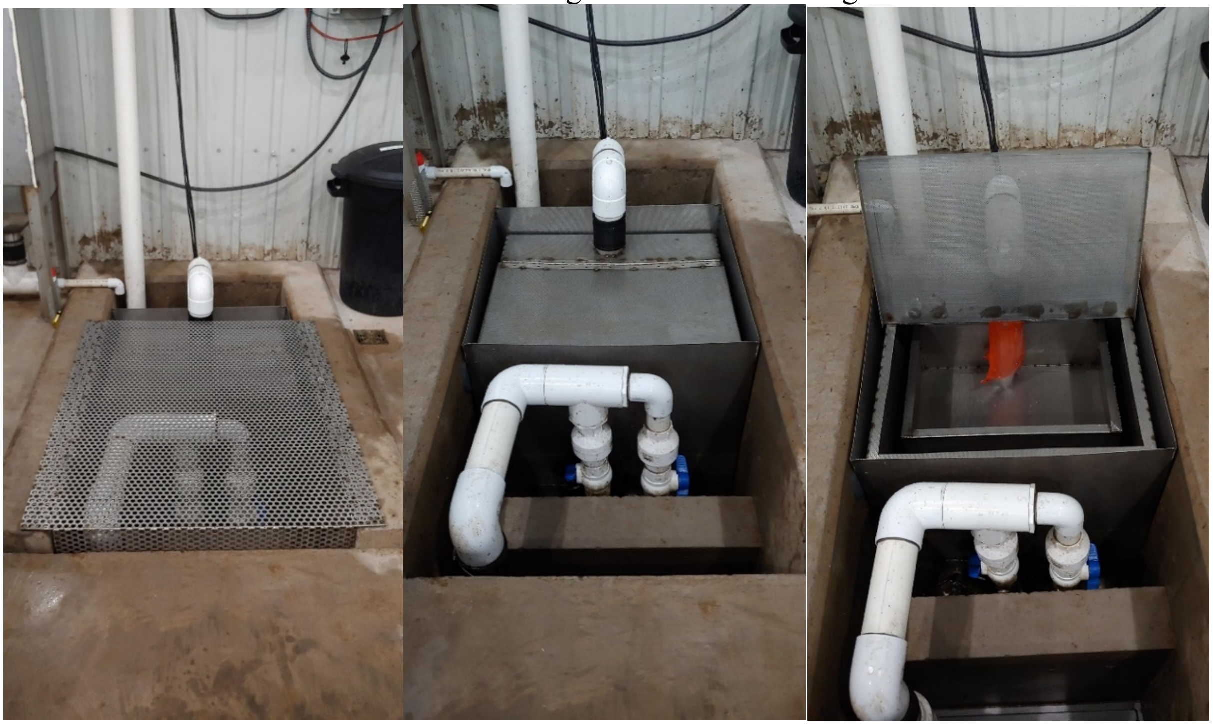


Image 5-17. Hatchery ER Containment Sump and Stainless Steel Containment Baskets

Two pumps controlled by float switches will be placed in the containment sump and water levels in the containment sump will be kept below containment sump overflow. One pump serves as the main pump and the second pump will be a back-up. Complete pump failure, or any other event causing the sump to flood, triggers a high-water alarm to alert staff of the problem.

Containment Point 7 – ER Containment Sump Overflow: Overflow from the ER Containment Sump will pass through a 1.5 mm perforated PVC pipe before being discharged into the Hatchery Floor Drains.

Containment Point 8 – AR Drum Filter: Water from the ER Containment Sump will be pumped to the drum filter on Hatchery RAS 2 located in the Hatchery AR. Water enters the drum filter and solids are collected on the 0.04 mm drum filter screen and removed from the water column. The resulting clear water recirculates through the RAS, while the collected solids flow to the Hatchery Main Containment Sump.

Containment Point 9 – Floor Drains: As shown in Image 5-11 (above), floor drains in the ER area are covered with 1.0 mm perforated PVC pipe to prevent eggs or first-feeder fry from entering the floor drains. These pipes are covered with solid PVC caps, providing additional containment in the ER. All floor drains flow to the Hatchery Main Containment Sump.

Containment Point 10 – Main Containment Sump Containment Box: Overflow from the RAS flows into a perforated (13 mm) stainless steel containment box inside the Main Containment Sump.

Containment Points 11 & 12 – Main Containment Sump Containment Baskets: Water from the Hatchery floor drains passes through two stainless steel 1.5 mm screens inside the Main Containment Sump.

Containment Point 13 – Main Containment Sump Containment Box: After passage through the two 1.5 mm screens (Containment Points 11& 12), effluent will be mixed with other water (primarily overflow from the RAS sumps) and pass through a perforated (13 mm) stainless steel screen (Image 5-18) before being discharged into the polishing pond located adjacent to the Hatchery (refer to Figure 5-4, above, for a diagram of water flow through the property).

The Main Containment Sump is located in a room adjacent to the Hatchery AR and all screens located in the Main Containment Sump are readily accessible.

Image 5-18. Hatchery Containment Sump Discharge Screen

5.6.4.1.2.2 *Additional ER Containment*

Chlorine pucks will be placed in the floor drains during spawning, egg handling, and transfer of fry. This would result in the inactivation or death of any milt, eggs, or fry in the unlikely event any of these entered the floor drains.

Effluent from the ER floor drains will be combined with the AR floor drain effluent and the effluent stream will be discharged through the Hatchery Main Containment Sump as described above.

5.6.4.1.3 *Hatchery Containment: Advanced Rearing*

When fry have reached approximately10 g they will be transferred from the ER to the Hatchery AR. The AR houses 15 tanks of 1.5 m^3 capacity (B-tanks) and 12 tanks of 14.8 m^3 capacity (C-tanks). The B-tanks will be used to rear fish from 10 g in size to approximately100 g, and C-tanks will be used to rear and hold broodstock from approximately 100 g to full adult size. Fish in the AR may reach 12,000 g.

There are nine containment points in place in the Hatchery AR (Table 5-8). Detailed descriptions follow.

Table 5-8. Hatchery AR Containment Points

Facility	Location	Containment Point	Location	Barrier Type	Barrier Material(s)	Perforation Size (mm)	Fish Sizes (g) in Unit
Hatchery Advanced Rearing (AR) B- (1.5 m^3 capacity) and C-(14.8 m^3 capacity) Tanks							
Hatchery	AR	1	B- and C-tanks	Tank cover nets	Polyethylene	13.0 - 25.4	10 - > 5000
Hatchery	AR	2	B-tank drains	PVC standpipe and cover	PVC and perforated plastic screen	5.0 -13.0	10 - > 5000
Hatchery	AR	3	C-tank side boxes	Screen	Polyethylene	5.0 -13.0	10 - > 5000
Hatchery	AR	4	C-tank bottom drains	Drain cover	Stainless Steel (SS)	5.0 - 25.0	10 - > 5000
Hatchery	AR	5	AR drum filter	Drum filter screen	SS frame with polyester micromesh	0.04	10 - > 5000
Hatchery	AR	6	Floor Drain	Perforated Covers	Bronze	8.00	10 - > 5000
Hatchery	AR	7	Main Containment Sump	Clear water overflow baskets	SS	13.00	10 - > 5000
Hatchery	AR	8	Main Containment Sump	Effluent baskets	SS	1.50	10 - > 5000
Hatchery	AR	9	Main Containment Sump	Screen	SS	13.00	10 - > 5000

Containment Point 1 – Tank Nets (Image 5-19): All tanks will be covered with mesh netting sized from 13.0 to 25.4 mm depending upon the size of fish housed in each tank.

Image 5-19. Example of Tank Netting in Hatchery AR

Containment Point 2 - B-Tank bottom drains: These tanks drain through a center floor drain that utilizes a single standpipe (Image 5-20) as the barrier to prevent release of fish. Standpipes are perforated with ~13 mm holes to allow feed and feces to exit the tank. Perforated screens will be used to cover the standpipes when small fish are being housed in the tanks with perforation sizes ranging from 5 mm upwards until no screen is required. The size will be chosen to prevent fish from entering the drain while optimizing removal of solids.

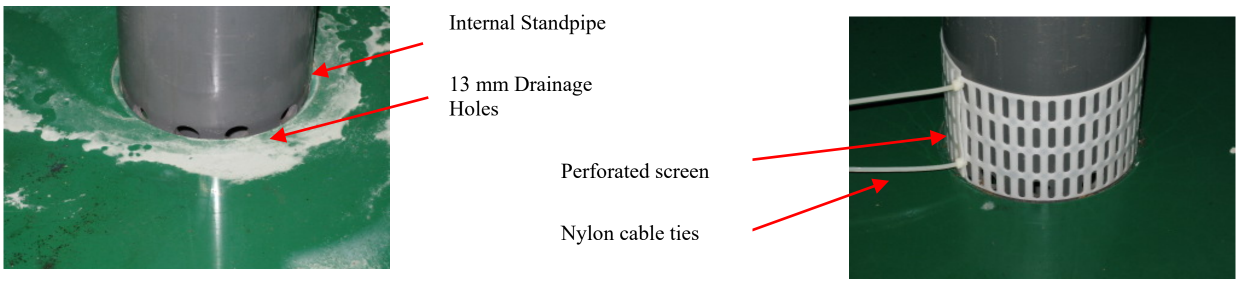


Image 5-20. Hatchery B-Tank Internal Standpipe and Cover

Containment Point 3 - C-Tank Side Boxes: > 90% of water will exit the C-tanks through side boxes equipped with screens (Image 5-21) to prevent fish from entering the side box. Screen sizes will range from approximately 5 mm to 13 mm and will be adjusted to optimize flows and exclude fish from the side box.

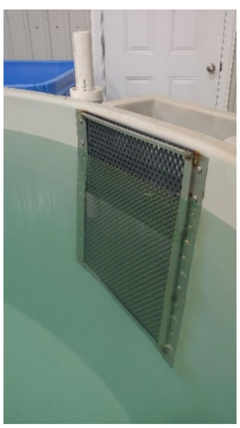


Image 5-21. C-Tank Side Box Screens

Image 5-22. C-Tank Bottom Drains

Containment Point 4 – C-Tank Bottom Drains: C-tanks will also be equipped with bottom drains used to remove feces and uneaten feed. The tank bottom drains will be covered with a plate perforated with varying sizes of holes (5.0 – 25.0 mm) depending on the size of fish housed in the tank (Image 5-22, above).

Containment Point 5 – AR Drum Filter: Recirculated water will flow from the B- and C-tanks into and through the 0.04 mm RAS drum filters and into the RAS units located in the AR. More than 99% of water entering the RAS is recirculated through the Hatchery. There are multiple containment barriers integrated into the RAS to prevent fish of any size from passing through the RAS alive.

Containment Point 6 – Floor Drains: The AR floor drains will be covered with bronze covers (Image 5-23) to prevent fish that reach the floor from entering the drain system. The openings are 8.0 mm in width and shorter than the smallest fish (approximately10 g) that will be housed in the AR

Image 5-23. Hatchery AR Floor Drain Cover

Containment Points 7 & 8 – Hatchery Main Containment Sump Effluent Baskets: All water that leaves the Hatchery passes through the main containment sump and through one of two sets of stainless steel containment baskets (Images 5-24A and 5-24B). Each set is comprised of a stainless steel basket that nests inside a stainless steel box with closing lid (Image 5-25).

Within the containment sump, overflow water from the RAS (i.e., clear water discharged from the RAS sump) will pass through a containment basket constructed with 13 mm perforations (Containment Point 7; Image 5-24A). Water collected in the AR floor drains and waste water effluent from the drum filters (i.e., removed solids) will also flow to the containment sump. The combined water flow will pass through two containment baskets constructed with 1.5 mm perforations (Image 5-24B) to prevent every life stage present in the Hatchery from escaping and entering the environment.

RAS Clear Water Overflow Containment Baskets Image 5-24A:

1: overflow from RAS 1

2: overflow from RAS 2

Effluent Containment Baskets Image 5-24B.

3: Drum filter effluent from RAS 1, water from floor drains & ER containment sump overflow

4: Drum filter effluent from RAS 2 and water from floor drains

Image 5-24. Stainless Steel Clear Water Overflow Containment Baskets (A) and RAS/Floor Drain Effluent Containment Baskets (B) Located in the Hatchery Main Containment Sump

Image 5-25. 1.5 mm Stainless Steel Containment Baskets Located in the Hatchery Main Containment Sump

Containment Point 9 – The final discharge point of water from the facility is the outlet drain of the Main Containment Sump (Image 5-26). The drain is covered with a cylindrical screen constructed of stainless steel with 13 mm perforations.

Image 5-26. Hatchery Main Containment Final Discharge Containment

5.6.4.1.3.1 *Additional Hatchery AR containment*

Solid waste removed by the drum filters flows to, and is collected in, the containment baskets located in the Hatchery AR containment sump (Image 5-24B). The collected solids will be removed daily and frozen on site until disposal. In the highly unlikely event that fish or eggs were to reach the drum filter, they will be captured with the solid waste stream.

B- and C-tanks are also equipped with a maintenance pipe that can be used to drain the tank for cleaning and maintenance. Water from these pipes will flow directly into the floor drains and then to the Hatchery main containment sump. As with all effluent captured in the floor drains, this water will pass through one of the two effluent containment baskets described above. Maintenance pipes will not be opened when fish are in the tanks.

5.6.4.2 *Grow Out Unit Containment*

Multiple, redundant containment mechanical (e.g., screens, filters, and nets) barriers are employed in the Grow Out Unit to prevent the escape of all life stages of fish. The containment methods and locations have been designed specifically for the operations and life stages that will be present in each area of the building. The combination of appropriately sized barriers placed in key locations makes the risk of escape extremely low. The details of the Grow-out containment barriers, including diagrams and images, follow.

5.6.4.2.1 *Grow Out Containment: Incubators*

Figure 5-8 provides a diagram of water flow and containment points that will be in place in the Grow Out ER/IR. Table 5-9 provides a list of the eight containment points that will be in place to prevent eggs and newly hatched alevin from escaping the Heath Stack incubators that will be used in the ER/IR to begin the production cycle. Most of the containment barriers used in the incubators are identical to those that will be used in the Hatchery ER and are summarized here. Details of the Incubator RAS and containment can be found in Section 5.6.4.1.1.1 (above).

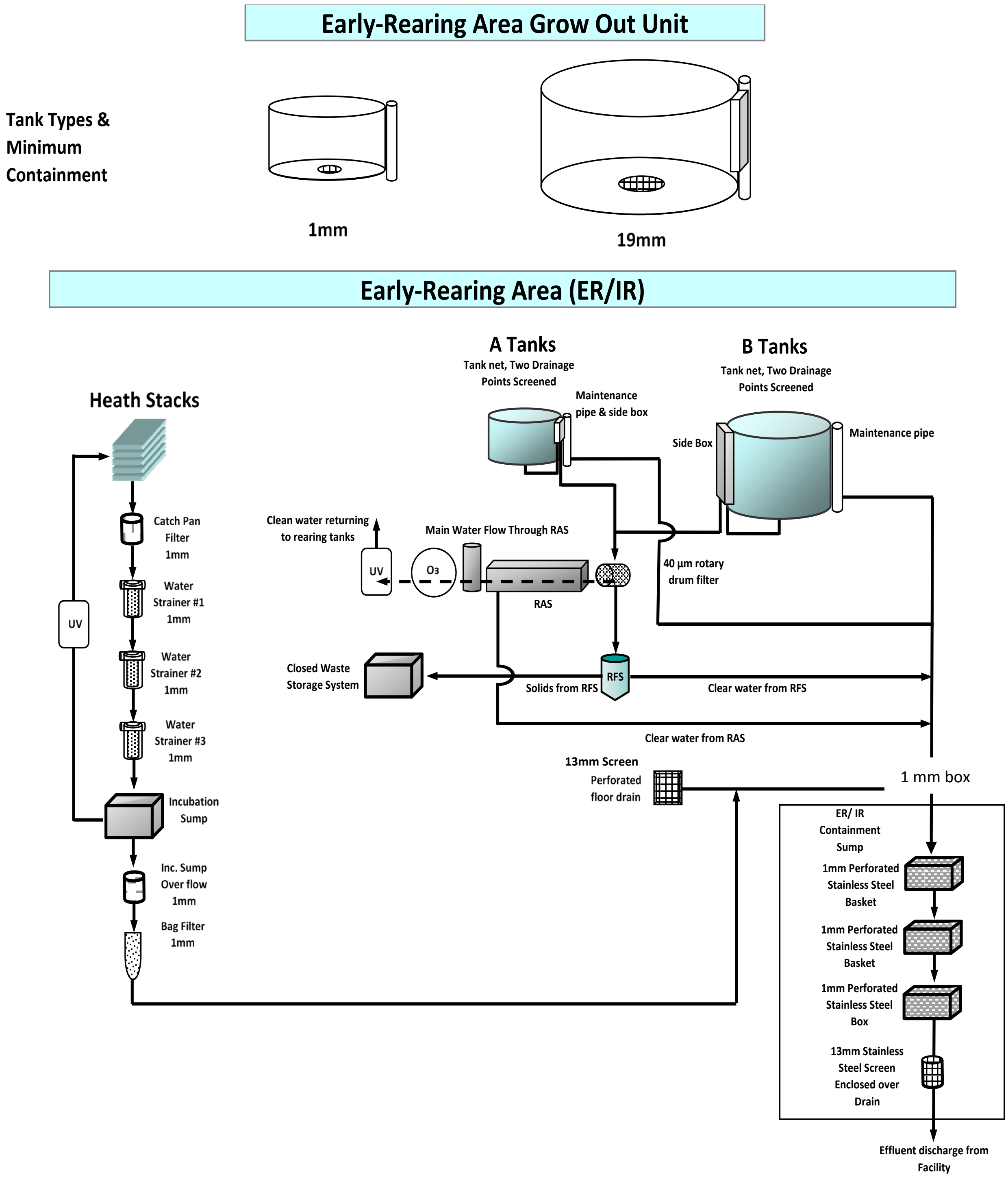


Figure 5-8. Schematic of Water Flow and Containment Points in the Grow Out ER/IR

Table 5-9. Grow Out Unit Incubator Containment Points

Facility	Area	Containment Point	Location	Barrier Type	Barrier Material(s)	Perforation Size (mm)	Fish Sizes (g) in Unit
Grow Out Incubators							
Grow Out	ER/IR	1	Heath Stack Egg Trays	Top and Bottom Screens	Molded plastic inserts with Polyester screen	1.50	5.0 mm - 0.1 g
Grow Out	ER/IR	2	Heath stack catchment pan	Strainer	PVC	1.00	5.0 mm - 0.1 g
Grow Out	ER/IR	3	Recirculation line	Strainer	Nylon	1.00	5.0 mm - 0.1 g
Grow Out	ER/IR	4	Recirculation line	Strainer	Nylon	1.00	5.0 mm - 0.1 g
Grow Out	ER/IR	5	Recirculation line	Strainer	Nylon	1.00	5.0 mm - 0.1 g
Grow Out	ER/IR	6	Incubation sump	Perforated Pipe	PVC	1.00	5.0 mm - 0.1 g
Grow Out	ER/IR	7	Incubation sump	Screen	Nylon	1.50	5.0 mm - 0.1 g
Grow Out	ER/IR	8	Floor drain	Perforated Covers	Stainless Steel	1.50	5.0 mm - 0.1 g

Containment Point 1: 1.5 mm polyester screens, smaller in size than salmon eggs or newly-hatched fry, sit on top and bottom of removable egg trays.

