U.S. Food and Drug Administration

# AquAdvantage® Salmon

## Environmental Assessment

In support of an approval of

a New Animal Drug Application related to AquAdvantage Salmon,

which are triploid, hemizygous, all-female Atlantic salmon (*Salmo salar*)

bearing a single copy of the α-form of the *opAFP-GHc2* recombinant DNA construct

at the α-locus in the EO-1α lineage

**November 12, 2015**

**Prepared by**

**Center for Veterinary Medicine**
**United States Food and Drug Administration**
**Department of Health and Human Services**

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## A NOTE TO THE READER

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## LIST OF ACRONYMS AND CONVENTIONS EMPLOYED

| | |
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| ~ | Approximately |
| ABRAC | Agricultural Biotechnology Research Advisory Committee |
| ABT | AquaBounty Technologies, Inc. (the sponsor) |
| AFP | antifreeze protein |
| bp | base-pair |
| CEQ | Council on Environmental Quality |
| CFIA | Canadian Food Inspection Agency |
| CFR | Code of Federal Regulations |
| CVM | Center for Veterinary Medicine |
| DFO | (Department of) Fisheries and Oceans [Canada] |
| cDNA | complementary deoxyribonucleic acid |
| DNA | deoxyribonucleic acid |
| DO | dissolved oxygen (concentration) |
| EA | environmental assessment |
| EIS | environmental impact statement |
| EC | Environment Canada |
| EO-1α | the integrated form of the AquAdvantage rDNA construct |
| EPA | U.S. Environmental Protection Agency |
| ERA | Early Rearing Area |
| ESA | Endangered Species Act |
| EU | European Union |
| FAO | Food and Agricultural Organization (of the United Nations) |
| FDA | U.S. Food and Drug Administration |
| FD&C Act | Federal Food, Drug, and Cosmetic Act |
| FONSI | Finding of No Significant Impact |
| FWS | U.S. Fish and Wildlife Service, Department of Interior |
| GE | Genetically engineered |
| GH | growth hormone |
| GFI | Guidance for Industry (CVM) |
| GOA | Grow Out Area |
| ISA / ISAV | infectious salmon anemia / infectious salmon anemia virus |
| mRNA | messenger ribonucleic acid |
| NAD | New Animal Drug |
| NADA | New Animal Drug Application |
| NEPA | National Environmental Policy Act |
| NMFS | National Marine Fisheries Service, National Oceanic and Atmospheric Administration, US Department of Commerce |
| NRC | National Research Council |
| OIE | World Organization for Animal Health |
| Op | ocean pout promoter regulatory region |
| OSTP | Office of Science and Technology Policy, Executive Office of the President |
| PEI | Prince Edward Island, Canada |
| rDNA | recombinant deoxyribonucleic acid |
| SOPs | Standard Operating Procedures |

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| SW | Sea Winter |
| USC | United States Code |
| USDA | U.S. Department of Agriculture |
| VMAC | Veterinary Medicine Advisory Committee |

## TECHNICAL TERMS*

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| Allele | Any alternative form of a gene that can occupy a particular chromosomal locus (see *heterozygous* or *homozygous*). |
| AquAdvantage construct | The recombinant DNA construct used to generate AquAdvantage Salmon, referred to as *opAFP-GHc2.* |
| AquaBounty Technologies Salmon (ABT salmon) | Any GE Atlantic salmon from the E0-1α lineage irrespective of ploidy, zygosity, or gender (i.e., the set of Atlantic salmon that includes diploid GE salmon that may be used as broodstock, as well as AquAdvantage Salmon). |
| AquAdvantage Salmon | The triploid, hemizygous, all-female Atlantic salmon from the E0-1α lineage GE Atlantic salmon subject to this application. They are a subset of ABT salmon. |
| Arctic char | A salmonid species related to Atlantic salmon, DNA sequences from which were used by the sponsor as part of the methodology for production of AquAdvantage Salmon. |
| Biological containment (bioconfinement) | Use of biological methods, such as induced sterilization (e.g., triploidy), to prevent gene flow and reproduction in the environment. |
| Chromosome | A physical structure consisting of DNA and supporting proteins called chromatin that carries hereditary information. |
| °C-day [min] | Compound unit of "time" (°C x days [min]) for relative determination of growth rate that accounts for the effect of water temperature. |
| Conspecific | An organism (plant or animal) of the same species. Herein, the term typicaly refers to wild or native Atlantic salmon, as well as salmon that may have been intentionally introduced or stocked into the environment. |
| Construct (gene or DNA construct) | A synthetically-assembled nucleic acid that frequently contains regulatory and coding sequences usually incorporated into the genome of an organism with the intended purpose of modifying its phenotype. |
| 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 | A mature haploid female germ cell extruded from the ovary at ovulation |
| Expression (gene) | The process by which the information encoded in a gene is used to direct the assembly of a protein molecule. |
| Flow cytometry | A technique for identifying and sorting cells and their components (e.g., DNA) by staining with a fluorescent dye and detecting the fluorescence usually by laser beam illumination. In this EA, flow cytometry is used to confirm ploidy. |

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| Gamete(s) | Mature male or female reproductive cell (sperm or ovum) with a haploid set of chromosomes. In animals, including fish, gametes are sperm and oöcytes (eggs). |
| GE Animal | Those animals modified by rDNA techniques, including the entire lineage of animals that contain the modification. The term GE animal can refer to both animals with heritable rDNA constructs and animals with non-heritable rDNA constructs (e.g., those modifications intended to be used as gene therapy). |
| Genome | The entire set of genetic instructions found in a cell. |
| Genotype | The genetic constitution of an organism or cell; it also refers to the specific set of alleles inherited at a particular locus. |
| GH Transgenic or GH genetically engineered (GE)Atlantic salmon | Atlantic salmon containing a growth hormone gene that was exogenously introduced via genetic engineering that may be closely related to AquAdvantage Salmon. |
| Haploid | A cell, tissue, or organism having a single set of chromosomes (as opposed to *diploid* or *triploid*). Haploid cells are generally found in gametes (sex cells) of higher organisms. |
| Hemizygous | An individual having only one copy (or allele) of a given pair of genes instead of the usual two. |
| Homozygous | The genetic status in which an individual inherits the same alleles for a particular gene from both parents. |
| Heterozygous | Having inherited different forms of a particular allele from each parent. A heterozygous genotype stands in contrast to a homozygous genotype, where an individual inherits identical forms of a particular gene (see *allele*) from each parent. |
| Milt | The sperm-containing secretion of the testes of male fish. Milt is analogous to semen in mammals. |
| Molecular Cloning | Cloning is the process of making identical copies of an organism, cell, or DNA sequence. 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. Then, each time the host cell divides, it replicates the foreign DNA sequence along with its own DNA. |
| Neomale | A genetically female fish converted to a phenotypic male by hormone treatment. |
| *opAFP-GHc2* | The AquAdvantage recombinant DNA construct Chinook salmon growth hormone (GH) gene and gene product, ocean pout and Chinook salmon–derived regulatory sequences, and a short synthetic linker. |
| Polymerase chain reaction | A standard technique to amplify copies of a DNA sequence often used to confirm genotype. |

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| Phenotype | An organism's actual observed properties, such as morphology, development, or behavior that derive predominantly from its genotype. The genetic contribution to the phenotype is called the genotype. Some traits are largely determined by the genotype, while other traits are largely determined by environmental factors. |
| Passive integrated transponder | Implantable radio-beacon for fish identification. |
| Plasmid | A plasmid is a small, often circular DNA molecule found in bacteria and other cells. Plasmids are separate from the bacterial chromosome and replicate independently of it. Plasmids are often used to make multiple copies of a recombinant DNA construct. |
| Ploidy | The number of complete sets of chromosomes contained within each cell of an organism (see *haploid*, *diploid*, and *triploid*). |
| Promoter | A sequence of DNA needed to regulate the expression of a gene, including whether the gene is transcribed or not. 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. |
| Raceway | A rectangular channel or tank with a continuous flow of water constructed or used for high-density fish production. It includes earthen channels as well as channels and tanks constructed of concrete, concrete block, timber, rock, fiberglass, or other materials where water flows in at one end and exits at the other end. |
| Recombinant DNA (rDNA construct) | DNA artificially constructed by combining genes from different organisms or by cloning chemically altered DNA, usually for the purpose of genetic manipulation. 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 may be copied or incorporated, and expressed. |
| Regulatory sequence | A nucleic acid sequence involved in regulating the expression of genes. |
| Salmonid | A ray-finned finfish of the family Salmonidae, a taxonomic group that includes salmon, trout, chars, freshwater whitefish and graylings. The family includes fish of the following genera, among others: *Salmo*, *Salvelinus*, and *Onchorhynchus*. |
| Sea Winter | Number of winters spent at sea (e.g., 1SW, 2SW). |
| Smolt | A freshwater juvenile Atlantic salmon that has undergone the physiological changes necessary to be able to survive in salt water. |
| Somatic (cell) | Any cell of the body except sperm and egg cells. Most somatic cells of higher organisms are diploid, meaning that they contain two sets of chromosomes, one inherited from each parent. |

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| Transgene | A 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 vector is any vehicle, often a virus or a plasmid that is used to ferry a desired DNA sequence into a host cell as part of a molecular cloning procedure. Depending on the purpose of the cloning procedure, the vector may assist in multiplying, isolating, or expressing the foreign DNA insert. |

*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 linked here; National Human Genome Research Institute, *Glossary of Genetic Terms* from the Human Genome Project, and the *Genetics Home Reference* at the National Library of Medicine within the National Institutes of Health.

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## 1. SUMMARY

AquaBounty Technologies, Inc. (ABT or the sponsor) has provided data and information in support of a New Animal Drug Application (NADA) for approval of a genetically engineered (GE) Atlantic salmon[1] to be produced and grown only under the conditions specified in the application. The resulting line of fish, referred to as AquAdvantage Salmon, is designed to exhibit a rapid-growth phenotype that allows it to reach smolt[2] size faster than non-GE farm raised salmon. This environmental assessment (EA) addresses the potential effects on the human environment of the United States as the result of the major federal action that would consist of the approval[3] of this specific NADA.

The AquAdvantage Salmon founder animal was generated in 1989 by micro-injecting a recombinant deoxyribonucleic acid (rDNA) construct, *opAFP-GHc2* containing the Chinook salmon growth hormone (GH) gene, ocean pout and Chinook salmon–derived regulatory sequences, and a short synthetic linker into the fertilized eggs of wild Atlantic salmon. Subsequent selection and breeding led to the establishment of the AquAdvantage Salmon line, which has been propagated for at least eight generations. Under the specific conditions specified in the NADA for AquAdvantage Salmon, these fish are defined as triploid[4], all-female populations that will be produced as eyed-eggs in the sponsor's facility on Prince Edward Island (PEI) in Canada; after confirming the genetic integrity of the broodstock used for producing the ultimate fish; and evaluating the effective induction of triploidy in the eyed-eggs, the eggs will be shipped to a grow-out facility in the highlands of Panama, where they will be reared to market size and harvested for processing. The conditions that would be established in the NADA, if approved, would not permit AquAdvantage Salmon to be produced or grown in the United States, or in net pens or cages, and would not permit live fish to be imported into the United States for processing.

As a part of the NADA review and approval process under the Federal Food, Drug, and Cosmetic Act (FD&C Act), 21 U.S.C. § 321 et seq., and consistent with the mandates in the National Environmental Policy Act of 1969 (NEPA), 42 USC § 4321 et seq., and the Food and Drug Administration's (FDA's) environmental impact considerations regulations (21 CFR Part 25), FDA's Center for Veterinary Medicine (CVM) has thoroughly evaluated the potential environmental impacts of this action (the approval of this specific NADA for AquAdvantage Salmon) and the no action alternative, issued a draft environmental assessment for public comment, incorporated relevant comments, and has prepared this final EA. FDA approvals for articles regulated under the new animal drug provisions of the FD&C Act may be subject

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

[2] 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.

[3] For the purposes of this document, the terms "action" and "approval" are used interchangeably.

[4] With reference to AquAdvantage Salmon, "triploid" means that, based on sampling, at least 95% of released eyed-eggs have three complete sets of chromosomes per cell with a probability of 0.95 (i.e., the probability that these eggs are not at least 95% triploid is less than 0.05) (see Section 7.4.1.2).

to a specific set of conditions that are proposed in the drug sponsor's NADA and would be conditions of an approved application. See 21 U.S.C. 360b(a)(1). Approvals by FDA of NADAs related to GE animals may be limited to a specific set of conditions enumerated and described in the NADA and the approval letter, with the GE animal remaining under FDA regulatory oversight as long as it is produced and marketed. FDA's approval of the AquAdvantage Salmon NADA would be for the specific set of conditions described in ABT's NADA and as enumerated in FDA's approval letter. No other conditions of production and use of AquAdvantage Salmon would be within the scope of the approval, or have been evaluated in this EA, as no others would be approved by FDA under this NADA. The approval of the NADA is therefore described as the preferred alternative. Any production or use outside the scope of the approval would be unapproved and, therefore, render the product unsafe under section 512(a) of the FD&C Act and adulterated under section 501(a)(5) of the FD&C Act. The sponsor must notify FDA about each proposed change in each condition established in an approved application, and obtain FDA approval of a supplemental application for the change where necessary. 21 CFR 514.8. Major and moderate changes to an approved NADA require the filing and review of a supplemental NADA. Approvals of such supplemental applications would constitute major agency actions and trigger additional environmental analyses under NEPA, unless otherwise excluded.

FDA has determined that for this action (i.e., approval of this NADA), conditions of use would include appropriate controls on the production of the AquAdvantage Salmon, including appropriate physical and biological containment to ensure the identity, quality, and purity of the animal lineage; these measures also serve to mitigate environmental risks. Under the specific conditions of the NADA for AquAdvantage Salmon, these fish are defined as triploid, all-female populations that would be produced as eyed-eggs at a single specific facility on Prince Edward Island (PEI) in Canada. Eyed-eggs would be shipped 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[5] use (e.g., preparation of eviscerated whole fish, fish fillets, steaks, etc.) in Panama prior to retail sale in the United States. The conditions that would be established in the NADA would require that there be processes in place to ensure the genetic integrity of the eggs, as well as the success of the process to produce triploidy if the application were to be approved. The conditions would specifically limit the production and grow-out of AquAdvantage Salmon to those two locations. In addition, the conditions would not include raising AquAdvantage Salmon in ocean net pens, or their production or growth in the United States.

Under an NADA approval for AquAdvantage Salmon, the sponsor would have on-going post-approval responsibilities to be further described in the FDA letter of of approval, should an approval be granted, including requirements to ensure that all facilities and equipment used in the production and grow-out of AquAdvantage Salmon conform to and are operated and administered as specified in the conditions of use in the approval. Any instance of lack of conformity of the facilities (including physical containment), including any equipment used to produce AquAdvantage Salmon, including that used to produce an all-female triploid population, would cause the approved article (i.e., the rDNA construct in the triploid hemizygous all-female AquAdvantage Salmon) to be adulterated. If the article is adulterated, any food bearing or containing the article would be adulterated under section 402(a)(2)(C)(ii) of FD&C Act. Adulterated food is subject to refusal of admission into the United States under section 801(a)(3)of the FD&C Act.

[5] For the purposes of this EA, "food" refers to food for humans and animals, including animal feed.

As part of the NADA review process under the FD&C Act, but separate from the environmental analysis, CVM has evaluated both the direct and indirect food safety of AquAdvantage Salmon (FDA, 2010), and any potential impacts of the rDNA insertion on animal safety. With respect to food safety, in 2010 FDA released its preliminary conclusion that food from AquAdvantage Salmon is as safe as food from non-GE farm-raised Atlantic salmon, and that there is a reasonable certainty of no harm from consumption of food from AquAdvantage Salmon. Further, FDA's preliminary conclusion was that no significant food consumption hazards or risks have been identified with respect to the phenotype of the AquAdvantage Salmon (FDA, 2010). In the event of an approval, this finding would be finalized and FDA would post a summary of its review at http://www.fda.gov/animalveterinary/products/approvedanimaldrugproducts/foiadrugsummaries/ucm056939.htm.

As the NADA approval action would only permit production and grow-out of AquAdvantage Salmon at facilities outside of the United States, the areas of the local surrounding environments that are most likely to be affected by the action lie largely within the sovereign authority of other countries (i.e., Canada and Panama). Because NEPA does not require analysis of impacts in foreign sovereign countries[6], effects on the local environments of Canada and Panama have not been considered and evaluated in this EA except insofar as it was necessary to do so in order to determine whether there would be significant effects on the environment of the United States due to the origination of exposure pathways from the production and grow-out facilities in Canada and Panama.[7]

In addition, social, economic, and cultural effects of the proposed action on the United States 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 United States. Under NEPA, social and economic effects must be considered only once it is determined that the proposed agency action significantly affects the physical environment. 40 CFR 1508.14.

FDA's approach to analysis in this EA is 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

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[6] See, e.g., Natural Resources Defense Council, Inc. v. Nuclear Regulatory Com., 647 F.2d 1345, 1366 (D.C. Cir. 1981); Consejo de Desarrollo Economico de Mexicali v. United States, 438 F. Supp. 2d 1207, 1234 (D. Nev. 2006), vacated and remanded on other grounds, 482 F.3d 1157 (9th Cir. 2007). CEQ has issued guidance on NEPA analyses for actions taking place within the U.S. that may have transboundary effects extending across the border and affecting another country's environment. This does not apply here because would be no effects that cross the border from the United States into other countries from AAS. https://ceq.doe.gov/nepa/regs/transguide.html. Canada and Panama exercise regulatory authority over ABT facilties in their respective countries. See Canadian Science Advisory Secretariat Summary of the Environmental and Indirect Human Health Risk Assessment of AquAdvantage Salmon (CSAS Summary), http://www.dfo-mpo.gc.ca/csas-sccs/Publications/ScR-RS/2013/2013_023-eng.html.

[7] Under Executive Order 12114, FDA considered whether the proposed action would have significant impacts on the environment of the global commons or of foreign nations not participating or otherwise involved in the action and, has determined that there would be no significant impacts. See http://www.fda.gov/AnimalVeterinary/DevelopmentApprovalProcess/GeneticEngineering/GeneticallyEngineeredAnimals/ucm466350.htm.

principles described above by the National Research Council (NRC, 2002) 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 diploid ABT salmon, and AquAdvantage Salmon, escaping, surviving, and becoming established in the environment, and then dispersing or migrating such that there might be an exposure pathway to the United States, and subsequently causing an adverse outcome (the risk) to the environment of the United States. These hazards are addressed for the production of eyed-eggs and grow-out to market size, within the framework of a conceptual risk assessment model, and the following series of risk-related questions:

1. What is the likelihood that AquAdvantage Salmon will escape the conditions of confinement?
2. What is the likelihood that AquAdvantage Salmon will survive and disperse if they escape the conditions of confinement?
3. What is the likelihood that AquAdvantage Salmon will reproduce and establish if they escape the conditions of confinement?
4. What are the likely consequences to, or effects on, the environment of the United States should AquAdvantage Salmon escape the conditions of confinement?

For the purposes of this environmental assessment, although AquAdvantage Salmon that will provide food for export into the United States is an all-female, triploid fish from the EO-1α lineage, this EA encompasses risks associated with all other lifestages (i.e., gametes through adults), and all of the zygosities and ploidies associated genotypes and phenotypes (i.e., diploids, triploids, hemizygotes, homozygotes females and masculinized females) that are required for the production of the triploid, all-female AquAdvantage salmon to be used for food. In general, when it is important for the purposes of assessing a specific environmental risk, we specify whether an animal is assumed to be reproductively competent, and the term "diploid ABT salmon" is used.

AquAdvantage Salmon and diploid ABT salmon would be produced and grown-out only in secure facilities with multiple and redundant forms of effective physical containment that have been verified and validated by FDA. Based on this analysis, FDA considers the likelihood that AquAdvantage Salmon and diploid ABT salmon could escape from containment, survive, and become established in the local environments of either the PEI or Panamanian facilities to be very low. This is consistent with the conclusions of Canadian authorities based on their qualitative Failure Mode Analysis of the physical barriers and operational procedures involving containment at both the PEI and Panamanian facilities. The Canadian officials concluded that the potential for both acute failure of physical containment and chronic release of AquAdvantage Salmon[8] is negligible at the PEI facility and low for the Panamanian facility, with at least reasonable certainty. Given this very low likelihood of escape, survival, and establishment in the environments local to the PEI and Panamanian facilities, it is also highly unlikely that AquAdvantage Salmon could disperse and migrate such that there would be an exposure pathway to the environment of the United States.

[8] The Canadian Science Response (DFO). (2013). Summary of the environmental and Indirect Human Health risk Assessment of AquAdvantage Salmon. DFO Can Sci. Divis. Sec. Sci. Respon. 2013/023) refers to all life stages as AquAdvantage Salmon. ("Although the proposed AquAdvantage Salmon product for export to Panama is all-female triploid eyed-eggs from the EO-1α line….other life stages (gametes through to sexually mature adults), genotypes (i.e., diploids, triloid, hemizygotes, homozyotes) and gender (females and masculinized females) are required for the production of the eyed-eggs and are therefore included in the risk assessment").

Should unintentional release occur, the environmental conditions in the geographic settings of the egg production and grow-out sites and farther afield (e.g., the tropical Pacific Ocean) would afford additional means of containment of any escaped eggs or fish, given that these conditions would be generally hostile to their long-term survival, reproduction, and establishment. In Canada, this is evidenced by the lack of Atlantic salmon in the vicinity of the egg production facility even though these fish are native to this area and have been intentionally stocked there in the past. These environmental conditions greatly limit, or in the case of Panama, essentially preclude the possibility of a complete exposure pathway by which diploid ABT salmon or AquAdvantage Salmon, could reach the United States.

In addition, because the production process for AquAdvantage Salmon ensures that populations produced will be triploid (effectively sterile), all-female animals, the possibility of AquAdvantage Salmon reproducing in the wild is likewise extremely remote. The greatest potential risk to the environment of the United States would occur in the event of the escape of diploid ABT broodstock from the PEI facility. These fish are likely reproductively competent, and some will be homozygous for the *opAFP-GHc2* gene. Given that growth enhanced Atlantic salmon in general do not have a reproductive advantage compared to non-GE Atlantic salmon, and sometimes are disadvantaged (Moreau and Fleming, 2011; Moreau et al. 2011a), it is expected that large numbers of fish would need to escape in order for there to be any potential chance of reproduction and establishment, and there is a very low probability of that occurring at the PEI egg production facility due to the small numbers of broodstock maintained at that facility, and the stringent physical containment at that site. In summary, the evidence collected and evaluated by FDA indicates that the proposed action on the NADA for AquAdvantage Salmon, including development, production, and grow-out of these GE salmon under the conditions specified in the application and as described in this EA, would not result in a significant impact on the quality of the human environment in the United States, including populations of endangered Atlantic salmon.

FDA has considered the no action alternative for this action, that is, a decision not to approve the NADA for AquAdvantage Salmon. There are two general likely scenarios to consider as a result of the no action alternative: (1) the sponsor would cease production of AquAdvantage Salmon, and (2) the sponsor would continue to rear AquAdvantage Salmon at the existing locations outside of the United States, and/or at new suitable locations outside the United States (and could decide to sell the eggs, fish, or the technology to producers outside the United States), with no intent to market food from these fish in the United States. There are no potential environmental impacts arising from the first general scenario. If no AquAdvantage Salmon are produced, there will be no production sites and no potential for escape or release of these fish to the environment, and therefore no effects on the environment of the United States. For the second general scenario, production of AquAdvantage Salmon at locations outside the United States for marketing outside the United States (i.e., outside the jurisdiction of FDA)[9], an assessment of potential effects on the environment becomes highly uncertain as the conditions and effects of those conditions

[9] This scenario, production of AquAdvantage Salmon outside the jurisdiction of the United States, is possible regardless of whether FDA approves the NADA. It appears more likely to occur if FDA does not approve the NADA because ABT would need to produce AquAdvantage Salmon outside FDA's jurisdiction, i.e., outside the U.S. without importing food from such fish into the U.S., if it wished to market food from its GE salmon without FDA regulation.

are not reasonably foreseeable. Because production of AquAdvantage Salmon would be possible at any number of locations worldwide, under different containment conditions and levels of regulatory oversight, and potentially within areas where native Atlantic salmon and other salmonid species are present, there are far too many variables and unknowns to define specific scenarios and perform a comprehensive risk assessment for them. A further set of unknowns includes the extent and nature of regulatory decisions in sovereign foreign countries with the authority to regulate either the technology of genetic engineering or the products thereof. Thus, it is impracticable to make any accurate predictions with respect to potential environmental impacts on the United States other than to state that should production occur with less restrictive physical or biological containment conditions than those specified in the NADA, adverse environmental impacts to the United States could be more likely to occur because escape, reproduction, establishment and migration of the AquAdvantage Salmon would be more likely. The same would be expected if production were to occur in locations where there would be less regulatory oversight than would occur under an FDA NADA approval.

FDA has not considered any cumulative impacts for this action because FDA believes there are no other past, present, or reasonably foreseeable future actions. The Council for Environmental Quality's NEPA regulations define cumulative impact as "the impact on the environment which results from the incremental impact of the present action when added to other past, present and reasonably foreseeable future actions . . . ." 40 CFR 1508.7. There would be no "incremental impact" because this would be the first NADA approval for AquAdvantage Salmon and FDA is not aware of any specific reasonably foreseeable future actions on NADAs for GE fish at this time.

As the result of the review of the materials submitted in support of an NADA for AquAdvantage Salmon, FDA has made a "no effect" determination under the Endangered Species Act (ESA), 16 USC § 1531 et seq. i.e., when produced and reared under the conditions specified in the application, and as described within this EA, AquAdvantage Salmon will not jeopardize the continued existence of United States populations of threatened or endangered Atlantic salmon, or result in the destruction or adverse modification of their critical habitat. The two federal agencies responsible for administering the ESA, the National Marine Fisheries Service (NMFS) of the National Oceanic and Atmospheric Administration (Department of Commerce) and the U.S. Fish and Wildlife Service (FWS) of the Department of Interior, have been provided with this "no effect" determination and the underlying information in support of it. Based on their statutory authorities and regulations, both of these agencies have either concurred with, or indicated no disagreement with, FDA's "no effect" determination. [see Appendix D]

# 2. PURPOSE AND NEED

## 2.1 Purpose and Need for Proposed Action

This EA was prepared as part of the regulatory considerations for approval of an NADA for AquAdvantage Salmon, a GE Atlantic salmon produced by AquaBounty Technologies, Inc. AquAdvantage Salmon contain an 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.

FDA regulates animals containing rDNA constructs under the new animal drug provisions of the FD&C Act, and analyzes potential environmental impacts of such animals as required under NEPA. An rDNA construct that is intended to affect the structure or function of a GE animal meets the statutory definition of a drug (see CVM Guidance for Industry (GFI) 187[10]), and generally must be approved by FDA prior to commercialization. Approvals of this type constitute "major Federal actions" for which FDA must meet environmental review requirements under NEPA and FDA's regulations, thus triggering the requirement to perform an environmental assessment (see subsequent discussion in Section 2.3.2).

FDA approvals for articles regulated under the new animal drug provisions of the FD&C Act may be for a specific set of conditions that are proposed in the drug sponsor's NADA, or that are required by FDA to mitigate potential risks. FDA reviewed safety and effectiveness under this NADA only under the specified conditions of use. The conditions that would be established in this NADA, if approved, would not include production or rearing of AquAdvantage Salmon in the United States, or in net pens or cages. The proposed action is limited to an NADA approval for a specific set of conditions, described in Section 5. Any modifications that the sponsor may propose to the conditions established in an approved application would require notification of FDA (21 CFR 514.8(b)). Major and moderate changes require the filing and review of a supplemental NADA (21 CFR 514.8(b)(2) and (3)). Approvals of such supplemental applications would constitute agency actions and trigger additional environmental analyses under NEPA (see 21 CFR 25.20(m)), unless otherwise excluded.

Sponsors must notify FDA of any modifications to the approved conditions of use, ranging from changes in labels to alterations of the conditions of husbandry. Major changes require a supplemental application that must be approved by the agency prior to implementation (21 CFR 514.8(b)(2)). In general, major changes would include any changes that have a substantial potential to adversely affect the identity and quality of the product as it relates to safety and effectiveness, including changes in the product, production process, quality controls, equipment, or facilities that have such a potential. FDA would consider the addition of a new egg production or grow-out facility (or expansions at an existing facility), changes

[10] Guidance for Industry (GFI) 187 (CVM, 2009), Regulation of Genetically Engineered Animals Containing Heritable Recombinant DNA Constructs. http://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM113903.pdf .

to the security or containment at an existing facility, or alterations of the approved product definition (see Section 4.1) to be major changes that relate to safety and effectiveness and therefore would require prior FDA approval of a supplemental application. Moderate changes have a moderate potential to have an adverse effect on factors that relate to the safety and effectiveness of the product, and also require submission of a supplemental application from the sponsor, but can be made 30 days after FDA's receipt of the supplement unless FDA informs the applicant that the change requires approval prior to distribution of the product made using the change (21 CFR 514.8(b)(3)).

FDA has determined that, among other things, the proposed approval for AquAdvantage Salmon would include the use of physical, biological, and geographical/geophysical forms of containment. For the proposed action (i.e., approval of an application for AquAdvantage Salmon), the conditions that would be established in the application, if approved, would limit production of eyed-eggs to a single specific facility on PEI, Canada, for delivery to a single specific land-based facility in Panama for grow-out (i.e., rearing to market size), with harvesting and processing (e.g., preparation of eviscerated fish, fish fillets, steaks, etc.) in Panama prior to retail sale in the United States. The specific limitations on the production and use (grow-out) of AquAdvantage Salmon, including the production of triploid, all-female fish populations are described in detail in Section 5.3 of this assessment.

## 2.2 Factors Influencing the Development of AquAdvantage Salmon

World-wide demand for protein production has increased significantly in the past decades (FAO, 2008b), and fish protein often comprises a significant portion of the daily dietary protein in many countries (FAO, 2008a; USDA/DHHS, 2010). The FAO estimates that globally, fish currently represent about 16.6 percent of animal protein and 6.5 percent of all protein for human consumption (FAO, 2012). The United States government now recommends that seafood-based protein sources be varied and increased in the American diet (USDA/DHHS, 2010). Unlike other sources of protein (e.g., beef, pork, poultry), fish, particularly cold-water finfish, provide a source of protein that is low in saturated fat and high in the omega-3 fatty acids, eicosapentaenoic acid and docosahexaenoic acid, nutrients that have been associated with improved health status (USDA/DHHS, 2010).