Containment Point 2: After flowing through the Heath Stack, water will flow into and through a PVC strainer with 1.0 mm slots.

Containment Points 3, 4, 5: After passing through the PVC strainer, water will flow through three, independent water strainers, each fitted with 1.0 mm mesh screens.

Containment Points 6, 7: After exiting the third in-line water strainer (Containment Point 5), water from the incubators will flow into the Incubation Sump. Although > 99% water is recirculated through the UV sterilizer and back into the incubators, a small amount of overflow water will be discharged into the ER/IR Containment Sump after passing through a 1.0 mm perforated PVC pipe (Containment Point 6) and 1.5 mm nylon screen (Containment Point 7).

Containment Point 8: Floor drains in the ER/IR will be covered with 1.5 mm perforated covers and can be capped with solid covers when operations dictate. The 1.5 mm covers will be small enough to prevent the passage of any fish or eggs which may find their way to the floor. Water that enters the floor drains will travel to the ER/IR Containment Sump.

5.6.4.2.2 *Grow-out Containment: ER/IR*

After eggs have hatched and fry have consumed most of their yolk sac, alevin will be transferred to the ER/IR. The ER/IR will house three tanks of 12.84 m^3 capacity (A-tanks), and three tanks of 70.7 m^3 capacity (B-tanks). Fish in the ER/IR will range in size from 0.1 g to approximately200 g.

Eleven containment barriers will be in place in the Grow Out ER/IR (Table 5-10) and are described in detail below. Figure 5-10 (above) provides a schematic view of water flow and containment points in the ER/IR.

Table 5-10. Grow Out ER/IR Containment Points

Facility	Area	Containment Point	Location	Barrier Type	Barrier Material(s)	Perforation Size (mm)	Fish Sizes (g) in Unit
Grow Out	ER/IR	1	A- and B-Tanks	Tank cover net	Polyethylene	9.0 - 13.0	0.1 -200
Grow Out	ER/IR	2	A- & B-Tank Side box	Screen	Stainless Steel (SS)	1.5 - 13.0	0.1 -200
Grow Out	ER/IR	3	A-tank bottom drain	Screen	SS	1.5 - 3.5	0.1 -200
Grow Out	ER/IR	3	B-tank bottom drain	Drain cover	SS	13.0	0.1 -200
Grow Out	ER/IR	4	ER/IR drum filter	Drum filter screen	SS frame with polyester micromesh	0.04	0.1 -200
Grow Out	ER/IR	5	Radial flow settler	Solids Collection	Fiberglass	Impermeable	0.1 -200
Grow Out	ER/IR	6	Waste Storage Tank	Concrete containment tank	Concrete	Impermeable	0.1 -200
Grow Out	ER/IR	7	Floor drains	Perforated cover	SS	1.5 or Solid	0.1 -200
Grow Out	ER/IR	8	ER/IR Containment Sump	Containment Basket	SS	1.5 - 6.30	0.1 -200
Grow Out	ER/IR	9	ER/IR Containment Sump	Containment Basket	SS	1.5-6.30	0.1 -200
Grow Out	ER/IR	10	ER/IR Containment Sump	Containment Box	SS	1.5 - 6.30	0.1 -200
Grow Out	ER/IR	11	ER/IR Containment Sump	Drain Screen	SS	13	0.1 -200

Containment Point 1 – Tank Nets: All tanks will be covered by mesh nets with openings of either 9.0 or 13.0 mm depending upon the size of fish in the tank.

Containment Point 2 – Tank Side Boxes: More than 90% of water leaves the tanks through side boxes equipped with screens appropriate to the size of fish housed in the tank. A-tank screens have 1.5 mm or 3.5 mm perforations and are changed as the size of fish changes; B-tank screens are 13 mm, which is small enough to prevent all fish housed in B-tanks from escaping.

Containment Point 3 – A-Tank Bottom Drain: The A-tanks will drain through a center floor drain covered by perforated stainless steel plates. Perforation sizes will range from 1.5 to 3.5 mm and can be changed to match the size of fish housed in the tank and optimize water flow and removal of solids from the tank.

Containment Point 3 – B-Tank Floor Drain: The bottom tank drain will be covered with perforated (1.5 – 3.5 mm) stainless steel plates.

Containment Point 4 – ER/IR Drum Filter: Water exiting the A- and B-tanks will flow to the ER/IR RAS. Greater than 99% of the water entering the RAS will be recirculated and pass through a 0.04 mm drum filter where solids will be removed from the effluent. Clear effluent will pass through the drum filter and into the RAS Unit for denitrification, degassing and oxygenation before being returned to the rearing tanks.

Containment Point 5 – Radial Flow Settler (RFS): The effluent slurry from the drum filter enters an RFS where solids settle out in a conical shaped fiberglass tank. Solids are pumped off the RFS into a contained septic tank. Clear water from the RFS flows into the containment baskets located in the containment sump. Water from the containment sump exits the facility through a PVC pipe and is discharged into a stone out wash located approximately 140 meters west of the Grow Out Unit. The water is filtered through the stone and flows approximately 40 m across a natural area populated with trees and undergrowth before eventually entering the Rollo Bay Brook. The amount of water entering the brook will vary with time of year and weather conditions. See Section 5.6.2.1.3 (above) for additional information on water discharge from the Grow Out Unit.

Containment Point 6 – Solid Waste Storage Tank: Collected solids from the RFS are pumped to a closed 9000 L concrete storage tank. The tank will be emptied as needed and the manure will be disposed of in a municipal waste treatment facility or applied to agricultural land as fertilizer depending on seasonal conditions.

Containment Point 7 - ER/IR Floor Drains: will be covered with 1.5 mm perforated covers or solid covers during egg handling operations to prevent eggs, fry, or juvenile fish from entering the floor drains. Effluent entering the ER/IR floor drains will be discharged into the ER/IR containment sump.

Containment Point 8, 9, 10 & 11 - ER/IR Containment Sump: Effluent from floor drains, clear water from the RFS, and clear water overflow from the RAS (i.e., water that is not recirculated within the facility) will all enter the ER/IR Containment Sump and must pass through two stainless steel baskets and a stainless steel box with perforations ranging from 1.5 to 6.3 mm, depending on the size of the fish in the system. Water from the ER/IR containment sump then passes through a stainless steel screen with 13 mm openings before flowing through a PVC pipe into a stone out wash located approximately 140 m west of the Grow Out Unit. The water is filtered through the stone out wash and flows approximately 40 m across a natural area populated with trees and undergrowth before eventually entering the Rollo Bay Brook. The amount of water entering the brook will vary with time of year and weather conditions. See Section 5.6.2.1.3 (above) for additional information on water discharge from the Grow Out Unit.

5.6.4.2.2.1 *A*dditional Containment: Grow Out ER

Each B- and C-tank will be equipped with a solid PVC maintenance pipe that can be used to drain the tank for cleaning and maintenance. Water from these pipes will enter directly into the floor drains and then to the ER/IR Containment Sump. Maintenance pipes will not be opened when fish are in the tanks.

5.6.4.2.3 *Grow Out Containment: AR*

The Grow-out AR will be used to rear fish from the time they leave the ER/IR at approximately 200 g to market weight of 5 kg. It will be organized into two identical sets of seven tanks: four tanks of 122.0 m^3 capacity (C- tanks) and three tanks of 274.1 m^3 capacity (D-tanks). A schematic diagram of the water flow and containment points in the Grow-out AR is provided in Figure 5-9 and the 12 containment points that will be in place for the Grow-out AR are identified in Table 5-11. Details about the containment points follow the table.

Table 5-11. Grow Out Unit AR C- and D-Tank Containment

Grow-out Advanced Rearing (AR) C- (122.0 m^3) and D- (274.1 m^3) Tanks							
Facility	**Area**	**Containment Point**	**Location**	**Barrier Type**	**Barrier Material(s)**	**Perforation Size (mm)**	**Fish Sizes (g) in Unit**
Grow Out	AR	1	All tanks	Tank cover nets	Polyethylene	38.00	200 - 5000
Grow Out	AR	2	Tank side box opening	Screen	Stainless Steel (SS)	25.00	200 - 5000
Grow Out	AR	3	Side box screen	Screen	SS	25.00	200 - 5000
Grow Out	AR	4	Tank floor drain cover	Screen	SS	15.00	200 - 5000
Grow Out	AR	5	AR drum filters	Drum filter screen	SS frame with polyester micromesh	0.06	200 - 5000
Grow Out	AR	6	Floor drains	Perforated drain covers	SS	8.00	200 - 5000
Grow Out	AR	7	AR containment sump	Vertical screen	SS	25.00	200 - 5000
Grow Out	AR	8	AR containment sump	Vertical screen	SS	25.00	200 - 5000
Grow Out	AR	9	AR containment sump	Vertical screen	SS	25.00	200 - 5000
Grow Out	AR	10	AR containment sump	Vertical screen	SS	25.00	200 - 5000
Grow Out	AR	11	Waste treatment Building	Radial flow settler (RFS)	Fiberglass	Impermeable	200 - 5000
Grow Out	AR	12	Waste treatment drum filter	Drum filter screen	SS frame with polyester micromesh	0.04	200 - 5000
Grow Out	AR	13	Manure storage	Concrete containment tank	Concrete	Impermeable	200 - 5000

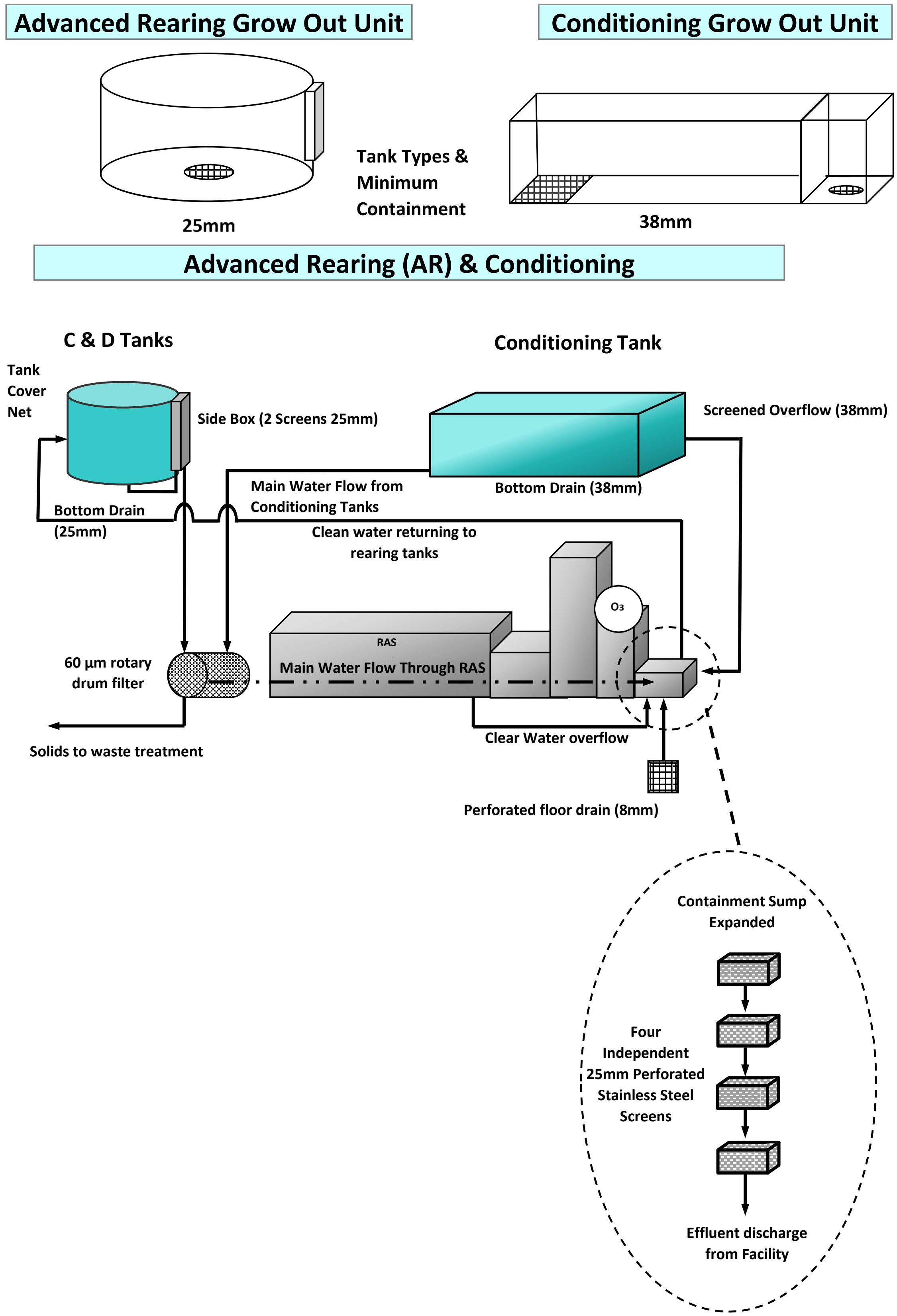


Figure 5-9. Schematic of Water Flow and Containment in the Grow-out AR

Water returning from the rearing tanks will pass through the drum filter and through the drum filter stainless-steel containment screen prior to entering the media bed. The media bed is divided into three compartments separated from one another by stainless-steel screens. Water passing through the final media bed is taken up by the pumps for reuse or discharged from the RAS through the overflow weirs. Overflow goes directly to the Grow Out AR containment sump.

Containment Point 1 – Tank Cover Nets: All tanks will be covered with 38 mm mesh nets.

Containment Point 2 – Tank Side Box Openings (Image 5-27): Most of the water from the tanks will return to the RAS by passing through side boxes located on each tank. The opening in the tank wall will be covered with a 25 mm stainless steel screen to prevent fish from escaping the tank.

Image 5-27. 25 mm screen installed in AR tank walls over the side box opening.

Containment Point 3 – Side Box Screen (Image 5-28): Water will leave the side box and flow to the RAS drum filter by passing through a second 25 mm stainless steel screen.

Image 5-28. Water exiting the AR tank side boxes flows through a second 25 mm screen on the way to the RAS drum filter.

Containment Point 4 –Grow Out AR Tank Floor Drain Cover (Image 5-29): All AR tanks will have center floor drains covered with perforated stainless steel plates. Perforations will be 15 mm, small enough to prevent any fish from entering the drain system but large enough to remove feces and uneaten food from the tank.

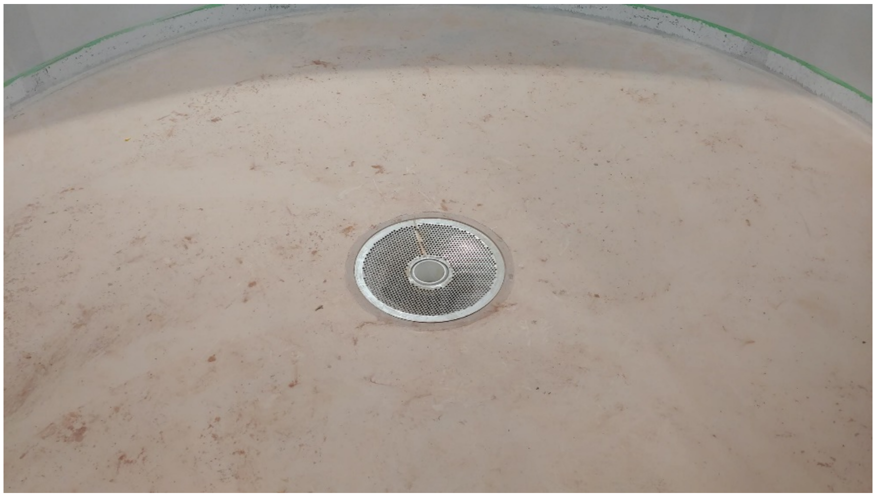


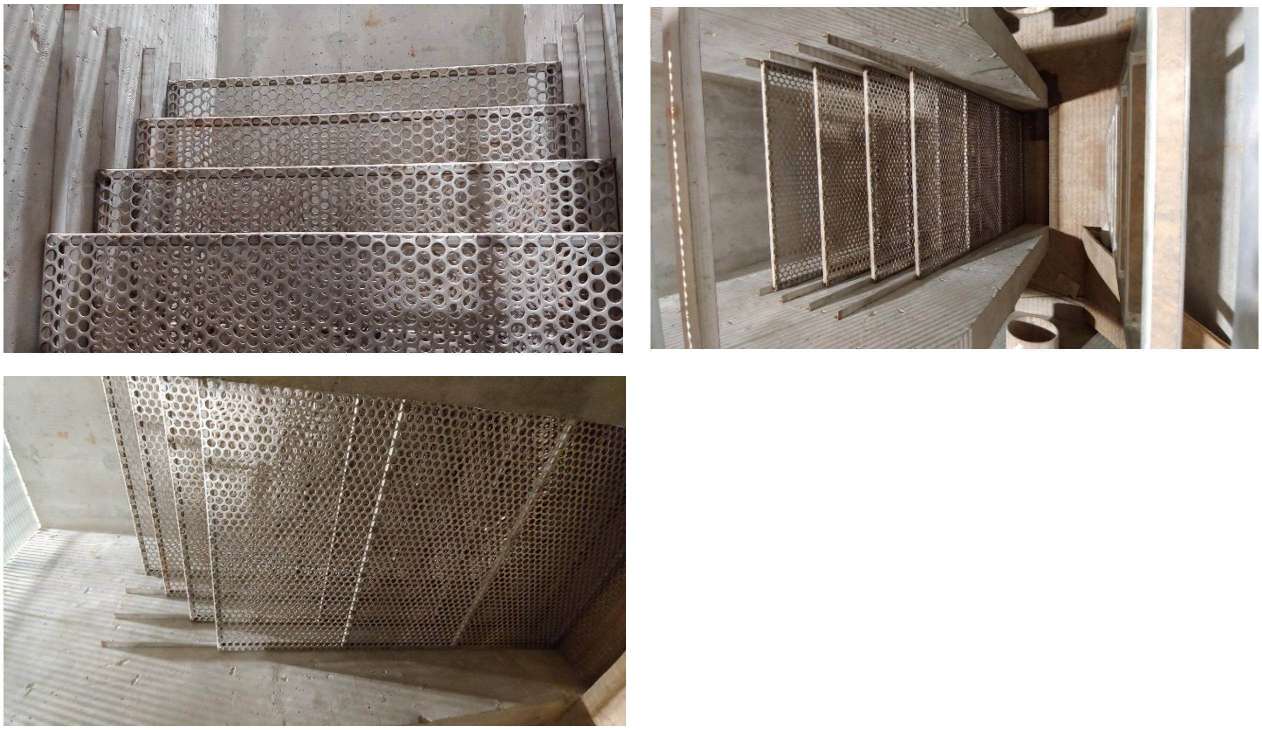
Image 5-29. Grow Out AR tank floor drain.

Containment Point 5 – AR Drum Filter: Recirculated water from the AR- and Conditioning-tanks flows to the RAS drum filters equipped with 0.06 mm screens. Clear effluent flows into the RAS Unit for denitrification, degassing and oxygenation prior to returning to the rearing tanks.

Containment Point 6 – AR Floor drains: Will be covered with 8 mm perforated grates (i.e., smaller than any fish grown in the AR). Effluent captured in the AR floor drains will pass through the AR Containment Sump before being discharged from the building.

Containment Points 7, 8, 9, 10 – AR Containment Sump: Effluent from the AR floor drains and clear water overflow from the AR RAS (i.e., water that is not recirculated) will flow to the AR Containment Sump. Water entering the sump will pass through a series of four perforated (25 mm) stainless steel screens (Image 5-30) before exiting the Grow Out Unit. Water flows through a PVC pipe into a stone out wash located approximately 140 m west of the Grow Out Unit. The water is filtered through the stone out wash and flows approximately 40 m across a natural area populated with trees and undergrowth before eventually entering the Rollo Bay Brook. The amount of water entering the brook will vary with time of year and weather conditions. See Section 5.6.2.1.3 (above) for additional information on water discharge from the Grow Out Unit.

Image 5-30: Three views of the Grow Out AR Containment Sump showing four perforated 25 mm screens.



Containment Point 11 – Waste Treatment Radial Flow Settlers: Solids removed by the AR drum filters will gravity flow through an underground pipe to the Waste Treatment Building (see Figure 5-4, above) where they will enter one of four RFS units to separate the stream into solids and clear effluent. A schematic of the waste treatment process is provided in Figure 5-10.

Containment Point 12 – Waste Treatment Drum Filter: Clear water discharged from the RFS units will pass through a drum filter in the Waste Treatment Building that will be equipped with a 0.04 mm screen. The solids will be removed and pumped back to the RFS to repeat the process. Clear water from the Waste Treatment drum filter will be discharged into an underground French drain located adjacent to the Waste Treatment Building.

Containment Point 13 – Manure Storage Tank: Solid waste collected in the Waste Treatment Building will be pumped to an underground 462 m^3 concrete tank. The solid waste will be stored and removed as required for disposal in a waste treatment facility or used as fertilizer for agricultural purposes according to provincial and federal regulations and guidelines.

5.6.4.2.3.1 *Additional Grow Out AR Containment*

AR tanks will also be equipped with a maintenance pipe drain that can be used to drain the tank during cleaning and maintenance procedures. Water that exits the tank via the maintenance pipe will travel directly to the floor drain line and then to the AR containment sump. Maintenance pipes will not be opened when fish are in the tank.

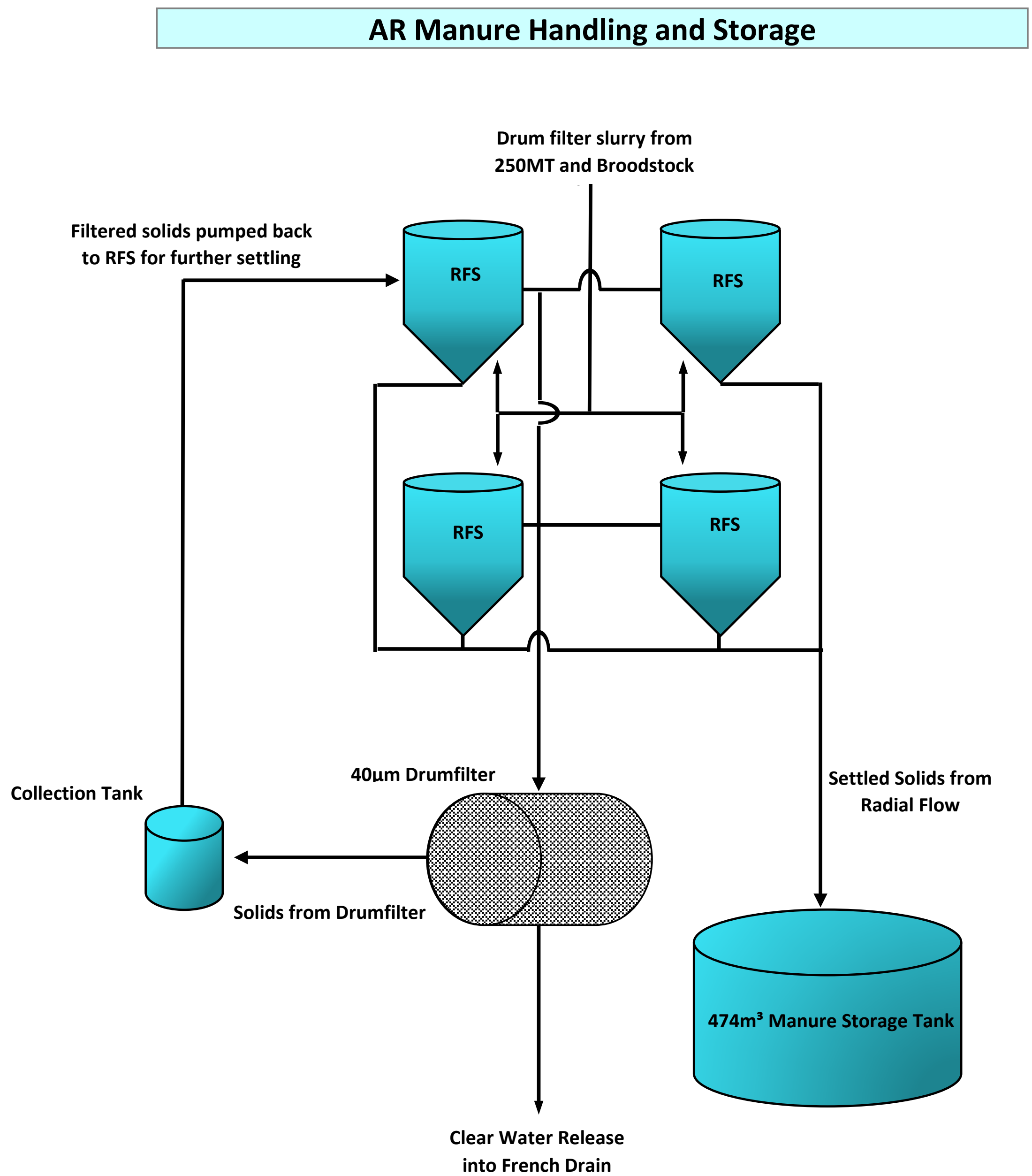


Figure 5-10. Schematic of the Waste Treatment Process Located in the Waste Treatment Building

5.6.4.3 *Conditioning Tank Containment*

The Conditioning area is used to prepare fish for harvest and operates on a flow-through water system. Market-sized fish (approximately 5000 g) will be moved from the AR D-tanks to the conditioning tanks approximately one week prior to harvest. The conditioning area is comprised of three rectangular concrete tanks, each measuring 11.58 m × 3.66 m × 1.98 m (L×W×H) with a capacity of 83.92 m^3. Incoming water for conditioning operations is directly from the wells. The

Conditioning area is in a separate room adjacent to the AR. Ten containment points will be in place in the Conditioning area (Table 5-12) and are described below.

Table 5-12. Conditioning tank containment points

Conditioning Tanks (E-Tanks) (83.92 m^3)							
Facility	**Area**	**Containment Point**	**Location**	**Barrier Type**	**Barrier Material(s)**	**Perforation Size (mm)**	**Fish Sizes (g) in Unit**
Grow Out	Conditioning	1	Tanks	Tank cover nets	Polyethylene	38.00	5000
Grow Out	Conditioning	2	Tank Outlet drains	Screen	Stainless Steel (SS)	38.00	5000
Grow Out	Conditioning	3	AR drum filters	Drum filter screen	SS frame with polyester micromesh	0.06	5000
Grow Out	Conditioning	4	Floor drains	Perforated drain covers	SS	8.00	5000
Grow Out	Conditioning	5	AR containment sump	Vertical screen	SS	25.00	5000
Grow Out	Conditioning	6	AR containment sump	Vertical screen	SS	25.00	5000
Grow Out	Conditioning	7	AR containment sump	Vertical screen	SS	25.00	5000
Grow Out	Conditioning	8	AR containment sump	Vertical screen	SS	25.00	5000
Grow Out	Conditioning	9	Waste treatment Building	Radial flow settler (RFS)	Fiberglass	Impermeable	200 - 5000
Grow Out	Conditioning	10	Waste treatment drum filter	Drum filter screen	SS frame with polyester micromesh	0.04	200 - 5000
Grow Out	Conditioning	11	Solid waste storage	Concrete containment tank	Concrete	Impermeable	200 - 5000

Containment Point 1 - Tank Nets: all conditioning tanks will be covered with 38 mm perforated nets.

Containment Point 2 - Tank Outlet Drains: most of the water from the conditioning tanks will return to the AR RAS by passing through a 38 mm screened outlet on a 3-inch PVC pipe that will deliver water from the conditioning tanks to the AR RAS. Conditioning tanks will also be fitted with a 3-in. overflow outlet that is covered with a 38 mm mesh screen to prevent fish from leaving the tank. Overflow from the conditioning tanks will pass through the overflow pipes into the AR floor drains. Fish in the Conditioning tanks are too large to pass through the 3-inch PVC pipe.

Containment Point 3 - AR RAS: The recirculated water from the AR and Conditioning tanks flows to the AR drum filters equipped with 0.06 mm screens. Solids are directed to the Waste Treatment Building and clear water effluent flows into the RAS Unit for denitrification, degassing and oxygenation before circulating through the AR tanks.

Containment Point 4 - Floor drains: Conditioning area floor drains will be covered with perforated (8 mm) stainless steel grates (i.e. the openings in the floor drain covers are smaller than any fish that will be present in the Conditioning area). Effluent captured in the floor drains will pass through the AR Containment Sump before being discharged into the environment.

Containment Points 5, 6, 7, 8 - AR Containment Sump Screens: effluent from the Conditioning area floor drains will join effluent from the AR floor drains and the AR RAS overflow (i.e., water not recirculated in the AR) in the AR containment sump. Water entering the containment sump will pass through a series of four perforated (25 mm) stainless steel screens (Image 5-30). After passing through the third screen, effluent is discharged into the Rollo Bay Brook.

Containment Point 9 – Waste Treatment Radial Flow Settlers: Solids removed by the AR drum filters will gravity flow through an underground pipe to the Waste Treatment Building (see Figure 5-6, above) where they will enter one of four RFS units to separate the stream into solids and clear effluent. A schematic of the waste treatment process is provided in Figure 5-12 (above).

Containment Point 10 – Waste Treatment Drum Filter: Clear water discharged from the RFS units will pass through a drum filter in the Waste Treatment Building that will be equipped with a 0.04 mm screen. The solids will be removed and pumped back to the RFS to repeat the process. Clear water from the Waste Treatment drum filter will be discharged into an underground French drain located adjacent to the Waste Treatment Building.

Containment Point 11– Manure Storage Tank: Solid waste collected in the Waste Treatment Building will be pumped to an underground 462 m^3 concrete tank. The solid waste will be stored and removed as required for disposal in a waste treatment facility or used as fertilizer for agricultural purposes according to provincial and federal regulations and guidelines.

5.6.4.3.1 *Extra precautions and procedural containment processes*

Because of the redundant layers of containment that will be in place there will never be a time when eggs, fry, or fish could go directly from a tank to the effluent discharge point, i.e., when one containment barrier is being cleaned, there are always several more in place. If large modifications are required to a containment system, either water flow will be redirected, or water will be shut off to the area being serviced so that no effluent is generated from that area. Some containment measures may not be employed if no fish are present in a given area. The circumstances in which a specific containment measure is not used will be defined in facility SOPs.

5.6.5 Site and Facility Security

Multiple systems are in place to monitor site security, prevent unapproved intrusion, avoid inadvertent escape of fish, and prevent loss of operational capacity. The Hatchery and Grow Out Units are equipped with independent back-up generators that satisfy power requirements in the event of an unexpected electrical outage. Culture tanks in all buildings will be monitored continuously for water level, DO levels, pH, temperature, carbon dioxide, and ozone.

Perimeter security: The service entrances to the site will be secured with a heavy chain during non-business hours. At night, the entire perimeter will be well-lit.

Exterior & interior entries: Exterior steel doors on all buildings are always locked. The primary visitor entrances in the Grow Out and Hatchery Units will only provide direct access to the administrative areas. Visitor access to the Hatchery Unit requires admittance by an ABT employee, and visitor access to the Grow Out Unit will require admission by an ABT employee or an intercom-interrogation and remote unlock. Access to fish rearing facilities in the Grow Out Unit will be further secured by an interior locked entry. Secondary access to the buildings will require a key and will be strictly limited to authorized staff.