As the worldwide demand for fish has increased, many of the world's fisheries have been fished at levels beyond their maximum sustainable yields. When a fishery's breeding stock drops below a sustainable level, the fish population in that area begins to disappear. Commercial fish currently at risk from overfishing include Chilean sea bass and bluefin tuna. Overfishing of wild Atlantic salmon in the Gulf of Maine was one of several factors that contributed to the placement of that fish species on the endangered species list in 2000.[11]

To meet increasing demand for fish protein in light of declining stocks and diminishing capture of wild fish, the use of commercial aquaculture—colloquially known as fish farming—has expanded significantly in recent years. Generally speaking, aquaculture includes the production or cultivation of fish and shellfish (e.g., shrimp, oysters) and aquatic plants (e.g., seaweed) up to market size, often under controlled conditions, and typically in ponds, tanks, cages, or raceways. Although fish grown using aquaculture are used for many

[11] See 65 Fed. Reg. 69, 459 (Nov. 17, 2000).

different purposes, including to support game fisheries and to rebuild wild populations, most farmed fish are raised for human consumption.[12]

Fish farming (or aquaculture) involves the use of many methods, such as sterility, triploidy, and other modifications of fish production that preserve the economic value of the genetics[13] that are being used, and that may provide additional economic or safety benefits. One such method involves diverting the animals' energy from reproductive development to growth (this is similar in intent to castration of male terrestrial animals raised for food, e.g., cattle, swine). A commonly employed method in aquaculture of diverting energy from reproductive development to growth is the induction of triploidy (for a more complete discussion of triploidy, see Section 5.3.1.1). In addition, the use of single sex populations, usually females, is a common practice in the aquaculture industry (Pandian, 1995). The production of an all-female population through the process of gynogenesis, has been used successfully in the aquaculture industry for many years (Dunham, 2004; Luo *et al*., 2011) and is well established for salmonids (Donaldson and Devlin, 1996).

A recent joint report by the World Bank, the Food and Agriculture Organization of the United Nations (FAO), and the International Policy Research Institute estimates that by 2030, aquaculture will provide close to two thirds of the food fish for global consumption as catches from wild capture fisheries level off and demand from an emerging global middle class substantially increases (World Bank, 2013). Modeling projects that the total global fish supply will increase from 154 million metric tons (MMT) in 2011 to 186 MMT in 2030. Aquaculture's share in the total global supply is predicted to increase from 52.8 MMT in 2008 (37.1%) to 93.6 MMT in 2030 (50.1%)(World Bank, 2013). For food fish, the contribution from aquaculture is projected to rise from 47.2 MMT in 2006 (42.2%) to 93.6 MMT in 2030 (61.7%).

The demand for farmed salmon has followed a trend similar to that of other fish species, increasing steadily year-by-year as new markets open (FAO, 2009). Commercial aquaculture was the source of about 69% of worldwide salmon production in 2006 (FAO, 2008b). During 2000-2004, Americans consumed an average of approximately 284,000 metric tons of salmon annually, of which two-thirds were farmed rather than wild caught (Knapp *et al*., 2007).In particular, demand for farm-raised Atlantic salmon has increased as the last commercial wild fishery for this species in the United States was closed in the 1980s: 99% of the Atlantic salmon consumed in the U.S. during 2000-2004 was farmed (Knapp *et al*., 2007) with almost all of that being supplied by aquaculture operations in Canada, Chile, Norway, and Scotland.

The Dietary Guidelines for Americans, 2010 (USDA/DHHS, 2010) specifically recommend that Americans increase the amount and variety of seafood consumed by choosing seafood in place of some meat and poultry. These guidelines indicate that consumption of seafood, which provides an average consumption of 250 mg per day of eicosapentaenoic acid and docosahexaenoic acid, is associated with reduced cardiac deaths among individuals with and without pre-existing cardiovascular disease, and thus recommend the consumption of higher

[12] See National Oceanic and Atmospheric Administration, What is Aquaculture? (available HERE).

[13] In this case, "genetics" refers to genomes that are being propagated to produce animals for actual food production. For example, the genetics of a particular population may include genes that encode for traits that allow for easy domestication, disease resistance, or rapid growth.

levels of seafood to help prevent heart disease. These recommendations are expected to further contribute to increased demands for seafood in the future.

The development of AquAdvantage Salmon is the end result of advances in genetic engineering within the past 30+ years. Recombinant DNA technology was first used to produce genetically engineered (GE) (or transgenic[14]) organisms in 1973 (Cohen *et al*., 1973). Although initial interest in GE animals centered primarily on mammals, by the late-1990s, genetically engineered carp, trout, loach, tilapia, catfish, and salmon had been produced (Brem *et al*., 1988; McEnvoy *et al*., 1988; Guyomard *et al*., 1989). The dominant interest in GE salmon and several other GE fish species has been to increase growth rate and feed conversion efficiency, which are principal drivers of production and the economic viability of commercial farming operations (for all production agriculture). The development of what is now known as AquAdvantage Salmon began in 1989 (Du *et al*., 1992b) and is the GE salmon that is closest to commercialization. (See Appendix B for additional background information on GE animals and genetic engineering.)

## 2.3 Relationship to Statutes, Regulations, and Policies

FDA regulates GE animals under the NADA provisions of the FD&C Act, 21 USC § 321 et seq. Major FDA actions such as an NADA approval trigger the requirements of NEPA and FDA's implementing regulations (21 CFR Part 25). This EA is intended to provide material assistance to FDA for making a decision to prepare either a finding of no significant impact (FONSI), or an environmental impact statement (EIS), as required by NEPA. The EA also addresses FDA's compliance with its obligations under the ESA.

### 2.3.1 Federal Food, Drug, and Cosmetic Act

FDA's authority over new animal drugs comes from the FD&C Act (21 USC § 321 et seq.). The definition of a drug, in section 201(g) of the FD&C Act, includes "articles (other than food) intended to affect the structure or any function of the body of man or other animals" (21 USC § 321(g)). The definition of "new animal drug" in section 201(v) of the FD&C Act includes that it is a drug intended for use in animals that is not generally recognized as safe and effective for use under the conditions prescribed, recommended, or suggested in the drug's labeling, and that has not been used to a material extent or for a material time (21 USC § 321(v)).

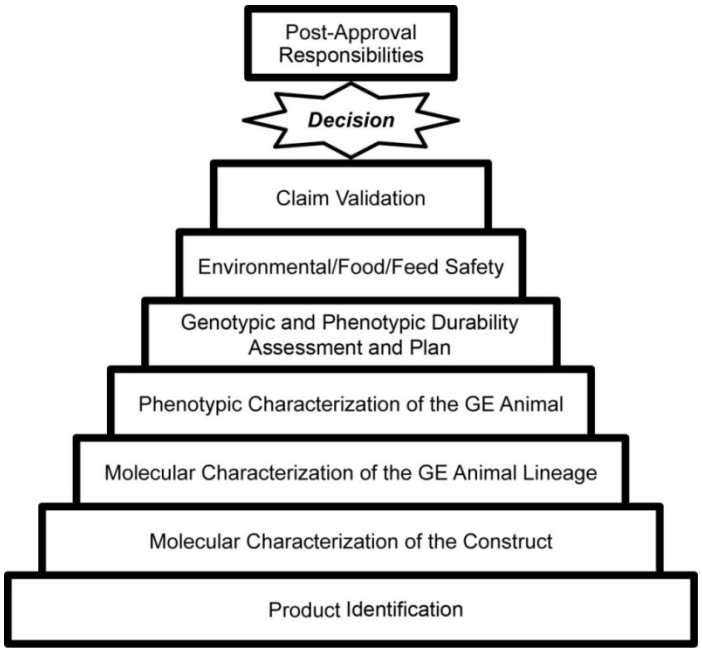
Generally under the FD&C Act, a new animal drug is "deemed unsafe" until FDA has approved an NADA for that particular use, unless the drug is only for investigational use and conforms to specified exemptions (see 21 USC §§ 360b(a)(1), (a)(3)), unless the drug is used in conformance with regulations promulgated under sections 512(a)(4) or (5) of the FD&C Act (21 USC § 360b(a)(4) or (5)), or unless it is a drug intended for minor uses or minor species that may be marketed under other provisions not applicable to GE animals (21 USC § 360b(a)(1)(B), 360b(a)(1)(C)).

[14] In general, FDA uses the term "genetically engineered" to refer to organisms containing either heritable or non-heritable rDNA constructs; others use the term "transgenic" to refer to similar organisms, particularly those bearing heritable rDNA constructs. These terms are used interchangeably in this EA, especially when citing other documents or scientific literature.

In order to clarify the applicability of NADA requirements and procedures to GE animals, FDA published Guidance for Industry (GFI) 187 (CVM, 2009), *Regulation of Genetically Engineered Animals Containing Heritable Recombinant DNA Constructs*. As outlined in this GFI, CVM has developed a risk-based, hierarchical approach to demonstration of safety and effectiveness that is consistent with the FD&C Act and its implementing regulations (see 21 CFR Parts 511 & 514). This approach, which is illustrated in Figure 1, begins with a product definition, and proceeds through a step-wise series of investigations to characterize the potential hazards associated with the rDNA construct, the lineage of the GE animal, and the durability of its genotype and phenotype. This information enables CVM to determine the likelihood and potential severity of impacts on animal or human health and the environment. Further information on FDA's regulation of GE animals is contained in Appendix C.

The sixth step of the hierarchical risk-based approach outlined in GFI 187 describes two assessments: (1) the evaluation of whether food derived from a GE animal is safe, and (2) whether approval of the NADA individually or cumulatively affects the environment.

**Figure 1. Regulatory Review Process for GE Animals**




Under the FD&C Act, FDA must consider food safety as part of its review of an NADA (21 USC § 360b(d)(2)). This includes the agency's review of GE animals containing heritable rDNA constructs, such as the AquAdvantage Salmon (see CVM GFI 187). Food from AquAdvantage Salmon is intended to enter the food supply and must be found safe; that is, there must be a reasonable certainty of no harm from consumption of such food. This is the same safety standard that applies to food from animals that have been treated with conventional new animal drugs (e.g., parasiticides). A food safety assessment has been performed by FDA for AquAdvantage Salmon as part of the NADA approval process under the FD&C Act and will not be repeated here. In 2010, FDA released a preliminary assessment that concluded that food from these salmon is as safe as food from non-GE salmon, and that there is a reasonable certainty of no harm from consumption of food from these fish (FDA, 2010). A summary of the Agency's food safety review was published on the

FDA website as Part VII of the Briefing Packet prepared for CVM's Veterinary Medicine Advisory Committee (VMAC). Additional updates will be included in the Freedom of Information Summary should the NADA be approved and will be posted on FDA's website at http://www.fda.gov/AnimalVeterinary/Products/ApprovedAnimalDrugProducts/FOIADrugSummaries/default.htm.

Post-approval oversight requirements under an NADA approval would be further described in the FDA Letter of Approval to the sponsor, should an approval be granted. These requirements would include ensuring that all facilities, equipment, and diploid ABT fish used in the production of AquAdvantage Salmon conform to or are operated, administered, or produced as specified in the approved application. Any instance of lack of conformity to these conditions would cause the approved article (i.e., the rDNA construct in the lineage of AquaAdvantage Salmon) to be adulterated. If the article is adulterated, any food bearing or containing the article would be adulterated. Section 402(a)(2)(C)(ii) of the FD&C Act. Adulterated food is subject to refusal of admission into the United States. Section 801(a)(3) of FD&C Act.

### 2.3.2 National Environmental Policy Act

NEPA requires federal agencies to prepare a detailed statement on, among other things, the environmental impact of proposed "major Federal actions significantly affecting the quality of the human environment." 42 USC § 4332(2)(C). NEPA also established the Council on Environmental Quality (CEQ), which has subsequently promulgated regulations implementing NEPA that apply to all federal agencies and are codified in 40 CFR Parts 1500 - 1508. These regulations mandate that an agency must prepare an EA to determine whether the environmental impacts of the action, if any, are significant enough to warrant further consideration through preparation of an EIS, except when the action is normally categorically excluded from the requirement to prepare an EA or when an agency has decided to prepare an EIS. 40 CFR 1501.3(a), 1501.4(b), 1508.4, 1508.9(a)(1). NEPA requires consideration of all potentially significant environmental impacts from the proposed action. 40 CFR 1508.8. From time to time, CEQ has also issued additional guidance to Federal agencies that augment its NEPA regulations, including the January 21, 2011 guidance addressing appropriate use of mitigation and monitoring in EAs and EISs (CEQ, 2011).

In consultation with CEQ, FDA has also promulgated its own regulations for implementing NEPA. These regulations describe sponsor obligations and the processes applicable to FDA for evaluating the potential environmental impacts of its actions, including approvals of NADAs. 21 CFR Part 25.

Social, economic, and cultural effects have not been analyzed and evaluated in this EA. Under NEPA, social and economic effects must be considered only once it has been determined that the proposed agency action significantly affects the physical environment. 40 CFR 1508.14; see *Olmstead Citizens for a Better Community v. U.S.*, 793 F.2d 201 (8th Cir. 1986) ("an impact statement generally should be necessary only when the federal action poses a threat to the physical resources of the area…."). See also *Metropolitan Edison Co. v. People Against Nuclear Energy*, 460 U.S. 766, 773-76 (1983). Our analysis in this EA has determined that the agency's action will not significantly affect the physical environment; therefore, economic and social effects on the United States have not been evaluated. In the event of a future supplemental application for AquAdvantage Salmon in which the scope of the NADA could include production or grow-out at locations within the United States and/or might otherwise rise to a level which might produce

significant effects on the physical environment, we may undertake an evaluation of interrelated social, economic and cultural effects, if appropriate.

As the NADA approval would only permit production and grow-out of AquAdvantage Salmon at facilities outside of the United States, the areas of the local surrounding environments that are most likely to be affected by the action lie largely within the sovereign authority of other countries (i.e., Canada and Panama). Because NEPA does not require an analysis of environmental effects in foreign sovereign countries,[15] the effects on the local environments of Canada and Panama have not been considered and evaluated in this EA, but have been considered by authorities in those countries, see Section 2.5 below. In order to determine whether there would be significant effects on the environment of the United States, we have, however, in this EA evaluated the exposure pathways that originate from the production and grow-out facilities in Canada and Panama.

### 2.3.3 Endangered Species Act

The endangered species listing for Atlantic salmon in the United States includes the Gulf of Maine distinct population segment (FWS, 2009). Section 7(a) of the ESA requires federal agencies to "insure that any action authorized, funded, or carried out by the agency" (the agency action) "is not likely to jeopardize" the continued existence (or result in the destruction or adverse modification of a designated critical habitat) of any species of fish, wildlife, or plants that have been determined to be threatened or endangered under Section 4 of the ESA (i.e., officially listed). One of the first steps in this process is a determination by the action agency (FDA in this case), usually based on a biological assessment such as this EA, as to whether the proposed action "may affect" listed species or critical habitat (FWS/NMFS, 1998). This determination is typically made through an informal consultation with one or both of the agencies responsible for administering the ESA[16]--NMFS and FWS. Depending on the proposed action, the action agency's determination with respect to whether the proposed action "may affect" listed species or critical habitat, and the outcome of the informal consultation, the consultation process may end altogether, or it may proceed to a formal stage.

FDA, having reviewed the materials submitted in support of an NADA for AquAdvantage Salmon, has determined that approval of an application for AquAdvantage Salmon will have no effect on Atlantic salmon (*Salmo salar*), Gulf of Maine Distinct Population Segment when produced and reared under the conditions that would be established in an approved application, and that are described within this EA. In addition, FDA has determined that this approval of AquAdvantage Salmon would not jeopardize the continued existence of a listed

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[15] CEQ has issued guidance on NEPA analyses for effects from actions taking place within the U.S. that may have transboundary effects extending across the border and affecting another country's environment. This does not apply here because there would be no effects from the NADA approval that cross the border from the United States into other countries https://ceq.doe.gov/nepa/regs/transguide.html.

As discussed in footnote 8, FDA has separately analyzed whether the proposed action would have significant impacts on the environment of the global commons or of foreign nations not participating or otherwise involved in the action and has determined that there would be no significant impacts.

[16] Typically only one of the agencies is involved in the process, but in the case of endangered Atlantic salmon, because the species has life stages that live in both freshwater and marine environments, both agencies share jurisdiction and participate in the process.

species or destroy or adversely modify designated critical habitat. Both NMFS and FWS have been provided with FDA's "no effect" determination and the underlying information in support of it. Depending on their statutory authorities and regulations, both agencies have either concurred with, or indicated no disagreement with, FDA's determination (see copies of letters from FWS and NMFS in Appendix D), thereby ending the informal consultation process for this particular agency action.

## 2.4 International Resolution

The North Atlantic Salmon Conservation Organization's (NASCO) Williamsburg Declaration is a non-binding resolution adopted by its members. The recognized decline in populations of wild Atlantic salmon stocks prompted the 1984 formation of NASCO through an inter-governmental Convention (The Convention for the Conservation of Salmon in the North Atlantic Ocean). Membership in NASCO, which is limited to governments, includes the United States, Canada, Denmark (in respect to the Faroe Islands and Greenland), the European Union (EU), Norway, and the Russian Federation. In June 2003, NASCO adopted the so-called Williamsburg Resolution[17], which is designed to minimize impacts of aquaculture introductions, transfers, and transgenics on the wild stocks of Atlantic salmon (NASCO, 2006). Article 7 of the Williamsburg Resolution states that the parties should apply the *Guidelines for Action on Transgenic Salmon* to protect against potential impacts from transgenic or genetically engineered salmonids on wild salmon stocks.

The portion of these Guidelines relevant to this EA (Williamsburg Resolution, Annex 5) states, "while there may be benefits from the introduction of such salmonids if, for example, they could not interbreed with wild stocks...," specific steps should be taken to ensure protection of the wild stocks, including utilization of "all possible actions to ensure that the use of transgenic salmonids, in any part of the NASCO Convention area, is confined to secure, self-contained, land-based facilities."

FDA has determined that the two facilities that will be used for production and grow-out of AquAdvantage Salmon as part of the action follow this recommendation in the NASCO guidelines in that they are secure, self-contained, land-based facilities (see Sections 5.4, 5.5 and 7.2 for additional information on these facilities and containment herein).

## 2.5 Foreign Regulatory Oversight

Under the proposed action, the production and use (grow-out) of AquAdvantage Salmon would only be permitted at locations outside of the United States. There will be additional regulatory oversight of both the egg production and grow-out facilities in Canada and Panama by federal and local authorities in these two nations. Both countries have legal authorities and processes in place for regulation of organisms containing rDNA constructs (i.e., genetically engineered or genetically modified organisms) for both research and commercialization. In addition, Canada has oversight authority over fish health issues and certification of status for exports of fish and fish eggs.

### 2.5.1 Canada

*Regulation of Products of Biotechnology*

[17] The Williamsburg Resolution was subsequently amended in June 2004 and June 2006.

In Canada, regulation of fish that are the product of biotechnology takes place under the New Substances Notification Regulations (Organisms) [NSNR (Organisms)] of the Canadian Environmental Protection Act, 1999 (CEPA)[18]. CEPA is the key authority for the Government of Canada to ensure that all new substances, including organisms, are assessed for their potential harm to the environment and human health. CEPA is administered by Environment Canada (EC) and Health Canada (HC). For fish products of biotechnology such as AquAdvantage Salmon, EC and HC have signed a Memorandum of Understanding assigning responsibilities to Fisheries and Oceans Canada (DFO) to assist in implementing the NSNR (Organisms) by conducting environmental and indirect human health risk assessments, and recommending any necessary measures to manage risks. The risk assessments evaluate whether the notified fish product of biotechnology is "CEPA toxic" as defined in Section 64 of CEPA 1999: a substance is toxic if it is entering or may enter the environment in a quantity or concentration or under conditions that

a. have or may have an immediate or long-term harmful effect on the environment or its biological diversity;
b. constitute or may constitute a danger to the environment on which life depends; or
c. constitute or may constitute a danger in Canada to human life or health.

Where it is suspected that a fish product of biotechnology is "CEPA toxic," EC and HC may impose control measures to minimize risk to the environment or human health, including

- controls on import and manufacture;
- the prohibition of import and manufacture; or
- prohibition of import or manufacture pending submission and assessment of additional information determined to be required by EC and HC.

A notification under the NSNR (Organisms) was submitted to EC for AquAdvantage Salmon by AquaBounty Canada Inc.[19] on April 30, 2013. This notification was to allow a change in production status from research and development to commercial manufacture. DFO conducted environmental and indirect human health risk assessments of AquAdvantage Salmon in order to make recommendations on any necessary risk management measures to EC to support a regulatory decision by the Minister of the Environment on AquAdvantage Salmon. The conclusions presented in DFO's preliminary comprehensive Environmental and Indirect Human Health Risk Assessment of AquAdvantage Salmon were peer reviewed by a group of scientific experts from DFO, HC, and EC, as well as external scientists with relevant expertise at a meeting held July 17-19, 2013 through the Canadian National Science Response Process. A consensus was reached amongst peer review participants on DFO's risk assessment conclusions and scientific advice. A summary of the risk assessment and associated conclusions is presented in Science Response 2013/023, published in November 2013, and publicly available on DFO's website on Aquatic Biotechnology Regulation[20].

With respect to exposure (i.e., unintentional release of fish), DFO used a qualitative Failure Mode Analysis to provide insight into the efficacy of all barriers and all operational

[18] Additional information is available at the following Canadian government websites:
http://www.ec.gc.ca/subsnouvelles-newsubs/default.asp?lang=En&n=E621534F-1
http://www.dfo-mpo.gc.ca/science/biotech-genom/regulation/regulatory-information-eng.htm

[19] Aqua Bounty Canada, Inc. is a subsidiary of Aqua Bounty Technologies, Inc.

[20] CSAS Summary, http://www.dfo-mpo.gc.ca/csas-sccs/Publications/ScR-RS/2013/2013_023-eng.html.

procedures involving containment. Consideration was given to (1) the potential for an acute failure of physical containment at either facility; (2) the potential for chronic release of fish at both the PEI and Panamanian facilities; as well as (3) the likelihood of release while in transit between the PEI and Panamanian facilities. DFO concluded that for the use scenario specified in the regulatory submission, which is identical to the conditions of use relevant to the Canadian facility that would be specified in an FDA NADA approval, exposure of AquAdvantage Salmon to the Canadian environment is expected to be negligible with a reasonable certainty. Taking into account this exposure assessment, it was further concluded that risk to the Canadian environment associated with the manufacture and production of AquAdvantage Salmon is low with reasonable certainty. A similar conclusion was made for the indirect human health risk assessment. Based on the outcomes of these assessments, DFO concluded that AquAdvantage Salmon are not "CEPA toxic."

Subsequent to the DFO risk assessment for AquAdvantage Salmon, a Significant New Activity (SNAc) Notice[21] was published in the Canadian Gazette Part I on November 23, 2013 by the Canadian Minister of the Environment. The SNAc Notice described the activities under which AquAdvantage Salmon could be used without the activity being considered a significant new activity. Among others, the activities allowed included (1) use of non-triploid fish within a contained facility[22] for producing triploid, all-female fish; (2) use of female, triploid fish with a contained facility for grow-out where fish are euthanized before leaving the contained facility: and (3) the export of female, triploid fish at the eyed-egg stage.

*Aquatic Animal Health Management*

The Government of Canada has developed a National Aquatic Animal Health Program (NAAHP) to bring Canada into compliance with international aquatic animal health management standards. The Canadian Food Inspection Agency (CFIA) and DFO share responsibilities for federal components of NAAHP. CFIA, as the lead agency for the NAAHP, provides program direction under the authority of the *Health of Animals Act*. CFIA is also responsible for aquaculture health surveillance. DFO is primarily responsible for providing scientific support for implementation of NAAHP. As of December 2011, the authority for international movement of fish (including salmonids) in Canada falls within the domain of the CFIA. DFO continues to regulate all interprovincial movement of salmonids. CFIA is responsible for certification of the health status of aquatic animal exports with respect to the risk of introduction or movement of an aquatic animal disease into a receiving country; however, it is important to note that it is not CFIA, but rather the importing country that sets the conditions for importation. CFIA will assess and determine if the Canadian aquatic animals are eligible for export (i.e., whether they meet the importing country's conditions). If import requirements can be met, the CFIA will issue an export certificate to allow the animals or products to enter the importing country. Anyone who owns or works with aquatic animals and knows of or suspects a reportable disease is required by Canadian law to notify CFIA. CFIA conducted an inspection of the PEI egg production facility in May 2013. CFIA found the facility's biosecurity plan to be adequate and no further mitigation measures were required to address pathogens of concern (see Section 5.4.2).

[21] SNAc Notice 16528, available here.

[22] A "contained facility" was defined as a land-based facility with walls, floor, and ceiling from which there is no release of the living organism at any life stage, and where, among other things, there are physical and chemical barriers in place to prevent the escape and survival of the organism. (see SNAc Notice for complete listing).

### 2.5.2 Panama

As authorized under Law 48 of August 2002[23], Panama operates a National Biosafety Commission that coordinates activities related to the biosafety of genetically modified organisms. Under the National Biosafety Commission, there are three Sectorial Biosafety Committees involved with review of applications for research and marketing of "genetically modified organisms" in the Republic of Panama: agriculture, health and the environment. Product approval and commercialization of AquAdvantage Salmon in Panama will primarily require involvement of the Sectorial Biosafety Committee for the agriculture sector, which includes members from relevant Panamanian institutions (e.g., Agricultural Development Ministry, Food Safety Authority, Authority of Aquatic Resources).

The health status of fish in Panama is under oversight of the Dirección Nacional de Salud Animal, the aquatic animal health division of the Ministry of Agriculture. Upon arrival in Panama, the eyed-eggs are transported to the grow-out-facility following direct transfer to ABT personnel. Once received at the grow-out facility, the eyed-eggs would be acclimated to ambient water temperature and pH, and after egg hatch, the alevin would be moved to the fry tanks, where they would remain until they would later be transferred to the grow-out tanks.

## 2.6 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 actually occur. These confinement approaches apply to GE fish as well as to non-GE fish (Kapuscinski, 2005). Three primary methods of confinement have been characterized (Mair *et al*., 2007):

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

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

3. 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*. (2007) are listed below along with a brief description of the containment measures that are to be used for the production, grow-out, and disposal of AquAdvantage Salmon. Sections 5 and 7 of this EA describe confinement and containment measures and how they specifically apply to AquAdvantage Salmon. These confinement measures have been incorporated as integral components of the NADA.

[23] See Appendix G.

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

   The primary form of preventing diploid ABT or AquAdvantage Salmon from entering the environment under the conditions established in the NADA, if approved, is the mandated use of redundant physical and physico-chemical barriers at the sites of egg production and grow-out. The salmon will further be prevented from surviving in the receiving environment because of naturally occurring geographic and geophysical conditions.

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

   In the highly unlikely event of escape from the Panama grow-out facility, gene flow from AquAdvantage Salmon would be prevented because the fish would be triploid females that are incapable of reproduction, either among themselves or with wild fish. In the event of escape from the broodstock facility on PEI, Sections 5 and 7 discuss why gene flow would be extremely unlikely.

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 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 will 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 containment 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.

FDA has determined that for the proposed action (i.e., approval of the NADA), the conditions of use should include controls on the production of the AquAdvantage Salmon, including appropriate physical and biological containment measures to ensure the identity, quality, and purity of the animal lineage, including the broodstock; these measures also serve to mitigate environmental risks. 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 periodic inspections by FDA, as well as by Canadian and Panamanian authorities.

As described in Section 5, multiple and redundant forms of containment are in effect at both the production and grow-out sites to effectively prevent the escape and establishment of

AquAdvantage Salmon. At the broodstock and egg production facility, the fish are fertile by design; containment depends primarily on multiple, redundant physical and geo-physical containment measures. In addition, as described later in this EA, the immediate environs of the egg production facility are inhospitable to early-life stages of these fish due to the high salinity of the local waters.

For the grow-out facility, in addition to effective physical (mechanical) containment, effective biological containment is present in the form of a population of salmon that is entirely female, triploid, and thus functionally sterile and unable to reproduce (see Sections 5.3.2 and 7.4.1). Likewise, the environment downstream of the sponsor's grow-out site is inhospitable to all life-stages of Atlantic salmon due to the high water temperatures, poor habitat, and abundant physical barriers that diminish the likelihood of survival, dispersal, and establishment in the receiving stream.

# 3. APPROACH TO ASSESSMENT

## 3.1 Introduction

As part of the overall process of developing an approach for the regulation of GE animals, FDA commissioned the NRC to evaluate "food, animal, and environmental safety issues with bioengineering animals and cloning that would be appropriate to address in any science-based regulatory scheme developed for these products." This resulted in a 2002 report entitled *Animal Biotechnology: Science Based Concerns* (NRC, 2002). This report did not specify or describe a method of risk assessment for GE animals, but rather identified risk issues associated with products of animal biotechnology. In particular, when considering environmental risks and associated risk analysis, the NRC report adapted principles of risk assessment described in two previous NRC reports on risk (NRC, 1983, 1996). The 1996 NRC report provided two important definitions: ***Hazard***: an act or phenomenon that has the potential to produce harm, and ***Risk***: the likelihood of harm resulting from exposure to the hazard.

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

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

Inherent in these definitions is the concept that both exposure and harm/hazards (i.e., adverse effects) are required components of risk, i.e., Risk = Exposure x Effects. Without either component (exposure or effect), there can be no risk.

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

1. identifying the potential harms regardless of likelihood;
2. identifying the potential hazards that might produce these harms;
3. defining what exposure means for a GE organism, as well as characterizing the likelihood of exposure;
4. quantifying the likelihood of harm given that exposure has occurred; and
5. combining the resulting probabilities to characterize risk.