Security & environmental monitoring: Motion-activated security cameras will be positioned for maximum surveillance of the site immediately surrounding the aquaculture facilities (Hatchery and Grow Out Units) and the generator and Waste Treatment Building (see Figure 5-2, above) and those cameras will be in continuous operation. Digital images will be recorded and stored for later retrieval and review. In addition, a series of magnetic door contacts, infrared motion detectors, and environmental sensors (e.g., power levels and water conditions) will be incorporated throughout the main buildings and utility buildings, all of which will be continuously monitored by a commercial security service.

Water supply: The four wells in operation will be contained inside concrete housings equipped with tamper-proof metal covers.

Remote notification of status: Alarms indicating suspected intrusion or any emergent change in environmental conditions (as noted above) during non-working hours will be conveyed by the security-monitoring service to facility staff via text message or email. In case of an alarm, on-call staff will receive telephone calls from the system to ensure proper and immediate response is initiated.

Disaster preparedness: In addition to the established SOPs that generally dictate day-to-day operations, specific plans for response to loss of operational capacity, breach of security, or catastrophic incidental occurrence will be formulated and documented in an Overview of Facility Operating Systems & Emergency Procedures. Among the items defined and described in the Overview will be the following:

- Operational descriptions of systems (i.e., supplies for water, electricity, oxygen and security monitoring);
- On-call responsibilities and emergency responses to system-supply failures;

- Contact information for service providers;
- Training, certification and emergency response checklists; and,
- Schematics of systems and supplies.

Ad hoc manned-security: If circumstances require, ABT will employ professional contract-security personnel who would remain on-site as needed. Contracted security personnel would surveille the property and have limited access to the central security-monitoring system in the main buildings. They would not have access to the egg hatching or fish rearing areas due to biosecurity concerns. These areas will remain locked-down and subject to surveillance by the motion-activated cameras and sensors composing the security network.

5.6.6 Operational Plans and Procedures

The Rollo Bay operations will be managed according to established SOPs based on the sponsor's successful operations at Bay Fortune and Indiana. The most important element of the containment system is well trained, knowledgeable staff who completely understand the operating systems and procedures, and who fully recognize the importance of following designated work procedures. The ABC and ABT management team are highly experienced with over 70 years of collective experience in commercial aquaculture. ABT has a full-time Director of Regulatory Compliance who ensures all aquaculture employees are fully trained and that Standard Operating Procedures (SOPs) are in place for all operations. ABT has operated its facilities at Bay Fortune for over 20 years and in Panama from 2008 to 2019 without a single escape of fish into the environment.

ABC will ensure the same levels of proficiency and quality control are in place at the Rollo Bay facility. Staff will be trained in all fish handling procedures related to their responsibilities, will be supplied with the equipment required to operate the facilities in a secure manner, will understand and follow the SOPs in place for all activities, and supporting documentation will be maintained.

SOPs for the Rollo Bay Hatchery and Grow Out Units have been developed using SOPs currently in use at the Bay Fortune broodstock facility and the ABT Grow Out facility in Indiana facility as templates. The SOPs for Rollo Bay have been modified based on experiences in current operations and to address the site-specific operational conditions and equipment present at the Rollo Bay facility. Rollo Bay SOPs have been developed to cover:

- Bio-security within the facility;
- Containment, including requirements for daily checks of critical containment barriers and procedures to follow in the unlikely event of a fish escape;
- Water quality maintenance and testing;
- Housing and management of fish populations;
- Handling, removal, and disposal of mortalities and waste;
- Actions to take in the event fish are found at a particular containment point;

- Procedures to follow when collection of waste requires bypassing or removing any given containment barrier;
- All routine fish handling and maintenance operations; and,
- Emergency response procedures for unanticipated events.

Among the many standard practices that will be in place for the Rollo Bay facilities, a few of those that deal with containment are noted here:

- When handling eggs (counting, fertilizing, pressure shocking, etc.) in the Hatchery Unit, care will be taken not to drop any eggs onto the floor. If, despite all precautions, an egg spillage does occur, chlorine pucks placed in the floor drains (standard procedure during egg handling in the Hatchery) will kill the eggs while they are in the floor drains. Eggs on the floor will be collected as and disposed of according to facility SOPs, and the floor will be cleaned with a 100 ppm bleach solution.
- All containment barriers will be checked daily by trained staff and cleaned or repaired as necessary. Required equipment will be available for routine cleaning and maintenance of the various containment barriers. For example, a new clean sock filter will be installed immediately after removing a soiled one; the soiled sock filter will then be cleaned to be ready for use.
- In the event fish are found in a containment point, they will be removed and disposed of in accordance with SOPs.
- All solid material collected in the ER/IR containment sumps will be placed in waste containers, frozen, and stored for later disposal in accordance with facility SOPs.
- Report forms will be in place to track daily activities, report anomalies and unexpected events (e.g., discovery of fish in a containment point), etc.
- Daily routine procedures for cleaning tanks will require that all debris be flushed out of the tanks and dead fish removed and disposed of according to facility SOPs.
- If it is suspected that fish have entered the drain in any facility, staff members will flush water through the lines to force the fish out of the drains and to the containment area; the containment area will be supervised by additional staff members during this procedure and fish will be retrieved and disposed of according to SOPs.
- As fish grow, barrier screens on tanks are changed to enable feces to flow from the tank while ensuring fish cannot escape the tank. Staff evaluate build-up of feces at the screen to determine when screens need to be changed. The appropriate size screen will be selected to allow excess feed and feces to exit the tank but prevent the smallest fish in the tank from exiting. When changes to tank effluent containment screens are required, all fish will be removed from the rearing Unit before making the changes. An SOP is in place detailing the process.

5.6.6.1 *Reporting*

Daily containment checklists have been incorporated into the Hatchery Unit SOPs for containment and will be incorporated into the Grow Out Unit Containment SOPs. For example, if a fish is found outside a containment barrier it will be noted in the comments section of the appropriate form. If fish are found in the containment sumps or a large breach in containment occurs or is suspected, the incident will be documented.

In the unlikely event that a complete loss of containment occurs, facility personnel will first identify and repair the containment failure and determine the extent of the breach. Recovery and euthanization of animals will begin immediately and regulatory authorities (FDA and Environment Canada) will be notified. ABT will coordinate any additional recovery and containment actions with those authorities.

Records of containment breaches have been maintained at the Bay Fortune facility since 2001 and records associated with established SOPs were maintained at the Panama facility from 2015 to 2019. The Conditions of Use defined by the FDA on AquAdvantage Salmon in the NADA requires AquaBounty to collect and periodically report data on the number of fish at any life stage that are found in containment barriers, outside of contained areas, and when any breach of containment occurs.

6 ACCESSIBLE ENVIRONMENT

6.1 Physical Site Characteristics

The Rollo Bay site, formerly known as Atlantic Sea Smolt, was purchased by ABC in 2016 from Snow Island Salmon Inc. The previous owners used the site to produce salmon smolts for sale to the local salmon aquaculture industry. At the time of purchase, the site had one permanent building used as a hatchery and a set of outdoor tanks for rearing smolts.

ABT acquired the location to expand production of AquAdvantage Salmon eyed-eggs to support ABT's growing global operations, and as a location for rearing AquAdvantage Salmon to market size. Although Canada will be the primary market for AquAdvantage Salmon grown for market at Rollo Bay, ABT is seeking approval of the Rollo Bay Grow Out Unit to enable export of AquAdvantage Salmon to the U.S. should ABT choose to do so.

The Rollo Bay site is located at 46 36 27N, 62 34 30W and lies in a predominantly agricultural area on approximately 70 acres bound by Route 307 (Bear River Road), which is a north-south highway that connects Highway 2 (Veteran's Memorial Highway) and Highway 16 (Northside Highway) (Figure 6-1). The site is in eastern PEI (Kings County) and is approximately 1.5 km north of the closest coastal waters. The site is approximately 7 km northwest of Souris, PEI (pop. 1,232), which is approximately 78 km northeast of the provincial capital of Charlottetown (pop. 38,174), and 12 km from the Sponsor's facility at Bay Fortune. The local economy is primarily dependent on farming, fishing-aquaculture, and tourism.

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 Prince Edward Island (https://atlanticadaptation.ca/en/islandora/object/acasa%3A775, accessed March 15, 2019). 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 (Figure 6-2).

Figure 6-1. Rollo Bay and the Surrounding Area, Including AquaBounty Bay Fortune Site

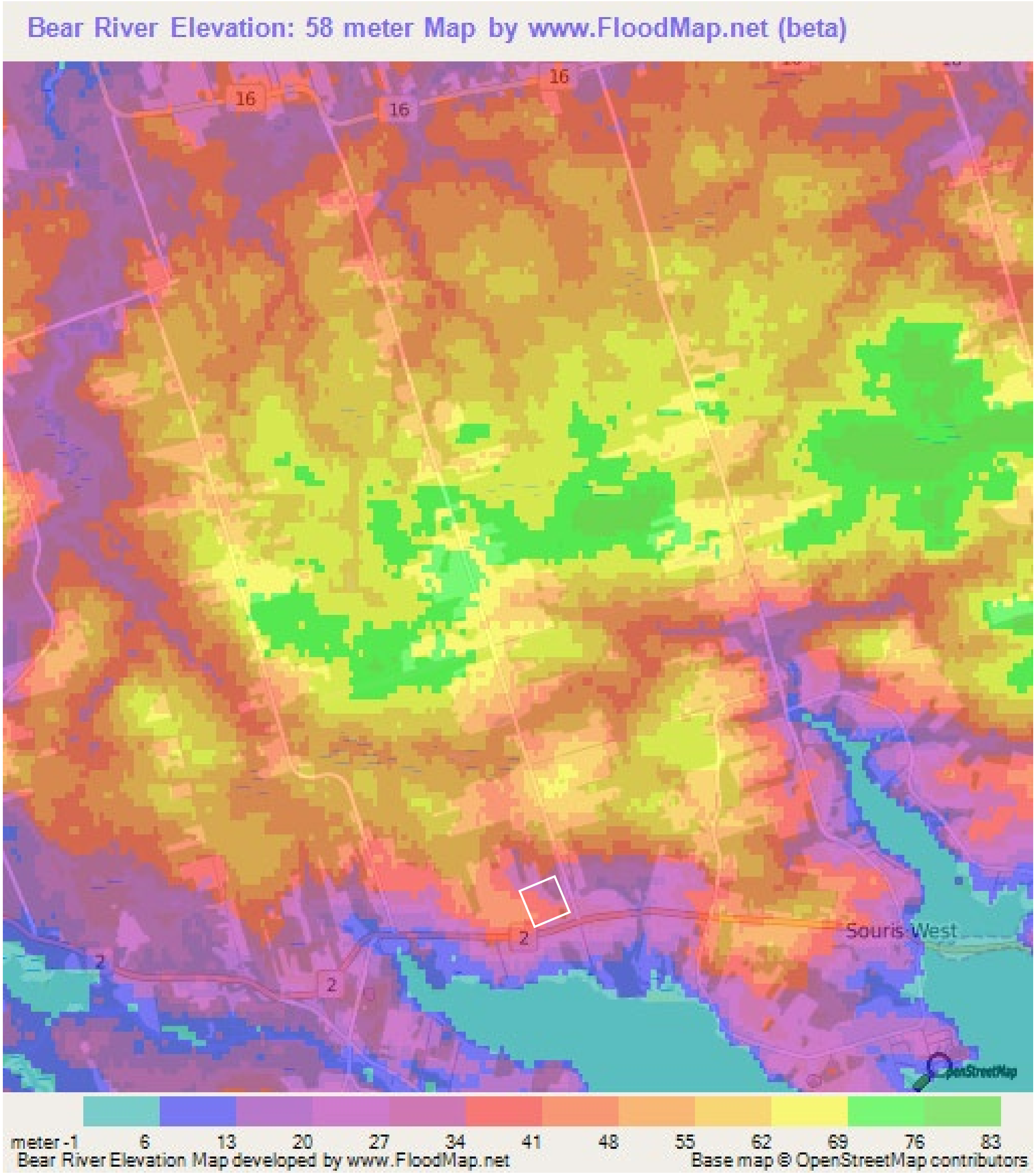


Figure 6-2. Coastal Topography near the Rollo Bay Facility

Approximate location of Rollo Bay site indicated as white box.

6.1.1 Risk of Natural Disaster

A description of historical natural disasters on PEI and the potential risk posed by future natural disasters to the sponsor's manufacturing facility at Bay Fortune was provided in the EA for NADA 141-454. Given the proximity of the Rollo Bay site to Bay Fortune, there is no additional information on the nature or frequency of natural disasters affecting PEI to report.

6.1.2 Rollo Bay Brook

A small stream with variable flow, known locally as the Rollo Bay Brook, runs through the Rollo Bay property and travels approximately 1.5 km from the property before entering the Northumberland Strait (Figure 6-3). The sponsor has measured water flows through the brook downstream of the Hatchery building ranging from 1086 L/minute to >8500 L/minute during heavy rain events. Water levels in the brook are quite shallow (Image 6-1) except during periods of heavy rain.

Figure 6-3. Rollo Bay Site, Rollo Bay Brook and Northumberland Strait

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. After leaving the Rollo Bay property, the brook flows approximately 1.5 km to before entering the Northumberland Strait. As can be seen in Figure 6-3, when the brook reaches the Northumberland Strait it spreads into a mini-delta and crosses a sandbar before entering the Strait. The shallow water depth at the sandbar, particularly during low tides, 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.

Image 6-1. Rollo Bay Brook Upstream from Hatchery Polishing Pond[21]

Some effluent from the site will be discharged into the Rollo Bay Brook (Section 5 contains detailed descriptions of water flow and effluent streams that enter the brook). 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 to 364 L/m from July through September and 546 L/m the rest of the year. There are no limits on maximum discharge volumes.

In test operations, discharge of the required minimum volumes has had minimal effect on the flow rate or depth of the brook.

[21] Picture taken on September 13, 2018

6.1.3 Northumberland Strait

The Northumberland Strait is a tidal water body between Prince Edward Island and the coast of eastern New Brunswick and northern Nova Scotia. The strait extends 225 km west-northwest to east-southeast from Richibucto Cape, New Brunswick, to Cape George, New Scotia, with a width of 13 - 43 km. It is 68 m deep at its eastern end but less than 20 m deep over a large central area. Pre-glacial and glacial valleys eroded into red sandstone and siltstone lead from both ends into the floor of the Gulf of St. Lawrence. The retreat of glacial ice from the strait and surrounding area about 13 000 years ago was followed by flooding by the sea. Soon after, isostatic uplift excluded the sea from the central area, which became an isthmus joining opposite coasts. By 5000 years ago, the rising sea level had flooded this link, establishing the strait, which has been deepening slowly.

A generally shallow depth causes strong tidal currents, water turbulence and a high concentration of suspended red silt and clay, which led early French colonists to name the strait "la mer rouge." 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 >25 °C and anoxic conditions have been reported in the Northumberland Strait near Souris, PEI 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).

High sediment loads, high summertime water temperatures and low DO, and high nitrate concentrations would make the waters of the Northumberland Strait less than ideal for long-term establishment of AquAdvantage Salmon or AquAdvantage Salmon broodstock. The coastal waters of Atlantic Canada vary in salinity but are reported to be around 33 to 34 ppt (Butler et al. 1996)[22], and 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 AquAdvantage Salmon or AquAdvantage Broodstock (i.e., those with the ability to osmoregulate) would have any prospect of surviving in the Northumberland Strait based on ambient salinity in the Strait. However, as discussed in Section 6.1.1, these 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.

6.2 Climate and Local Conditions

The climate at the Rollo Bay site is generally damp, with average annual rainfall of 87 cm and average annual snowfall of 340 cm; the average temperature is -7ºC in January and 19ºC in July. Average minimum and maximum daily temperatures by month for Charlottetown have ranged from -12.6 to 13.8ºC and -3.3 to 23.2ºC, respectively, over the past 30 years (Table 6-1).

[22] accessed at http://waves-vagues.dfo-mpo.gc.ca/Library/240630.pdf , February 23, 2019

Table 6-1. Weather Data for Charlottetown, PEI

Month[b]	Avg[a] Daily Temp (°C)		Avg Precipitation	
	Min	Max	Amt (cm)	Days
Jan	-12.6	-3.3	10.6	19
Feb	-12.4	-3.3	8.6	16
Mar	-7.1	0.9	9.2	16
Apr	-1.4	6.7	8.8	15
May	4.0	14.1	9.8	15
Jun	9.6	19.6	9.3	13
Jul	13.8	23.2	8.6	12
Aug	13.5	22.6	8.7	11
Sep	9.1	18.0	9.5	14
Oct	3.8	11.8	10.9	15
Nov	-1.1	5.7	11.1	17
Dec	-8.1	-0.1	12.3	21

[a]Abbreviations: Amt, amount; Avg, average; Max, maximum; Min, minimum. Values are based on monthly averages for the 30-year period
[b]http://www.theweathernetwork.com/statistics/summary/cl8300300/cape0005; accessed January 23, 2018

6.3 Biological/Ecological Properties

6.3.1 PEI Marine Aquatic Environment

In 2003, the Department of Fisheries and Oceans (DFO) Gulf Region initiated the development of a monitoring program called the Community Aquatic Monitoring Program (CAMP). One of the program goals was to help determine the ecological health of estuaries and coastal shorelines in the southern Gulf of St. Lawrence (sGSL) (Weldon et al. 2008). In 2007 (the last year for which a published report is available), a total of 25 locations were included in the program, including 18 in New Brunswick, five in Nova Scotia, and seven in PEI. Three of the PEI locations - Basin Head, Montague and Brudenell River, and Murray River - are on the East coast of the island in the general vicinity of the AquaBounty operations.