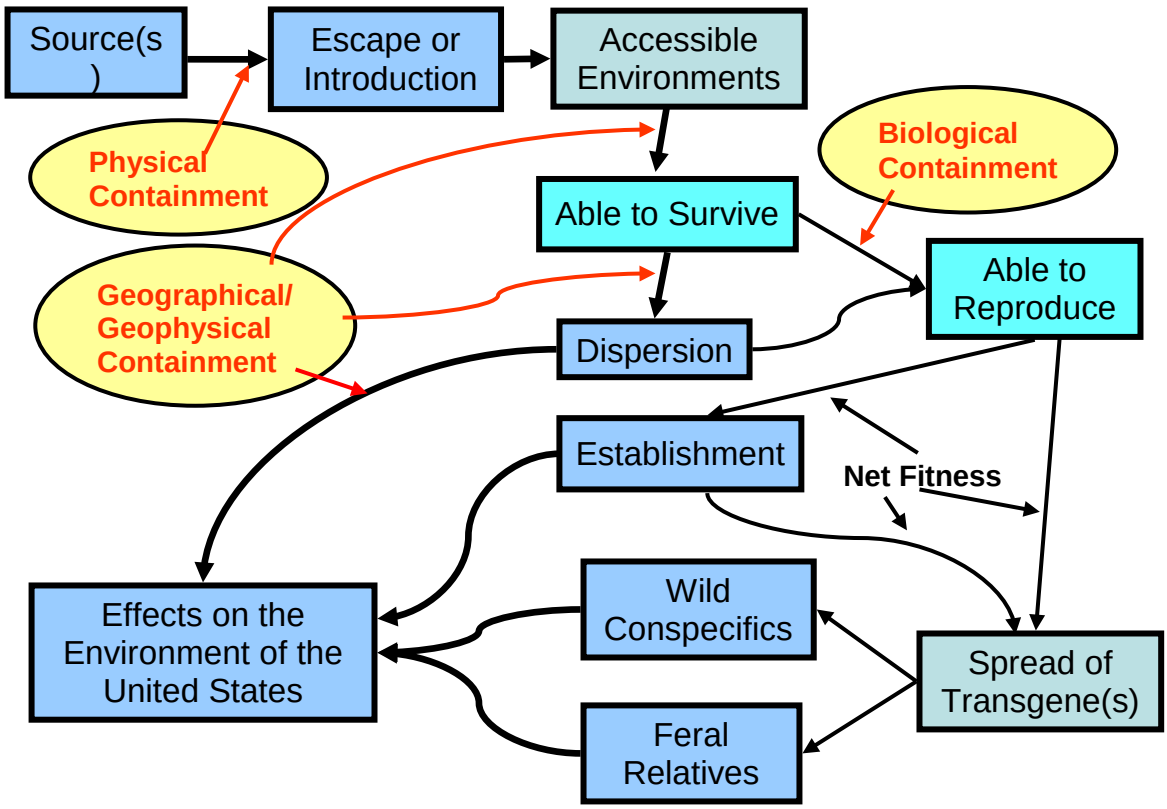
Consistent with the other parts of FDA's review process for GE animals (see Section 2.3.1 and Appendix C), FDA's approach in this EA is one based on an evaluation of exposure pathways, hazards, and risk. The environmental analysis of consequences in the EA conceptually incorporates the principles described above by the NRC (NRC, 2002) as well as the U.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 and diploid ABT salmon escaping, surviving and becoming established in the environment, dispersing or migrating (i.e., evaluating whether there is an exposure pathway to the United States), and subsequently causing an adverse outcome (the risk). These hazards are addressed for the production of eyed-eggs and grow-out to market size, within the framework of a conceptual risk assessment model and a series of risk-related questions (see next section). This analysis and its outcomes are discussed in the Environmental Consequences section of this EA.

### 3.2 Risk-Related Questions

FDA has developed a general conceptual model (Figure 2) for analyzing exposure pathways, hazards, effects, and risks based on the principles outlined in the previous section.

**Figure 2. Conceptual Model for Risk Assessment**




In order for FDA to make an informed decision regarding what may occur as a result of the proposed action, the critical risk-related issues are the likelihood of the GE organism surviving and becoming established in the environment (the pathway by which exposure in the United States could occur) and the outcome or consequences of this establishment on the environment of the United States. As a framework for evaluating these issues, we have thus developed this EA around the following cascaded risk-related questions[24]:

[24] For the purposes of this environmental assessment, although AquAdvantage Salmon that will provide food for export into the United States is an all-female, triploid fish from the EO-1α lineage, this EA encompasses risks associated with all other lifestages (i.e., gametes through adults), and all of the zygosities and ploidies associated genotypes and phenotypes (i.e., diploids, triploids, hemizygotes, homozygotes females and masculinized females) that are required for the production of the triploid, all-female AquAdvantage salmon to be used for food. In general, when it is important for the purposes of assessing a specific environmental risk, we specify whether an animal is assumed to be reproductively competent, the term "diploid ABT salmon" is used.

1. What is the likelihood that AquAdvantage Salmon will escape the conditions of confinement?
2. What is the likelihood that AquAdvantage Salmon will survive and disperse if they escape the conditions of confinement?
3. What is the likelihood that AquAdvantage Salmon will reproduce and establish if they escape the conditions of confinement?
4. What are the likely consequences to, or effects on, the environment of the United States should AquAdvantage Salmon escape the conditions of confinement?

#### 3.2.1 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. For example, treatment with 10-15 mg/L chlorine for 15-30 minutes is effective in killing fish in fresh water (ABRAC, 1995). 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 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. With respect to risk management/mitigation, the Performance Standards state that, although the number of independent containment measures[25] is site- and project-specific, they should generally range from three to five.

#### 3.2.2 Likelihood of Survival, Dispersal, Reproduction, and Establishment in the Unconfined Environment (Pathway for Exposure in the United States)

In order 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 measureable 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 in order for escape or release to present a hazard. Therefore, for the purposes of assessing risks of GE animals in the

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

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:

1. the effect of the transgene on the "fitness" of the animal within the ecosystem into which it is released (i.e., survival and reproduction within the ecosystem);

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

3. the stability and resiliency of the receiving community[26].

The likelihood of establishment is dependent on all three parameters; however, the ability of the GE animal to escape is considered the most important of these because without escape (or intentional release) there can be no 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 of the phenotypic attributes of an animal that affect survival and reproduction, and ultimately how the individual's genetics contribute to future generations of the animal's population. In general, animals are adapted to a specific niche in the ecosystem (i.e., habitat and ecological role) and exhibit maximal "fitness" for that environment. In terms of population and community dynamics, if escaped GE animals have a greater overall net fitness than other animals occupying the same niche in the receiving environment (including wild relatives or farmed domesticated animals of the same species), they may eventually replace them and become established in that community. On the other hand, if the GE animals are less fit, they will either not survive in the receiving environment, or the engineered trait will eventually be removed (by virtue of selection) from the receiving population. For purposes of assessing risk associated with GE animals, it is critical to characterize the fitness of GE animals in relation to the appropriate comparator animal(s), whether wild or domesticated, and compare the two in the context of expected environment(s) in which either population of animals can be or will be found.

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

In addition to the animal's "fitness," in order for escapees to survive and ultimately reproduce, the ecosystem in which they arrive must be suitable with respect to food, habitat, and environmental conditions (e.g., temperature and, for fish, salinity and water quality). Often the presence of conspecifics[27] or species closely related to the GE escapee in accessible ecosystems implies that a suitable environment exists (provided that the fitness

[26] 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, 1984).

[27] 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*).

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.

### 3.2.3 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 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 non-GE domesticated (farmed) salmon and wild salmon, see Appendix A.

The scale and frequency of introductions of GE fish into a particular 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 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 GE organisms 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 (Snow *et al*., 2005; NRC, 2002; NRC, 2004; Devlin *et al*., 2006; Devlin *et al*., 2015). Although primarily hypothetical to date, general risks identified by one of these groups (Snow *et al*., 2005) include the following:

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

The Snow *et al*. report (2005) 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. Specifically with respect to aquatic GE animals, the Snow *et al*. (2005) report 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 non-GE conspecifics, competitors, prey, or predators. Further consideration of these effects in relation to AquAdvantage Salmon is presented in Section 7.5.

# 4. ALTERNATIVES INCLUDING THE PROPOSED ACTION

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

The preferred alternative was developed through years of discussions between FDA and the sponsor during which time potential risks were identified. As the result of those interactions, FDA and the sponsor developed the conditions for production and grow-out of AquAdvantage Salmon that were ultimately included in the NADA that ABT submitted for AquAdvantage Salmon, and that would be an integral part of the conditions established in the NADA, if approved. Those conditions are discussed in detail in the subsequent sections, beginning with a description of the AquAdvantage Salmon and its fitness relative to other farmed Atlantic salmon. The preferred alternative then goes on to describe the containment conditions inherent in the biology of the GE animal and the specific conditions of use that would be established in the approved application.

The "no action" alternative considers the environmental ramifications of not approving the NADA for AquAdvantage Salmon.

## 4.1 Proposed Action (Preferred Alternative) - Approval of AquAdvantage Salmon under Specific Production and Grow-Out Conditions

The action evaluated in this EA is the approval of the NADA for AquAdvantage Salmon submitted by the sponsor, which would permit only the commercial production of eyed-eggs for AquAdvantage Salmon at the sponsor's facility on PEI, and the grow-out of AquAdvantage Salmon at the sponsor's facility in Panama. No other conditions of production and use of AquAdvantage Salmon would be within the scope of this approval,[28] as no others would be approved by FDA under this NADA. The approval of the NADA is therefore described as the preferred alternative. Any production or use outside the scope of the approval would be unapproved and, therefore, would render the product unsafe under section 512(a) of the FD&C Act and adulterated under section 501(a)(5) of the FD&C Act.

Any changes and/or additions to the conditions of production and use for AquAdvantage Salmon would require notification of FDA. FDA would consider production in a new facility to be a major change that would require a supplemental NADA approval prior to implementation. Any supplemental approval would constitute a new agency action triggering additional environmental analysis under NEPA (see 21 CFR 25.20(m)) to address the potential and cumulative impacts of any proposed changes and/or additions.

### 4.1.1 Product Definition

For the purposes of an NADA approval, an rDNA construct contained in a GE animal is "defined" in terms of its identity, the claim made for it (i.e., its effectiveness), and any

[28] Several additional alternatives, including rearing of AquAdvantage Salmon under other production conditions (e.g., ocean net pens), were considered but rejected for further evaluation (see EA Section 4.3).

limitations and/or conditions placed on the resulting GE animals and their use. The following is the product definition for the rDNA construct in AquAdvantage Salmon:

*Product Identity*

A single copy of the α-form of the *opAFP-GHc2* recombinant DNA construct at the α-locus in the EO-1α lineage of triploid, hemizygous, all-female Atlantic salmon (*Salmo salar*) known as AquAdvantage Salmon.

*Claim*

Significantly more AquAdvantage Salmon grow to at least 100 g within 2,700 °C-days than their comparators.

*Limitations for Use*

AquAdvantage Salmon are produced as eyed-eggs and grown-out only in physically-contained freshwater culture facilities specified in an FDA-approved application.

The following warnings also apply to AquAdvantage Salmon and would be required to be prominent on product labeling accompanying all life stages of AquAdvantage Salmon up to the time of harvest:

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

The product label must also contain a statement that eggs and fry[29] are not for resale.

### 4.2 No Action Alternative: Denial of NADA Approval

The no action alternative as applied to the NADA for AquAdvantage Salmon would be the decision by FDA not to approve the application. FDA is required to approve an application for a new animal drug product when it is found to meet the FD&C Act approval standard, including that it is safe and effective for its intended use (21 USC § 360b(d)(1)).

Should FDA decide not to approve the NADA for AquAdvantage Salmon, the outcomes that could result fall into one of two general likely scenarios: (1) the sponsor would cease production or maintenance of AquAdvantage Salmon; or (2) the sponsor would continue to raise AquAdvantage Salmon at the existing locations outside the United States and/or at new suitable locations outside of the United States (and/or to sell the eggs, fish or the

[29] Fry are included because a portion of the eyed-eggs may hatch during transport from Canada to Panama.

technology to producers outside the United States) with no intent to market food from these fish in the United States, i.e., outside of FDA jurisdiction.[30]

Under the second scenario, production and grow-out of AquAdvantage Salmon could occur almost anywhere that (1) suitable water quality and temperature conditions for Atlantic salmon currently exist (or could be artificially engineered or controlled), and (2) regulatory approvals could be gained from the sovereign bodies governing those regions. This could potentially include any of the marine locations where Atlantic salmon are currently commercially grown (e.g., Canada, Chile, China, Norway, and Scotland) in net pens or cages, but also non-traditional freshwater locations where adequate water conditions occur naturally (e.g., temperatures are low enough and dissolved oxygen (DO) concentrations are high enough), or have been physically altered, to support Atlantic salmon survival and growth. Grow-out in freshwater locations could potentially occur in net pens or cages in ponds and lakes, in flow-through tanks and/or raceways, or in recirculating systems.[31]

In summary, there are two general scenarios that have been evaluated as a consequence of the no action alternative: (1) complete termination of the production of AquAdvantage Salmon, and (2) production and marketing of AquAdvantage Salmon outside of the United States and outside of FDA jurisdiction.

### 4.3 Alternatives Considered But Rejected For Further Evaluation

The approval of an NADA is strictly limited to the set of conditions of use enumerated and described in the NADA. For AquAdvantage Salmon, this set of conditions includes several forms of physical, biological, and geographical/geophysical containments that the sponsor has included in the fish themselves (i.e., triploidy and female populations) or as a part of the facilities where the diploid ABT salmon are raised, and fish eggs are produced, or the fish grown to market size (i.e., screening, filters, netting, etc.). The methods of containment go well beyond those normally applied to non-GE farm-raised salmon and other fish grown for either commercial food production or stock enhancement purposes.

Currently, almost all Atlantic salmon grown for food production worldwide are reared from the smolt stage to market size in net pens or cages that are located in the coastal marine environment (the major producers of farmed Atlantic Salmon include Canada, Chile, Norway, and Scotland). Due to concerns over escapees and their potential effects on wild populations of fish, and other potential interactions between farmed fish and wild populations (e.g., disease and parasite transfer), some have advocated for the use of recirculation systems or other closed containment systems for the commercial rearing of Atlantic salmon.

In this context, three potential alternatives for rearing and grow-out of AquAdvantage Salmon were considered during the preparation of this EA: (1) ocean or open water net pens/cages; (2) water-based, closed containment systems such as floating fiberglass tanks; and (3) land-based, closed recirculation systems. Although all three of these are potentially

[30] ABT could seek to import food derived from AquAdvantage Salmon into the U.S. without an NADA approval if it first obtained FDA establishment of an import tolerance, 21 U.S.C. 360b(a)(6);this would require review under NEPA.

[31] This scenario is possible regardless of whether FDA approves the NADA. It appears more likely to occur if FDA does not approve the NADA, because ABT would need to produce AquAdvantage Salmon outside FDA's jurisdiction, i.e., outside the U.S. without importing such fish into the U.S., if it wished to market its GE salmon without FDA regulation.

viable alternatives, they were ultimately excluded from further evaluation in this EA. FDA did not consider the use of net pen/cage technologies to be an appropriate alternative for consideration at this time because ocean net pens or cages deployed in coastal marine locations have not proved to be consistently effective in preventing farmed salmon escapes to date and would not ensure sufficient primary physical/mechanical containment of AquAdvantage Salmon without further technological development. In addition, these would be significant increases in the uncertainty associated with possible outcomes should AquAdvantage Salmon escape from ocean net pens in significant numbers. We reached a similar conclusion for water-based, closed containment systems such as floating fiberglass tanks. These systems are quite new and still undergoing development. Because their performance in ensuring containment under commercial operating conditions has not been extensively documented, the uncertainty associated with this technology is currently considered unacceptable.

Land-based recirculating aquaculture systems (RAS), although potentially highly effective in insuring adequate physical containment of fish under commercial rearing conditions, were not evaluated further in this EA. RAS are typically designed and operated with 90 to 99% of the water recirculated on a daily basis, that is, 1 to 10% of the water volume is discharged from the system each day and replaced with new (make-up) water. Except for the amount of water being discharged on a daily basis, RAS and flow-through aquaculture systems such as the ones to be used for production and grow-out of AquAdvantage Salmon are similar, and the types of physical containment used in them are generally the same because, regardless of the type of rearing system used, it is important to confine the eggs, fry and/or fish to the production units (e.g., incubation chambers, tanks, etc.). In addition, due to high equipment and operating costs and water quality considerations, these systems are very rarely designed for and operated with zero effluent discharge (i.e., 100% recirculation of system water). Thus, almost all aquaculture systems are operated as flow-through systems at some time. As a result, the potential for escape of fish and fish eggs is not significantly different for RAS than for flow-through aquaculture systems such as the ones to be used for production and grow-out of AquAdvantage Salmon. Therefore, recirculating systems do not provide any significant advantage over flow-through systems for the two escape/release scenarios considered most likely to occur at the PEI and Panamanian facilities: (1) fish escape through complete containment failure resulting from a natural disaster, and (2) malicious intentional release of fish through a facility break-in and act of vandalism (see Sections 7.2.1.1 and 7.2.1.2 for further discussion). In both of these scenarios, risk of escape or release of salmon would be similar when rearing fish in recirculating systems as when rearing them in flow-through systems.

## 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 conditions that would be established in the NADA, if approved, 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 presented in Appendix E. Additional background information on GE animals and genetic engineering is contained in Appendix B, while 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 consequences 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

In general, because the essential nature of the salmon has not changed as a result of the introduction of the AquAdvantage construct, an AquAdvantage Salmon is still an Atlantic salmon (see *Is AquAdvantage Salmon an Atlantic salmon?*; page 63 of the FDA Briefing Packet; FDA, 2010). The empirical confirmation that an AquAdvantage Salmon is, in fact, an Atlantic salmon is demonstrated by referring to the FDA Regulatory Fish Encyclopedia (RFE). The RFE is a searchable compendium of some 1,700 species of fin- and shell-fish developed by FDA scientists at the Seafood Products Research Center (Seattle District), and the Center for Food Safety and Applied Nutrition (CFSAN) to help federal, state, and local officials and purchasers of seafood identify species substitution and economic deception in the marketplace[32].

"Fingerprints" based on protein-banding patterns in Isoelectric Focusing (IEF) gels have been developed for 57 specimens from 39 species within the RFE to provide a chemical taxonomy based on characteristic patterns that can be used in species identification. The following FDA study has evaluated AquAdvantage Salmon tissue using the RFE standardized approach: *Comparison of Growth-Hormone Fish* (FDA Report Transgenic Fish Atlantic salmon *Salmo salar* Edible Tissue with the FDA/CFSAN RFE Standard for Non-Transgenic dated 3 December 2004). The goal of this FDA study was to determine whether there were differences in the IEF and 2-dimensional gel electrophoresis fingerprints between non-GE Atlantic salmon and AquAdvantage Salmon. The IEF and 2-dimensional gel results showed no appreciable differences in banding patterns. In addition, FDA employed its DNA barcode species identification analysis used in all FDA regional laboratories to determine whether it would identify AquAdvantage Salmon as Atlantic salmon. Frozen AquAdvantage Salmon skin-on filet was obtained from the sponsor. Two subsamples were used for DNA barcode analysis (Handry et al., 2011), and CFSAN's SOP for FDA Analysis: DNA Based Fish Identification (Barcoding) Method: Version 2: November 2011. http://www.fda.gov/Food/FoodScienceResearch/DNASeafoodIdentification/ucm237391.htm#SOP

**Conclusion:** FDA found that AquAdvantage Salmon matched two FDA reference standards for Atlantic salmon based on the cytochrome *c* oxidase 1 mitochondrial gene currently used

[32] Available HERE.

by the agency for species identification, and the DNA barcoding methodology, and thus concludes that an AquAdvantage Salmon is an Atlantic salmon.

## 5.2 Phenotypic Characterization of AquAdvantage Salmon

This section discusses the phenotype of AquAdvantage Salmon and diploid ABT salmon relative to non-GE farm-raised Atlantic salmon to help characterize its fitness. 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 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 wild-type Atlantic salmon generally encompasses those of AquAdvantage Salmon and diploid ABT salmon.

### 5.2.1 Comparative Studies

FDA has evaluated multiple studies conducted by the sponsor comparing farm-raised Atlantic salmon to AquAdvantage Salmon. When appropriate, we have also considered data and information published in peer-reviewed journals, which may include comparisons to wild Atlantic salmon. In a few instances, when potentially relevant, we have included results from studies that have been conducted of other GE fish including diploid, mixed-sex GE GH Atlantic salmon, and other species of salmon, most notably coho salmon. 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 Briefing Packet, Weight of Evidence determination).[33]

#### *5.2.1.1 Nutritional and Hormonal Composition*

The nutritional and hormonal composition of AquAdvantage Salmon muscle and skin is similar to that of present-day farm-raised Atlantic salmon (see human food safety evaluation in the FDA Briefing Packet; FDA, 2010).

#### *5.2.1.2 Gross Anatomy, Histopathology, and Clinical Chemistry*

The gross anatomy, histopathology, and clinical chemistry of male and female, triploid ABT salmon and size-matched, non-GE 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 ABT salmon and the non-GE comparators, with the number of abnormal findings being greater for triploid fish (both GE and non-GE) 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 non-GE salmon or

[33] FDA notes that 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 promoter of the same species (Mori,T.and R. Devlin 1999)).

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 the 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.

In the same comparator-controlled study, no severe malformations were noted among the AquAdvantage Salmon and diploid ABT salmon enrolled. Irregularities in the fins and gill structure of triploid AquAdvantage Salmon as well as triploid non-GE 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 non-transgenic salmonids, most commonly affecting cartilaginous and boney structures (Brown and Nuñez, 1998), 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* (Bæverfjord *et al*., 1996; Vägsholm and Djupvik, 1998; Silverstone and Hammell, 2002; Fjelldal *et al*., 2012) *S. trutta*, (Poynton, 1987), *O. mykiss* (Mbuthia, 1994 as cited by Silverstone and Hammell, 2002); Madsen & Dalsgaard, 1999), 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 non-GE 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, but have also been reported in the culture of other fish species.

Neither intensive selection for growth nor inbreeding depression are deemed responsible for these morphologic irregularities (Bæverfjord *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 then

diploids (mean %, 22.0 vs 42.7 and 24.4 vs 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).

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.

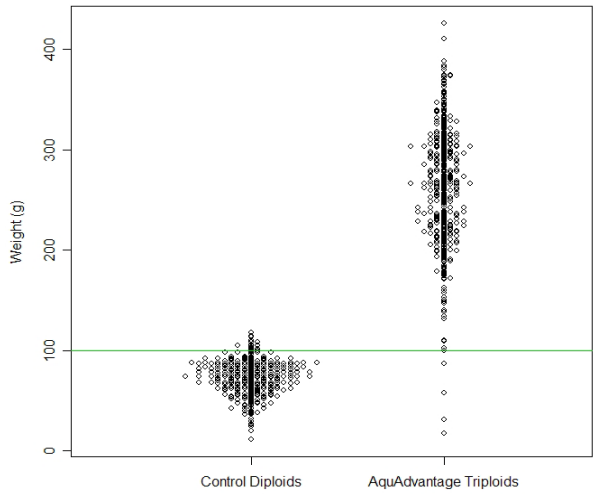
Recently, Tibbetts *et al*. (2013) have reported on the growth and nutrient utilization of GE GH Atlantic salmon (both diploid and triploid) fed a practical grower diet (see following section for a description of results related to growth). This study included a skeletal bone analysis, as well as an 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 nontransgenics; 3.7% for transgenics) than diploids (0.3% for nontransgenics; 0.9% for transgenics). These results are very similar to those presented by Fjelldal and Hansen (2010) for vertebral deformities in diploid and triploid non-GE 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.

#### *5.2.1.3 Growth Rates*

The main difference between AquAdvantage Salmon and non-GE 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 GE 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 non-GE comparators during the first year of life (Du *et al*., 1992b). A comparator-controlled study of growth performance in $F_6$-generation AquAdvantage Salmon has confirmed their significant growth advantage over a period of ~2,700°C-day in both average size (261.0 g *vs*. 72.6 g for diploid controls) and proportion of animals larger than 100 g (98.6% *vs*. 4.9% for diploid controls). Data from this study are summarized in Figure 3.

**Figure 3. Weight of AquAdvantage Salmon and Comparators at 2700 °C-days**

| | Number of Fish | Weight (g) | | Fish Weighing > 100 g | |
|---|---|---|---|---|---|
| | | Mean | Standard Error | Number | Percent |
| Control Salmon | 306 | 72.6 | 1.02 | 15 | 4.9 |
| AquAdvantage Salmon | 369 | 261 | 3.29 | 364 | 98.6 |




Tibbetts et al. (2013) have recently reported on the growth and nutrient utilization of GE GH Atlantic salmon (with a single copy of the EO-1α gene construct), both diploid and triploid, compared to full-sibling, size-matched non-GE Atlantic salmon, both diploid and triploid. GE salmon consumed a significantly higher amount of feed on a daily basis, resulting in a three-fold increase in target weight gain in 40% of the time of non-GE fish. GH genetically engineered Atlantic salmon also had enhanced specific growth rates (%/day), higher thermal growth coefficients ($g^{⅓}$/degree day), better feed conversion ratios, and higher nitrogen retention efficiencies. As a result, the overall total amount of feed required to produce the same fish biomass was reduced by 25% in GE fish. Feed intake was lower in triploid GE salmon compared to diploid GE salmon, but feed efficiency, digestibility and nutrient retention efficiencies were equal to those of GE diploids. In addition, without exception, GE triploids out-performed their related non-GE counterparts regardless of ploidy.

### 5.2.2 Other Phenotype and Fitness Characteristics

Rapid-growth phenotypes, including those produced in domesticated Atlantic salmon though selective breeding, appear to share several key physiological and behavioral attributes regardless of breeding methodology, including the following: 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 farmed Atlantic salmon, Fleming *et al*., 2002; in early-generation, GH transgenic Atlantic salmon, see Abrahams & Sutterlin, 1999 and Cook *et al*., 2000a; in growth-

accelerated GE fishes, see 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 & Hallerman (1991):

- Metabolic rate;
- Range of tolerance values for physical factors;
- Behavior;
- Resource or substrate use; and
- 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 AquAdvantage Salmon, their potential for survival and establishment, and their interactions with other organisms and the ecosystem.

#### *5.2.2.1 Metabolic Rates*

Metabolic rates influence the components of the overall energy budget for an individual; the components of the energy budget in turn influence an individual's impact on nutrient and energy flows, and other organisms. The distinguishing feature of 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*) treated with time-release GH implants (Johnsson & 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 GE 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*., 2000b).

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 non-GE comparators, GH transgenic Atlantic salmon had lower initial energy reserves, 2.1 to 2.6-fold greater feed consumption, and a propensity to deplete body protein, dry matter, lipids, and energy more quickly during starvation (Cook *et al*., 2000a & 2000b). Routine oxygen uptake in GH transgenic 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 & Sutterlin, 1999).

Although these GH transgenic 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).

#### *5.2.2.2 Tolerance of Physical Factors*

Tolerance of physical factors such as temperature, salinity, pH, etc. potentially can 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 specifically, studies have shown that oxygen consumption in adult GH transgenic Atlantic salmon is higher than in non-GE comparators (Abrahams & Sutterlin, 1999; Cook *et al*., 2000a; Cook *et al*., 2000b; Deitch *et al*., 2006). In contrast, oxygen consumption of eyed embryos, newly hatched larvae (alevins), and first-feeding juveniles (fry) is similar to that of non-GE salmon (Moreau, 2011). The increased requirement for oxygen in adults would engender a reduced tolerance for diminished oxygen content in general, and a reduced capacity for survival when the DO concentration is critically low, which is more likely to occur when water temperatures are elevated,[34] compared to their non-GE counterparts in the wild. In experiments with GH transgenic Atlantic salmon, oxygen uptake was independent of oxygen concentration above 10 mg/L, but started to decrease at approximately 6 mg/L DO in GE fish versus 4 mg/L DO in control fish (Stevens *et al*., 1998). Although under conditions of high dissolved oxygen, GE salmon are not at a disadvantage compared to controls, as oxygen demand is readily satisfied,[35] escape into water with a DO level less than approximately 6 mg/L would place the GH transgenic Atlantic salmon at a physiological disadvantage.

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

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

[35] Growth hormone appears to have a role in osmoregulation in anadromous salmonids (Down *et al*., 1989; Powers, 1989). During migration from fresh water to sea water, levels of GH are elevated, leading to an increase in sodium exclusion at the gills. Migrating GE smolt would therefore be likely to avoid predation better than wild smolt upon entering sea water because they would adjust faster to the saline environment and thereby escape estuarine and coastal predation (Hindar, 1993). Other factors (discussed in subsequent sections) tend to increase the predation risk for GE fish.

increased cardiac output and, ultimately, cardiac failure, at high temperatures that are not lethal to diploids.

Studies on GH-transgenic coho salmon indicate that growth of these fish is stimulated to a greater extent by higher temperatures than the growth of wild-type fish, suggesting to the study authors that the optimal thermal conditions for GH-enhanced coho salmon might be higher than for the wild-type (Lõhmus *et al*., 2010). However, the growth of GH-transgenic alevins decreased at a temperature of 14°C and above, and the growth of transgenic juveniles was almost identical at 16 and 18°C, suggesting the temperature optima for growth for these GH-transgenic coho salmon is 18°C for early life stages.

#### *5.2.2.3 Behavior*

Behaviors associated with swimming, feeding, reproduction, territorial defense, migration, or other developmental events could be affected by genetic engineering. The ecological impacts of these changes in behaviors could affect life history patterns, population dynamics, and species interactions (ABRAC, 1995).

In nature, swimming performance is important in foraging and predator avoidance. GH transgenic Atlantic salmon did not differ from wild counterparts in critical swimming speed (Stevens *et al*., 1998); however, they did demonstrate twice the movement rate of wild-type fish (Abrahams & Sutterlin, 1999).

GH also increases appetite in various species of salmonids (Abrahams & 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 transgenic Atlantic salmon would spend significantly more time feeding in the presence of a predator than non-GE salmon, indicating that they possess a higher tolerance for predation risk.

The differences between GE 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; based on experimental studies, a 25% difference in size has been suggested as necessary to overcome the resident advantage in Atlantic salmon (Metcalfe *et al*., 2003).

The effect of triploidy on wild-type phenotype is also important to consider as AquAdvantage *S*almon 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*., 2000a), 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.

Under laboratory conditions, GH-transgenic 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*., 2004), more likely to attack novel prey (Sundström *et al*., 2004), and better at using lower quality food

(Raven *et al*., 2006) when compared to wild relatives. 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. The extent to which this information on GE coho salmon can predict the behavior of GE Atlantic salmon is also unknown.

#### *5.2.2.4 Resource or Substrate Use*

Changes in resource or substrate use might occur through direct or indirect impact of transferred genes, either via interbreeding or genetic engineering. An example of an indirect impact is the potential for fast growing fish, including fish bearing a GH gene construct, to alter food webs; their increased size at a given age can lead to increases in size of their selected prey (Kapuscinski & Hallerman, 1990). As previously mentioned, GH increases appetite; however, Cook *et al*. (2000c) have also found that feed conversion efficiency was improved by 10% in GH transgenic Atlantic salmon suggesting some potential offset in the need for food.