Monitoring activities took place monthly from May through September and included collection and counting of aquatic species, measuring water temperature, salinity, and levels of several nutrients in the water. Nearly 600,000 fish and crustaceans representing 34 different species were collected in 2007 and the most abundant species were similar across the three provinces. The most common species were sand shrimp (*Crangon septemspinosa*), mummichog (*Fundulus heteroclitus)*, killifish (*Fundulus diaphanous*), 4-spine stickleback (*Apeltes quadracus*) and Atlantic silverside (*Menidia menidia*).

At the three east coast PEI sites, sand shrimp were the most common species collected at Basin Head and Montague and Brudenell River, the two closest sites to where the Rollo Bay Brook discharges into the Northumberland Strait (approximately 10 and 15 miles, respectively). The two species of *Fundulus* (mummichog and killifish) were the second most commonly identified organisms. These fish are tolerant of low DO, low pH, and wide fluctuations in salinity and water temperature (Weis 2002).

Water temperatures across the three sites ranged from 8.2 °C to 22.0 °C, salinity ranged from 23 to 29 ppt, and DO ranged from 5.8 to 9.9 mg/L. Salmonids are known for requiring more dissolved oxygen than many other fish. Shepherd and Bromage (1988) state that the DO content of water in a salmonid farm should never drop below 6 mg/L. Similarly, Stead and Laird (2002) suggest that DO levels should never fall below 5 mg/L; for good growth of salmonids, a minimum of 7 mg/L is essential. From July through August, the three surveyed locations experienced DO levels on the low end of requirements for salmonids. Detailed information on the temperature and DO preferences and requirements for Atlantic salmon is provided in Section 5.4.2 and Appendix A.3 of this EA.

Restocking and habitat enhancement have been pursued with some success in the Province and in 2013, salmon occupied approximately 26 rivers (of 71 total) on PEI. However, the two rivers closest to Rollo Bay, the Souris and Fortune, do not contain resident Atlantic salmon populations (Cairns and MacFarlane 2015)

6.3.2 Endangered Populations

6.3.2.1 *Canadian Protected Environmental Areas*

Canada maintains a network of federally-protected environmental areas including Marine Wildlife Areas, Marine Protected Areas, Migratory Bird Sanctuaries, Marine Protected Areas, and National Marine Conservation Areas. Provinces may also designate protected areas for conservation of nature, the ecosystem, and cultural values, most of which have some marine component.

Fourteen population segments of wild Atlantic salmon have been defined by geographical limits and are assigned a status dependent on population assessments. The locations of these population segments are illustrated in Figure 6-4. The Committee on the Status of Endangered Wildlife in Canada (COSEWIC) has listed five of these population segments as endangered, one as threatened and four as special concern[23]. The Rollo Bay facility is approximately 64 nautical miles (NM)[24] from Cheticamp, Nova Scotia, the approximate closest point within the area for the Gaspe-Southern Gulf of St. Lawrence population of Atlantic salmon, which is listed as an area of special concern; ~400 NM from South Coast of Newfoundland population segment that is listed as threatened; and, ~580 NM from the Inner Bay of Fundy population which is listed as Endangered.

[23] http://www.dfo-mpo.gc.ca/species-especes/sara-lep/identify-eng.html, accessed 25 March 2018

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

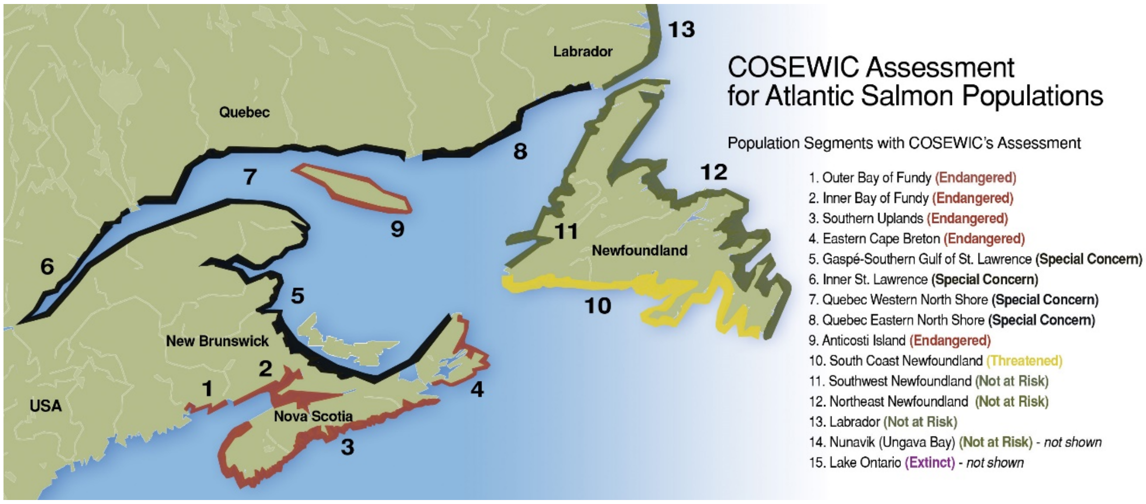


Figure 6-4. Location of Atlantic Salmon Populations in Maritime Canada[25]

6.3.2.2 *U.S. Populations of Endangered Atlantic Salmon*

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 affected the temperature of the water in the ocean at the depths at which Atlantic salmon are found (2-10 m 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% of the rivers in New England that historically supported salmon. They are in "critical condition" in the remaining 16%. In 2004, only 60 - 113 individual fish were counted in the eight rivers in Maine that support Atlantic salmon. In 2000, the National Oceanic and Atmospheric Administration's (NOAA) Fisheries Services and the U.S. Fish and Wildlife Service (FWS) listed the Gulf of Maine Distinct Population Segment of Atlantic salmon as "endangered" under the Endangered Species Act. That designation was extended in 2009 to include fish in several rivers in Maine. Populations in Canada have also declined. In the 1970s,

[25] http://www.oldsalmon.ca/issues.php?id=4, accessed 25 March 2018

approximately 1.5 million salmon returned to their natal rivers in Eastern Canada; by 2004, that number had dropped to approximately 350,000 (Knapp et al. 2007).

7 ENVIRONMENTAL CONSEQUENCES

This section discusses the potential effects of the proposed action, including potential effects on populations of Atlantic salmon listed as endangered in the State of Maine and populations of threatened and endangered species in Atlantic Canada.

7.1 Scope and Approach to the Analyses of Effects

Given that risk mitigations in the form of several different types of containment or confinement (i.e., physical, biological, and geographical/geophysical) would be in place at the Rollo Bay facilities, the analyses of potential effects or impacts focuses primarily on the adequacy and redundancy of these containment measures for their intended purposes to prevent escapes and reproduction that would affect the environment of the U.S. This and additional information on the accessible environment (Section 6) is used to determine whether there are complete exposure pathways that could potentially lead to environmental impacts.

7.2 Question 1: What is the likelihood that AquAdvantage Salmon or AquAdvantage Broodstock will escape the conditions of confinement?

As discussed in Section 3, the likelihood of escape would depend primarily on the extent and adequacy of physical (mechanical) containment at the facility. 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 proposed facility in Rollo Bay. As a result of multiple and redundant forms of effective physical confinement, it can be concluded that the likelihood of escape of AquAdvantage Salmon is extremely low. The following discussion provides the reasoning for this conclusion.

To ensure containment, a redundant, multi-level strategy has been used. Physical containment for the Hatchery and Grow Out Units are described Sections 5.6.3.1 and 5.6.3.2, respectively. Operational protocols and procedures are in place for inspections of critical containment barriers, which are to be conducted daily; for responding to emergencies (such as an interruption of the water supply); 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 Rollo Bay Hatchery Unit to the Charlottetown airport when eggs are shipped to Indiana, and transfer of eyed-eggs from the Hatchery Unit to the Grow Out Unit at the Rollo Bay facility takes place in covered containers following SOPs. Harvest of AquAdvantage Salmon for market is also subject to SOPs that ensure no live fish leave the Grow Out Unit. These measures are described in Sections 5.5.3 and 5.5.4, respectively.

7.2.1 Physical Containment at the Rollo Bay facility

Physical containment at the Rollo Bay facility is described in detail in Section 5.6.3. The Hatchery and Grow Out Units are operated as recirculating aquaculture systems except for the Conditioning area of the Grow Out Unit, which will be operated under partial recirculating conditions. These conditions mean that the discharge of water, and concomitant potential for fish escape, is minimal. The entire process is housed within self-contained buildings, so there is no risk of escape or movement of fish through predation by wildlife.

A minimum of nine points of physical containment are in place within each area of the Hatchery and the Grow Out Units where eggs, alevin, or larger fish are handled and housed. Containment barriers are 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 Hatchery RAS operates at > 99% efficiency, which in itself is a significant containment barrier, and all effluent passes through one or two containment sumps (Hatchery AR and Hatchery ER, respectively) before final discharge from the Hatchery Unit. During egg handling operations, chlorine pucks will be placed in floor drains to provide a chemical barrier in the event eggs are dropped and enter the floor drain system.

The Grow Out Unit includes facilities for egg handling and incubation, rearing AquAdvantage Salmon from first-feeding alevin to market weight fish, and conditioning of market-ready fish. More than 99% of water used in the building is recirculated, further reducing the likelihood of fish at any life stage escaping. All solid waste is pumped to permanent concrete storage tanks where it is held until the tanks are full and the solid waste moved to an offsite waste treatment facility or used for agricultural purposes (land application) according to provincial and federal regulations and guidelines.

Discharge water from the Grow Out Unit flows through a through a 20" PVC pipe into a stone out-wash approximately 140 m west of the Grow Out building. The water is filtered through the stone and flows approximately 40 m across a natural area populated with trees and undergrowth before eventually entering the Rollo Bay Brook.

These multiple and redundant barriers prevent the escape of any life stages of AquAdvantage Salmon from the facility.

7.2.2 Issues Affecting Containment and Security

7.2.2.1 *Natural Disasters*

Potential causes of weather-related natural disaster for the sponsor's facility at Bay Fortune, PEI were described in detail in the 2015 EA (Section 7.2.1.1.4). The Rollo Bay facility is approximately 12 km by highway from the Bay Fortune facility and the risk of a catastrophic weather event at Rollo Bay is, if anything, lower than the risk at Bay Fortune due to the higher elevation and increased distance to ocean waters at Rollo Bay compared to Bay Fortune. At the lowest point, the Rollo Bay site is approximately 19 m above sea level and there is no report of a storm surge greater than 1.37 m with the sea level rising to 4.23 m on the south shore of PEI (https://atlanticadaptation.ca/en/islandora/object/acasa%3A623), accessed March 21, 2019). Due to the topography of the area (see Figure 6-2, above), water will drain away from the facilities and flooding is not a concern around the Rollo Bay facility.

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 to allow full operation of the facilities for a period of days. Even if a complete power failure occurred, 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 the facility, the presence of multiple, redundant containment measures makes it very unlikely fish could escape the facilities or enter the Rollo Bay Brook. In the event of a weather event severe enough to damage the entire facility, it is 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.

7.2.2.2 *Physical Security*

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).

Information about physical security measures at the Rollo Bay site has been described in Section 5.6.5. Measures include restricted entry to the site, perimeter and exterior lighting, enclosure of operations in locked buildings, and main entrance access only to administrative areas. Motion-activated security cameras will be positioned for maximum surveillance of the site immediately surrounding the main buildings and associated utilities and will be in continuous operation. Digital images will be recorded and stored for later retrieval and review.

In addition, a series of magnetic door contacts, infrared motion detectors, and environmental sensors (e.g., power levels and water conditions) will be incorporated throughout the main buildings and utility buildings, all of which will be continuously monitored by a commercial security service.

Access by predators is eliminated because the entire facility is indoors. In addition to the physical security measures, there are SOPs in place to address containment failure and security issues. Employees have undergone training and the facility will be subject to routine inspection by Canadian officials.

7.2.2.3 *Malicious Intentional Release*

Given the redundancy in physical containment measures and the low probability of occurrence of severe natural disasters in the area, the most likely event leading to introduction of AquAdvantage Salmon to the environment surrounding the Rollo Bay facility would be an intentional malicious release. ABT is aware that unauthorized access to the site may represent a potential hazard and has taken appropriate steps to reduce the possibility this will occur. As described in Section 5.6.5 and above, there are extensive security measures, equipment, and plans in place to ensure that the probability of such an event would be extremely low.

7.2.3 Conclusions for the Rollo Bay Facility

The probability that AquAdvantage Salmon would escape from the Rollo Bay facility is extremely small due to the presence of multiple, independent forms of physical (mechanical) containment, augmented by chemical containment when eggs are handled in the Hatchery Unit. In the unlikely event fish were to escape through the effluent, the likelihood of any life-stage migrating to the brook would be low due to the additional physical barriers where effluent is released, including solid concrete storage tanks, a stone out-wash, a septic leach field and a French drain. Backup systems are in place in the event of equipment failures or a natural disaster, and site security measures are in place to prevent malicious activities.

7.2.4 Transportation of Eggs from Rollo Bay Hatchery

As described in Section 5.5.3, eyed-eggs of AquAdvantage Salmon are currently shipped from Bay Fortune to Indiana via air freight with subsequent ground-shipment to the grow out facilities. This will continue to be the case when eggs are shipped to Indiana from Rollo Bay. When shipped, multiple containment measures are in place for AquAdvantage Salmon 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. Unintentional escape of AquAdvantage Salmon eggs is therefore particularly unlikely.

7.2.5 Disposal of Fish and Fish Wastes

As discussed in the 2015 EA, disposal of AquAdvantage Salmon (including non-viable eggs, mortalities, and culls) and the non-viable waste material associated with the production, processing, and consumption of the fish (e.g., feces, fish pieces) would not require different handling from that used for wild or domesticated wildtype fish: 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.

Fish wastes and uneaten feed (biosolids) will be removed from the effluent at the Rollo Bay Grow Out Unit by mechanical filtration through drum filters in the ER/IR and AR Containment Sumps. Further separation of solids is achieved using settling cones located in the ER/IR Containment Sump and at the Waste Treatment Building. Solid waste from the Grow Out ER/IR will be stored in an underground concrete septic tank (see Section 5.6.3.1.4 and Figure 5-4) and solid waste from the Grow Out AR will be stored in a concrete manure storage tank located in the Waste Treatment Building (see Section 5.6.3.1.5 and Figure 5-4). Solid waste will be removed from the storage tanks as they fill and used as fertilizer in land applications conducted in compliance with Canadian and Provincial laws.

Biosolids from the Hatchery Unit are collected from stainless steel baskets located in the Hatchery AR containment sump and frozen (see Section 5.6.4.1.3.1). 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.

Market-ready AquAdvantage Salmon will be killed at the facility, placed on ice, and then transported to an appropriate processing plant (no processing agreements are in place at this time). The specific method by which the fish wastes generated through processing (i.e. entrails) will be disposed of will be in accordance with applicable Canadian and Provincial laws. As discussed in the 2015 EA, no specific hazards or risks have been identified in conjunction with mortalities and fish wastes. The integrated EO-1α construct is not inherently hazardous and is not expected to be mobilized through waste disposal; therefore, disposal of dead fish and fish wastes will not present a risk to the environment.

For many of the same reasons described above, specifically a lack of any specific hazards associated with non-live AquAdvantage Salmon or parts thereof, no effects on the environment are expected due to disposal of any unconsumed parts or pieces of AquAdvantage Salmon that are processed as food.

7.2.6 Conclusions for Question 1

For the NADA supplements, production of eyed-eggs and grow out of AquAdvantage Salmon is proposed to be conducted only in land-based facilities with redundant physical containment measures. Eyed-eggs produced at Rollo Bay that will be transported to the ABT facility in Indiana will be under the control of ABT from the Hatchery Unit to the shipper. Transfer of eyed-eggs from the Hatchery Unit to the Rollo Bay Grow Out Unit will be done in closed containers.

There are multiple and redundant physical and mechanical barriers in place in the water systems at the Rollo Bay facility 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 AquAdvantage Salmon. The facility has a minimum of nine mechanical barriers in place for all internal flow streams that release water to the outside environment. This level of containment is consistent with recommendations in the ABRAC Performance Standards (ABRAC, 1995).

Physical containment is also augmented by chemical containment (chlorine pucks) in the Incubator section of the Hatchery Unit, and exterior physical barriers in the effluent discharge stream. In addition to the physical and chemical containment barriers in place, physical security and containment to prevent unintentional releases of salmon due to natural disasters or intentional releases due to malicious activities are in place.