#### *5.2.2.5 Impact of Disease and Parasites*

If a GE organism were to have improved resistance to disease or parasites, in theory it could out-compete its non-GE counterparts. Based on an evaluation of general health records, tank records, fish necropsies, and study data, we have found no evidence that AquAdvantage Salmon have any altered resistance to disease or parasites. A limited study of 20 gram AquAdvantage Salmon was performed by the sponsor to determine if the presence of the AquAdvantage gene construct alters the disease resistance of these fish to furunculosis (a disease caused by *Aeromonas salmonicida*) compared to size matched non-GE salmon. Although there was an earlier peak in the mortality of AquAdvantage Salmon following challenge, overall there was no obvious difference in mortality profiles between the two fish groups.

An analysis of general mortality data for AquAdvantage Salmon, diploid ABT salmon, and non-GE Atlantic salmon at the ABT PEI facility over the period from 2007 through 2012 shows similar rates of mortality between the two groups for six year classes of fish, indicating that AquAdvantage Salmon and diploid ABT salmon do not have an altered susceptibility to disease.

An outbreak of infectious salmon anemia (ISA) occurred in the PEI facility during the third quarter of 2009, see Section 5.4.2 for additional details. During this outbreak, no consistent difference in disease occurrence was noted between GE and non-GE 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 non-GE salmon (21.7%) than for GE salmon (both AquAdvantage and diploid ABT salmon) (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 both GE (both AquAdvantage and diploid ABT salmon) and comparator fish (0.88% versus 0.83%) for animals that were held in the Early Rearing Area (ERA) of the 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 non-GE comparators before the studies were discontinued.

No currently notifiable diseases or disease agents for finfish per Canadian or international (World Organisation for Animal Health (OIE)) requirements have been detected in recent years at the PEI facility as a result of periodic inspections by the DFO Fish Health Unit for the period from 2010 through 2014 and by CFIA in 2102, 2013, and 2014. Pathogens encompassed by these inspections included several viruses and filterable replicating agents, such as ISAV, plus other common fish pathogens. See Section 5.4.2 for additional details. FDA examined the facility's records related to the ISA outbreak during an inspection in June 2012 (see Appendix F), and found extensive documentation of the outbreak and diagnosis of ISAV as the causative agent. ABT's response to the outbreak was found 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.

Aside from the information presented above for AquAdvantage Salmon and diploid ABT salmon, there is limited data on disease resistance in other GE fish. Jhingan *et al*. (2003) have studied resistance to the bacterial pathogen *Vibrio anguillrium* in diploid and triploid coho salmon (*Onchorhyncus kisutch*) that are transgenic for growth hormone. They found that resistance (as measured by cumulative mortality) was not affected in transgenic fish relative to their non-transgenic counterparts when they were infected at the fry stage, but was lower in transgenic fish when infected near smolting (i.e, transgenic fish had higher mortality rates). Vaccination against vibriosis provided equal protection to both transgenics and non-transgenic fish. Triploid fish showed a lower resistance to vibriosis than their diploid counterparts.

#### *5.2.2.6 Morphology and Limits to Growth Maximization*

Changes in the morphology of the organism (e.g., size, shape, and color) could alter species interactions (ABRAC, 1995); however, it should be noted that accelerated growth, or increased body size, is not an assured outcome for GE salmon in nature. The rapid-growth phenotype is expressed only if supported by sufficient food, as has been shown in both genetically engineered coho salmon (Devlin *et al*., 2004b; Sundström *et al*., 2007) and GH transgenic Atlantic salmon (Cook *et al*., 2000b; 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 recent experiments of Moreau *et al*. (2011b) on GH transgenic 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 non-transgenic individuals were equally likely to be dominant in competitions for foraging territory. In the previous investigations of Abrahams & 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 transgenic 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 & Sutterlin, 1999).

These considerable differences in growth and feeding behavior between non-GE salmon, whether wild-type 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-transgenic coho salmon exhibited greater predation and ~3-fold greater fork-length than

age-matched wild type conspecifics; when reared under naturalized stream conditions, they exhibited more modest predation activity and were only 20% longer than controls (Sundström *et al*., 2007).

#### *5.2.2.7 Reproduction*

Changes in the age at maturation, fecundity, and sterility could alter population and community dynamics and interfere with the reproduction of related organisms (ABRAC, 1995). Due to their enhanced growth rate, diploid ABT salmon broodstock could be expected to achieve reproductive maturity in a shorter time-frame than their non-GE siblings. Because many animals, including Atlantic salmon, select mates based upon male body size, diploid GE males exhibiting larger-than-average body size potentially might have an advantage over their wild counterparts.

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

Oke *et al*., 2013 have recently reported on the hybridization of diploid GH-transgenic Atlantic salmon with closely related wild diploid brown trout (*Salmo trutta*). Experimental crosses produced in the laboratory using gametes from diploid fish resulted in transgenic hybrids (i.e., hybrids with the GH EO-1α transgene) that were viable[36] and grew more rapidly than GE salmon and other non-transgenic crosses in hatchery-like conditions. In stream mesocosms designed to emulate natural conditions, transgenic hybrids appeared to express competitive dominance and suppressed growth of transgenic and non-transgenic salmon. The researchers did not investigate the fertility of the transgenic hybrids or the

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

viability of any progeny resulting from hybrid backcrosses[37] to either Atlantic salmon or brown trout; however, they did identify and discuss several lines of evidence from the literature that combine to suggest that introgression of the transgene into the brown trout genome via backcrossing is unlikely. The implications of these observations (i.e., viable hybrids) for risk of establishment and further introgression are mitigated, however, as it has long been observed that progeny resulting from backcrosses of Atlantic salmon X brown trout hybrids are either non-viable, or triploid and therefore effectively sterile (Galbreath and Thorgaard 1995). Thus, there is virtually no potential for any further introgression of the transgene into brown trout or Atlantic salmon genomes via backcrossing.

In terms of hybridization and reproduction in general, the potential relevance of the findings discussed above to the proposed agency action are effectively limited to the PEI egg production site where broodstock are located; however, it is significant that despite being widely introduced into parts of Canada and the United States, there are no brown trout in PEI waters (see Section 6.1.1.3)[38]. In Panama, only triploid (functionally sterile), female AquAdvantage Salmon would be raised for commercial grow-out, and as will be discussed later in this document, there are no male Atlantic salmon or brown trout present at this location (Section 6.1.2.3), so reproduction there is precluded.

#### *5.2.2.8 Life History*

Changes in embryonic and larval development, metamorphosis, and life span could alter life-history patterns as well as population and community dynamics (ABRAC, 1995). GH constructs in salmonids have been shown to influence larval developmental rate (in coho salmon, Devlin *et al*., 1995b & 2004a) and smoltification (in Atlantic salmon, Saunders *et al*., 1998; in four species of Pacific salmon, Devlin *et al*., 1995a). Saunders *et al*. (1998) found that diploid GH transgenic Atlantic salmon reached smolt size sooner than normal and the smoltification process was not inhibited by high temperatures (19ºC) or constant light. Somewhat unexpectedly, Moreau and Fleming (2011) 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.

#### *5.2.2.9 Acute Stress Response*

Physiological responses to stress could be altered by GH transgene expression potentially resulting in changes in fitness and phenotype. Cnaani *et al*. (2013) have recently investigated the effects of stress on diploid GH transgenic Atlantic salmon, non-GE triploid Atlantic salmon, and what the authors refer to as wild-type Atlantic salmon. Groups of fish were subjected to either no stress (control), one-week of fasting, or low dissolved oxygen (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-transgenic salmon showed greater responses to stress than the two other genotypes, with the triploid fish producing intermediate responses. Wild-type fish maintained homeostasis more effectively than

[37] 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.

[38] Brown trout are not native to North America but have been introduced from Europe since the late 1800s. Today they are found in all Canadian provinces *except* PEI, Manitoba, and the Northwest Territories.

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-transgenic and non-GE triploid Atlantic salmon in the wild.

## 5.3 Conditions of Production and Use

### 5.3.1 AquAdvantage Salmon Egg Production Plan

The commercial production of eyed-eggs of AquAdvantage Salmon would occur only at a single facility on PEI where broodstock are currently held. The following discussion presents the general characteristics of the production process, followed by a detailed description of the specific production facility.

#### *5.3.1.1 Reproductive Biology of AquAdvantage Broodstock*

The production of AquAdvantage Salmon eyed-eggs requires the development of AquAdvantage broodstock, which are neomales (i.e., genetic females) homozygous for EO-1$\alpha$ (i.e., they have two copies of the genetic construct), through a process involving two methodologies for the manipulation of salmonid reproductive biology: gynogenesis and sex reversal. Milt from AquAdvantage broodstock is used to fertilize eggs from non-GE, female Atlantic salmon, and the fertilized eggs are pressure shocked to induce triploidy. The result of this process is a triploid, eyed-egg that will produce a sterile female Atlantic salmon that is hemizygous for EO-1$\alpha$ (i.e., it has only one copy of the genetic construct).

In order to produce broodstock for AquAdvantage Salmon, individual diploid ABT females homozygous for EO-1$\alpha$ are subjected to gynogenesis, a reproductive method that generates a larger population of homozygous females, which are then sex-reversed via treatment with androgen. The resulting neomales are genotypic females that produce sperm, which can only produce female offspring when crossed with a true female. The original source of homozygous females derives from matings between male (T-, XY) and female (T-, XX) AquAdvantage Salmon, and the identification of homozygous animals (TT, XY & TT, XX) that produce 100% AquAdvantage Salmon when back-crossed.

The process of gynogenesis involves the destruction of the genetic component in fish sperm, use of those "empty" sperm for egg activation, and restoration of a diploid state in the activated egg by forced retention of the second polar body. All of the offspring from this process are genetic females with a full complement of maternal DNA. The induction of gynogenesis in Atlantic salmon is a proven methodology that has most often been accomplished by destruction of sperm DNA via ultraviolet (UV)-irradiation, followed by the use of pressure- or heat-shock to prevent loss of the second polar body (Refstie, 1983; Quillet & Gaignon, 1990; Johnstone & Stet, 1995; Slettan *et al*., 1997). To avoid any contribution of genetic material from sperm that may inadvertently escape destruction during irradiation, a different fish species can be used for egg activation. Thus, the sperm that escape destruction will produce either non-viable offspring or hybrid progeny that can be distinguished visually. In the process applicable to AquAdvantage Salmon, gynogenesis is performed by using UV-irradiated milt from Arctic char (*Salvelinus alpinus*), followed by pressure shock to restore diploidy. Any salmon-char hybrids that may be produced are easy to distinguish from pure salmon due to a distinct difference in their coloration pattern.

Atlantic salmon have an XY system of sexual determination such that females are homogametic (XX) and males are heterogametic (XY). Many fish species experience a labile period after hatch when intentional exposure to sufficient levels of androgen or estrogen can

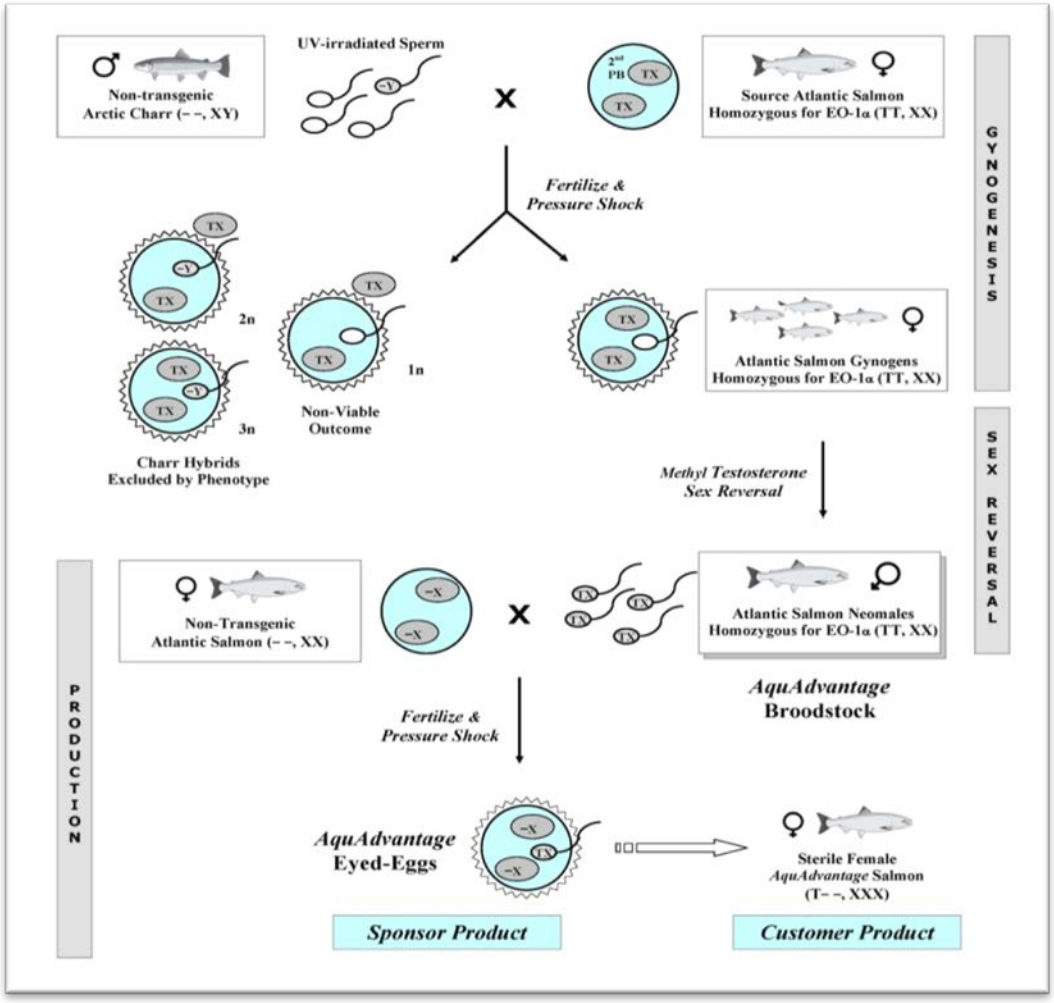
influence phenotypic sexual maturity (Pandian & Sheela, 1995). A genetic female can be induced to develop as a phenotypic male, or so-called neomale (XX), the milt from which will produce only genetically female offspring when crossed with a true female (XX). The monosex nature of the progeny derived from neomale-female matings has been demonstrated in several salmonid species, including Atlantic salmon and rainbow trout (Johnstone & Youngson, 1984; Johnstone *et al*., 1978; Johnstone & MacLachlan, 1994; Lee *et al*., 2004). In the AquAdvantage Salmon production process, 17$\alpha$-methyltestosterone administered in the diet is used to produce AquAdvantage neomales. In the claim validation study conducted by the sponsor, the animal subjects enrolled were derived from 20 non-GE Atlantic salmon females that were crossed with nine hemizygous AquAdvantage neomales: the sex of 180 progeny tested for confirmatory purposes was determined to be female.

The reason for generation of an all-female population, which is subsequently sex-reversed, is that it is tedious and time-consuming to distinguish neomales from true males following 17$\alpha$-methyltestosterone treatment of a mixed-sex population. Consequently, gynogenesis is used to produce an all-female population of salmon homozygous for EO-1$\alpha$, which will generate *only* the homozygous GE neomales required for eyed-egg production when they are treated subsequently with 17$\alpha$-methyltestosterone.[39]

The homozygous AquAdvantage neomales are mated with non-GE females to produce egg populations that are 100% hemizygous AquAdvantage females. Triploidy in the eggs is then induced by pressure shock to render the animal sterile. The reproductive biology of broodstock and eyed-egg production is summarized schematically in Figure 4.

[39] As noted by Piferrer *et al*. (2009), sex reversal is commonly used in the commercial production of rainbow trout per EU Directive 96/22/CE (26 April 1996).

**Figure 4. Reproductive Biology of AquAdvantage Broodstock and Eyed-Egg Production [40]**




5.3.1.2 Technical Details and Logistics of Commercial Production

[40] For AquAdvantage broodstock development, eggs from a female salmon homozygous for EO-1 ☐ are fertilized with UV-irradiated char sperm, and forced retention of the second polar body (PB) is accomplished by pressure shock [**Note:** As shown, the 2nd PB is disproportionately large to allow for indication of genotype]. Salmon-char hybrids that develop from any sperm that retain viable DNA are identified and removed from the gynogenetic population desired. For production, eggs from non-GE Atlantic salmon are fertilized with milt from neomales homozygous for EO-1 ☐ and pressure shocked to induce triploidy to ensure that the eyed-eggs sold into commerce could only generate sterile female AquAdvantage Salmon. Abbreviations: ***T-***, hemizygous transgenic; ***TT***, homozygous transgenic; **--**, non-transgenic; ***XX***, genetic female; ***XY***, genetic male.

The activities comprising the technical and logistic details of AquAdvantage Salmon production are discussed below and summarized schematically in Figure 5.

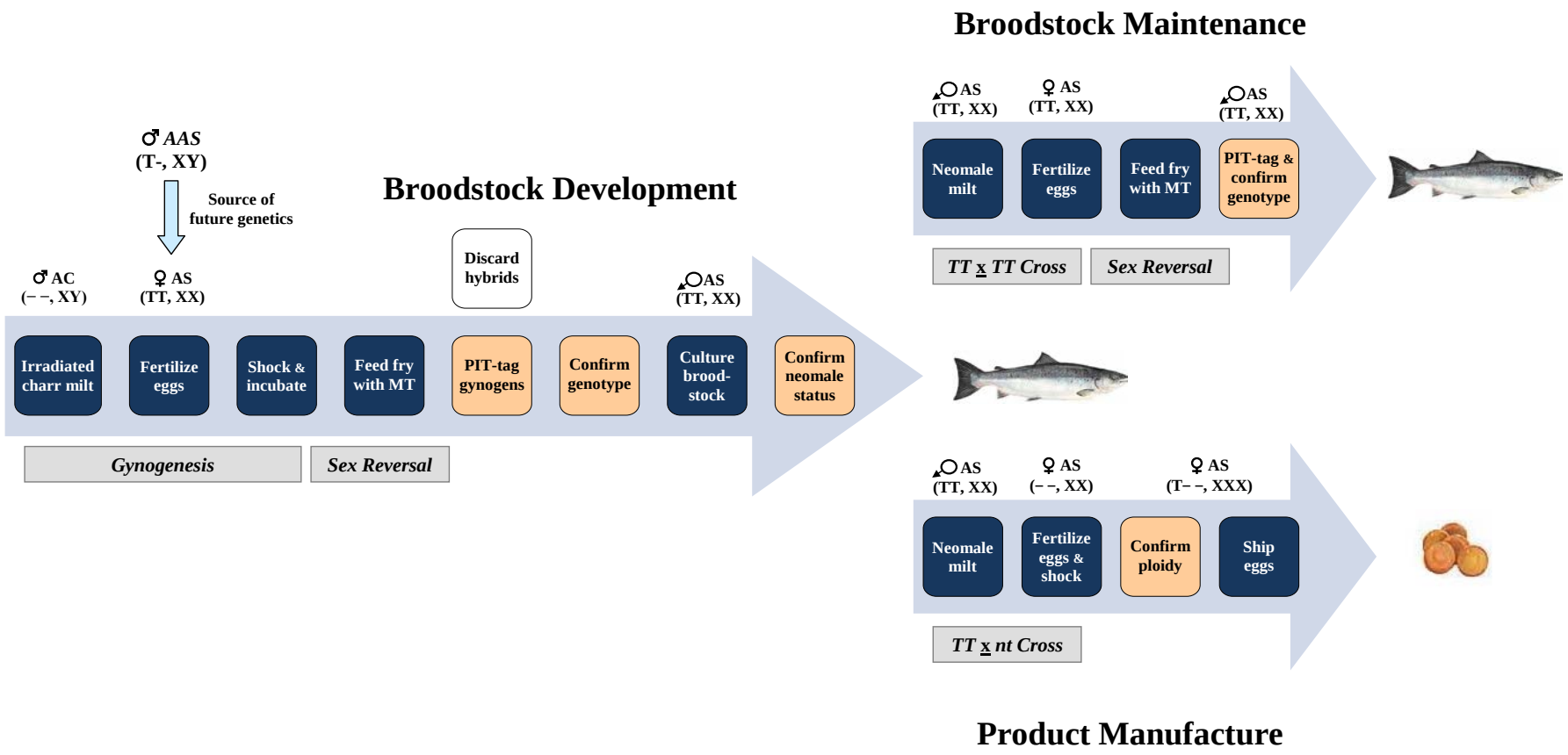
***Development of AquAdvantage broodstock for egg production:*** Eggs collected from sexually mature, genetic-female diploid ABT salmon homozygous for EO-1α (TT, XX), in which the identity and integrity of the AquAdvantage gene construct has been confirmed using diagnostic methods, are fertilized with irradiated milt from Arctic char, pressure shocked, and incubated until hatch. The fry (TT, XX) are sex-reversed using 17α-methyltestosterone, then graded and tagged with a passive integrated transponder at a body weight of ~10-20 g, at which time any salmon-char hybrids in the population would be identified for disposal. These neomale broodstock (TT, XX) are reared to sexual maturity, when their neomale status would be confirmed by spermiation (the release of mature spermatozoa).

***Maintenance of AquAdvantage broodstock for commercial egg production:*** Subsequent generations of broodstock can be derived from existing neomales homozygous for EO-1α by using the milt from those animals to fertilize eggs from true females homozygous for EO-1α (TT, XX); the offspring will be sex-reversed, graded, tagged, and subject to molecular-diagnostic confirmation of genotype prior to their qualification for use in future spawnings.

***Production of AquAdvantage Salmon eyed-eggs for commercial sale*:** Eggs from non-GE female Atlantic salmon (--, XX) will be fertilized with the milt from neomale broodstock (TT, XX), and the fertilized eggs (T-, XX) will be pressure shocked to induce triploidy (T--, XXX). The eyed-eggs will be incubated in Heath stack incubators (~10,000 eggs/tray **x** 12-16 trays) or upwelling jars (100-200,000 eggs) for 325-400°C-days, at which time batch-wise sampling will be performed to confirm the successful induction of triploidy via flow cytometry prior to release for commercial sale.[41] Confirmation of triploidy is discussed further in Section 5.3.2.3 below.

[41] ***Triploidy*** is induced in fin-fish to inhibit their sexual development and render them "sterile." Pressure shock has exhibited an average efficiency of 99.8% in inducing triploidy in AquAdvantage Salmon eggs at commercial scale. Although almost all of the AquAdvantage Salmon being cultured for retail sale as food would have no reproductive capacity, triploidy is not 100% effective in producing infertility (*see* Sections 5.3.2.4 and 7.4.1.3), and reference to "sterile" AquAdvantage Salmon in this document should be interpreted in that context. The production of ***monosex*** (i.e., all-female) populations of AquAdvantage Salmon, which is accomplished through a biological process that is 100% efficient, would be used to further diminish the possibility that AquAdvantage Salmon could become established in the wild in the event of escape from physical containment.

**Figure 5. Technical Details & Logistics of Commercial Production ***




* Abbreviations for genotypes are defined in the footnote to Figure 4.
NB: This figure was obtained from ABT. Male AS and female AS salmon are referred to as diploid ABT male or female salmon respectively in this EA.

### 5.3.2 Biological Containment Applied to AquAdvantage Salmon

Biological containment can serve as an effective risk mitigation measure by both (a) preventing any possibility of reproduction at the grow-out site, thus greatly reducing the risk of escape and/or release of gametes, embryos, or larval stages, and (b) greatly reducing or eliminating the possibility of reproduction of the GE organisms if they accidentally escape. Under the specific conditions of an approved NADA for AquAdvantage Salmon, two forms of complementary biological containment into the fish population through the egg production process would be required: an all-female (monosex) genotype and triploidy (effective sterility). As discussed further below in Section 5.3.2.3, although very highly effective (99.8% in the case of AquAdvantage Salmon), the current process used for inducing triploidy is not perfect (i.e., not 100% effective). Therefore, a second inherent form of reproductive containment, which ensures that AquAdvantage Salmon are all females through the design of a production process using gynogenesis[42] (see Figures 4 and 5), and which in this case is 100% effective (i.e., production of males is not possible), is required. Although the production method could have been designed to produce an all-male fish population, the production of females is preferred because triploid males, although sterile, can still engage in spawning behavior with diploid females in the wild, thereby leading to the reduced reproductive success of the wild females.

#### *5.3.2.1 Production of All-Female Eggs*

The eyed-eggs of AquAdvantage Salmon that are produced are 100% female. As described previously in Section 5.3.1.2, this is accomplished by fertilizing eggs from non-GE female salmon with milt from GE neomale broodstock (i.e., sex-reversed genotypic females) produced via gynogenesis. No "true" male fish will be used in the process thereby completely insuring the production of monosex, all-female populations of AquAdvantage Salmon for grow-out. The production of monosex populations prevents AquAdvantage **x** AquAdvantage reproduction outside of the PEI egg production facility.

#### *5.3.2.2 Induction of Triploidy in AquAdvantage Salmon Eggs*

The other complementary means of biological containment to be required for AquAdvantage Salmon is the functional sterility of fish produced by triploidy. Thus, even if these fish were to escape the grow-out facility and survive in the environment, they would not be able to reproduce. The induction of triploidy is the only accepted method currently available for sterilizing fish on a commercial scale.

Triploid fish have three sets of chromosomes in their somatic cells, rather than the two sets in the normal diploid state. Benfey (2001) describes two fundamental effects of triploidy on fish physiology: (1) the size of the somatic cells increases to accommodate the extra genetic material, but the number of cells decreases so that triploid fish are no larger overall than diploids; and, (2) gametogenesis and gonadal development is so severely impaired that triploids are sterile. Triploidy is generally induced by either thermal or hydrostatic pressure shock of the eggs within the first hour after fertilization. Hydrostatic pressure shock is more easily controlled and therefore preferred (Benfey, 2001); this is the method

[42] As described previously in Section 5.3.1.1 and shown in Figure 4, the only true male fish (i.e., neomales excluded), that would be used directly in the production of AquAdvantage Salmon eggs and broodstock are non-transgenic Arctic char. Neomales must be sacrificed in order to obtain milt for spawning.

that is used to generate triploid AquAdvantage Salmon. Pressure shock treatment for five minutes shortly (300ºC-min) after fertilization has been used successfully to induce triploidy in five year-classes of Atlantic salmon in New Brunswick, Canada (O'Flynn *et al*., 1997). The preferred method for verification of effective induction of triploidy is flow cytometry, because it is rapid and yields unambiguous results (Benfey, 2001). This process is the same as that used during the production of eyed-eggs at the production facility. Following pressure treatment, the eggs are water-hardened. The very high efficiency of the induction process (> 99%) ensures that very few diploid eggs with possible future reproductive potential would be shipped to Panama for grow-out (see following section).

#### *5.3.2.3 Reliability of Inducing Triploidy in AquAdvantage Salmon Eggs*

The use of triploidy greatly reduces, but does not eliminate, all environmental risks that are dependent upon reproductive capacity. The assurance of risk-mitigation by this particular measure is complicated by several factors: its reliability; its effectiveness in inducing sterility; residual spawning behavior in sterile males; and the survivability of sterile triploids should they be released in sufficient numbers to compete with diploid conspecifics of other species (CEQ-OSTP, 2001). The first three factors are addressed below, while overall survival ability of sterile triploids has been addressed in Section 7.3.

The major variables influencing the effectiveness of pressure shock in inducing triploidy are the following, in order of decreasing importance: timing; intensity; and duration of shock (Felip *et al*., 1997). Although method optimization for effective induction varies by species (Piferrer *et al*., 2009), laboratory-scale efficiencies of 100% that have been reported for Atlantic salmon (Benfey & Sutterlin, 1984) are not likely to be attained on a commercial scale (McGeachey *et al*., 1995), although as indicated below, ABT's success rate for achieving triploidy has been demonstrated to be quite high, 99.8% on average.

The sponsor has determined the effectiveness of the pressure shock method and conditions used for the induction of triploidy at the PEI production facility through a method validation study. In this study, one-to-one crosses were established with eggs from non-GE female Atlantic salmon and milt from ABT males hemizygous for EO-1$\alpha$. The fertilized eggs from each cross were apportioned into five replicate groups: one diploid control group that was not subjected to pressure shock, and four treated replicates that were pressure shocked (9,500 psi for five minutes at 300ºC-min post-fertilization). Ploidy analysis was performed on a sub-sample of 350 eyed-eggs collected from each of the treated replicates from five different crosses using flow cytometry; the efficiency of triploid induction was determined for a total of 20 independent pressure-shocked groups. The results of the initial method validation studies indicate that conditions used in the production facility can reliably produce batches of eggs that are on average 99.8% triploid. The range for individual batches was 98.9 to 100%, with 100% triploidy in 14 of the 20 batches. These results have been confirmed in additional validation studies using high-capacity pressure chambers, in which the percentage of triploids for 10 independent crosses (n = 200 eggs per cross) also averaged 99.8%, with 100% triploidy in six crosses and 99.5% triploidy in the other four crosses.

One of the conditions for post-approval quality control that would be required is the continued demonstration of the effectiveness of triploid induction in a statistically-appropriate sample of eyed-eggs from the production stream using established methods and procedures that require strict performance of controls and interpretability of analysis. The sponsor will be required to conduct composite sampling of individual upwelling chambers, which comprise multiple batches of pressure-shocked eggs, before any egg shipments occur. The acceptance criterion for releasing a batch of eyed-eggs for shipment

for grow-out is such that the probability would be less than 0.05 that these eggs are not at least 95% triploid. Egg batches that fail to meet this test criterion would be re-tested and destroyed upon confirmed failure.[43]

#### *5.3.2.4 Effectiveness of Triploidy in Inducing Sterility*

The degree of functional sterility in triploids varies depending upon the species and sex (Kapuscinski, 2005) and appears to be more complete in triploid females than triploid males (Thorgaard & Allen, 1992; Benfey, 1999; Piferrer *et al*., 2009; Benfey, 2015). Triploid females rarely produce eggs (i.e., do not reach ovulation), and if they do, the eggs usually are very few, undeveloped and unfertilizable (Piferrer *et al*., 2009). As described by Benfey (2015), most triploid oögonia fail to proceed to the oöcyte stage and, as a result, there are few (if any) ovarian follicles that develop to a stage of functional steroid biosynthesis. Because triploid females retain the endocrine profiles of early juvenile fish, any oocytes that do complete vitellogenesis will not be released due to the lack of endocrine signaling for final maturation and ovulation.

In reviewing data on approximately 26 fish and shellfish species being investigated in Japan, Arai (2001) noted that triploid males exhibit greater gonadal development than females and display secondary sex characteristics and sometimes spawning behavior, which females do not exhibit. Benfey (1999, 2015) cites several reports, including the studies of Johnstone *et al.* described below, of the occasional production of mature oocytes by triploid female fish of different species, which are able to produce small numbers of mature, post-meiotic cells. The growth of these cells progresses at such a slow rate that they are not observed at the normal time of sexual maturation in diploids.

The most relevant studies with respect to triploidization and sterility have been conducted by Johnstone and coworkers (Johnstone *et al*., 1991; Johnstone, 1992) on Atlantic salmon. This work indicated that three of approximately 3,000 female triploids (0.1%) underwent maturation after two years' time. When fertilized with normal sperm, eggs stripped from these three triploid females were markedly variable in size, and most underwent little obvious development (Johnstone, 1992). Approximately 10% of the eggs from two of the three triploids developed to the eyed-egg stage; however, the embryos were clearly malformed and none survived beyond hatching. Based on these study results, Johnstone concluded that the expectation that triploid Atlantic salmon females are functionally sterile has therefore been confirmed. Lee and Donaldson (2001) have reported that triploid coho salmon (sex not stated) in Japan and older triploid fish (of unidentified species) have sometimes been found to be fertile; however, no specific data were shown, and no references were reported to verify this report. In research with Arctic char (*Salvelinus alpinus*), few of the triploid females developed ovaries, fecundity was low, and the fertilized eggs from the triploid females did not hatch (Gillet *et al*., 2001) suggesting that reproduction was functionally precluded.

[43] Quality control is dependent upon the statistically-appropriate sampling of large populations; samplings are chosen in such a way that the measure of effectiveness determined is a probable *minimum* value for induction efficiency. Actual efficiencies might, in fact, be 100% or very close to that value, since the probability of an alternative (i.e., non-triploid) outcome under effective induction conditions is exceedingly low. Proof of 100%-efficient induction is an unrealistic benchmark that would require analysis of *every* egg regardless of the production-scale used, the impracticality of which is obvious in that the analysis requires destruction of the egg itself.

In order to ensure the highest probability of sterility and reproductive containment, the production of triploids is usually used in combination with a process that produces monosex fish, such as gynogenesis. This is the approach to be used for production of AquAdvantage Salmon. Because triploid females do not exhibit residual spawning behavior and are much less likely to have mature gonads than triploid males, the production of triploid all-female populations is considered to be the most effective form of biological containment applicable to GE fish in order to protect wild populations (Donaldson & Devlin, 1996; Arai, 2001; Mair *et al*., 2007; Benfey, 2015).

## 5.4 Egg Production on PEI: Facility Description, Containment, and Security

Production of AquAdvantage Salmon eggs occurs only at a single site: the sponsor's land-based, freshwater aquaculture facility[44] on the northeast side of PEI, which as of late November 2013 has been approved under applicable Canadian regulations for the commercial production, and export, of female, triploid eyed-eggs of AquAdvantage Salmon (see Section 2.5.1). Canadian government inspections of the facility for various purposes over the past 15+ years have shown it to be compliant with appropriate containment practices. Since 1996, the ABT facility on PEI has been subject to oversight by Fisheries and Oceans Canada (DFO) and Environment Canada (EC) for its use in research and development involving GE fish, and more recently by the Canadian Food Inspection Agency (CFIA) with respect to its compartmentalization program. In terms of containment, DFO inspections characterized the facility as being "as escape-proof as one can reasonably expect"[45] and, as described in Section 2.5.1, based on a qualitative Failure Mode Analysis, DFO has concluded that the potential for an acute failure of physical containment at the PEI facility is negligible with reasonable certainty. DFO has also concluded that the potential for chronic release of any life stage of AquAdvantage Salmon[46] from the PEI facility is negligible with high certainty. Based on the information described below, the conclusions of this EA are in complete agreement with the DFO assessment.

Two independent FDA inspections of the PEI facility have found it to be as described by the sponsor and in compliance with applicable manufacturing establishment requirements. See Appendix F, Inspections and Site Visit Summaries.

### 5.4.1 Location and Operations

The PEI facility is sited near the northeast coast of the island, close to the Fortune River, a coastal estuary, at a location approximately one mile inland from its confluence with a bay connected to the Gulf of St. Lawrence (Atlantic Ocean). The site of operations includes a main building, storage facility, and several ancillary structures. These buildings sit at an

[44] The PEI facility is owned and operated by AquaBounty Canada, a wholly-owned subsidiary of AquaBounty Technologies, Inc. (ABT). Some services in support of AquAdvantage Salmon production and development activities at the PEI facility are provided under a Collaborative Research Agreement with the Center for Aquaculture Technologies, Inc., a spin-off of ABT's research and development organization that was sold to Tethys Ocean. The PEI facility remains under the direct control and management of ABT.

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

[46] See footnote 8.

elevation of approximately 20 to 25 feet above water level; the distance to the estuary is approximately 120 feet at its closest point. The main building comprises approximately 9,240 sq ft used for aquaculture operations and approximately 3,020 sq ft used for laboratory, office, and living space.

Aquaculture operations are conducted in two principal areas: (1) the Early-Rearing Area (ERA) for eggs, alevin, and fry; and (2) the Grow-Out Area (GOA) for fry and smolt, as well as longer-term cultivation of juveniles and broodstock. As indicated in Table 1, the ERA and GOA contain tanks of several different volumes that provide for maintenance and rearing of fish of different sizes. The size of internal containment screening used on these tanks varies with fish size (Table 1).

**Table 1. Tank volumes, fish/egg sizes and containment screening sizes for the PEI facility**

| Culture Tank | Culture Tank | Fish Size [b] | Fish Size [b] | Containment Screening [d] | | | |
|---|---|---|---|---|---|---|---|
| Tank Type (Area) | Volume (L)[a] | ~BW (g) | ~FL (mm) | Number[c] | mm | inch | |
| G (GOA) | 200 | 0.1 – 100 | 20 - 200 | 1 | 1.6 | 0.0625 | 1/16 |
| A, B & D (ERA) | 160 | 0.1 – 100 | 20 - 200 | 1 | 0.8 | 0.030 | 1/32 |
| C (ERA) | 1,500 | ≥ 0.1 | ≥ 20 | 2-3 | 0.8 | 0.030 | 1/32 |
| E (GOA) | 1,500 | ≥ 10 | ≥ 100 | 3 | 3.2 | 0.125 | 1/8 |
| F (GOA) | 11,300 | ≥ 100 | ≥ 200 | 1 | 12.7 | 0.5 | 1/2 |
| Egg Incubators (Heath stacks) | 10,000 eggs/tray | *Na* | 5 mm (egg diameter) | Top & Bottom | | Standard Mesh | |
| - | Effluent | - | - | Sock filter | | 0.8 mm (0.030 in) | |

BW = body weight; FL = fork length; ERA = early rearing area; GOA = grow-out area
***a.*** Maximum operational volume; ***b.*** Size-range of fish in body weight and fork length typically housed (0.1 g, alevin; 10 g, fry; 100 g, smolt); ***c.*** Minimum number of internal tank screens (C & E groups have additional screens deriving from tank-insert use in the former and a design difference in the latter); ***d.*** Minimum size of the opening in containment screening used (Note: screen size is increased as the fish grow to facilitate wash-out of feces and unconsumed feed).

The ERA is made up of 32 C tanks of 1,500 L capacity and 73 A, B, and D tanks with a 160 L capacity, all of which are fitted with an internal standpipe and mesh-net covering to ensure containment. The ERA also contains a number of separate units (Heath stacks or upwelling chambers) for egg incubation. The GOA includes 12 E tanks of 1,500 L capacity and 24 F (large grow-out) tanks with an 11,300 L capacity that are also outfitted with mesh netting. A variety of other physical barriers and containment practices have been established to ensure that none of the fish life stages escape from the facility into the local environment, see further description below.

A site description, detailed containment diagram, and procedures governing husbandry practice and maintenance have been provided to FDA, which (as noted previously) has conducted two separate on-site inspections that identified no material deficiencies relevant to use of the facility for production of AquAdvantage Salmon, see Appendix F.

### 5.4.2 Disease Status of Facility

During the third quarter of 2009, a disease outbreak later determined to be ISA occurred at the PEI facility. Prior to this, the PEI facility had been considered "disease free" for many years based on periodic inspections and testing by Canadian authorities. The ISA outbreak

was first detected in fish in the GOA and later spread to fish in parts of the ERA. Once the presence of the ISAV was confirmed, ABT notified DFO. CFIA was notified shortly thereafter.

ABT responded to the ISA outbreak by implementing standard Atlantic salmon mitigation strategies appropriate for this disease in its facility (e.g., extirpation of all affected individuals, and implementation of an ISA detection and monitoring program). All fish displaying any characteristic of poor health or high viral load, most of the broodstock, and other non-essential fish were culled from the facility. In the GOA, only asymptomatic AquAdvantage broodstock and a few non-GE females were retained, while the ERA was completely depopulated and decontaminated. Subsequently, quarantine areas were constructed within the GOA to house and isolate important AquAdvantage broodstock that had potentially been exposed to ISAV. The ERA and GOA have also been permanently and physically separated into two distinct, biosecure facilities. Ultraviolet (UV) lights were installed to disinfect both the incoming well water as well as the recirculated water within both the ERA and GOA. Ozone treatment was added to disinfect water recirculated within the ERA.

All year classes of fish produced since the 2009 ISA outbreak have tested negative for ISAV when assayed using the most sensitive quantitative real time polymerase chain reaction (qPCR) diagnostic assay available. Since November 2009, there has been no detectable evidence of ISA disease in the PEI facility. All mortalities in the GOA have been necropsied and examined for signs of ISAV. No mortalities with clinical signs of ISAV have been observed. Samples of fertilized eggs, fry, and blood from fish in the ERA have been collected periodically since the ERA was depopulated and decontaminated in October 2009. No ISAV positive samples (fry, whole blood, or mortalities) have been detected by any method in the ERA during that time.

Samples of water entering tanks as well as samples of the facility effluent have been collected monthly since October 2009 and tested for the presence of ISAV using qPCR. None of the water or effluent samples have ever tested positive for ISAV.

As of late November, 2014, no notifiable fish diseases or disease agents under Canadian or OIE requirements, had been detected in fish or eggs from either the ERA or the GOA of the PEI facility since before the ISA outbreak in 2009. Negative results have been found in all subsequent inspections of each area, including several inspections conducted by the DFO Fish Health Unit in the years from 2010 to 2014 that specifically tested for ISAV and other pathogens. The most recent laboratory reports issued by DFO for the ERA and GOA indicate negative findings for the following pathogens:

- Infectious salmon anemia virus (ISAV)
- Viral hemorrhagic septicemia virus
- Infectious hematopoietic necrosis virus
- Infectious pancreatic necrosis virus
- *Aeromonas salmonicida*
- *Yersinia ruckeri*
- *Myxobolus cerebralis*

- *Ceratomyxa shasta*

The Canadian Food Inspection Agency (CFIA) inspected the PEI facility in May of 2013 and assessed the introduction risk for a number of exposure pathways and pathogens, including ISA, EHN, IPN, IHN, and VHS, among others. The overall introduction risk was found to be minimal for ISA, EHN, IHN, and VHS (these pathogens received the lowest possible rating), and acceptable for all other pathogens. The facility report concluded that no mitigation measures were needed. In addition, no amendments were requested for the facility's biosecurity plan.

After a multi-year process in which CFIA approved the PEI facility biosecurity plan, conducted an epidemiological assessment, performed facility inspections, and conducted diagnostic testing for various diseases and agents, in November 2014, CFIA officially recognized the PEI facility as two compartments under its supervision stating "Your premises had been evaluated and, while in compliance with compartmentalization program requirements, has been determined to present minimal risk of the introduction of ISA, VHS, IPN, *Myxobolus cerebralis*, *Ceratomyxa sahsta*, *Gyrodactylus salaris*, eHN and OMV diseases in *Salmo salar* and *Onchorhynchus mykiss* associated with movements to and from other countries."

FDA conducted an inspection of the PEI facility in June of 2012 with one of its primary goals to examine facility records, SOPs, and responses by the sponsor in relation to the outbreak of ISA that occurred in the fall of 2009. As a result of the inspection, FDA concluded that (1) the results of the diagnostic evaluations are consistent with the detection of ISAV; and (2) appropriate biosecurity measures were taken in response to the outbreak, including installation of UV and ozone water treatment systems. See Appendix F for additonal information on this inspection. In the event of an NADA approval, post-approval reporting requirements include reporting of any future presumptive or confirmed disease.

#### 5.4.3 Physical Containment at the PEI Egg Production Site

Physical containment refers to measures or barriers implemented on-site to prevent the movement or escape of fish from the facility. Containment measures can include the use of mechanical devices, either stationary or moving (e.g., tanks, screens, filters, covers, nets, etc.), or the use of lethal temperatures or chemicals to prevent uncontrolled escape. For example, treatment with 10-15 mg/L chlorine for 15-30 minutes is effective in killing fish in fresh water (ABRAC, 1995). 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 are also important to prevent unauthorized access, control movement of authorized personnel, and prevent access by predators. The sponsor has developed and employs an extensive number of SOPs that govern physical containment as well as every other significant activity that occurs at the site. All containment equipment is inspected by facility staff on a daily basis and a form is completed documenting the results of this inspection. These records are subsequently reviewed by facility management. FDA's inspections have included review of the SOPs, the SOP for physical containment in particular, and verification of the processes described therein. No deviations were found by FDA.

The potential for accidental escape could derive from any of the following components of the water system: influent water and makeup water; effluent and draw-down water; and waste slurries collected when filters are backwashed, screens scrubbed, or rearing units cleaned by siphoning (ABRAC, 1995). At the PEI facility, except for solid wastes that go into a septic

tank, all waters and wastes are discharged to the environment through a single, controlled effluent (Figure 6).

A number of redundant measures have been implemented at the egg production facility on PEI to provide physical containment of the AquAdvantage Salmon eggs and the AquAdvantage broodstock that would be used to produce them. In general, the physical containment measures or barriers ensure entrapment of eggs or fish at the immediate source of housing for cultivation (i.e., via tank covers or nets), and redundancy in screening and filtration of the water flow paths (e.g., pipes and floor drains) into which fish could potentially gain access. These measures, which are employed in a redundant manner and at multiple locations, are summarized in Table 2. A schematic of the containment system components and water flow within the facility is provided in Figure 6. This figure shows containment features for both the early rearing area (eggs, alevins, fry) and grow-out area (fry, smolts, broodstock). Table 3 further describes these containment components and the level of containment that they provide to various egg, fry and fish holding structures with the PEI facility.

As indicated in Table 3, all areas of the facility have at least four independent, sequential forms of physical containment on their water systems, while many areas, including the egg incubation units and their discharges, have five or more forms of containment in series before the water is discharged[47]. Ultimately, all effluent from the facility is combined together and passes through an exterior containment sump before it is discharged from the facility to a nearby drainage ditch, which ultimately empties into the Fortune River. Within this sump, all effluent must pass through a series of three, independent stainless steel screens with hole-opening diameters of 6.2, 10, and 13 mm, respectively. Each screen may be removed individually for cleaning or replacement, with the other two screens remaining in place at all times for redundant containment. In addition, the PEI facility also utilizes chlorine pucks in the floor drains during the spawning process as a means of killing any embryos that are "lost" as a result of spills or accidents.

**Table 2. Key Components of Physical Containment at the PEI Egg Production Facility**

| Purpose | Feature or Component |
|---|---|
| **Primary Containment** | |
| To prevent escape through rearing unit or egg incubator water overflow | Perforated metal screens on tank bottoms |
| | Screens and/or slots on stand pipes, top and bottom (where appropriate for size of fish to be contained) |
| | Incubator tray screens |

[47] For egg incubation units, this number only includes forms of containment (e.g., screens) that have a mesh size or design that would ensure containment of eggs. For example, downstream filters or screens that have a large screen opening and thus would only be effective in preventing the passage of fry or larger life stages of fish, are not counted as forms of containment for eggs or the egg incubation units.

| Purpose | Feature or Component |
|---|---|
| To prevent escape over the side of a tank or from an egg incubator | Screened tank overflows<br>Cover nets<br>Jump fences<br>Tank covers<br>Incubator (Heath stack) tray screens<br>Upwelling chamber screens |
| To prevent downstream passage of newly fertilized eggs and/or gametes | Chemically lethal environment (chlorine puck) in spawning area drain |
| | Perforated metal drain cover in spawning area |
| | Closed septic system |
| **Secondary Containment** | |
| To prevent entry of fish into drains and effluent piping | Floor drain covers, solid or mesh |
| | Incubator-stack catchment box with screening |
| | Waste de-watering sieve box |
| To prevent downstream passage of fish within the drains & piping | Barrier screens within drains and piping |
| | Drum filter |
| **Tertiary and Quaternary Containment** | |
| To prevent downstream passage of fish within the drains and piping | Stainless steel barrier screens within drains of various sizes & locations |
| | Stainless steel filter baskets within the ERA Containment sump |
| | Catchment box with screening for some of the tanks within the GOA |
| | Mesh filter on ERA drum filter gray water |
| | Heat exchanger on main ERA tank effluent |
| | Three independent stainless steel screens within the exterior containment sump |
| **Waste Treatment** | |
| | Sock filters, containment screens, basket-sieve for straining waste material from the ERA tanks |
| | Chlorine kill solution (5 mL Javex containing 0.52 grams sodium hypochlorite per liter of water) |
| | Chlorine pucks in floor drains |

## Figure 6. Schematic of Physical Containment Components at the PEI Egg Production Facility

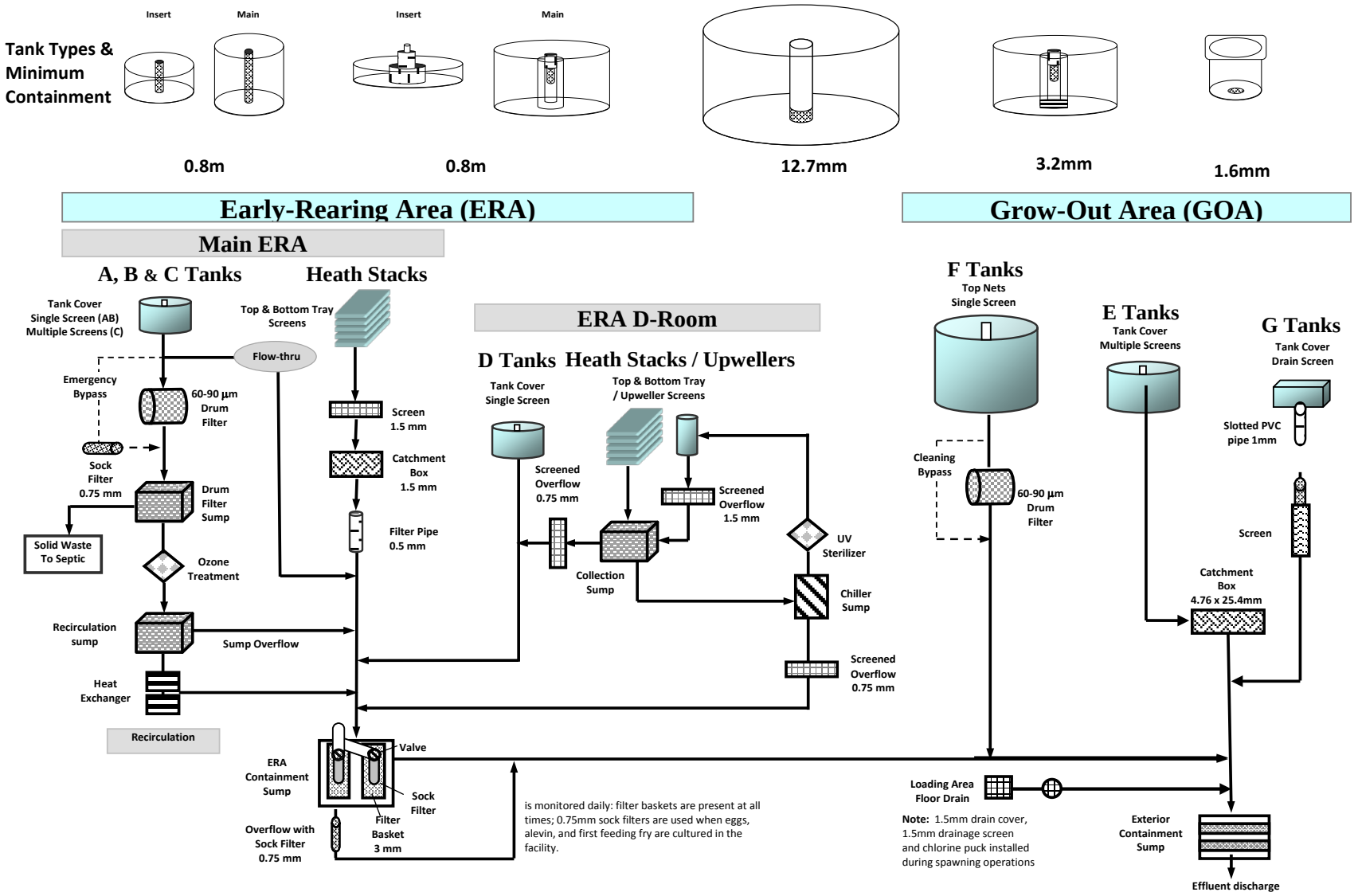


**Table 3. Containment Components and Level of Containment for the PEI Egg Production Facility Area**

| Area | Containment level | Component | Details |
|---|---|---|---|
| **ERA - Heath Stacks** | 1 | Screened Trays | Each drawer is a 1.5 mm screened compartment (top and bottom). The water must pass through both of these screens at each level as it descends towards the drain. |
| | 2 | Vertical Screen | Effluent is screened through a vertical 1.5 mm perforated PVC screen. |
| | 3 | Catchment box | Effluent is filtered through 1.5 mm perforated catchment box filter with a horizontal screen. |
| | 4 | Filter Pipe | Effluent is filtered through a 0.5mm slotted PVC capped pipe. |
| | 5 | ERA containment sump sock filters | When smaller hatchlings and first feeding fry are present sock filters are attached to the drain pipe outlets. The socks used have 1.5 and 0.75 mm mesh as appropriate for the fish size. |
| | 6 | ERA containment sump | Three drain lines feed water into two stainless steel perforated baskets with 3 mm punched holes and 30 cm high sides. When cleaning a containment baskets and/or sock filter, water flow is restricted while clean socks are put in place. The sump overflow is fitted with a 0.75 mm sock filter. |
| **ERA - Upwelling Units** | 1 | Screened inflow and overflow | Each unit is equipped with a 1.5 mm screened disk on both top and bottom. The water passes through the bottom screen when entering the upwelling unit and exits through the top screen. |
| | 2 | Effluent screen | Effluent is screened through a 0.75 mm sock filter. |
| | 3 | Overflow screen | Overflow from collection sumps are filtered through a 0.75 mm sock filter. |
| | 4 | ERA containment sump sock filters | When smaller hatchlings and first feeding fry are present sock filters are attached to the drain pipe outlets. The socks used have 1.5 and 0.75 mm mesh as appropriate for the fish size. |
| | 5 | ERA containment sump | Three drain lines feed water into two stainless steel perforated baskets with 3 mm punched holes and 30 cm high sides. When cleaning a containment baskets and/or sock filter, water flow is restricted while clean socks are put in place. The sump overflow is fitted with a 0.75 mm sock filter. |

| Area | Containment level | Component | Details |
|---|---|---|---|
| **ERA - Early Rearing Tanks** | 1 | Slotted tank stand pipe | Covered slotted standpipe with progressively wider slots as fish size increases; deep C tanks have an additional barrier |
| | 2 | Septic tank for solids collection | The solids (plus some water) from ERA drains are separated out by the drum filter and then pass into the drum filter solid waste septic tank. |
| | 3 | ERA containment sump sock filters | When smaller hatchlings and first feeding fry are present sock filters are attached to the drain pipe outlets. The socks used have 1.5 and 0.75 mm mesh as appropriate for the fish size. |
| | 4 | ERA containment sump | Three drain lines feed water into two stainless steel perforated baskets with 3 mm punched holes and 30 cm high sides. When cleaning a containment baskets and/or sock filter, water flow is restricted while clean socks are put in place. The sump overflow is fitted with a 0.75 mm sock filter. |
| **GOA - Large Circulars (F Tanks)** | 1 | External stand pipe screens and standpipe cover | Screened ports or drilled holes dependent upon fish size. The top of the standpipe is fitted with a solid PVC cap. |
| | 2 | Facility Containment Screen # 1 | All effluent passes through a 6.2 mm punched stainless steel basket screen. |
| | 3 | Facility Containment Screen # 2 | All effluent passes through a 10 mm flat punched stainless steel basket screen. |
| | 4 | Facility Containment Screen # 3 | All effluent passes through a 13 mm flat punched stainless steel screen. |
| **GOA - Small Circulars (E Tanks)** | 1 | Internal stand pipe screens and stand pipe cover | Screened ports or drilled holes dependent upon fish size. The top of the standpipe is fitted with a solid PVC cap |
| | 2 | External stand pipe screens | Baskets with appropriate screening are located within exterior standpipe. Appropriate size screen is fitted to top of the external standpipe drain above the water level |
| | 3 | E Tank Quarantine Containment Screen | Slotted (4.76 x 25.4mm) stainless steel basket or 13 mm perforated stainless steel basket in place prior to water entering the floor drain |
| | 4 | Facility Containment Screen # 1 | All effluent passes through a 6.2 mm punched stainless steel basket screen. |

| Area | Containment level | Component | Details |
|---|---|---|---|
| | 5 | Facility Containment Screen # 2 | All effluent passes through a 10 mm flat punched stainless steel basket screen. |
| | 6 | Facility Containment Screen # 3 | All effluent passes through a 13 mm flat punched stainless steel screen. |
| **GOA - Deep Swede (G Tanks)** | 1 | Tank drain screen | Perforated metal or plastic screens with progressively larger perforations or a covered slotted standpipe with progressively wider slots as fish size increases. |
| | 2 | PVC Filter Pipe | A length of 2" slotted PVC pipe fitted with an end cap. Minimum slot size is 1mm. |
| | 3 | Sock filter on shared drain outlet | A 1.5 mm mesh sock filter is fitted to the outlet of the shared drain pipe of the G tanks. |
| | 4 | Slotted PVC screened sieve box | 1.5mm slotted PVC screen. |
| | 5 | Facility Containment Screen # 1 | All effluent passes through a 6.2 mm punched stainless steel basket screen. |
| | 6 | Facility Containment Screen # 2 | All effluent passes through a 10 mm punched stainless steel basket screen. |
| | 7 | Facility Containment Screen # 3 | All effluent passes through a 13 mm flat punched stainless steel screen. |
| **GOA - Loading Area Floor Drain** | 1 | Floor Drain Covers | Normal drain covers are perforated steel plates with 7.0 mm openings. During spawning or packaging eggs for shipment, a 1.5 mm drain cover screen is installed. |
| | 2 | Floor Drain Screen | An addition drainage screen is installed consisting of 1.5mm perforations |
| | 3 | Chlorine Puck | Installed under the floor drain screen while spawning. |
| | 4 | Facility Containment Screen # 1 | All effluent passes through a 6.2 mm punched stainless steel basket screen. |
| | 5 | Facility Containment Screen # 2 | All effluent passes through a 10 mm punched stainless steel basket screen. |
| | 6 | Facility Containment Screen # 3 | All effluent passes through a 13 mm flat punched stainless steel screen. |

### 5.4.4 Security at the PEI Egg Production Facility

Multiple and redundant forms of security are present at the PEI site to prevent malicious activities and unauthorized access to operational structures, GE fish, and associated broodstock. Site security includes the following:

- ***Perimeter security:*** An eight-foot-high, heavy-gauge, galvanized chain-link fence of commercial quality completely surrounds the property, inclusive of freshwater well-heads, back-up generators, liquid oxygen containment, and the storage facility. A service entry adjacent to the storage building is secured by a double-swing, chain-link gate except when service access to the property is required. A roll-away, chain-link gate spanning the main entry to the property adjacent to the main building is secured during non-business hours. At night, the entire perimeter remains well-lit.

- ***Outside entries:*** Windows on the lower-level of the main building are barred, and all exterior steel-doors on the main and storage buildings are dead-bolted. Entry into the main building requires a key or intercom-interrogation and remote unlocking by facility staff. Within the main building, access to the first-floor aquaculture facility is further protected by a cipher-locked, interior entry.

- ***Security monitoring:*** The manufacturing site is protected by exterior-interior security cameras and a system of multiple interior sensors (contact & motion) that is professionally monitored 24/7 for detection of and response to unapproved intrusion or loss of operational capacity (power, water levels and dissolved oxygen concentrations). Eight motion-activated security cameras are positioned for maximum surveillance of the property immediately surrounding the main building. Additional interior cameras cover entrances and key work areas. These cameras are in continuous operation and automatically capture digital images that are stored for later retrieval. Magnetic door-contacts and interior motion-detectors deployed throughout the main building, storage facility, and out-buildings comprise a network of zones that are monitored by a commercial security service.

- ***Water supply & pump-house:*** The primary well and pumping facilities (one primary, two back-ups) that supply the PEI facility are securely enclosed in a steel containment structure.

- ***Remote notification of status:*** Environmental alarms notify staff of any significant changes in facility operational conditions (e.g., water levels, dissolved oxygen levels). Security alarms indicating suspected intrusion during non-working hours are conveyed by the security service to senior facility staff via numeric page; in addition, direct telephone contact with the facility manager or other on-call staff are to be pursued until successfully made, so that clear communication of the event occurs and proper and immediate response is managed.

- ***Additional security:*** As conditions warrant, the sponsor may employ professional security personnel to remain on-site during non-business hours. In addition to their direct surveillance of the property, these personnel would have access to the central, security-monitoring system in the main building, but would not have access to the facility at-large, which would remain locked-down and subject to the network of electronic sensors and motion-activated cameras comprising that system. An apartment in the main building provides for additional surveillance by staff living on-site. Personnel employed by the sponsor are present at the site 24 hours per day, seven days per week.