ABT also employs SOPs that govern physical containment, as well as every other significant activity that occurs at the site. In addition, a strong operations management plan is in place at the Rollo Bay site, comprising policies and procedures that meet the recommendations for an integrated confinement system for GE organisms as summarized in Section 3 and in Table 7-1 below.

Any breakdown of 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 Canadian and Provincial officials.

The combination of these factors results in an extremely low likelihood that any life stage of AquAdvantage Salmon present at the Rollo Bay facility could escape into the wild and cause effects on the environment. This aligns with the conclusions of Canadian regulators from the Department of Fisheries and Oceans (DFO) and ECCC following a pre-approval inspection of the Rollo Bay site and facilities in June 2018[26]. The Canadian regulators determined there was "[a] high degree of certainty associated with the physical, biological and operational containment of EO-1α Salmon 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.

Table 7-1. Implementation of an Integrated Confinement System for AquAdvantage Salmon

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

* Confinement System based on Kapuscinski (2005)

[26] DFO. 2019. Environmental and Indirect Human Health Risk Assessments for the Manufacture and Grow-out of EO-1α Salmon, including the AquAdvantage® Salmon, at a Land-Based and Contained Facility near Rollo Bay, PEI. DFO Can. Sci. Advis. Sec. Sci. Advis. Rep.2019/014 (http://www.dfo-mpo.gc.ca/csas-sccs/Publications/SAR-AS/2019/2019_014-eng.pdf).

7.3 Question 2: What is the likelihood that AquAdvantage Salmon or AquAdvantage Broodstock will survive and disperse if they escape the conditions of confinement?

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). Section 5.6.3 provided a detailed analysis of the containment measures in place at Rollo Bay and the very low likelihood of their escape has been addressed in responding to the first risk question (Section 7.2). Consequently, in the very unlikely event that any life stages of AquAdvantage Salmon or AquAdvantage Broodstock were to escape the Rollo Bay facility, the likelihood of survival and dispersal is a function of two complementary sets of parameters: their phenotype and fitness and the specific geographical and geophysical containment in the accessible environment.

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 rearing. Furthermore, unless deemed to be 100% effective under all reasonably foreseeable circumstances, containment of this type would normally be considered secondary to other containment measures, including the physical containment measures that have been described in detail in this EA.

As an overall statement, the dispersal of AquAdvantage Salmon or AquAdvantage Broodstock would depend on how many escaped and survived, their fitness and physiological characteristics, and their reproductive potential. This section will focus on the geographical/geophysical factors and physiological/fitness factors that would affect survival and dispersal. The reproductive factor will be considered in question 3 (Section 7.4).

7.3.1 Geographical/Geophysical Containment

The Rollo Bay facility is surrounded by farmland and pasture and the only aquatic access to the local marine environment is the Rollo Bay Brook, described in detail in Section 6.1.1. 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 to 364 L/m from July through September and 546 L/m the rest of the year. There are no limits on 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 population of brook trout and could potentially support escaped AquAdvantage Salmon or AquAdvantage Broodstock. The likelihood of survival would be affected by the environmental conditions at the time of escape and the life stage of fish that escaped.

In addition to the barriers inherent to the receiving environment, in Section 5.6.3.1, ABT has created multiple physical barriers that prevent or significantly slow the movement of water, and therefore fish, into Rollo Bay Brook. These include concrete tanks for storage of solid wastes, a stone out wash field located approximately 40 m from the brook through which all water discharged from the Grow Out Unit must pass before entering the brook; an underground leach field, and a French drain that channels clear water discharge from the Waste Treatment Building into the field next to the Waste Treatment Building.

As described in detail in Section 6.1.2, the Northumberland Strait, located approximately 1.5 km downstream from the Rollo Bay facility (Figure 6-3), is a tidal water body between PEI and the coast of eastern New Brunswick and northern Nova Scotia. A generally shallow depth causes strong tidal currents, water turbulence and a high concentration of suspended red silt and clay in the strait, 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 Section 5.4.2, conditions such as those are much less than optimal for GH modified Atlantic salmon.

In order for escapees to survive, the accessible ecosystem must meet their needs for food, habitat, and environmental cues for reproduction. The existing presence of conspecifics or species closely related to the GE escapee in accessible ecosystems indicates that a suitable environment does exist (Kapuscinski *et al*., 2007). Brook trout and rainbow trout do occur in streams in the general vicinity of the Rollo Bay site on PEI (Guignion et al. 2010). However, Atlantic salmon are not currently present in the Rollo Bay watershed or any nearby watershed (Cairns and McFarlane, 2015), although they were once periodically stocked in the area over the years from 1907-1937, and perhaps later (Cairns et al. 2010). This information suggests that the local environment is potentially suitable for survival of salmonids, although as will be discussed subsequently in Sections 7.4 and 7.5, the potential for reproduction and establishment of Atlantic salmon in the vicinity is considered very low.

7.3.2 Fitness and Physiological Characteristics

Although the Rollo Bay Brook environment would at times be favorable to the initial survival of escaped fish, as described in Sections 5.2 – 5.4 and Appendix A, the physiologic and behavioral changes observed in GH transgenic fish would reduce the likelihood of AquAdvantage Salmon or AquAdvantage Broodstock surviving and dispersing in the wild.

In an optimized production environment Atlantic salmon expressing a growth hormone gene, including AquAdvantage Salmon relatives (Du et al. 1992) and EO-1α 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-1α salmon would provide to salmon that escaped the confines of a production system and entered the environment. The absence of enhanced growth in EO-1α first feeding fry, the growth stage at which rapid growth might be expected to provide a significant advantage in a natural setting, has been noted (Levesque et al. 2008; Moreau et al. 2014). There is similar evidence from AquAdvantage

Salmon relatives (Cook et al. 2000a), coho salmon (Devlin et al. 2004a), and rainbow trout (Crossin et al. 2015).

Although the growth-enhancing effect of the EO-1α transgene is delayed until after first feeding is underway, GH salmonids, including AquAdvantage Salmon 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). Along with the impact of predation, in food constrained environments GH salmonids, including EO-1α salmon, do not always exhibit superior growth relative to wildtype comparators (Crossin et al. 2015; Leggatt et al. 2017b; Moreau et al. 2011b).

The development of EO-1α 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 growth hormone 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 not unreasonable 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 factors that reduce the fitness of EO-1α salmon, factors associated with the requirement to rear EO-1α salmon in contained aquaculture systems 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). AquAdvantage Salmon have been bred and reared in captivity for 12 generations and that alone reduces the risk of survival and dispersal posed by an escape.

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). This low survival 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 have adapted to being fed on 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 they 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 Rollo Bay facility, AquAdvantage Salmon and AquAdvantage Broodstock might not transition to a wild prey diet and thus would be susceptible to starvation and early mortality.

7.3.3 Conclusions for Question 2

As described above, the aquatic pathway from the Rollo Bay facility to the Northumberland Strait first requires escaped or released fish to enter the Rollo Bay Brook. ABT has engineered the water flow pathways from the Rollo Bay facility to the brook to be inhospitable to all life-stages of AquAdvantage Salmon and AquAdvantage Broodstock, making it quite unlikely for an escaped fish (or fish egg) to reach the brook. In the unlikely event escaped fish were able to enter the brook or released fish were introduced into the brook, it is quite possible escaped or released fish could survive there, at least in the short term, based on the current presence of brook trout in this stream. If escaped/released fish were able to migrate downstream and exit the brook, they would enter the Northumberland Strait, a body of water that is turbid and can be highly inhospitable to salmonids at various times in the year due to high temperature and low DO levels. Consequently, while the receiving environment may allow short-term survival and possible dispersal, it is not a highly favorable environment and does not appear to be a habitat suitable for long-term survival or establishment as evidenced by the absence of Atlantic salmon in the local watersheds despite years of stocking and attempts in the past to establish populations in the area.

As documented above, several of the physiological and fitness attributes displayed by GH modified Atlantic salmon, including AquAdvantage Salmon and AquAdvantage Broodstock, would reduce the ability of GH salmon to survive or become established in the natural environment. Additionally, the transition from a farmed environment to a natural environment poses its own challenges to survival and reproduction of non-transgenic Atlantic salmon and would likely have at least as much of an impact on AquAdvantage Salmon or AquAdvantage Broodstock. As a result, in the highly unlikely event of an escape from the Rollo Bay facility, the escaping fish would be unlikely to survive long enough to adapt to the environment and disperse into the broader environment (e.g., other PEI watersheds where Atlantic salmon are currently established).

7.4 Question 3: What is the likelihood that AquAdvantage Salmon or AquAdvantage Broodstock will reproduce and establish if they escape the conditions of confinement?

As described in Section 3.2, there will be three types of transgenic fish housed at Rollo Bay: AquAdvantage Salmon, transgenic neomales, and transgenic diploid females. The largest number of fish (approximately 100,000) that will be housed at Rollo Bay will be the AquAdvantage Salmon reared for harvest in the Grow Out Unit. Consequently, in the highly unlikely event of an escape, the most likely fish to escape would be AquAdvantage Salmon. These fish are hemizygous, i.e. have one copy of the GH gene, are all female, and are triploid. Because they are triploid and therefore effectively sterile[27], in the unlikely event any were to escape, they could not successfully mate with any other fish. As documented in Section 5.5.5 of this EA, ABT routinely achieves >99% triploidy (Table 5-2, above), and thus the vast majority of fish housed at Rollo Bay will be incapable of reproducing successfully (i.e., producing viable offspring).

As described in Section 5.5.5, when at capacity, there will be approximately 100,000 AquAdvantage Salmon present in the Rollo Bay Grow Out Unit. Based on the average rate of diploidy in AquAdvantage Salmon eggs (0.08%; Table 5-2), fewer than 100 salmon in the Grow Out Unit would be diploid and therefore potentially capable of reproducing. These fish would carry only one copy of the GH gene, which reduces the potential genetic impact by 50%. Because such fish would have undergone the same treatment as the triploid counterparts (i.e. eggs were pressure shocked at 10,000 psi), it is unknown if they would be capable of reproduction. However, even if they were reproductively competent, it is unlikely they would reproduce or establish populations if they were to escape because of the small total number of fish, the decrease in reproductive fitness relative to native populations of Atlantic salmon (see Sections 5.3 and 5.4), and the limited opportunities for interactions with native populations (see below).

The Hatchery Unit also houses approximately 300 neomales. Neomales are diploid, homozygous (two copies of the GH gene) genetic females that have been masculinized by introducing testosterone into their diet from first feeding (Section 5.5.1.2). Neomales produce viable milt but cannot release it naturally due to the lack of a functional vas deferens (sperm duct). Additional discussion can be found in Section 7.5.1.1.1 of the 2015 EA. Neomales are used to fertilize the eggs of non-transgenic females in order to produce AquAdvantage Salmon eggs, but they must be sacrificed, and their gonads harvested to do so. Consequently, in the highly unlikely event that neomales were to escape, they could not reproduce with or transmit any of their genes to local salmonids.

The only other reproductively functional transgenic fish that will be housed at the Rollo Bay facility are diploid, homozygous females used to maintain AquAdvantage Broodstock production

[27] The effectiveness of triploidy in inducing sterility is discussed in Sections 5.3.2.4 and 7.4.1.3 of the 2015 EA.

(see Section 5.3 and Figure 5 in the 2015 EA). These fish are capable of spawning and could potentially transmit the GH gene to local populations. Given that GH transgenic Atlantic salmon in general do not have a reproductive advantage compared to non-GE Atlantic salmon, and sometimes are disadvantaged (see Section 5.2.2.7 of the 2015 EA; Moreau et al., 2011a; Moreau and Fleming, 2011), it is expected that a significant number of these reproductively competent diploid female fish would need to escape in order for there to be any potential chance of reproduction and establishment.

Similar reproductive studies on GH transgenic coho salmon, although not necessarily representative of diploid GH salmon (including diploid AquAdvantage Broodstock), 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 behaviors 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.

Given the very small number (≤ 20) of reproductively competent GH females that will be housed at Rollo Bay and the many redundant points of physical and chemical containment that are in place, the likelihood of any GH females escaping is quite low. However, if GH females were to escape the Rollo Bay facility, and if they were as reproductively competent as wildtype salmon, there would be no impact on local populations unless the transgenic fish were able to breed with local Atlantic salmon. In 2013, Atlantic salmon occupied approximately 26 rivers (of 71 total) on PEI. However, the two rivers closest to Rollo Bay, the Souris and Fortune, do not contain resident Atlantic salmon populations, and the closest river with a resident population is approximately 50 km from where the Rollo Bay Brook enters the Northumberland Strait (Cairns and MacFarlane 2015). Although salmon do travel long distances, in order to successfully breed, an escaped diploid transgenic female would have to travel at least 50 km, arrive at a time when spawning males were present, and successfully compete with native Atlantic salmon females.

The only "true" genotypic Atlantic salmon males expected to be held at the Rollo Bay facility[28] would be non-GE wildtype males used to maintain the wildtype population there. These wildtype males are needed to fertilize the eggs of female wildtype Broodstock to produce wildtype Broodstock (see Section 5.3 of the 2015 EA). The number of wildtype males present in the Hatchery Unit will be limited (<300), and these fish will be housed separately (i.e., in different tanks) from the AquAdvantage Broodstock and also in an entirely different building from the AquAdvantage Salmon, thus the possibility for them to escape and spawn with reproductively

[28] Non-GE wildtype males have been brought in as necessary for spawning from the nearby Bay Fortune facility. However, in the future ABT anticipates that up to 300 wildtype males may be held at the Rollo Bay facility as egg production is increased on a year-round basis.

competent (diploid) AquAdvantage females is extremely remote. This would require a mass escape under some type of disaster scenario (e.g., tornado or tsunami) in which the survival of any released fish would likely be precluded anyway.

Aside from Atlantic salmon, two other salmonids species, brook trout (*Salvelinus fontinalis*) and non-native rainbow trout[29] (*Onchorynchus mykiss*) are found in PEI streams (Guignion et al. 2010). Of these, only brook trout are found in the Rollo Bay Brook that runs through the property where the Hatchery and Grow Out Units are located. Laboratory crosses of male brook trout with female Atlantic salmon have been shown to produce small numbers of viable fry (1-5%) (Gray et al. 1993; Sutterlin et al. 1977); however, more importantly, ABT 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). This leads to a conclusion that there would be no successful reproduction with the native brook trout even in the event of an escape of AquAdvantage Salmon or AquAdvantage Broodstock.

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. This scenario would require the periodic escape or release of large numbers of fish, such as sometimes occurs from net pens in the marine environment. This is not a realistic possibility for the Rollo Bay facility due to the small population sizes relative to those used for grow out in net pens, as well as the highly redundant containment and security measures employed at the site.

7.4.1 Conclusions for Question 3

The two largest groups of transgenic fish that will be housed at the Rollo Bay facility are the all-female, hemizygous, triploid AquAdvantage Salmon (~100,000 fish) and a much smaller number (~ 300) of homozygous, diploid GH neomale broodstock fish. As described above, both genotypes are unable to successfully reproduce and therefore could not become established in the natural environment.

No more than 20 reproductively competent GH females and 100 diploid (i.e., non-triploid) AquAdvantage Salmon (all females) are expected to be housed at Rollo Bay in the Hatchery and Grow Out Units, respectively. Given the multiple levels of redundant physical and chemical containment that will be in place at the Rollo Bay facility, it is highly unlikely that any of these fish would escape. If they did, and were able to pass through or over the exterior physical barriers engineered by ABT (concrete storage tanks, stone out wash area, underground leach field, and underground French drain) to reach and survive in the Rollo Bay Brook long enough to reach the Northumberland Strait, it is unlikely they would encounter wild Atlantic salmon with which to interact and/or mate. Based on the absence of populations in the area, this would require a migration of 50 km or more. In addition, the physiological, reproductive, and behavioral

[29] Rainbow trout are native to western North American and were introduced to PEI in 1925 (DFO undated)

changes that result from the introduction of the GH genes would reduce the fitness of the transgenic fish; and it has been shown that fish grown in a hatchery environment are at a competitive disadvantage to wildtype fish, regardless of genotype. These combinations of factors make the risk of reproduction and establishment quite low.

Given the available information and weight of evidence, it can be concluded that there is a small likelihood that AquAdvantage Salmon and AquAdvantage Broodstock would reproduce and establish self-sustaining populations if they escaped from the facility in Rollo Bay. As explained below, this is the conclusion that was also reached by Canadian regulators when they reviewed the Rollo Bay location and facilitr.

In July 2018, ABT submitted a New Substance Notification (NSN) to Environment and Climate Change Canada (ECCC) proposing production of eyed-eggs and grow out and rearing out of AquAdvantage Salmon at the Rollo Bay site. In March 2019, after reviewing the NSN and physically inspecting the Rollo Bay site and units, ECCC concluded that the physical and chemical containment measures that will be used at Rollo Bay result in a low potential for exposure to the environment and authorized ABT operations at the site.