## 5.5 Grow-Out in Panama: Facility Description, Containment, and Security

### 5.5.1 Location and Operations

If approved, under the conditions that would be established in the NADA, commercial rearing and grow-out of eyed-eggs of AquAdvantage Salmon will occur at only one site: the sponsor's land-based, freshwater aquaculture facility in the western highlands of Panama. This site sits near a high-gradient river at an elevation of approximately 5,000 feet above sea level in the upper portion of the river's watershed. This river eventually combines with several others and ultimately discharges into the Pacific Ocean at a location many miles downstream from the facility.

The Panamanian facility, which is designed for rearing AquAdvantage Salmon from the eyed-egg stage to market-size, comprises a small building that is used for egg incubation, fry-tank housing, quarantine, feed storage and office space, and four outdoor culture (grow-out) tanks. Other components of the facility include water-intake structures, header tanks, low-head oxygenators (LHO), containment structures and devices, and four sedimentation ponds. A site description and detailed containment diagram were provided by the sponsor to FDA. FDA staff, accompanied by a NMFS aquaculture expert, have conducted a site visit of the Panamanian facility that identified no material deficiencies relevant to use of the facility for grow-out of AquAdvantage Salmon. See Appendix F, Inspection and Site Visit Summaries.

Eyed-eggs would be received at the site, acclimated to ambient water temperature and pH, and then incubated in trays within a vertical Heath stack incubator. The tray incubator is to be operated on a 100% recirculating basis during early egg incubation, and then once the eggs hatch, the recirculation mode is to be gradually transitioned to continuous flow-through. After the eggs hatch, the alevins (yolk-sac fry) will be moved to the fry tanks, where they will remain until transferred to the grow-out tanks.

The fry-tank building contains six fiberglass tanks, each with a capacity of 3 $m^3$. Water flow in the fry tanks is regulated at 2-2.5 L/min-kg biomass. The primary water supply for the fry tanks derives from a spring located north of the site that is delivered through two 6-inch pipes, which converge prior to entering an oxygenation tank. The tank is equipped with a water-level control sensor and alarm, and two small LHOs that are supplied with pure oxygen via hoses from liquid-oxygen cylinders. Oxygen is injected into the spring water, which then flows by gravity to the fry tanks. Water flow to the fry tanks is controlled by means of valves located on the incoming supply line to each tank. In the event of an interruption in spring-water flow, a secondary, emergency water line can be employed. The water intakes are inspected on a daily basis, or more frequently during inclement weather.

When the fish reach an average size exceeding 25 g, they will be transferred from the fry tanks to the grow-out tanks, and initially will be stocked into two of the four grow-out tanks. Subsequently, as they grow and biomass approaches 35 kg/$m^3$, the fish will be distributed among all four grow-out tanks, where they will remain until they reach market size (1-3 kg body weight). The individual grow-out tanks have a maximum capacity of 100 $m^3$, but are operated at a maximum volume of 85 $m^3$. Densities in the grow-out tanks will be maintained at values below 35 kg/$m^3$ for optimal water quality and growth conditions, with water flow that supports complete turnover of tank capacity approximately once every hour. Water leaving the grow-out tanks flows through the slotted drain-screen and is discharged into a concrete containment sump, from which it flows into an excavated earthen drainage canal.

The primary water supply for the grow-out tanks derives from an intake canal that diverts water from a local river. Water flows to a basin, which in turn supplies a very large LHO. The water is then gravity-fed to the grow-out tanks through a 16-inch pipe. Water flows are adjusted by two valves located on the incoming water supply to each tank. The intake canal is inspected weekly and cleaned when debris accumulates.

Fish can be harvested at different weights to test different markets and product presentations. The fish will be harvested by netting, euthanized on-site using ice water, and shipped by truck to a local plant for processing and shipment to local and export markets. No live fish will leave the grow-out facility. The fish may be marketed in different presentations (e.g., whole on ice, whole-dressed on ice, fillet on ice, or frozen or smoked fillet). Fish exported to the United States will be shipped in refrigerated containers by established wholesalers subject to Panamanian law, and subject to all applicable U.S. regulations.

### 5.5.2 Physical Containment at the Panama Grow-Out Facility

Multiple forms of physical containment are in place to prevent the escape of fish at the grow-out facility, primarily in the form of filters and screens wherever water flows in the system. Security is provided by surrounding the fry tanks and grow-out tanks with netting and fencing topped with barbed-wire to deter human or animal intrusion. An additional level of physical containment is provided by several downstream hydro-electric plants, which also serve to prevent passage of any escaped fish to downstream sections of the river or the Pacific Ocean (see Section 6.1.2). Key components of containment are summarized in Table 4. A schematic of the containment system elements is provided in Figure 7, supplemented with further information on the individual containment components in Table 5. All containment equipment (e.g., screen, filters, standpipes) is inspected at least once per day for signs of breaches or failures, as well as signs of fish escape.

The egg incubation system consists of an eight-tray Heath stack incubator that is operated in recirculation mode with zero discharge until eggs begin to hatch. Once flow-through operation begins, all effluent from the tray incubator system is routed through a single in-line micro (50µm) filter cartridge, then after being combined with effluent from the fry tanks, through a series of in-line sock filters and interchangeable screens associated with the fry tank trap (see Figure 7).

The fry tanks are assembled with an upper insert containing an interior standpipe that controls the water level. The standpipe is covered by a 1 mm screen when fry are being fed the smallest feed sizes, and a 1.5 mm screen when they graduate to larger feed sizes. An exterior screen with a 1.5 mm slot-aperture is placed outside the interior standpipe screen. The lower (primary) tank is equipped with a basket screen (3 or 6 mm) and top screen (3, 6 or 12 mm). In addition, inside the standpipe, affixed by screws to the base of a basket screen, is a permanent metal screen with 5 mm openings that prevent fish of larger diameter from leaving the tank. All water leaving the fry tanks must first pass through a 500 µm (0.5 mm) sock filter, then through an enclosed fry tank trap containing one or more interchangeable screens (0.5 to 3.0 mm mesh opening). Effluent from the fry trap is then routed through a secondary filter sock (0.5 mm) and discharged into the primary drainage canal. Any one of the filters or screens can easily be changed or cleaned, leaving several additional physical barriers in place at all times for containment. Operational protocols are in place for inspecting critical containment barriers and other common daily procedures, see Section 5.7.

Each grow-out tank is equipped with a rigid, polyvinyl chloride (PVC) drain-screen plate having slots of 0.9 cm aperture that is anchored by screws to the one-and-only drain opening of the tank. Fish are transferred from fry tanks to the grow-out tanks when 100% of the animals are more than 1 cm in diameter, so that no animals can pass through the drain-screen plate.

Drainage from both the fry and grow-out tanks enters the drainage canal and flows through a second concrete containment sump equipped with a 12 mm steel screen-plate, which is anchored in such a way that all water passing through the sump is screened. Downstream of the sump, the water flows into a sequential series of four in-line settling (sedimentation) ponds, each with a single concrete outlet structure that that is equipped with two metal screens, one a 12 mm pre-screen, the other a 6 mm primary screen. Either screen can be changed or removed for cleaning without a loss of containment for the pond. From these ponds, the water is discharged into a local river.

The fry tanks and building containing them, as well as the outdoor grow-out tanks, are covered with netting to prevent predation and fish movement by birds in the area and to ensure that "jumpers" (i.e., fish attempting to escape their tanks by jumping clear of the water) will not be successful. In particular, the grow-out tanks are sealed horizontally and vertically inside a cage comprised of netting supported by a rigid structure. Escape from the tanks by jumping, or removal of fish by avian predators, would therefore be essentially impossible.

In summary, there are highly effective forms of physical containment in place for all life stages of AquAdvantage Salmon at the Panama facility. As described in Table 5, there are a minimum of 14 sequential physical barriers in place between the fry tanks and the local river that will confine AquAdvantage Salmon to the grow-out site; Similarly, 11 sequential physical barriers are installed in the outflow from the grow-out tanks to the local river. For fish eggs and fry tank inserts, there are five separate forms of containment in the effluent flow stream. In addition, netting is in place on all tanks to prevent the fish from being actively removed from containment by predators or passively removed in the event of any overflow of the water level.

## Table 4. Key Components of Physical Containment at the Panama Grow-Out Facility

| Purpose | Feature or Component |
|---|---|
| **Primary Containment** – To prevent escape of eggs from the Heath stacks | Each incubation tray is screened on the top and bottom. The water must pass through both of these screens at each level as it descends toward a single drain. |
| | Micro filter cartridge (50 µm) screens all effluent exiting from the Heath stacks. |
| To prevent escape from the fry tanks via water | Center standpipe cut below tank rim to ensure water level is always below rim.<br>Netting stretched taut over top of tank to prevent fish from escaping even if tank is overflowing. |
| | Collar-sleeve screens inserted into top of standpipes to prevent fish from entering standpipe by swimming. |
| | Metal screen inside standpipe at base of basket screen impedes fish that enter standpipe (by jumping) from leaving the tank. |
| | Rigid circular plastic screens surrounding the center standpipes. |
| | Porous gravel floor around each tank allows downward percolation of overflow water but traps any fish in the overflow. |
| To prevent escape from the fry tanks by avian predators | The building is covered and sealed by netting. |
| | Netting stretched taut over the top of each tank. |
| To prevent escape from the grow-out tanks via water | A single external (so no fish can jump into it) standpipe cut below tank rim to ensure water level is always below rim. |
| | A 1 cm thick, rigid PVC slotted drain plate affixed by screws to the only drain in the tank. |
| | Porous gravel floor around each tank allows downward percolation of overflow water but traps any fish in the overflow. |
| To prevent escape from the grow-out tanks by avian predators | Each tank is entirely covered by netting stretched over and around the tank on a rigid support structure. |
| | Netting stretched taut over the top of each tank. |
| **Secondary Containment** – To prevent escape of eggs from Heath stacks into drain | Two separate sock filters (0.75 and 0.5 mm) screen the effluent. |
| | A fry trap with one or more interchangeable screens that filters all effluent. |
| To prevent escape from fry tanks into drains | Two separate sock filters (0.75 and 0.5 mm) screen the effluent. |
| | A fry trap with one or more interchangeable screens that filters all effluent. |
| To prevent escape from grow-out tanks into drains | Sealed metal cage (affixed to ground) through which all effluent from grow-out tanks must pass before entering the drain canal. |
| To prevent escaped fish from passing through the drain canal to the sedimentation ponds | Concrete structure and containment sump through which all water must pass. |
| | Rigid metal screen affixed to bottom of containment sump through which all water must pass. |
| **Tertiary and Quaternary Containment** – To prevent escaped fish from passing from one sedimentation pond to another | Rigid metal screens on the outlet of each pond (two screens per pond so that one is always in place in the event the other is removed for cleaning). |

| Purpose | Feature or Component |
|---|---|
| To prevent escaped fish from entering the river from the drain canal | Four sedimentation ponds in series, each with two outlet screens in series. |

**Figure 7. Schematic of Physical Containment Components at the Panama Grow-Out Facility**

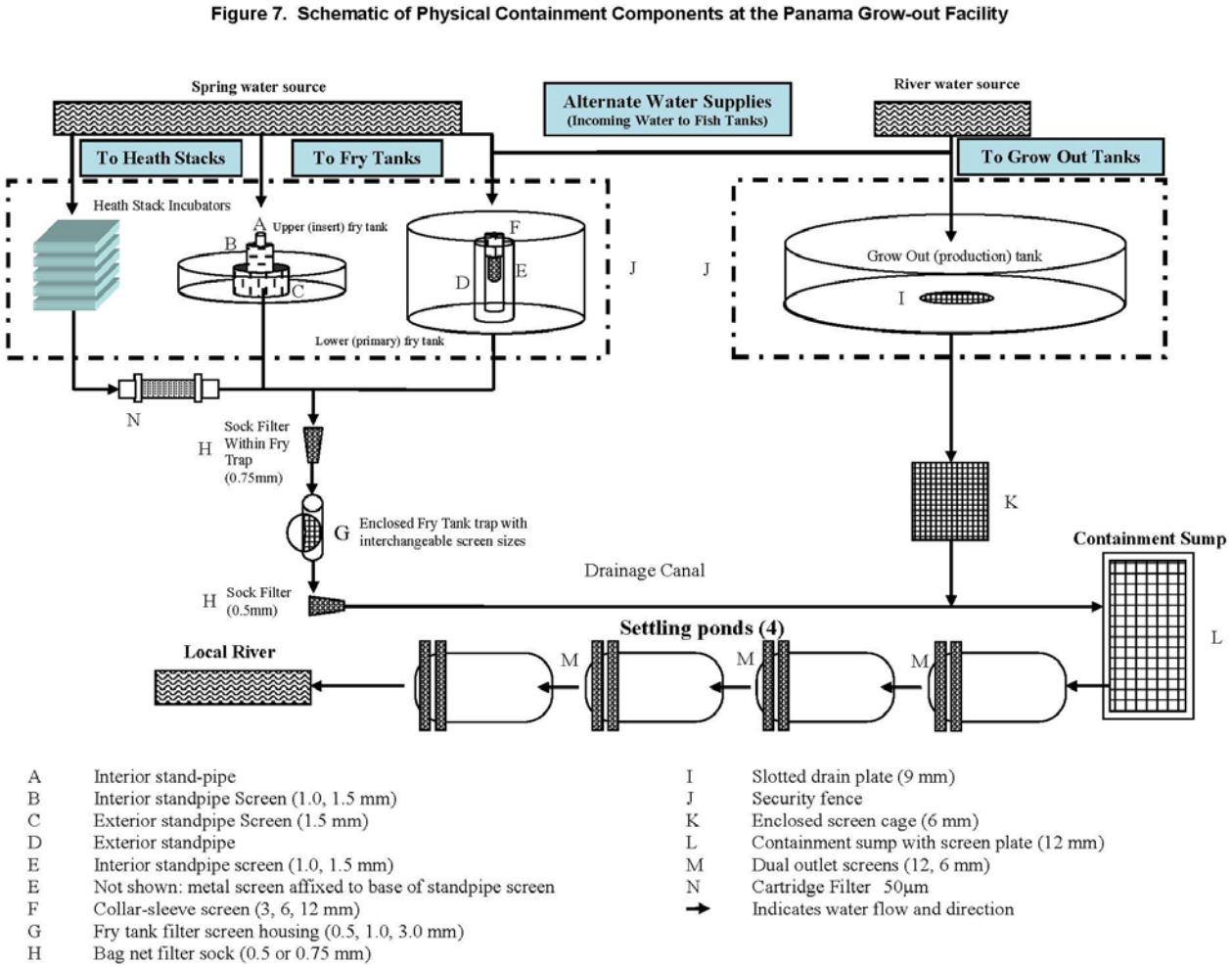


**Table 5. Containment Components and Level of Containment for the Panama Grow-Out Facility**

| Area | Containment level | Component | Details |
|---|---|---|---|
| Hatchery - Heath Stacks | 1 | Screened Trays | Each drawer is a 1.5 mm screened compartment (top and bottom). The water must pass through both of these screens at each level as it descends towards the drain. |
| | 2 | Cartridge Filter | All effluent is screened through an enclosed 50µm cartridge filter. |
| | 3 | Sock Filter | All effluent is filtered through a 0.75 mm sock filter. |
| | 4 | Fry Trap | All effluent is filtered through a 1.0 mm perforated PVC screen. |
| | 5 | Sock Filter | All effluent is filtered through a 0.5 mm sock filter. |
| Fry Tank Inserts | 1 | Exterior Standpipe Surround | When alevin are present in the fry tank inserts, this barrier is set up around the exterior standpipe, with 1.5 mm perforations. |
| | 2 | Exterior Standpipe | Water draining from the tank must pass through a 4” PVC pipe with 1 mm perforations. |
| | 3 | Sock Filter | All effluent is filtered through a 0.75 mm sock filter. |
| | 4 | Fry Trap | All effluent is filtered through a 1.0 mm perforated PVC screen. |
| | 5 | Sock Filter | All effluent is filtered through a 0.5 mm sock filter. |
| Fry Tanks | 1 | Interior standpipe Collar | Collar-sleeve (3, 6, or 12 mm mesh) on top of center standpipe to prevent fish from swimming into the standpipe. |
| | 2 | Interior Standpipe Filter | Interior standpipe filter constructed of 4'' PVC pie (1.0 mm slot openings). The bottom end is covered with metal screen screwed to the base of the standpipe filter (1.5 mm perforations), which prevents fish that may find their way into the interior standpipe from escaping the tank and entering the drain pipe. |

| Area | Containment level | Component | Details |
|---|---|---|---|
| | 3 | Sock Filter | All effluent is filtered through a 0.75 mm sock filter. |
| | 4 | Fry Trap | All effluent is filtered through a 1.0, 3.0 or 12.0 mm perforated PVC screen or metallic screen. Screen size is dependent of the size of the fish located in the fry tanks. |
| | 5 | Sock Filter | All effluent is filtered through a 0.5 mm sock filter. |
| | 6 | Containment Sump | Concrete containment sump, which receives and screens all drainage effluent from the fish tanks (fry tanks and growout tanks). A stainless steel screen with 12 mm perforations is in place. |
| | 7 | Settling Pond 1: Barrier 1 | Metal prescreen with 12 mm perforations. |
| Fry Tanks (*continued*) | 8 | Settling Pond 1: Barrier 2 | Metal screen with 6mm perforations |
| | 9 | Settling Pond 2: Barrier 1 | Metal prescreen with 12 mm perforations. |
| | 10 | Settling Pond 2: Barrier 2 | Metal screen with 6mm perforations |
| | 11 | Settling Pond 3: Barrier 1 | Metal prescreen with 12 mm perforations. |
| | 12 | Settling Pond 3: Barrier 2 | Metal screen with 6mm perforations |
| | 13 | Settling Pond 4: Barrier 1 | Metal prescreen with 12 mm perforations. |
| | 14 | Settling Pond 4: Barrier 2 | Metal screen with 6mm perforations |
| Grow Out Tanks | 1 | Drain Plate | FRP drain plate (with 0.9 cm slots) screwed to the floor at the center of each grow out tank. |

| Area | Containment level | Component | Details |
|---|---|---|---|
| | 2 | Containment Cage | Stainless steel mesh (6 mm) containment cage through which all drainage water from the growout tanks is filtered before entering primary drainage canal. The containment cage has a metallic lid which can be removed to inspect the interior of the cage. |
| | 3 | Containment Sump | Concrete containment sump, which receives and screens all drainage effluent from the fish tanks (fry tanks and growout tanks). A stainless steel screen with 12mm perforations is in place. |
| | 4 | Settling Pond 1: Barrier 1 | Metal prescreen with 12 mm perforations. |
| | 5 | Settling Pond 1: Barrier 2 | Metal screen with 6mm perforations |
| | 6 | Settling Pond 2: Barrier 1 | Metal prescreen with 12 mm perforations. |
| | 7 | Settling Pond 2: Barrier 2 | Metal screen with 6mm perforations |
| | 8 | Settling Pond 3: Barrier 1 | Metal prescreen with 12 mm perforations. |
| | 9 | Settling Pond 3: Barrier 2 | Metal screen with 6mm perforations |
| | 10 | Settling Pond 4: Barrier 1 | Metal prescreen with 12 mm perforations. |
| | 11 | Settling Pond 4: Barrier 2 | Metal screen with 6mm perforations |

### 5.5.3 Security at the Panama Grow-out Facility

As is the case for the PEI production site, there are multiple and redundant forms of security at the Panama grow-out facility to prevent unauthorized access and malicious activities. The facilities at this site are secured as follows:

- The site is located in a remote area with very limited access.
- Entry onto the site requires passage via a securely-gated footbridge.

- The perimeter of grow-out facility is surrounded by a security fence with barbed wire.
- Entrance gates to the fenced area are securely locked.
- The area is protected by guard dogs.

## 5.6 Labeling, Packaging, and Shipping

If approved, a condition that would be established in the application would be that the product being shipped from the production site on PEI to the Panama grow-out site would be limited to eyed-eggs, which are the life-stage most efficiently, effectively, and safely transported.

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

A bilingual (English and Spanish) Product Label printed on tear- and water-resistant paper would be affixed to both the egg crate and shipping container; this label shows the product name and provides information on the product identity, claim, limitations, warnings, and handling instructions of immediate importance to the end-user. A bilingual Package Insert comprising detailed handling recommendations and important information regarding performance, animal safety, and environmental considerations also would be included. The shipment would be identified as "Eggs & Fry[48]" that is "Not for Resale." The following additional warnings (or facsimile thereof) would also appear on the Product Label:

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

Product prepared for shipment would be transported by motor vehicle to a local international airport by ABT staff, where direct control would be assumed (through prior arrangement) by a freight-forwarder. The freight-forwarder would arrange, manage, and personally monitor air-freight shipment of the product to Panama (inclusive of permits & customs requirements), where control would be returned to ABT personnel waiting on the ground.

During handling, transport and opening, the container would be maintained in an upright position; and upon receipt, egg temperature would be determined to assess the need for

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

equilibration to the receiving temperature if the difference between the two exceeds 4 ºC. The equilibrated eggs would be held in fresh water at 2-8 ºC and ≥ 7 mg/L DO.

All tanks holding AquAdvantage Salmon at the Panama grow-out facility would be required to be marked with the product label.

## 5.7 Operational Plans and Procedures

Both the egg production and grow-out sites are managed according to established SOPs and protocols that cover day-to-day operations; in addition, a specific written plan describing operating systems and emergency procedures for addressing responses to loss of operational capacity (e.g., power loss, pump failure), breach of security, or catastrophic incident occurrence has been developed for the egg production facility on PEI. This *emergency procedures* manual provides information with regard to the following:

- Operational descriptions of systems-supplies for water, electricity, oxygen and security monitoring;
- On-call responsibilities and emergency responses to system-supply failures;
- Priority listings for fish inventory;
- Contact information for service providers;
- Training, certification and emergency response checklists; and
- Schematics of water supply and effluent systems.

For the grow-out facility in Panama, there are operational protocols in place for daily management of AquAdvantage Salmon (eggs, fry, and fingerlings), disposal of dead and discarded fish, and other routine activities. In addition, procedures are in place for a twice-daily inspection of critical containment barriers, and responding to emergencies (such as an interruption of the water supply). A contingency plan is also in place describing actions to be taken in the unlikely event of a fish escape.

# 6. ACCESSIBLE ENVIRONMENTS

In order to assess exposure pathways that could potentially lead to impacts on the environment of the United States, this section discusses the physical environments in the vicinity of the sponsor's egg-production and grow-out sites in PEI and Panama, respectively.

## 6.1 Site Characteristics of the Proposed Action (Preferred Alternative)

The accessible environments discussed in this EA are those surrounding the two facilities where AquAdvantage Salmon would be produced as eggs and grown to market size. Although specific effects on the local environments of Canada and Panama have not been evaluated in this EA, the egg production and grow-out facilities and the physical environments in the vicinity are considered as a potential source of exposure (i.e., an exposure pathway) for AquAdvantage Salmon or diploid ABT salmon to reach and impact the environment of the United States.

### 6.1.1 PEI Egg Production Site

Production of AquAdvantage Salmon eyed-eggs would only occur at the land-based, freshwater aquaculture facility located on the northeast side of Prince Edward Island near the Fortune River. This section discusses various aspects of the environment in which the facility is situated.

#### *6.1.1.1 Climate and Local Conditions*

The climate at the egg production facility is generally damp with an average yearly rainfall of 87 cm and an average yearly snowfall of 340 cm; average temperature is -7ºC in January and 19ºC in July. Climate data for the nearest PEI location with available data is shown in Table 6. Over the past 30 years, average daily minimum and maximum temperatures by-month have ranged from -12.4 to 13.8ºC and -3.3 to 23.2ºC, respectively.

**Table 6. Weather Data for the Egg Production Site Environment ***

| Month | Average Daily Min Temp (ºC) | Average Daily Max Temp (ºC) | Average Rainfall Amt (cm) | Average Rain Days |
|---|---|---|---|---|
| Jan | -12.6 | -3.3 | 10.6 | 18.8 |
| Feb | -12.4 | -3.3 | 8.6 | 16.1 |
| Mar | -7.1 | 0.9 | 9.2 | 16.0 |
| Apr | -1.4 | 6.7 | 8.8 | 15.4 |
| May | 4.0 | 14.1 | 9.8 | 14.7 |
| Jun | 9.6 | 19.6 | 9.3 | 12.8 |
| Jul | 13.8 | 23.2 | 8.6 | 12.4 |
| Aug | 13.5 | 22.6 | 8.7 | 11.3 |
| Sep | 9.1 | 18.0 | 9.5 | 13.7 |
| Oct | 3.8 | 11.8 | 10.9 | 15.0 |
| Nov | -1.1 | 5.7 | 11.1 | 17.5 |
| Dec | -8.1 | -0.1 | 12.3 | 20.6 |

**Amt**, amount; **Avg,** average**, Max**, maximum; **Min**, minimum. Values are based on monthly averages for the 30-year period 1971-2000. Mean number of rain days = mean number of days with at least 0.2 mm of precipitation (rain and/or snow).

During the spring, summer and fall, temperatures in the waters adjacent to the facility are suitable for salmon survival; however, water temperatures during the winter months are typically very low, with surface ice being common. The temperature of the local Fortune River estuarine waters ranges from -2 to 2ºC in the winter, with a typical ice cover of 0.3-0.6 m. The ice cover limits the growth of marine life by acting as a barrier to both oxygen and light. Salmon would tend to avoid these conditions by either (a) remaining in fresh water (i.e., rivers or lakes) where minimum water temperatures do not fall below 0°C, or (b) migrating offshore to ocean waters where such low temperatures and ice can be avoided. Consequently, local coastal conditions would be inhospitable to salmonids during the coldest periods of winter.

Salinity in the Fortune River estuary system adjacent to the PEI facility varies with the tide, distance from the outflow, and time of year. Despite these variations, the water remains quite saline, with common salinity values exceeding 21 ppt (and up to ~30 ppt). These salinity levels would preclude survival of all pre-smolt stages of Atlantic salmon.

#### *6.1.1.2 Occurrence of Natural Disasters*

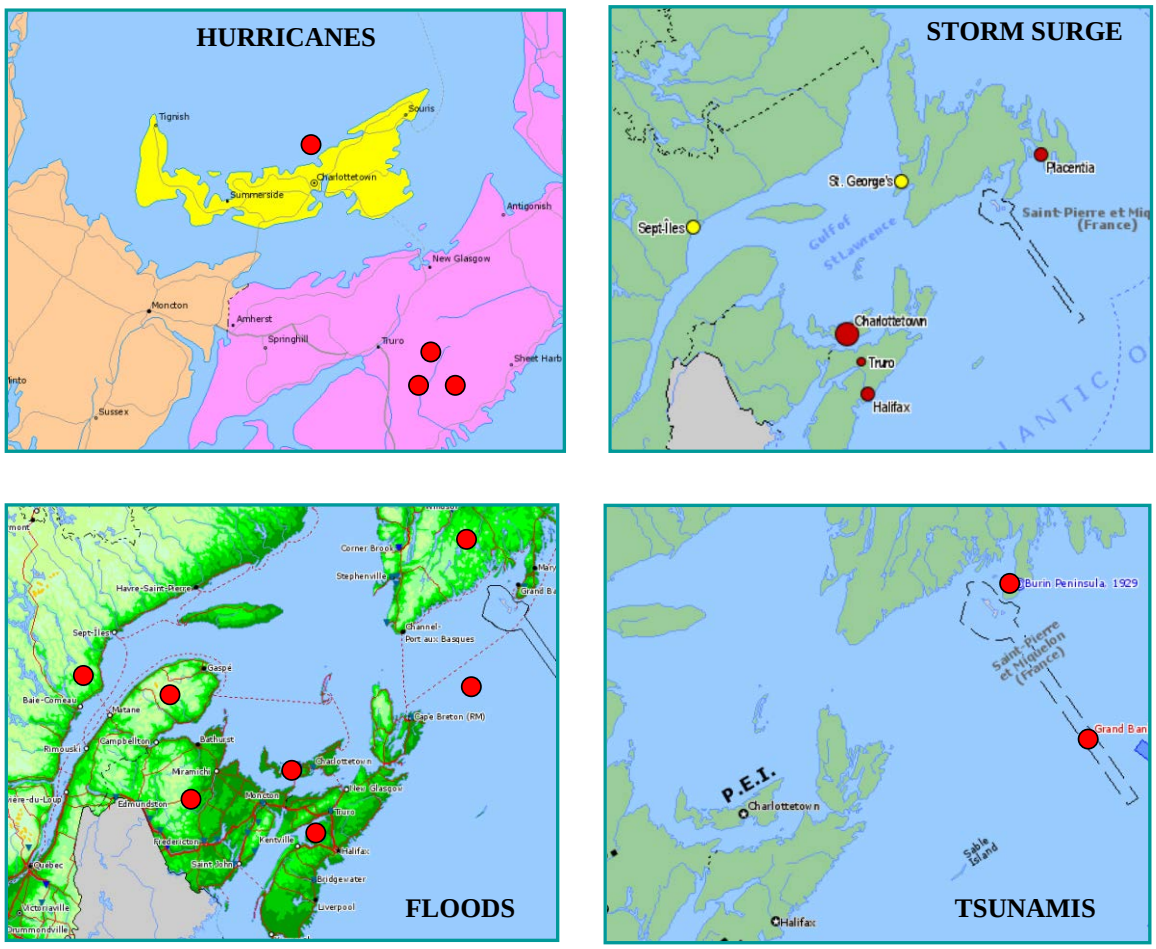
Although Prince Edward Island is frequently affected by outcomes such as power outages, rain and snow storms from December until April, it has rarely been subject to significant weather-related damage. As shown in Figure 8, Natural Resources Canada has reported that only four major hurricanes are reported to have occurred in the vicinity of PEI prior to 2000[49]. The winter of 2003-2004 was an exception: in September 2003, high winds (~90 mph) associated with Hurricane Juan devastated central Nova Scotia, killing eight people and causing an estimated $200 million (Canadian dollars) in losses that extended into Prince Edward Island; and, in February 2004, a blizzard nicknamed "White Juan" brought a record one-day snowfall of ~40 inches that briefly crippled the area. Neither of these events had an effect on the operations of the PEI facility.

Flooding and severe storm surges, on the other hand, occur with regularity in the vicinity of Charlottetown on the south side of PEI (the egg production facility is located on the northeast side). A storm surge of 3.6 m above the mean sea level occurs approximately once every 40 years in the southern Gulf of St. Lawrence (Lemmen *et al*., 2008). One with a height of 4.22 m above mean sea level was recorded at Charlottetown in January 2000. At the present rate of sea-level rise, by the year 2100 a storm surge of 3.6 m elevation above the present sea level would be expected to occur annually in the Gulf of St. Lawrence (Lemmen *et al*., 2008).

Two tsunamis have been reported east of Nova Scotia in the vicinity of southern Newfoundland and the Grand Banks, and one tornado has been reported in coastal New Brunswick northwest of Moncton (map not shown). No avalanches, earthquakes, forest fires, hailstorms, landslides, or volcanic eruptions have been reported for PEI or the Canadian Maritime Provinces.

[49] *See*, http://atlas.nrcan.gc.ca/site/english/maps/environment/naturalhazards for access to this and related weather-related history through 1999.

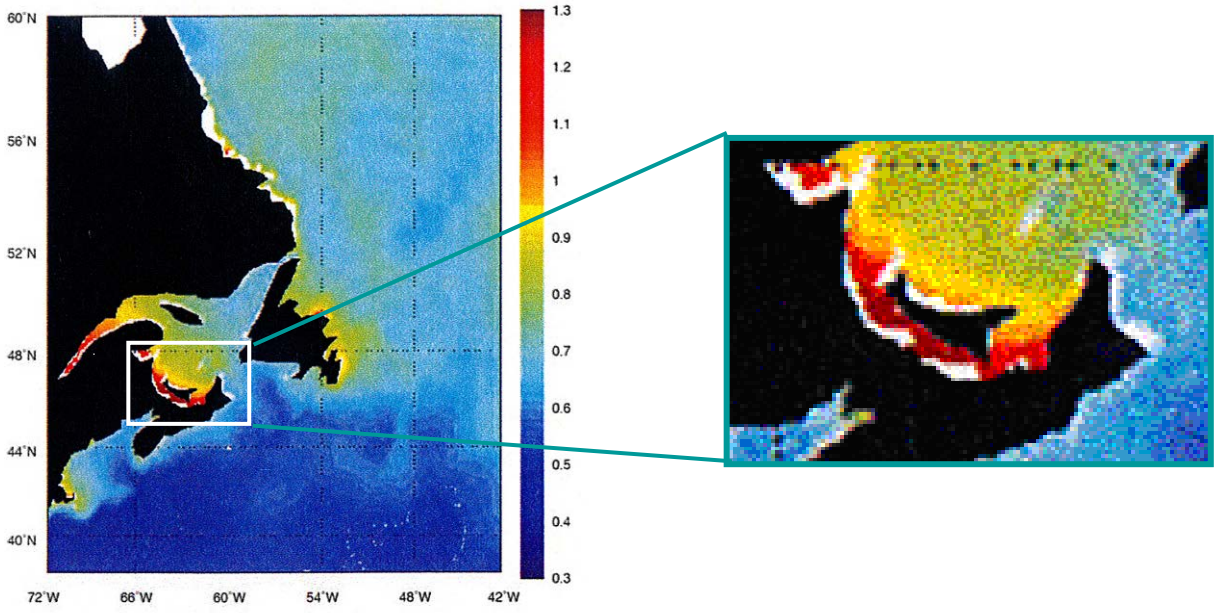
**Figure 8. Occurrence of Natural Hazards in Proximity to PEI ***




* With the exception of *Storm Surge*, where circle size is indicative of frequency (small, medium, large = low, medium, high) and circle color is indicative of severity (green, yellow, red = low, medium, high), all other circles are location indicators for single events reported by National Resources Canada through 1999. **Note:** The red dots indicating location of weather-related events have been significantly increased in size for ease of identification; their exact locations may differ slightly from those in the original graphic on the National Resources Canada website.

Storm surge and flooding in Charlottetown are expected to increase in both frequency and severity due to climate change and rising sea level over the coming decades (McCulloch *et al*., 2002). It is important to note, however, as shown in Figure 9, the south-facing shore of northeastern PEI (which includes the area where the PEI facility is located) is much less subject to these effects than are the southwest coast on the Northumberland Strait and the northwest coast on the Gulf of St. Lawrence (Lemmen *et al*., 2008).

**Figure 9. Variability of Storm Surge for the Atlantic Coast of Canada**




* Left-most figure of Atlantic Canada abstracted from Lemmen *et al*., 2008, p. 132.

#### *6.1.1.3 Biological/Ecological Properties*

The local environment near the ABT facility has numerous shallow bays, broad estuaries, and short rivers that contain an abundance of favorable habitat for diadromous fishes, those species that use both marine and freshwater habitats at some time during their lifecycle. Fish common to the area include the following: mackerel; herring; eel; gaspereau (e.g., alewife & blueback herring); silverside; smelt; and, salmonids. The salmonid fishes found on PEI include the following: Atlantic salmon (*S. salar*) and brook trout (*Salvelinus fontinalis*), which are native to the region; and, rainbow trout (*O. mykiss*), which were introduced into the region in 1925. Brown trout (*Salmo trutta*), though not native to North America but widely introduced into Canada and the United States, do not occur on PEI (DFO, undated)[50]. Commercially important crustaceans include lobster and snow crab; bivalves (e.g., mussels, oysters, soft-shelled & bar clams, quahogs) are also fished commercially.

Populations of Atlantic salmon are no longer found in the Fortune River, the estuary near the PEI facility. Although reported to occur there naturally in the late 1800s and stocked in this river periodically from 1907 to at least 1937, and perhaps later (Cairns *et al*., 2010), they disappeared at some point thereafter and were not present in surveys conducted there in the 1980s, in 2001, and in 2008 (Cairns *et al*., 2010; Guignion, 2009; Guignion *et al*., 2010). The Fortune River was once well known for it salmon run, but during the 1980s when

[50] Underwater World - Trout in Canada's Atlantic Provinces (accessible at http://www.dfo-mpo.gc.ca/science/ publications/uww-msm/articles/trout-truites-eng.htm). In addition, PEI is one of three Canadian provinces (along with Manitoba and the Northwest Territories) where brown trout are not found (Nova Scotia Fisheries and Aquaculture, Species Fact Sheet – Brown Trout; accessible at http://www.novascotia.ca/fish/sportfishing/species/brn.shtml)

more than 150 beaver dams were removed, no evidence of Atlantic salmon were found over three years of sampling (Guignion *et al*., 2010). Although present in 19 other streams on PEI, rainbow trout are also not reported to occur in the Fortune River, although brook trout are (Guignion *et al*., 2010).

With respect to Atlantic salmon, between 1971 and 1985, the estimated abundance of 1-SW fish in North America fluctuated between 0.8-1.7 MM fish annually; between 1995 and 2006, the estimated abundance declined to about 0.4-0.7 MM fish. When pronounced declines in abundance were observed in the 1980s, a wide range of management measures were introduced for conservation purposes. The closures of commercial fisheries, which began in 1972 in strategic intercepting and terminal fisheries, were expanded in 1984 to include all the commercial fisheries of the Canadian Maritime Provinces (which includes New Brunswick, Nova Scotia, and Prince Edward Island) and portions of Québec (DFO, 2009). Also in 1984, mandatory catch and release in the recreational fisheries of all large salmon was introduced in the Maritime Provinces and insular Newfoundland. Closure of all commercial fisheries for Atlantic salmon was expanded to all of eastern Canada in 2000. The most severe declines in Atlantic salmon abundance in Canada have been reported in the 32 rivers of the Inner Bay of Fundy, where Atlantic salmon have been designated as "endangered" by the Committee on the Status of Endangered Wildlife in Canada and listed under the Species at Risk Act. In PEI, Atlantic salmon populations are listed as "may be at risk" (Guignion *et al*., 2010). The factors contributing directly to reduced marine survival of Atlantic salmon remain largely unknown, although significant factors affecting survival in fresh water include acid rain, poaching, habitat alteration, and agricultural activities.

Over-exploitation, competition from non-native rainbow trout, and other factors have contributed to the elimination of natural Atlantic salmon runs in the environs of the PEI production site; however, the current primary limitations to population recovery are believed to be stream sedimentation caused by agriculture and other land-use activities and blockages from beaver dams (Cairns *et al*., 2010; Guignion, 2009). Man-made and beaver blockages in the Grovepine branch of the Fortune River have caused summer temperaures to exceed tolerable levels for salmonids and oxygen levels to likewise fall below minimum accepted concentrations (Guignion, 2009). As a result, water quality is compromised in much of the main branch of the Fortune River, down to the head of tide. Restocking and habitat enhancement in PEI streams and rivers have been attempted with limited success. As a practical matter, no wild salmon populations remain, and future returns of salmon to local rivers are dependent on hatchery stocking of smolts raised semi-naturally in open impoundments.

### 6.1.2 Panama Grow-Out Site

The land-based, grow-out site is located at high elevation in Panama adjacent to a river within a major watershed that flows from north to south into the Pacific Ocean. Dams associated with three operational hydro-electric facilities divert a significant portion of the aggregate water flow from the river for power generation, returning effluent to the watershed further downstream. During the 4-5 month dry season, up to 100% of the water flow in the river may be diverted for this purpose. Water diversion occurs through canals that provide a poor habitat for salmonids because of a low gradient and high sedimentation rate, which results in a poor bottom substrate and low food availability (see further discussion below). Four additional hydro-electric facilities are currently planned for the watershed. These existing (and planned) facilities, and the water diversion structures (i.e., dams & canals) associated with them, constitute a significant, but not complete, barrier to fish migration to the Pacific Ocean.

#### *6.1.2.1 Climate and Local Conditions*

Air and water temperatures were determined at a series of points along the course of the local river from its highland origins (Point 11) to its lowland return to the Pacific Ocean (Point 1) in September 2009. These values, which are shown in Table 7, vary little from month-to-month and are representative of year-round conditions.

**Table 7. Air and Water Temperatures in the River Adjacent to the Grow-Out Facility**

| **Point** | **Elevation** (m) | **Air Temp** (°C) | **Water Temp** (°C) |
|---|---|---|---|
| 1 | 13 | 28.9 | 26.4 |
| 2 | 91 | 31.9 | 28.1 |
| 3 | 250 | 29.4 | 26.0 |
| 4 | 347 | 28.6 | 25.8 |
| 5 | 649 | 24.3 | 22.6 |
| 6 | 995 | 21.6 | 19.3 |
| 7 | 1024 | 21.6 | 19.0 |
| 8 | 1086 | 21.7 | 20.7 |
| 9 | 1278 | 20.7 | 18.8 |
| 10 | 1792 | 17.2 | 15.1 |
| 11 | 1850 | 18.1 | 15.8 |

The watershed, and rivers and streams discharging into it, receive average-annual rainfall of 398 cm, 91.8% of which occurs during the rainy season. During the dry season, precipitation is markedly less, but streams and rivers do not go dry. Generation of hydro-electric power continues to dominate water use (> 93%), followed by agricultural and industrial demands. As shown in Table 8, average-monthly air temperatures at higher elevation in the watershed range from 16.8 to 19.6ºC over the course of the year (minimum: 11.6-14.8ºC; maximum: 23.3-28.8ºC; World Meteorological Society). Data collected over a period of nine years for the nearby region indicate that average-daily temperature ranges from only 17.6 to 20.6ºC regardless of the month of the year (data from www.worldclimate.com).

**Table 8. Weather Data in the Higher-Elevation Vicinity of the Grow-Out Facility***

| | **Jan** | **Feb** | **Mar** | **Apr** | **May** | **Jun** | **Jul** | **Aug** | **Sep** | **Oct** | **Nov** | **Dec** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Avg Temp (ºC) | 18. | 18. | 19. | 19. | 19.2 | 18.9 | 16.8 | 18.2 | 18.5 | 18.2 | 18.1 | 19.0 |
| Min Temp (ºC) | 11. | 13. | 13. | 14. | 13.1 | 14.0 | 13.8 | 14.2 | 13.9 | 14.3 | 13.6 | 12.4 |
| Max Temp (ºC) | 23. | 27. | 27. | 28. | 28.1 | 26.5 | 26.1 | 27.7 | 27.4 | 28.2 | 27.0 | 26.0 |
| **Humidity** (%) | 56. | 59. | 60. | 64. | 80.0 | 78.5 | 77.6 | 83.2 | 84.0 | 85.3 | 82.8 | 58.9 |
| Days with Rain | 1 | 5 | 4 | 9 | 21 | 17 | 24 | 30 | 24 | 25 | 18 | 10 |
| Days without Rain | 30 | 24 | 27 | 21 | 10 | 14 | 7 | 1 | 6 | 6 | 12 | 21 |
| Total Rain $mo^{-1}$ | 0.4 | 1.9 | 2.9 | 9.1 | 104. | 32.9 | 78.8 | 101. | 79.6 | 89.7 | 53.9 | 8.2 |
| Total Rain $yr^{-1}$ (cm) | 0.4 | 2.3 | 5.2 | 14. | 118. | 151. | 230. | 331. | 413. | 503. | 557. | 569. |

* Data from a private weather station in the immediate vicinity of the facility. Abbreviations: ***Avg***, average; ***d***, days; ***Max***, maximum; ***Min***, minimum.

Data recorded at two locations near sea level also show very little variation during the year. As shown in Table 9, average-monthly minimum and maximum daily temperatures ranged from 18.8 - 21.6ºC and 31.7 - 36.3ºC, respectively, over 30 years for which data are available.

**Table 9. Weather Data for the Near Sea-Level Locations***

| Month | Avg-Daily Min Temp (ºC) | Avg-Daily Max Temp (ºC) | Avg Rainall Amt (cm) | Avg Rain Days |
|---|---|---|---|---|
| Jan | 18.8 | 34.5 | 3.3 | 2.8 |
| Feb | 19.3 | 35.6 | 1.9 | 1.7 |
| Mar | 19.9 | 36.3 | 3.6 | 3.2 |
| Apr | 21.1 | 36.3 | 10.3 | 6.7 |
| May | 21.6 | 33.8 | 29.7 | 16.3 |
| Jun | 21.5 | 32.5 | 32.3 | 16.3 |
| Jul | 21.2 | 32.7 | 29.0 | 15.4 |
| Aug | 20.9 | 32.4 | 34.0 | 18.1 |
| Sep | 21.1 | 32.0 | 40.7 | 19.9 |
| Oct | 21.1 | 31.7 | 40.1 | 21.3 |
| Nov | 20.7 | 31.9 | 30.0 | 15.7 |
| Dec | 19.3 | 33.1 | 7.7 | 6.4 |

* Abbreviations: ***Amt***, amount; ***Avg***, average; ***Max***, maximum; ***Min***, minimum. Data are the aggregate monthly averages for the 30-year period from 1971 to 2000. Average number of rain days = average number of days with at least 0.1 mm of rainfall.

In addition to temperature, other physical and chemical parameters affect the likelihood of survival and propagation of fish and wildlife in the major rivers of the watershed. Values for these chemical and physical parameters are presented in Table 10.

**Table 10. Chemical & Physical Parameters in the Major Rivers of the Watershed***

| Parameter | Units | Upper | Mid-basin | Lower |
|---|---|---|---|---|
| Avg Annual Rainfall | (cm) | 300 | 300 | 600 |
| Avg Annual Rainfall Volume | ($m^3$) | 1.43 | 5.54 | 50.8 |
| Avg Water Temperature | (ºC) | 14-15 | 24.9 - 25.2 | 23.6 - 25.8 |
| Dissolved Oxygen Content | (mg/L) | 7.6 - 8.4 | 7.0 - 7.2 | 7.8 - 8.0 |
| Transported Sediment | (Ton/yr) | 1058 | *NA* | 116,000 |
| Turbidity | (NTU) | 1.6 - 23.0 | 1.4 - 4.0 | 1.4 - 6.0 |
| Total Solids | (mg/L) | 74.1 – 80.6 | 45.1 - 90.0 | 84.6 - 117.0 |

* Abbreviations: ***Avg***, average; ***NA***, not available; ***NTU***, nephelometric turbidity units.

The upper part of the local river has favorable conditions for establishing salmonid populations: temperature, DO, and turbidity are all within their tolerances. These conditions change in the mid- and lower-parts of the river where water temperatures exceed the upper lethal limit (~23ºC) that has been identified for Atlantic salmon (see Appendix A.3 and Section 7.3.1.2 for additional information on their temperature tolerance). High sedimentation loads downstream further diminish the quality of the local environment for salmon survival.

#### *6.1.2.2 Occurrence of Natural Disasters*

No substantial record of weather-related disasters is available for the grow-out site in Panama given that the area has been (and remains) largely uninhabited. Recent history, however, makes clear that the most likely threat derives from the risk of flooding. In that regard, Panama in its entirety experienced unprecedented rainfall in late November 2008 due to an unusual weather pattern. This rainfall produced a flood estimated as a 50- to 100-year event that occurred in the general area of the Panama grow-out site. This flood damaged, and in some cases destroyed, bridges, roads, and buildings within the general watershed. Because the grow-out facility is sited at a higher elevation than the associated flood plain and approximately five meters above the normal (non-flood stage) level of the river, no serious damage to the facility was produced by this event, and no problems of significance to aquaculture operations occurred. The flooded river did not come close to either the grow-out tanks or the fry building, and at its peak flood stage was still approximately 2 meters below the floor of the grow-out tanks. Notably, as described subsequently, there were no fish present in the facility at the time.

Prior to this flooding event, which had no significant effect on the Panama grow-out facility, another weather-related event in August 2009 resulted in the "loss" (death) of all AquAdvantage Salmon on site at the time due to asphyxiation (lack of oxygen). This event was caused when the water supply line to the facility was severed by a falling tree during a rain and wind storm, which stopped the flow of water, and thus dissolved oxygen, to the tanks. All fish, fingerlings at the time they died, were buried on site in a landfill in accordance with Panamanian regulations. A number of corrective measures were subsequently taken to ensure there would be no reoccurrence of this event or anything similar. Among others, these measures included (1) installation of redundant water supply piping; (2) installation of DO monitors, alarms, and backup oxygenation systems; (3) additional staff training; and (4) development of operational protocols for responding to emergencies and other events.

There is a potentially active volcano, Volcán Barú, in the general vicinity of the Panama grow-out facility, therefore, the possibility of an eruption and its effects on the facility have also been considered. Background information for this evaluation comes from a volcano-hazards assessment for Volcán Barú conducted by staff of the the U.S. Geological Survey and University of Panamá (Sherrod *et al*., 2008). The most recent eruption of Volcán Barú occurred 400-500 years ago, but analysis of samples using radiocarbon-dating indicates there have been several other eruptions during the past 1,600 years. It is likely that the volcano will erupt again, preceded by some premonitory period of seismic activity and subtle ground deformation that may last for day or months. In the past, eruptive episodes typically have included widespread tephra (fragmented material such as ash, cinders, or larger volcanic bombs or blocks that are released during an eruption) fallout, pyroclastic flows (a mixture of hot gasses and volcanic rock, up to 1,500°F, that behaves like a fluid staying close to the ground, and that can travel at speeds of up to 300 mph), and lahars (mudflows made up of volcanic debris that look and behave much like flowing concrete). Prehistoric eruptions have repeatedly spread tephra blankets more than 100 km downwind and deposited thicknesses of ash and cinders of 10 to 20 cm at distances 10 to 15 km downwind (to the west). Pyroclastic flows have been common during eruptions of the volcano. The type most common, block-and ash flows, has originated by the collapse of hot lava from the steep slopes of the summit lava dome. Block-and-ash flows of the past 1,600 years have descended westward from the summit area in a direction away from the ABT grow-out facility.

According to Sherrod *et al.* (2008), the hazard with the greatest threat to human life (and for damage to the grow-out facility) will be pyroclastic flows that accompany renewed dome growth during future eruptions of Volcán Barú. If eruptions are at the summit, the pyroclastic flows will move westward and away from the facility, owing to the configuration of the volcano's westward-opening amphitheater. If the summit dome were to erupt north or east of the summit, its products could overtop the amphitheater rim and conceivably reach the river valley where the grow-out facility is located. If this were the case, damage to the facility could be extensive, and the temperatures of the pyroclastic flows would likely incinerate all organic materials (including the fish in the grow-out facility) in its path.

Lahars are another potential volcanic hazard to the grow-out facility. The proximal lahar-hazard zone extends 2 to 7 km outward from the volcano summit, an area which does not include the ABT facility, but large debris avalanches and lahars can sometimes travel 10 to 20 km away from the volcano and flow over a much broader area that could potentially include the facility. Lahars, like floods, follow river valleys. Because many of the rivers in the vicinity of the volcano are contained within steep-sided canyons and valleys, the damage from any lahars that occur will generally be limited to the canyon or valley itself; however, because the ABT facility is located within one of these steep river valleys, it is possible the facility could be severely damaged or destroyed in the unlikely event that a lahar occurs in this area.

#### *6.1.2.3 Biological/Ecological Properties*

A diversity of macroinvertebrates exists in the local river, including mayflies, stoneflies, and other organisms that would be prey for salmon; these macroinvertebrates, however, are not abundant. Predators would include birds, especially kingfishers and herons, and mammals, especially nutria (*Myocastor coypus*), a large semi-aquatic rodent. There are few natural predatory fish in the area. Freshwater tarpon (*Tarpon prochilodus)* occur in the warmer waters of the lower basin. There may also be a population of rainbow trout (*Onchorhynchus mykiss*) that could prey on salmon due to introductions in the upper river basin. These rainbow trout were intentionally stocked in Panama in 1969, and are reported to constitute an established, naturally reproducing population (Welcomme, 1988); however, although reported to occur in this vicinity, their prevalence has not been well documented in the area near the PEI grow-out facility.

Except for rainbow trout, there are no known reports of other salmonid species, including brown trout (*Salmo trutta*), occurring in the local watershed, although brown trout have reportedly been introduced into Panama at some time in the past and have had reproducing populations there (Welcomme, 1988). Brown trout, although not native to Panama or the Americas, have been introduced widely throughout the world, and are closely related phylogenetically to the Atlantic salmon (they are both in the same genus). Viable hybrids can be produced if diploid brown trout and diploid Atlantic salmon interbreed, although the progeny of any backcrosses are expected to be non-viable or triploid (see discussion in Section 5.2.2.7). The same is not true for crosses between diploid Atlantic salmon and diploid rainbow trout; they are not expected to produce viable hybrids because although both of these species are salmonids, they are not in the same genus and are not closely related phylogenetically[51]. As a result, the hybrid offspring resulting from crosses of diploid

[51] Rainbow trout are currently classified in the genus *Oncorhynchus* (the same genus as Pacific salmon species), but were formerly in the genus *Salmo* (the same genus as Atlantic salmon and brown trout) before being reclassified in 1992.

Atlantic salmon and diploid rainbow trout either die before hatching or die shortly thereafter (Refstie and Gjedrem, 1975; Sutterlin *et al*., 1977; Blanc and Chevassus, 1979; Blanc and Chevassus, 1982; Gray *et al*., 1993).

In the upper-basin, vegetation on the river banks is scarce, and the substrate tends to consist of medium to very large round stones, rocks, and boulders due to the high gradient and flow conditions.

The natural physiography of the river basin reflects the high volume of water that flows through it during the rainy season; there are no areas of waterfalls or natural barriers to fish passage. The river has been, and will continue to be, used for hydro-electric energy generation. Although fish can navigate the upper part of the river, a large dam presents an obstacle to fish passage, especially during the dry season.

In summary, although conditions in the immediate vicinity of the grow-out facility could potentially support all life stages of salmonids, physical barriers, sub-optimal habitat, and lethally-high water temperatures likely would prevent the long-term survival and establishment of Atlantic salmon in the river downstream of the facility.

## 6.2 Site Characteristics of the No Action Alternative

There are two general likely scenarios to consider as a consequence of the no action alternative (decision not to approve the NADA for AquAdvantage Salmon): (1) cessation of activities on the part of the sponsor resulting in no production of AquAdvantage Salmon anywhere, and (2) continued production of AquAdvantage Salmon at the existing locations in Canada and Panama and/or at new suitable locations outside of the United States (or sale of the fish or the technology to producers outside the United States) with no intent to directly market food from the fish in the United States. We have not attempted to assign relative probabilities to either scenario.

The first scenario presented in the no action alternative, termination of the production of AquAdvantage Salmon, by definition would yield no sites of production and no effects on environments in the United States.

For the second scenario, production of AquAdvantage Salmon at locations outside the United States for marketing outside the United States (i.e., outside the jurisdiction of FDA), a large number of production sites could be envisioned, depending on market conditions, the economics of production and other factors. These sites could be distributed to widely dispersed locations with highly variable physical, biological and chemical characteristics, be few in number, close together, with similar characteristics, or possess any combination of these characteristics. They could range from ocean net pens to highly-contained, land-based systems identical to the ones described in the AquAdvantage Salmon NADA. Production could occur at freshwater and marine sites located around the world as long as ambient water quality (e.g., dissolved oxygen levels) and water temperature conditions were suitable for survival and growth of Atlantic salmon (see Appendix A and Section 5.2.2.2), or where these conditions can be controlled to be within a suitable range using appropriate technology (e.g., water chillers and aerators). Locations could include those in which Atlantic salmon are native (e.g., Canada, Scandinavia, and northern Europe) and/or those where they are not (e.g., Chile, Australia, New Zealand). In addition, the locations could also include those in which Atlantic salmon are not normally reared (e.g., tropical highland locations with sufficient coldwater) and/or at inland or indoor locations using freshwater recirculation systems in which production of non-GE salmon would be at an economic

disadvantage compared to traditional marine grow-out locations where net pens and cages are used.

# 7. ENVIRONMENTAL CONSEQUENCES

This section discusses the potential effects of the proposed action, including potential effects on populations of Atlantic salmon that are listed as endangered in the State of Maine. It includes potential effects on the United States of the proposed action as well as the no action alternative.[52]

It is important to note that the FDA action is limited to an approval for a specific set of conditions of use. As previously stated, any modifications that the sponsor may propose to the conditions established in this application, should it be approved, would require notification to FDA. Major and moderate changes would require the filing and review of a supplemental NADA. Approvals of such supplemental applications would constitute agency actions and trigger additional environmental analyses under NEPA.

## 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) must be in place at the two facilities to be used for the production and grow-out of AquAdvantage Salmon, the analyses of the effects focus 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 United States. This and additional information on the accessible environments (Section 6) are used to determine whether there are complete exposure pathways from the sites of egg production and grow-out to the United States.

Information included in this evaluation comes from the sponsor, the 2008 and 2012 FDA inspections of the PEI egg production facility (Appendix F), the FDA site visit to the Panama grow-out facility (Appendix F), and the sponsor's study report on the induction of triploidy. Subsequently, the risk-related questions identified earlier in Section 3.2 are addressed to evaluate the potential for significant environmental effects to occur in the United States as a result of an NADA approval of AquAdvantage Salmon under the specified conditions of use (i.e., breeding and egg production in PEI, grow-out in Panama). Similar analyses are conducted for the no action alternative considered.

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

### 7.2.1 Proposed Action (Preferred Alternative)

As discussed in Section 3, the likelihood of escape would depend primarily on the extent and adequacy of physical (mechanical) containment at each facility. GE fish are considered to pose little risk to native populations if they are adequately contained (Mair *et al*., 2007;

[52] For the purposes of this environmental assessment, although AquAdvantage Salmon that will provide food for export into the United States is an all-female, triploid fish from the EO-1α lineage, this EA encompasses risks associated with all other lifestages (i.e., gametes through adults), and all of the zygosities and ploidies associated genotypes and phenotypes (i.e., diploids, triploids, hemizygotes, homozygotes females and masculinized females) that are required for the production of the triploid, all-female AquAdvantage salmon to be used for food. In general, when it is important for the purposes of assessing a specific environmental risk, we specify whether an animal is assumed to be reproductively competent, the term "diploid ABT salmon" is used.

Wong and Van Eenennaam, 2008). Confinement of GE fish in closed, land-based facilities is considered optimal in order to ensure an acceptably low risk of escape (Mair *et al*., 2007). Such is the case for both the AquAdvantage Salmon egg production facility and the grow-out facility. As a result of multiple and redundant forms of effective physical confinement at both facilities, FDA concludes that the likelihood of escape of any life stage of AquAdvantage Salmon or diploid ABT salmon is extremely low. This conclusion is in agreement with a DFO risk assessment based in part on a Failure Mode Analysis, in which DFO concluded that the potential for an acute or chronic failure of physical containment at the PEI facility is negligible (see Section 2.5.1). The following discussion provides the reasoning for FDA's conclusion.

Physical containment for egg production and grow-out is described in Sections 5.4.2 and 5.5.1 of this EA, respectively. Key components of physical containment for the PEI facility are identified and illustrated in Tables 2 and 3 and Figure 6, and in Tables 4 and 5 and Figure 7 for the Panama facility. In addition, Section 2.6 describes the redundant, multi-level strategy used to ensure containment at both facilities. Several SOPs are in place at the PEI facility to help ensure containment, including an SOP that addresses physical containment of GE salmonids, which describes the containment components present in the PEI facility, documents procedures for ensuring containment, and contains forms for documentation of daily and annual inspections of containment equipment. There are also SOPs in place to address biosecurity, disinfection, decontamination, and other disease-related issues. Operational protocols and procedures are also in place in Panama for inspections of critical containment barriers, which are conducted twice daily, and for responding to emergencies (such as an interruption of the water supply). There is also a contingency plan in place to address the unlikely possibility of a fish escape.

For AquAdvantage Salmon, both the production of eyed-eggs and the grow-out of the fish would be conducted in land-based facilities with redundant containment measures, with point-to-point control of shipping and land-based materials transfer. These measures have been described in detail in Sections 5.4 and 5.5; additional information and discussion is provided below for each facility and location. Measures would include 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; passive and active measures to insure physical security; redundant back-up power generation; and the historical absence of natural disasters that could render these measures ineffective.

#### *7.2.1.1 Egg Production (Prince Edward Island Facility)*

##### 7.2.1.1.1 Physical Containment at the PEI Facility

Physical containment at the PEI facility is described in detail in Section 5.4.1, Tables 2 and 3, and Figure 6. The adequacy of physical containment at the sponsor's PEI facility was initially addressed in FDA's evaluation of the sponsor's 2001 EA (prepared in support of investigational studies related to AquAdvantage Salmon), and subsequently in the FDA facility site inspections conducted in October 2008 and June 2012 (see descriptions below and in Appendix F). All areas of the PEI facility have at least four independent forms of physical or mechanical containment. The areas of highest concern with respect to potential escape (i.e., egg incubation units and fry rearing tanks) have five or six separate, independent forms of physical containment.