In the Joint Assessment Report issued by ECCC and Health Canada (https://www.canada.ca/content/dam/eccc/documents/pdf/pded/new-substances-organisms/Aquadvantage-salmon-summary.pdf, the reviewers offered this summary of the environmental risk posed by AquAdvantage Salmon and AquAdvantage Broodstock at Rollo Bay:

"Should there be an inadvertent release, conditions may be favourable for survival and dispersal of EO-1α Salmon if released into the drainage brook that runs through the Rollo Bay facility; however, they would need to survive in the drainage brook, migrate to and survive in marine ecosystems, migrate to spawning grounds of wild populations at the same time as wild fish, then successfully reproduce. The closest stream with wild Atlantic Salmon populations is within 50 km of the Rollo Bay facility. Given that it is unlikely that all these conditions are present at the same time, it is therefore unlikely that any EO-1α Salmon would be able to mate with wild salmon."

7.5 Question 4: What are the likely consequences to, or effects on, the environment of the United States should AquAdvantage Salmon or AquAdvantage Broodstock escape the conditions of confinement?

The environmental risk posed by GE organisms is similar to that posed by any introduced species and is a function of the fitness of the introduced organism, its interactions with other organisms, role in ecosystem processes, and potential for dispersal and persistence (Kapuscinski and Hallerman 1991). Moreau (2014) reviewed sources of uncertainty in risk assessments of GH Atlantic and coho salmon. Among his observations were that variations in phenotype and characteristics within a species depended not only on the presence of the transgene but were also strongly influenced by background genotype, gene-environment interactions, and/or life-history stage, especially in artificial laboratory environments where juvenile fish were studied.

In the very unlikely event of an escape, AquAdvantage Salmon and AquAdvantage Broodstock are expected to occupy the same ecological niche as wild and domestic Atlantic salmon, competing for food, shelter, and other resources. Although AquAdvantage Salmon and AquAdvantage Broodstock would have one key increased fitness attribute relative to their wild and domesticated counterparts (i.e., more rapid growth to smolt stage), in many other respects, their fitness would be reduced (e.g., increased need for food, increased DO 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, as ABT is not aware that any growth enhanced GE animal has ever been released into the wild. These potential outcomes, and their likelihoods, are discussed below.

This EA has documented that physical/chemical containment is very stringent for both the Hatchery and Grow Out Units located at the Rollo Bay facility, and escapes from either Unit is highly unlikely (see Sections 5.6.3 and 7.2). In the event, however unlikely, that escapes should occur, biological containment would be imposed on the numerically most prevalent population of fish housed at Rollo Bay, the all-female, triploid AquAdvantage Salmon, and on the diploid EO-1α neomales, the second most prevalent population of transgenic fish that will be housed at the Rollo Bay Hatchery.

As has also been described in this EA (see Section 7.3.1), geographical and geophysical containment factors present in the environment would also provide important barriers to long-term survival, dispersal, and establishment of AquAdvantage Salmon or AquAdvantage Broodstock in the marine environment of Atlantic Canada. These barriers would also reduce the potential for escaped AquAdvantage Salmon or AquAdvantage Broodstock to impact the environment of the U.S.

It should also be noted that the scale and frequency of introductions of GE fish into 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). As previously discussed, the probability of escape from the Rollo Bay facility is very low due to multiple and redundant physical containment measures.

7.5.1 Exposure Pathways for Effects on the United States

The only likely scenarios for escape or release of AquAdvantage Salmon to the local environment with subsequent effects on the environment of the U.S. are: (1) accidental escape of a large number of reproductively competent AquAdvantage Broodstock, specifically a large number of GH females, or diploid AquAdvantage Salmon that did not undergo triploidization during pressure shock treatment (estimated to be approximately 0.08% based on testing to date, Table 5-2), to the adjacent Rollo Bay Brook; or (2) malicious intentional release through a break-in and act of vandalism or eco-terrorism.

As documented in this EA (see Sections 5.6 and 7.2.2), the use of multiple and redundant containment barriers at the Rollo Bay facility makes Scenario 1 improbable and would only result from a complete failure of all physical containment systems due to a catastrophic event, and then only if the escaped fish were able to pass successfully through the multiple physical barriers ABT has put in place to restrict movement from the facility to the Rollo Bay Brook. Likewise, because of redundancies in security and containment measures at Rollo Bay, Scenario 2 is also an unlikely event. Regardless of the scenario, the very small number of reproductively competent AquAdvantage Broodstock (<20) and diploid AquAdvantage Salmon (<100) that will be present at Rollo Bay at any one time precludes the potential for a mass release of large numbers of reproductively competent transgenic fish from Rollo Bay facility.

As discussed in Section 7.3, depending upon the time of year at which an escape or release occurred, escaped GH females could potentially survive in the local environment. However, as described in Section 7.4, the likelihood of escaped GH females encountering wildtype local Atlantic salmon would be limited due to the reduced numbers of Atlantic salmon present in the rivers of PEI, the closest of which is approximately 50 km from the Rollo Bay site. In the unlikely event they encountered local salmon populations, in order to reproduce and potentially establish, the encounter would have to occur during spawning season and the very small number of GH females that might be present would have to compete with a population of local, well-adapted female Atlantic salmon.

For these reasons, it is highly unlikely that escaped or released GH females would be able to reproduce and establish in the local environment. The geographical and geophysical barriers that were described in Section 7.3.1 and the physiological/fitness of GH transgenic salmon described in Section 7.3.2, would also reduce the likelihood of escaped/released GH females migrating away from the local PEI environment and potentially establishing in a more distant location.

In the highly unlikely event of an escape or release of AquAdvantage Salmon or AquAdvantage Broodstock from the Rollo Bay facility, possible interactions with wild Atlantic salmon could theoretically include competition for resources (e.g. spawning habitat, food), interbreeding (and resulting gene flow and expression), and disease populations. Because there are no populations of wild or stocked Atlantic salmon in either the Fortune or Souris rivers, the two rivers closest to the Rollo Bay location, interactions of AquAdvantage Salmon or AquAdvantage Broodstock with wild Atlantic salmon would be highly unlikely. Interactions with wild Atlantic salmon would require either significant migrations along the PEI coastline to rivers where populations of wild Atlantic salmon still occur, or migrations out into the Northumberland Strait or Gulf of St. Lawrence.

As described in this section and Section 7.4, the potential for gene flow, i.e. the transmission of the EO-1α construct carried by all AquAdvantage Salmon and AquAdvantage Broodstock, is extremely limited due to the very small numbers of reproductively competent AquAdvantage Salmon and AquAdvantage Broodstock that will be present at Rollo Bay. The potential is further limited by the low possibility of encountering wild Atlantic salmon during spawning. Additional factors limiting the potential for gene flow were discussed in detail in the 2015 EA, Section 7.5.1.1.1, and provide further evidence that in the unlikely event of an escape or release of GH females from Rollo Bay, the potential impact on the environment of the U.S. would be extremely small.

Disease transmission to wild populations in the event of escape is another theoretical outcome to be considered in relation to the Rollo Bay facility. Although disease transmission is often a concern for aquaculture facilities, ABT does not expect it to be an issue at Rollo Bay for three reasons. First, there are no data to suggest that AquAdvantage Salmon or AquAdvantage Broodstock are more susceptible to disease than non-GE salmon and thus more likely to be affected by disease (see Section 5.4.5). Second, and more importantly, all of the fish present at Rollo Bay originated from the nearby ABT Bay Fortune facility and there have been no positive findings of any Canadian or OIE notifiable diseases or disease agents in any of the fish-holding areas of the PEI facility as determined in a series of inspections by Canadian Fish Health Officials over the past several years (see Section 5.4.5). Third, the Rollo Bay facility contain state-of-the-art equipment for water treatment, will be managed using strict biosecurity protocols, and will undergo periodic inspections by Canadian authorities. The Rollo Bay facility will also undergo periodic FDA inspections to verify that this remains the case. Therefore, disease transmission from the Rollo Bay facility, or from the fish therein as a result of escape/release, is highly unlikely.

Resource competition is another potential risk for wild Atlantic salmon in the event of an escape or release of GE salmon from the Rollo Bay facility. This could include competition for habitat (e.g., spawning substrate, over-wintering sites), food, or mating. Because they grow faster, there has been a suggestion that AquAdvantage Salmon or diploid Atlantic salmon might be more aggressive and thus out-compete their wild counterparts for resources.

Research on GH transgenic Atlantic salmon in laboratory experiments indicates these fish are more likely to feed in the presence of a predator than non-GE controls (Abrahams and Sutterlin 1999). Also, during pre-smolt growth these GE salmon consume 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); however, the availability of food and specific environmental conditions also influence behavior and competition for resources. Moreau et al. (2011b) found that, under food-limited conditions in simulated aquatic environments (i.e., stream microcosms), conditions expected to be much more representative of those in the natural environments than was the case for the previously mentioned laboratory studies, the presence of the growth hormone gene construct in these GE fish does not influence territorial dominance or growth or survival of first-feeding fry at high or low fry densities. In the simulated stream environments, GE and non-GE individuals were equally likely to be dominant (Moreau 2014).

Snow *et al*. (2005) have presented six major environmental concerns or impacts that may be associated with, or affected by, GE organisms (Table 7-2). Two of these processes, persistence without cultivation (i.e., reproduction and establishment) and interbreeding with related taxa (i.e., reproduction with wild Atlantic salmon) have been discussed above. The remaining four processes are addressed in Table 7-2; some are not applicable to GE animals in general or specifically to GE fish. Each of these processes and their theoretical ecological consequences, which, to date, remain largely undocumented and hypothetical, are presented in relation to their prospective applicability to AquAdvantage Salmon or AquAdvantage Broodstock. No significant risks associated with production of AquAdvantage Salmon at the Rollo Bay facility have been identified.

Table 7-2. Potential Environmental Concerns/Impacts for GE Organisms

Process*	Potential Ecological Consequence	Risk Associated with AquAdvantage Salmon or diploid ABT Atlantic salmon, including AquAdvantage Broodstock in PEI
Persistence without cultivation	Transgenic organisms able to spread and maintain self-sustaining populations could disrupt biotic communities & ecosystems, leading to a loss of biological diversity.	NO SIGNIFICANT RISK See discussion in text.
Interbreeding with related taxa	Incorporation of transgenes could result in greater invasiveness or loss of biodiversity, depending on particular transgenic trait and gene flow from generation to generation.	NO SIGNIFICANT RISK See discussion in text.
Horizontal gene flow	Non-sexual gene transfer is common in some microbes but rare in plants & animals; ecological consequence would depend on particular transgenic trait and gene flow.	NO SIGNIFICANT RISK. The integrated EO-1α construct (transgene) is incapable of being passed thru non-sexual means.
Change in viral disease	In virus-resistant transgenic organisms, genetic recombination could lead to increased virulence of viral disease and undesirable effects on natural hosts.	NO SIGNIFICANT RISK. The EO-1α construct has no viral component; this type of recombination is not possible.
Evolution of resistance	Pesticide resistance leading to greater reliance on damaging chemicals or other controls for insects, weeds, and other pests.	Not applicable for fish.

**Process and General Consequences information derives from Snow et al. (2005).*

7.5.2 Effects on Populations of Endangered Atlantic Salmon in the United States

As described in Section 6.3.2, populations of endangered Atlantic salmon are present in the Gulf of Maine and in rivers in the northern part of the state of Maine. It is highly unlikely that AquAdvantage Salmon or AquAdvantage Broodstock would affect those populations for the reasons previously discussed: physical containment at the Rollo Bay facility is very stringent and it is highly unlikely that fish would escape; in the highly unlikely event of escape, the surrounding environmental conditions are not conducive to long-term survival and establishment, as evidenced by the lack of self-sustaining salmon populations in the rivers and coastal areas of PEI that used to possess plentiful salmon runs. In addition, the fitness of AquAdvantage Salmon and AquAdvantage Broodstock appears to be low in the wild and very few, i.e. ≤ 20 EO-1α females used in the broodstock program and an unknown but very limited number (estimated at <100) of AquAdvantage Salmon that were not converted to triploids, would be reproductively competent. Finally, in the highly unlikely event any fish were to escape, they would not carry disease from the Hatchery or Grow Out Units. The possibility for effects to occur on endangered Atlantic salmon populations in Maine is further reduced by the great distance between PEI and the waters of Maine (several hundred miles by sea) and other areas of the north Atlantic Ocean where the Maine Atlantic salmon populations might migrate to as part of their life cycle.

In order to migrate to waters of the U.S., any surviving AquAdvantage Salmon or AquAdvantage Broodstock that have escaped from the Rollo Bay facility would have to complete a significant long-distance migration. There is no reason to expect any of these escaped/released AquAdvantage Salmon to undertake a migration to waters of the U.S. given that these fish are produced from domesticated hatchery stocks, as are farmed Atlantic salmon. 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 AquAdvantage Salmon and broodstock behave similarly, and they would be expected to because of their domesticated genetic background, these salmon should remain in the general vicinity of the Rollo Bay facility in the event of an escape or release.

Even if AquAdvantage Salmon or AquAdvantage Broodstock were to undertake such a migration, it is unlikely that any significant numbers would survive the journey. Based on recent return rate data for U.S. 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). Triploidy has been shown to further reduce survival/recapture rates of salmon in the field (O'Flynn *et al*., 1997). In fact, a study of the controlled release of micro-tagged triploid and diploid groups of Atlantic salmon (both mixed-sex and all-female groups) on the western coast of Ireland found that the return rate of triploid salmon, both to the coast and fresh water, was substantially reduced compared to diploid salmon (Cotter *et al*., 2000a). In another study on Atlantic salmon, that of Wilkins *et al*. (2001), recapture rates for triploids were reduced by an additional 76 to 88% compared to diploids, suggesting that overall marine mortality rates for triploids would likely exceed 99% and could in some cases be greater than 99.9%. Mortality rates for AquAdvantage Salmon and AquAdvantage Broodstock 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. Thus, even if a migration of escaped or released salmon were to occur, few if any of these fish would likely survive the migration to waters of the U.S.

7.5.3 Effects Due to Escape/Release During Transportation

As discussed above in Section 5.5.3, escape of AquAdvantage eggs during transport from PEI to Indiana is not reasonably foreseeable. Any release of eggs during shipment would be the result of accidental release due to a major incident during transport. Due to the fragile nature of salmonid eggs and the unlikelihood of the eggs ending up in a suitable habitat for survival (i.e., cold freshwater with sufficient DO), survival of eggs through and after a significant shipping incident, such as a trucking accident or plane crash, is remote. As a result, no effects on the environment are anticipated.

7.5.4 Conclusions for Question 4

Multiple factors have been described and analyzed that address the potential impacts of escape or release of AquAdvantage Salmon or AquAdvantage Broodstock on the environment of the U.S., including stocks of endangered wild Atlantic salmon in Maine. Adequate data and information exist to conduct this analysis, and none indicates that escape or release of AquAdvantage Salmon or AquAdvantage Broodstock would result in significant effects on the environment of the U.S.

7.6 Cumulative Impacts

As previously stated, this EA supports supplements to the AquAdvantage Salmon NADA to allow production of AquAdvantage Salmon eyed-eggs, housing of AquAdvantage Broodstock, and grow out of AquAdvantage Salmon, in two discrete units (Hatchery and Grow Out) of the ABT Rollo Bay facility located near Rollo Bay, PEI. All other specific production and use conditions for AquAdvantage Salmon approved under original NADA and the NADA Supplement for the Indiana grow out facility remain in effect.

The EAs prepared for the original NADA and the Indiana facility supplement concluded that the production and grow out of AquAdvantage Salmon under the specified conditions, namely production at the ABT site in Bay Fortune, PEI and grow out at ABT sites in Panama and Indiana, would not result in significant impacts on the environment of the U.S. The current EA presents evidence that operations at the ABT site in Rollo Bay will not result in significant impacts on the environment of the U.S. Therefore, the cumulative impact of adding the Hatchery and Grow-out Units in Rollo Bay, PEI, is negligible for the environment of the U.S.

7.7 Production of non-GE (non-transgenic) Atlantic salmon eggs

For business reasons ABT may occasionally undertake production of non-GE Atlantic salmon eggs, for sale to external parties, which could occur along-side of AquAdvantage Salmon production in the Rollo Bay Hatchery Unit. ABT has identified three potential exposure pathways associated with production of non-GE Atlantic salmon at the Rollo Bay Hatchery Unit that could theoretically present risks to the environment of the United States, particularly if GE salmon eggs (i.e., AquAdvantage Salmon or AquAdvantage Broodstock eggs) and non-GE salmon eggs were to be comingled or either group of eggs were to be mislabeled prior to shipment. The first potential exposure pathway to the environment of the United States is the escape or unintentional release of the non-GE eggs from the Rollo Bay Hatchery Unit. The second pathway is the shipment of these non-GE eggs to Canadian Atlantic salmon aquaculture facilities for smolt production and subsequent grow out[30]. This pathway represents a potential risk if there were to be comingling or mislabeling of AquAdvantage Salmon and non-GE Atlantic salmon eggs, and after shipment of the mislabeled GE salmon eggs or the comingled non-GE and GE salmon eggs there was an inadvertent escape/release of AquAdvantage Salmon

[30] Once smolts are produced, which is expected to occur in freshwater land-based facilities, these smolts could be grown out to market size at the same facility or subsequently moved to net pen farms in the marine environment.

eggs, smolts, or posts-molts. The third pathway is the shipment of these non-GE eggs to ABT's Indiana Grow Out facility for rearing to market size.

For the first exposure pathway, the likelihood for this event to occur is no greater than for AquAdvantage Salmon eggs, which as previously described in this EA (Sections 5.6.4 and 7.2) is a highly unlikely event because of the numerous and redundant physical and operational containment measures currently in place. Therefore, the risks to the United States environment from this exposure pathway are considered negligible. However, even if the non-GE eggs were to escape or be released to the local environment, it is highly unlikely that a population on non-GE salmon would establish there as evidenced by the current lack of Atlantic salmon populations in the nearby watersheds on the northeast coast of PEI (see Sections 7.4 and 7.5 above). But even if a population of non-GE Atlantic salmon were to establish there, there should be no impacts to the environment of the United States because these fish are not genetically engineered (i.e., they are similar to the wild type Atlantic salmon in the United States) and because of the great distance to the United States (see discussion in Section 7.5 above). For the second exposure pathway, the risks of an unintentional release of AquAdvantage Salmon eggs or older life stages have been addressed by the implementation of a number of operational procedures and controls at the Rollo Bay Hatchery Unit. These include the testing of all batches of non-GE eggs produced to insure they are of the proper non-GE genotype prior to egg shipment, and protocols to prevent comingling and/or mislabeling of egg batches. These operational procedures are discussed further below. For the third exposure pathway, the risks to the environment of the United States are no greater than from shipment of AquAdvantage Salmon eggs to the Indiana Grow Out facility, a scenario that has already been evaluated in the 2018 EA and resulted in an FDA finding of no significant impact. However, the operational procedures that have been put into place should preclude the possibility that non-GE Atlantic salmon eggs would be shipped to this facility unintentionally[31].

The environmental exposure and risks of this production scenario (i.e., co-production of non-GE Atlantic salmon eggs) have also been considered and evaluated by DFO in its Science Advisory Report 2019/014 and by ECCC and HC in their April 2019 Joint Assessment Report. For this co-production scenario (i.e., Scenario A), DFO determined "the potential for human error in shipping eggs increases potential exposure. Consequently, the likelihood of exposure of EO-1α Salmon to the Canadian environment is ranked low[32], and therefore results in low to moderate risk of EO-1α Salmon to the Canadian environment".

In its Science Advisory Report, DFO recommended several actions to be taken for production of non-transgenic salmon to mitigate the potential for human error that might result in the mixing of transgenic and non-transgenic eggs under Scenario A. These recommendations included:

[31] For business reasons, intentional shipments of non-GE Atlantic salmon have been made to the Indiana Grow Out facility in the past and may continue in the future.

[32] This compares to an exposure likelihood ranked as "negligible" for Scenario B in which non-transgenic eggs are not sold to external parties.

a. Physical separation of the production cycle for non-transgenic eggs (from egg fertilization to the end of the egg shipping process) so that it occurs in a location where there is no production of transgenic fish (i.e., in a different building or in a physically separate area within a building);
b. No overlap in time between transgenic and non-transgenic spawning events, and between egg shipping events;
c. Production undertaken by staff trained on all applicable SOPs;
d. A statistically appropriate sampling methodology for validation of a non-transgenic genotype, as close to the time of shipping as possible, and for all shipments;
e. Labeling inside and outside of shipping boxes to indicate contents, and shipping of eggs as soon as possible following validation.

In the ECCC and HC Joint Assessment Report, several operational procedures were recommended for ABT to implement to prevent the accidental mixing of transgenic and non-transgenic egg batches, should the company decide to produce non-transgenic eggs for sale. These operational procedures include:

a. temporal separation of egg production for transgenic and non-transgenic eggs;
b. physical separation of the two types of eggs;
c. highly sensitive genetic testing procedures to validate egg genotypes; and
d. clear labeling protocols.

Based on these procedures and other information, the Joint Assessment Report characterized environmental risks associated with AquAdvantage Salmon for use in commercial, contained, land-based aquaculture to be low because "there is low potential for exposure, especially in light of additional measures for maintaining separation between transgenic and non-transgenic eggs".

ABT has already developed and implemented all of the operational procedures identified by ECCC and HC for risk mitigation during production of non-GE Atlantic salmon eggs. ABT will test all batches of non-GE eggs to insure they are of the proper genotype prior to any shipments of these eggs. Under these conditions, ABT believes that production of non-GE Atlantic salmon eggs at the Rollo Bay Hatchery Unit, for sale to external parties, and along-side of production of AquAdvantage Salmon there, will not result in significant impacts to the environment of the United States.

8 SUMMARY AND CONCLUSIONS

Using a risk-based approach, this environmental assessment has found no evidence that approval of two supplements to NADA 141-454 to allow production of AquAdvantage eyed-eggs and grow out of AquAdvantage Salmon at ABT's land-based facility in Rollo Bay, PEI, would result in significant impacts on the environment of the U.S. There is also no evidence that cumulative effects will occur through the supplemental NADA approvals. The findings are summarized by the following list of questions and answers, addressing the proposed facility in Rollo Bay:

8.1 What is the likelihood that AquAdvantage Salmon or the AquAdvantage Broodstock used to produce AquAdvantage Salmon eyed-eggs will escape the conditions of confinement?

Due to the presence of multiple, redundant and effective physical containment measures at the Rollo Bay site, the likelihood of AquAdvantage Salmon and AquAdvantage Broodstock, including the reproductively competent diploid GH female broodstock, escaping into the environment is very low. This is case for all life stages of salmon from eggs to adults.

8.2 What is the likelihood that AquAdvantage Salmon or the AquAdvantage Broodstock used to produce AquAdvantage Salmon eyed-eggs will survive and disperse if they escape the conditions of confinement?

In the unlikely event of an escape or release, there is a possibility that AquAdvantage Salmon or AquAdvantage Broodstock could survive in the local environment, specifically the Rollo Bay Brook. The brook currently hosts a population of brook trout (*Salvelinus fontinalis*) and at times may be hospitable to AquAdvantage Salmon or AquAdvantage Broodstock. The likelihood of survival would be dependent upon the specific environmental conditions at the time of the escape/release and the life stage(s) of escaped/released fish.

In the event any escaped/released eggs or fish survived and were able to migrate approximately 1.5 km downstream to the Northumberland Strait, the high salinity marine environment there would likely preclude pre-smolt fish from surviving. AquAdvantage Salmon or AquAdvantage Broodstock that survived the transition from fresh water to sea water would enter an environment that is less than ideal for salmonids. As described in Sections 6.3.1 and 7.3.1, the waters of the Northumberland Strait are shallow and turbid and, in the summer, can reach temperatures that are harmful or lethal to Atlantic salmon.

Given the challenges posed by local environmental conditions, it is concluded there is only a low to moderate likelihood that AquAdvantage Salmon or AquAdvantage Broodstock would survive there over the long-term or disperse if they were to escape or be released from the Rollo Bay facility. This is evidenced by the lack of Atlantic salmon population in the local watersheds despite efforts in the past to reestablish population there.

8.3 What is the likelihood that AquAdvantage Salmon or the AquAdvantage Broodstock used to produce AquAdvantage Salmon eyed-eggs will reproduce and establish if they escape the conditions of confinement?

In the unlikely event that any AquAdvantage Salmon or AquAdvantage Broodstock escaped or were released, the likelihood they would reproduce or become established is very low. This conclusion is based upon several factors: 1) the very small number (≤ 120 total including diploid AquAdvantage Broodstock and those AquAdvantage Salmon that did not undergo triploidization during egg pressure treatment) of reproductively competent transgenic fish (all of which are females) that will be housed at Rollo Bay; 2) the challenging environmental factors they would encounter upon escape or release; and, 3) the small likelihood of encountering spawning Atlantic salmon males in the local environment or nearby watersheds.

8.4 What are the likely consequences to, or effects on, the environment of the United States should AquAdvantage Salmon or the broodstock used to produce AquAdvantage Salmon eyed-eggs escape the conditions of confinement?

The collective information on the potential for survival, dispersal, reproduction and establishment indicates that no effects are expected on the environment of the U.S. (including populations of endangered wild Atlantic salmon in Maine) from production at the Hatchery and Grow Out Units in Rollo Bay.

In summary, the evidence presented indicates that the production of AquAdvantage eyed-eggs in the Hatchery Unit, and grow out of AquAdvantage Salmon in the Grow Out Unit, at the ABT facility near Rollo Bay, PEI under the conditions that would be established in the supplements to NADA 141-454, if approved, and as described in this EA, would not result in significant effects on the quality of the human environment in the U.S., including populations of endangered Atlantic salmon.

9 MITIGATION MEASURES

Because the proposed action would not have a significant effect on the environment, no additional mitigation measures will be required beyond those already incorporated for containment.

10 AGENCIES AND PERSONS CONSULTED

This EA was prepared with input and assistance from members of the Environmental Safety Team and others in the Office of New Animal Drug Evaluation in FDA's Center for Veterinary Medicine.

11 LIST OF PREPARERS

This document was prepared by Aqua Bounty Technologies, Inc. (Mark Walton) and Exponent, Inc. (Jane Staveley)

12 CERTIFICATION

The undersigned official certifies that the information presented in this Environmental Assessment is true, accurate, and complete to the best of their knowledge.

________________________________ October 29, 2019

Mark Walton, Ph.D. Date

Chief Technology Officer
AquaBounty Technologies, Inc.

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APPENDIX A BACKGROUND ON THE BIOLOGY OF THE ATLANTIC SALMON

Appendix A. 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 wildtype Atlantic salmon, 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 AquAdvantage Salmon can be evaluated.

A.1 Historic and Current Geographic Range

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.

A.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 *et al.*, 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 *et al.*, 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 down-river 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. 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, which are referred to as parr marks, aid in camouflaging the young fish, which are preyed upon by other fish, as well as 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 eight 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 10-22 cm long at this point in their development (OECD, 2017).

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[1]. 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 generally leave Maine rivers 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 fin fish, shell fish (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 3 to 17 kg and be up to 76 cm 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 11 kg.

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

[1] http://www.fishwatch.gov/profiles/atlantic-salmon, accessed 12/19/2017.

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 and the females 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 salt water 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[2] 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 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: 1-SW salmon may allocate 50% of their energy for spawning compared to 70% for older salmon (Jonsson *et al.*, 1991; 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 (Jonsson *et al.*, 1996; Thorpe *et al.*, 1984). Although absolute fecundity varies greatly among individuals due 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

[2] The sperm-containing secretion of the testes of male fish. Analogous to semen in mammals.

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, 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).

A.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), and the upper lethal temperature for salmon has been cited as being 23ºC (Stead and Laird, 2002). In a study examining the tolerance and resistance to thermal stress in juvenile Atlantic salmon, fish were acclimated for two weeks to various temperatures (5, 10, 15, 20, 25 and 27ºC) then temperatures were raised or lowered by 1ºC per hour to estimate the incipient lethal temperature. 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 27ºC initially demonstrated the highest incipient lethal level at 27.8 ± 2ºC; for these fish, the lower mean incipient lethal level was 2.2 ± 4ºC. Temperature limits for feeding increased slightly with acclimation temperature to upper- and lower-mean values of 22.5 ± 0.3ºC and 7.0 ± 0.3ºC, respectively. The fish acclimated to 25ºC and 27ºC did not feed, while fish acclimated to the lower temperatures fed normally at 21.6-22ºC (Elliott, 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 ~23ºC and would eventually starve. For farmed salmon the feeding and activity range for smaller Atlantic salmon (i.e., < 100 g) in fresh water was favorable up to ~23ºC, with mortality occurring at ~26ºC}. For larger Atlantic salmon, upper temperatures for feeding and activity in saltwater have been reported as ~ 20ºC with mortality occurring at ~22ºC (Elliott, 1991; Willoughby, 1999. Elliott (1991) noted that little is known about the upper temperature limits for survival of Atlantic salmon in the wild and available data revealed tolerances similar to those observed in laboratory studies. Other experimental studies indicate the optimum temperatures for growth of young Atlantic salmon to be in the range of 16-19ºC {Elliott, 1981 #2206. Elliott (1991) noted that little is known about the upper temperature limits for survival of Atlantic salmon in the wild and available data revealed tolerances similar to those observed in laboratory studies. Other experimental studies indicate the optimum temperatures for growth of young Atlantic salmon to be in the range of 16-19ºC {Elliott, 1981 #2206).

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." Research has shown the DO content of water in a salmonid farm should not drop below 5-6 mg/L and be maintained at a minimum of 7 mg/L for good growth, and that carbon dioxide (which influences the pH of the water) starts to be a problem for salmonids above 15 mg/L , and for good growth rates (Shepherd and Bromage, 1988; Stead and Laird, 2002).

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).

A.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 National Oceanic and Atmospheric Services’ (NOAA) Fisheries Services and the U.S. Fish and Wildlife Service listed the Gulf of Maine Distinct Population Segment of Atlantic salmon as “endangered” under the Endangered Species. That designation was extended in 2009 to include fish in several rivers in Maine Act (https://ecos.fws.gov/ecp0/profile/speciesProfile?spcode=E07L accessed 2/20/2019). 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 (Knapp *et al.*, 2007).

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 U.S. 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[3]. In addition, as previously mentioned, the United States is a member of the North Atlantic Salmon Conservation Organization (www.nasco.int), a group dedicated to the conservation, restoration and management of Atlantic salmon.

A.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 (krill), amphipods (scud), copepods, and crab larvae. Large juveniles prey mostly upon fish.

A.6 Domesticated and Wild Salmon

General practices used in salmon aquaculture are presented in this section; specific production and grow-out practices for AquAdvantage Salmon are described in Section 5 of the Rollo Bay EA. This section of the appendix discusses information about the interaction of domestic salmon with their wild

[3] Available at http://www.nmfs.noaa.gov/pr/pdfs/20160329_atlantic_salmon_draft_recovery_plan.pdf , accessed 12/19/2017.

counterparts to provide context for predicting how AquAdvantage Salmon might fare in the unlikely event that they would be released into the wild.

A.7 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 United States.

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 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.*, 2011). 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.

A.8 Interactions between Wildtype (non-GE) Farmed 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 are:

- Transfer of exotic pathogens or amplification of endemic pathogen loads (McVicar, 1997; Saunders, 1991)

- Genetic disturbance caused by transmission of fitness-reducing alleles (Frankham, 1995; Ryman and Utter, 1987), disruption of locally-evolved allelic combinations (McGinnity *et al.*, 2003; Ryman *et al.*, 1995; Templeton, 1986), or “swamping” of the native gene pool (Sægrov *et al.*, 1997)
- Direct competition for environmental resources, such as habitat, food, or mating opportunities (Fleming *et al.*, 2000; McGinnity *et al.*, 1997)
- 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 AquAdvantage Salmon, each of these potential interactions is discussed in more detail below.

A.8.1 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 (Inglis *et al.*, 1991; Johnsen and Jensen, 1994). 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
- 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 on the other hand 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 Jenser, 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 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 (White, 1940). 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.

A.8.2 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 and 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 (Bermingham *et al.*, 1991; Bourke *et al.*, 1997; King *et al.*, 2001; McConnell *et al.*, 1995; Ståhl, 1987; Taggart *et al.*, 1995). 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, 2003). 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).

A.8.3 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, (Clifford *et al.*, 1998; Webb *et al.*, 1993)(Webb et al., 1993; Clifford et al., 1998) and then at a fraction of the spawning rate of wild Atlantic salmon (Clifford *et al.*, 1998; Fleming, 1996). Social dominance and unsuccessful mate competition have been identified as two primary factors for these observations.

Although socially dominant in culture environments, farmed Atlantic salmon are subordinate in nature. Salmon form dominance hierarchies around foraging opportunities and farmed salmon establish their social status in confinement where foraging opportunities differ significantly from those in the wild. In nature, despite the potential dominance effect of fish size, wild fish have a "resident advantage" that deters larger, non-native fish from evicting territory holders from their home ground.

Farmed salmon have been shown to compete poorly for mates and spawning locations. Males are particularly disadvantaged in both access to mating opportunities and breeding success (Fleming *et al.*, 2000) and farmed females enter rivers out-of-phase with wild salmon and make fewer, poorly-covered nests, breed for a shorter period of time, and retain more eggs than wildtype counterparts (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 returning adults has been reported to be only 2–19% of that achieved by wild Atlantic salmon (Clifford *et al.*, 1998; Fleming *et al.*, 2000; McGinnity *et al.*, 2003). Additionally, the loss of 68% of eggs deposited during spawning is a further barrier to successful introgression or establishment of escaped farmed salmon within or co-existent with natural populations (McGinnity *et al.*, 2003).

A.8.4 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[4] 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).

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[4] "Feral" refers to animals that have escaped from domestication and become wild.

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 1%. Less than 2% 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).

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