Currently, most eggs at the PEI facility are being incubated using Heath stack incubators. When scaled up production is needed, egg incubation takes place in large (23 L) upwelling

chambers instead of (or in addition to) the Heath stack incubators. The physical containment conditions for these upwelling units are equivalent to, or exceed, physical containment conditions currently in place for egg incubation (see Section 5.4.1, Table 3, and Figure 6).

#### 7.2.1.1.2 FDA 2008 Inspection of PEI Broodstock and Hatchery Facility

FDA conducted an inspection of the sponsor's PEI broodstock and hatchery facility from October 7 - 9, 2008 as a limited directed inspection under Compliance Program Guidance Manual (CPGM) 7368.001 (a pre-approval inspection conducted as part of the NADA process). The FDA inspector was accompanied by three experts in aquaculture or biotechnology from CVM. The facility was found to be in compliance with FDA regulations; no Form FDA 483[53] was issued at the conclusion of the inspection.

*Background*

An EA was submitted by the sponsor in December 2001 to address potential environmental effects as a result of investigational activities related to AquAdvantage Salmon at the PEI facility. This EA resulted in preparation of a FONSI by FDA for investigational studies under the investigational new animal drug (INAD) file. Section 4.0 of the 2001 EA described the various passive and active forms of containment present at the sponsor's Canadian facility in PEI, Canada. Passive containment includes physical-biological containment afforded by the surrounding environment (e.g., temperature, salinity, predators), while active containment describes the presence of physical barriers in the facility design (e.g., screens, nets) to prevent the escape or accidental release of fish and fish eggs to the outside environment.

Appendix IV of the 2001 EA contained SOPs in place at the facility relating to secure containment. The most relevant of the SOPs addressed physical containment of GE salmonids. It contained a schematic figure of the containment equipment in place in the facility's early rearing annex and grow out area, and the associated key to the components in the figure. The containment level (i.e., primary, secondary, etc.) for each component was described. According to the figure and key, all areas of the sponsor's facility have at least three independent forms of mechanical containment and some areas, including the egg incubation units and their discharges, have as many as four.

*Actions and Findings*

During the 2008 inspection, FDA requested the most recent version of the sponsor's SOP addressing physical containment at the facility. Review of this SOP and the schematic therein reflected physical additions and modifications made to the facility several years prior to the inspection, including enlargement of the early rearing area and changes in the sizes, shapes, and arrangement of tanks in certain parts of the facility. At the time, all areas of the facility were found to have at least two independent levels of containment and some had three or four[54]. Components shown and described in the SOP that provide containment include the following:

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

[54] The inspection report reported a minimum of two forms of mechanical containment, but counted the primary and secondary screens in the effluent containment sump as only one form. Here these two

**Early Rearing Area**

- Screened trays (egg incubators)
- PVC screening
- Catchment box screening
- Sock filters on drainage pipes
- Containment sump with stainless steel perforated basket filters/screens
- Floor drain covers
- 60 micron drum filter and septic tank for solids removal
- Tank covers, slotted stand pipes, and overflow screens

**Grow-Out Area**

- External standpipe screens
- Standpipe covers
- Top nets or surround nets for each tank
- Floor drain covers (perforated steel plate; 1.5 or 7.0 mm)
- Chlorine puck in floor drain sump (during spawning of fish)
- Effluent containment sump with primary and secondary screening

The types and general locations of the containment components described in the SOP were verified by visual observations during the inspection of the PEI facility. Photographs were also taken of many of the key components. A detailed piping and instrument drawing was not available for the water/wastewater distribution system; therefore, it was not possible to verify the specific location and presence of each piece of equipment with a containment function. However, all components of the containment system that were observed during the inspection appeared to be in good operational condition and functioning as designed. Records that the sponsor maintained relative to inspection of hatchery effluent screens and containment equipment indicated that these components were being inspected internally by the sponsor on a regular basis, usually at least once per day.

The Canadian governmental authorities charged with responsibility for the regulatory oversight of the research and development and the commercial deployment of transgenic aquatic organisms are Environment Canada (EC) and DFO. Inspections of the PEI facility by DFO occurred in 1996 and 2001. Reports from both DFO inspections found the facility "is as 'escape-proof' as one can reasonable expect." (Appendix F) Regulatory oversight for containment is now under the oversight of EC. Both DFO and EC inspected the PEI facility in 2013 in conjunction with the DFO environmental risk assessment for AquAdvantage Salmon and the regulatory decision by EC to publish a Significant New Activity Notice for AquAdvantage Salmon in November 2013 (see Section 2.5.1).

As a result of the 2008 FDA inspection, no concerns or issues for follow-up or correction were identified. Since then, additional containment and isolation measures have been implemented by the sponsor at the PEI facility, and are reflected in Figure 6. A key upgrade is the addition of a third stainless steel containment screen in the facility's external containment sump, through which all of the facility's effluent must flow before being discharged. With this additional screen in place, all areas of the facility have at least four independent, sequential forms of physical containment on their water systems, while many

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stainless steel screens are considered to be independent forms of containment as they are physically distinct. More recently, a third stainless steel screen has been added to the effluent containment sump (Figure 6), adding another independent level of containment.

areas, including the egg incubation units and their discharges, have five or more forms of containment in series before the water is discharged.

#### 7.2.1.1.3 FDA 2012 Inspection of PEI Broodstock and Hatchery Facility

FDA conducted a follow-up inspection of the PEI facility in June of 2012 with two primary goals:

(1) to examine facility records, SOPs, and responses in relation to an outbreak of ISA that occurred in the fall of 2009 after the previous facility inspection was conducted, and

(2) to examine and evaluate the effectiveness of physical containment equipment and operational procedures within the PEI facility to prevent the escape of AquAdvantage Salmon[55] eggs, fry, juveniles and adults.

As a result of the inspection, FDA concluded that (1) the results of the diagnostic evaluations are consistent with the detection of ISAV; (2) appropriate biosecurity measures were taken in response to the outbreak, including installation of UV and ozone water treatment systems; and (3) effective and redundant physical containment equipment are present within the facility to prevent the escape of Atlantic salmon eggs, fry, juveniles and adults. The agency also confirmed that an additional stainless steel filter screen was added in the exterior containment sump (to supplement the two existing stainless steel screens) to ensure there are always at least two forms of physical containment in this sump in the event that one of the other filter screens needs to be removed for cleaning.

The FDA inspector was accompanied by experts in aquaculture and biotechnology from CVM. The facility was found to be in compliance with FDA regulations, and no Form FDA 483 was issued at the conclusion of the inspection. See Appendix F for further details.

#### 7.2.1.1.4 Issues Affecting Containment and Security

*Natural Disasters*

In some cases, containment may be adversely affected by natural disasters such as floods, storms, earthquakes, etc.; therefore, it is important to consider the potential for these events to occur and take them into account when locating and designing facilities for GE fish. Information on the potential occurrence of natural disasters (e.g., hurricanes, storm surge, floods, tsunamis, and tornados) in the vicinity of PEI was presented in Section 6.1.1.2. Based on past history, these are all rare or extremely rare events. Storm surges and flooding have been reported elsewhere on PEI, particularly in the vicinity of Charlottetown, but flooding has not been an issue in the specific area where the egg production facility is located on the northeast side of the island. This facility is situated approximately 25 feet above sea level at its highest point and sits approximately 120 feet inland from a tidal river/estuary.

It is highly unlikely that storm- or hurricane-induced surges or tidal waves would directly impact the PEI facility or subject it to flooding as there are rip-rap (rock) barriers across much of the river mouth at its confluence with the Gulf, which is approximately one mile away. Even in the remote event that flooding did occur in the area, all of the fish tanks at

[55] Although the inspection report does not say so explicitly, this examination and evaluation also included diploid ABT salmon.

the PEI facility are located indoors, are raised, and have top netting, which would further preclude the escape of fish. The conditions at the PEI site are in general conformance with recommendations in the ABRAC Performance Standards for research facilities holding GE fish and shellfish. Flooding at the site is not expected to be an issue because of the facility's location.

*Physical Security*

Physical security measures and equipment are important 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 GE fish offsite for outdoors projects (ABRAC, 1995). In addition to physical security, there may also be the need for alarms, stand-by power, and an operational plan that addresses training, traffic control, record keeping, and an emergency response plan.

The physical security measures in place at the PEI facility are extensive (see Section 5.4.2) and were verified by FDA during the two PEI facility inspections and/or through subsequent submissions from the sponsor. Measures include perimeter fencing, remote monitoring systems (surveillance cameras), redundant locking systems, etc. These security measures are believed to be adequate to address the concerns listed above with respect to unauthorized entry; access by predators is not an issue at this facility as it is totally enclosed. The sponsor is aware that unauthorized access to these sites may represent a potential hazard and has taken appropriate steps to reduce the possibility this will occur. In addition to the physical security measures, the sponsor has a written operational plan and SOPs in place at the PEI facility to address containment failure and security issues. Employees at the facility undergo training and the facility is subject to periodic audits by Canadian authorities.

*Malicious Intentional Release*

Given the redundancy in physical containment measures and the low probability of occurrence of natural disasters in the area, perhaps the most likely event leading to introduction of AquAdvantage Salmon or diploid ABT salmon to the environment surrounding the PEI facility would be an intentional malicious release. The probability of such an occurrence is low, however, as described in Section 5.4.1 and above, as there are extensive security measures, equipment, and contingency plans in place to limit unauthorized access.

#### 7.2.1.1.5 Conclusions for the PEI Facility

The probability that any life stages of AquAdvantage Salmon and diploid ABT salmon would escape from the PEI egg production facility is extremely small due to the presence of multiple, independent, and redundant forms of physical (mechanical) containment. This containment has been evaluated and verified through inspections by FDA and Canadian authorities, including a Failure Mode Analysis conducted by DFO, which led to the conclusion that the potential for acute or chronic failure of physical containment was negligible. FDA concludes that physical security and containment is sufficient to ensure that it is highly unlikely there would be any unintentional releases of AquAdvantage Salmon or diploid ABT salmon due to equipment failures, natural disasters or malicious activities at the PEI facility. This facility is subject to future inspections by FDA and Canadian authorities.

### *7.2.1.2 Fish Grow-out (Panama Facility)*

#### 7.2.1.2.1 Physical Containment at the Panama Facility

Physical containment at the Panama facility is described in detail in Section 5.5.1, Tables 4 and 5, and Figure 7. The Panama grow-out facility includes small tanks for rearing fry and juveniles, plus large tanks for growing fish to market size (see Figure 7). The fry tanks contain either interior or exterior stand pipes, plus a series of two to three mechanical fine mesh screens (1 – 1.5 mm for small fry; 3 – 12 mm for larger fry and juveniles) made of metal to prevent fish from escaping. In addition, all water from these tanks must pass through two sock filters (one 0.75 mm; the other 0.5 mm) and a fry trap prior to entering a drainage canal that collects all water from the facility and sends it to a series of four settling ponds (and from there to a nearby river). Thus, at a minimum, five levels of physical containment are present for these early life stages of AquAdvantage Salmon and diploid ABT.

Grow-out (production) tanks have external stand pipes (to control the water height) and drain water through a slotted (0.9 cm), rigid PVC drainage plate in the tank bottom. The drainage plate and slots serve as the initial and primary form of physical containment for the fish in these tanks.

Water from the grow-out tanks is routed to a drainage canal that also collects water from the fry tanks and other facility operations. There is an additional mechanical (12 mm) screen within a concrete containment sump that filters water from the drainage canal prior to it entering the series of four settling ponds. Each of the four ponds has two rigid metal screens on its outlet, one with 12 mm perforations and one with 6 mm perforations. These larger screens act as effective barriers to larger fry, juveniles and adults, but are not expected to preclude passage of small fry (or eggs). Taken as a whole, conservatively counting the series of four settling ponds with duplicate screens as only a single form of containment, there are four independent forms of physical containment applicable to fish reared in the grow-out tanks.

Although not present at the time of the site visit in November 2009 (see below), Heath stack egg incubation units have subsequently been added within the Panama facility. The physical containment conditions for the incubation units is similar to, and no less effective than, those currently in place for egg incubation at the PEI facility, offering a minimum of five independent levels of containment, see Section 5.5.1, Table 5, and Figure 7.

Additional containment in the form of tank netting and chain link security fencing is present to limit access by potential predators and unauthorized personnel.

#### 7.2.1.2.2 Site Visit of the Panama Grow-Out Facility

From November 10 to 12, 2009, a site visit of the sponsor's grow-out facility in Panama was conducted by two FDA experts in aquaculture and biotechnology, accompanied by a fisheries scientist from NMFS. This site visit was conducted primarily to verify that the conditions of rearing and containment at the grow-out facility were as described in the sponsor's submissions, and to evaluate any other factors that could influence the potential for escape. A secondary objective of the visit was to observe and gain information on the local environment, including portions of the river adjacent to and downstream of the grow-out facility, to help ascertain whether AquAdvantage Salmon would be likely to survive and establish in the unlikely event of an escape from the grow-out facility.

Based on observations made and information gathered during the site visit, the descriptions and schematics provided by the sponsor on the Panama grow-out facility, the river and

surrounding environment were accurately represented. There are a minimum of four or five levels of containment between the fry tanks and grow-out tanks and the river, respectively. This includes a very conservative counting of the series of four downstream settling ponds (each with two outlet screens) as only a single level of containment, rather than eight.

Visual observations of the river adjacent to the sponsor's grow-out facility indicate a very high gradient profile with high current velocity and substrate consisting predominately of large rocks and boulders. Except in terms of water temperature, the river habitat in the vicinity of the sponsor's facility does not appear to be favorable to Atlantic salmon, or most other fish species for that matter, although it would not necessarily preclude survival and possibly establishment (if the salmon were reproductively competent). Although populations of rainbow trout have been reported to inhabit the river as a result of intentional stocking by the Panamanian government as far back as 1969 (see Section 6.1.2.3), the abundance of these trout has not been well documented, and none were observed by the visiting U.S. government staff during the site visit.

#### 7.2.1.2.3 Issues Affecting Containment and Security

*Natural Disasters*

The grow-out facility in Panama is potentially subject to flooding conditions from a nearby river. The area receives a significant amount of annual rainfall, approximately 570 cm or 224 inches per year (Table 7), with much of it coming in the wet summer months. There was a significant flood of the river in November 2008 that caused extensive damage at locations downstream of the grow-out facility. The facility itself, however, was not directly affected by flood waters and sustained no serious damage (see Section 6.1.2.2). The only incidental damage was sustained as a result of debris that clogged the metal intake screens filtering water from the river as it enters the concrete water distribution canal. Several months prior to this during a storm, the main water line to the facility was severed resulting in the death of all fish in the facility, but no escapes (Section 6.1.2.2). In the time since these incidents occurred, redundant intake piping has been added, and many of the pipes have been moved underground, to prevent any future occurrences. Considering that the 2008 flooding was among the worst to ever occur in the area, it seems improbable that the grow-out facility would be impacted by future events of this type in a manner that could cause accidental release of GE fish. In addition, all tanks in the facility have appropriately sized top netting to prevent fish escape in the unlikely event that flooding would occur on the grounds of the facility.

Damage to the Panama grow-out facility cannot be ruled out in the unlikely event of a volcanic eruption (see Section 6.1.2.2); however, the facility is not located in a high risk area for either pyroclastic flows or lahars, and any eruption is expected to be preceded well in advance by seismic activity and other warning signs. If the facility were to be impacted by either a pyroclastic flow or lahar, because it is located in a river canyon, it would likely be severely damaged or destroyed, most likely resulting in the release of AquAdvantage Salmon. Survival of any released fish under these conditions is highly unlikely due to either extreme temperatures (temperatures in pyroclastic flows and surges commonly exceed several hundred degrees Celsius) or impact and burial (lahars can travel many tens of kilometers downvalley at speeds of tens of kilometers per hour and typically leave behind deposits of muddy sand and gravel several meters or more in thickness).

*Physical Security*

The ABRAC Performance Standards 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 offsite for outdoors 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 with respect to physical security measures at the Panama grow-out facility has been described in Section 5.5.2. Measures include a remote location, restricted entry to the site, security fencing, guard dogs, and local surveillance. Access by predators is controlled by fencing, top nets on all tanks, and heavy duty stainless steel screening on tank effluents. Based on the information provided by the sponsor, as well as observations made by FDA personnel during the Panama facility site visit, the physical security measures in place appear to be adequate to address the concerns listed in the ABRAC Performance Standards.

As is the case for the PEI facility, in addition to the physical security measures, the sponsor has operational protocols in place at the Panama facility to address containment failure and security issues. Employees at the Panama facility currently undergo training and the facility is subject to periodic audits by Panamanian authorities (See Appendix G) and would be subject to continued inspections by FDA.

*Malicious Intentional Release*

Given the redundancy in physical containment measures and the low probability of occurrence of natural disasters in the area, the most likely event leading to introduction of AquAdvantage Salmon to the environment surrounding the Panama facility would be an intentional malicious release. The sponsor is aware that unauthorized access to these sites may represent a potential hazard and has taken appropriate steps to reduce the possibility this will occur. As described in Section 5.5.2 and above, there are extensive security measures, equipment, and plans in place to ensure that the probability of occurrence of such an event would be extremely low.

#### 7.2.1.2.4 Conclusions for the Panama Facility

The probability that AquAdvantage Salmon would escape from the Panama grow-out facility is extremely small due to the presence of multiple, independent forms of physical (mechanical) containment. This containment has been verified by FDA through a site visit. The facility is also subject to regulatory oversight by Panamanian authorities. Physical security and containment is adequate and acceptable to ensure that it is highly unlikely there would be any unintentional escapes or releases of AquAdvantage Salmon due to equipment failures, natural disasters or malicious activities.

### *7.2.1.3 Transportation of Eggs from PEI to Panama*

Section 5.6 briefly describes shipping from Canada to Panama as occurring via air freight with subsequent ground-shipment to the grow-out facility. Notably, due to the biology of Atlantic salmon reproduction, eggs are only expected to be available for shipment during limited seasons of the year. Furthermore, due to the modest size of the Panama grow-out facility, only a very small number of shipments are expected annually. 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.

#### *7.2.1.4 Disposal of Fish and Fish Wastes*

Disposal of ABT salmon (including non-viable eggs, mortalities, and culls) and the non-viable waste material associated with the production, processing, and consumption of AquAdvantage Salmon (e.g., feces, fish pieces) would not require handling that is different from that used for wild or domesticated non-GE 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.
In PEI, mortalities and culls requiring disposal will be stored frozen until they are incinerated offsite in a local facility. In Panama, fish mortalities will be deposited in 1-m deep, on-site burial pits that are located no nearer than 25 m from the local river. Each pit will be filled with alternating layers of dead fish and quicklime until buried fish are within 0.2 – 0.3 m of the soil surface, at which point, the top layer of fish will be covered with quicklime and a compacted layer of topsoil.

Fish wastes (biosolids) from the PEI facility are subject to extensive treatment prior to discharge to the local estuary. In Panama, biosolids from the grow-out tanks will be removed from the facility's effluent in a series of four sedimentation ponds prior to discharge of this effluent to a nearby river.

Fish processing (i.e., production of fillets) will occur at a commercial processing plant that is located within a short drive of the grow-out facility in Panama. AquAdvantage Salmon will be killed at the grow-out facility, placed on ice, and then transported to the processing plant for filleting. The specific method by which the fish wastes generated through processing (i.e., heads, bones, and entrails) will be disposed of will be in accordance with applicable Panamanian laws. 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. In addition, the disposal exposure pathways originating in Canada and Panama are considered incomplete, that is, none of the dead fish or fish wastes will migrate to the United States as a result of disposal, and thus are not expected to result in any effects on the environment of the United States.

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 of the United States are expected due to disposal of any unconsumed parts or pieces of AquAdvantage Salmon that have been imported from Panama to the United States as food.

### 7.2.2 Conclusions for Question 1

For AquAdvantage Salmon and diploid ABT salmon, both the production of eyed-eggs and the grow-out of the fish are to be conducted *only* in land-based facilities with redundant physical containment measures and with point-to-point control of shipping and land-based materials transfer. There are multiple and redundant physical and mechanical barriers in place in the water systems at the PEI egg production and Panama grow-out facilities to prevent the accidental release of eggs and/or fish to nearby aquatic environments. These barriers have been designed specifically to prevent the escape of different life stages of AquAdvantage Salmon and diploid ABT salmon. Both facilities have a minimum of three to five mechanical barriers in place for all internal flow streams that release water to the

environment. This level of containment is consistent with recommendations in the ABRAC Performance Standards and has been verified by an FDA inspection or site visit.

FDA considers the likelihood that any life stage of AquAdvantage Salmon or diploid ABT salmon could escape from confinement at these sites to be very low. In addition, FDA has made the determination that physical security and containment to prevent unintentional releases of salmon due to natural disasters or intentional releases due to malicious activities are acceptable at both sites. The containment measures described above for the sites of egg production and grow-out include strictly physical measures (e.g., screens, covers, filters), as well as physico-chemical measures (e.g., chlorine).

The sponsor also employs SOPs that govern physical containment, as well as every other significant activity that occurs at these sites. In addition, strong operations management plans are in place at the PEI and Panama sites, comprising policies and procedures that meet the recommendations for an integrated confinement system for GE organisms as summarized in Table 11.

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; and redundant passive and active measures to ensure physical security, and continued inspections by local and U.S. officials.

The combination of all of these factors results in an extremely low likelihood that even a single AquAdvantage Salmon or diploid ABT salmon could escape into the wild and cause effects on the environment of the United States.

**Table 11. Implementation of an Integrated Confinement System for AquAdvantage Salmon and diploid ABT salmon ***

| Recommended element | Egg Production | Grow-out |
|---|---|---|
| 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 | ✓ | ✓ |
| * After Kapuscinski, 2005 | | |

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

#### *7.3.1 Proposed Action (Preferred Alternative)*

In the very unlikely event that any life stage of AquAdvantage Salmon or diploid ABT salmon escaped, the likelihood of survival and dispersal is a function of two complementary sets of parameters: their phenotype and fitness (e.g., tolerance to physico-chemical parameters such as temperature and dissolved oxygen), and the specific geographical and geophysical containment in the accessible environment that are a function of the specific location and environment conditions at the site of escape. We define geographical and geophysical containment 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. We further note that unless deemed to be 100% effective under all reasonably foreseeable circumstances, containment of this type would normally be considered to be secondary to other containment measures.

Geographical/geophysical containment would be present at both the production and grow-out sites for this application is discussed separately below for both the PEI broodstock and Panama grow-out sites. As an overall statement, the spread of AquAdvantage Salmon or diploid ABT salmon (or any fish) would depend upon how many escaped and survived, their characteristics, and their reproductive potential. The very low likelihood of their escape has been addressed in responding to the first risk question. The phenotypic qualities introduced above include reproductive potential, which is a function not only of their survival rate and

fertility, but also environmental conditions affecting reproduction in the accessible ecosystem(s). For example, 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).

The environmental conditions in the geographic settings of the egg production and grow-out sites would afford additional means of containment of any escaped eggs or fish, given that these conditions would be generally hostile to their survival, growth, and reproduction. These conditions would greatly limit or preclude the possibility of a complete exposure pathway by which AquAdvantage Salmon or diploid ABT salmon could reach the United States. For the reasons discussed in the following sections, FDA has concluded that the geographical and geophysical settings of the AquAdvantage Salmon egg production and grow-out sites make the likelihood of environmental impacts on the United States from survival and dispersal of AquAdvantage Salmon and diploid ABT salmon extremely low.

#### *7.3.1.1 PEI Egg Production Facility*

##### 7.3.1.1.1 Geographical/Geophysical Containment for the PEI Facility

The breeding facility lies on the southern shore of a tidal river close to its confluence with the Gulf of Lawrence (Atlantic Ocean) on the northeast side of Prince Edward Island. Water from the facility, including effluent from all floor drains, fish tanks and egg incubators, eventually discharges to this river. At the time of year fish would be spawned at the facility-- November and December-- environmental conditions in the vicinity of the facility would not be conducive to early life stages of these fish (eggs, fry and pre-smolts), although they are generally conducive to adult Atlantic salmon. Water temperatures in the winter months are typically very low (less than 0 ºC) and the water has a relatively high salinity, in the range of 21 parts per thousand (ppt)[56]. Therefore, it is highly unlikely that early life stages of any Atlantic salmon at the facility would be able to survive these environmental conditions if they were able to escape the multiple levels of physical containment in place. Although not as applicable to older fish, it is still unlikely that adults raised entirely in fresh water would be able to survive the sudden, abrupt transition from their low salinity, freshwater environment to a moderately high salinity, brackish water environment. Survivability is discussed further below in Section 7.3.1.1.3.

As a result of intentional stocking efforts, hatchery-reared Atlantic salmon inhabit the ocean waters surrounding PEI and several watersheds on the island (Cairns *et al*., 2010), although they are not known to currently populate the waters near the egg production site (Guignion, 2009). In fact, the particular watershed in which the PEI facility is located has not had populations of Atlantic salmon (either wild-type or from hatchery-reared fish) for many years (Cairns *et al*., 2010; Guignion *et al*., 2010). Thus, although the local environment might provide a suitable habitat for at least some life stages of AquAdvantage Salmon and diploid ABT salmon during part of the year, environmental conditions do not appear to be suitable for the long-term establishment of populations in the area.

##### 7.3.1.1.2 Phenotype and Fitness of AquAdvantage Salmon

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

Detailed analyses of the phenotype of the various life stages of AquAdvantage Salmon and diploid ABT salmon (see Section 5.2 of this EA and the Briefing Packet; FDA, 2010) indicate that the introduction of the EO-1α construct did not have deleterious effects on the health of the salmon, including their ability to resist infection. Although an outbreak of ISAV occurred at the PEI facility in 2009, there is no indication from the morbidity and mortality data for this outbreak, and for subsequent year classes of fish, that AquAdvantage Salmon or diploid ABT salmon are any more susceptible to this or any other disease than non-GE Atlantic salmon.

"Fitness" (e.g., oxygen requirements, swimming speed, metabolic scope, etc.) was not explicitly evaluated in the studies submitted to the agency in support of phenotypic characterization and animal safety. Reports on these fitness characteristics from peer-reviewed journals on GH transgenic Atlantic salmon (described in Section 5.2), however, indicate that changes in the observed phenotype consistent with the presence of the EO-1α construct appear to result in decreased fitness. This decreased fitness would be expected to reduce the chances for survival and establishment should AquAdvantage Salmon and diploid ABT salmon escape from commercial production facilities.

#### 7.3.1.1.3 Analysis of Survivability

In the unlikely event of escape, the survival of escaped AquAdvantage Salmon or diploid ABT salmon would be a function of the life stage(s) escaping and the location in which escape occurred. As cited in Section 7.3.1.1.2 immediately above, aside from the apparent lack of effect on disease resistance, the available information on the phenotype of adult AquAdvantage Salmon or diploid ABT salmon suggests their fitness may be reduced compared to non-GE Atlantic salmon. This reduction in fitness of adult animals, however, is not expected to be compromised to such an extent that survival would be affected greatly, at least on a short-term basis. In contrast, embryos and early life stages (i.e., alevin), would not be expected to survive the conditions of high salinity (and very low water temperature depending on the time of year) in the local accessible aquatic environments of PEI if they were to escape confinement. Because broodstock spawning occurs in the late fall and early winter months, prevailing temperature conditions in the local estuary would be at their worst for survival in the local environment if eggs or early life stages were to escape confinement at this time.

There are no specific data addressing survivability for older stages (post-smolts to adult) of AquAdvantage Salmon or diploid ABT salmon should they escape confinement in PEI and enter nearby estuarine and marine environments. When hatchery-reared non-GE salmon smolts are raised exclusively in fresh water and are not transferred to seawater after they have undergone physiological adaptation, they will undergo "desmoltification" and lose their tolerance to salinity (Lundqvist and Fridberg 1982; McCormick *et al*., 1998). It is generally believed that direct transfer of these fish from fresh water to seawater during, or after, desmoltification may result in increased mortality and/or poor growth (Arnesen *et al*., 2003). For salmon that have desmoltified and have undergone a complete loss of hypo-osmoregulatory capacity (i.e., adaptation to seawater), there is an expectation that the osmotic shock resulting from a rapid transfer from freshwater to saltwater (or estuarine) conditions would severely curtail survival. Applied AquAdvantage Salmon or diploid ABT salmon, this loss of salinity tolerance would be expected to result in rapid death if these salmon were to escape and enter the local tidal river (estuary) or nearby ocean where the salinity is high (i.e., >22‰)[57] relative to that in the freshwater tanks in which they had

[57] ‰ = parts per thousand

been raised (<1‰). Nonetheless, because there are no specific data to indicate that AquAdvantage Salmon or diploid ABT salmon undergo desmoltification if they remain in fresh water, we have made the conservative assumption herein that older post-smolt life stages of these salmon could survive if they escape physical containment at the PEI egg production facility and enter the local estuary.

This assumption must be tempered by the considerable additional remaining environmental-climatological impediments to survival. Among these is the substantial failure of intentional efforts to re-establish Atlantic salmon in their native habitat (in conditions resembling those surrounding PEI). In fact, as noted by the Council on Environmental Quality (CEQ) and Office of Science and Technology Policy of the Executive Office of the President (OSTP), escapes of farmed Atlantic salmon have not resulted in established populations in North America (CEQ-OSTP, 2001), despite the fact that they are reared commercially on both the East and West coasts of North America.

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 production site on PEI; however, rainbow trout are not currently present in the Fortune River watershed in which the PEI facility is located (Guignion et al., 2010). Atlantic salmon are not currently present in the Fortune River watershed or any nearby watersheds, 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.

In terms of feeding and survival, farmed Atlantic salmon that have escaped ocean net pens often remain in the vicinity of the fish farm from which they have escaped and continue to feed on feed pellets that passs through the pen netting (Soto *et al*., 2001). This would not be an option for AquAdvantage Salmon or diploid ABT salmon if they escaped the PEI facility as there are no fish farms anywhere nearby. Further, although not extensively studied to date, the survival of escaped and released farm salmon has been found to be low (Whoriskey *et al*., 2006; Hansen, 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; see Section 7.5.1.1.1). 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 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 ~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 AquAdvantage Salmon or diploid ABT salmon may not transition to a wild prey diet in the unlikely event they were to escape the PEI facility, and thus would be susceptible to starvation and early mortality. Additional factors would further reduce the likelihood of their survival and dispersal, including reduced swimming ability and predator avoidance that would likely increase their predation mortality (see Section

5.2.2.5). These attributes suggest that AquAdvantage Salmon and diploid ABT salmon would not be particularly fit for the local PEI environment, even if they were to escape.

FDA has therefore concluded that it is unlikely that early life stages of AquAdvantage Salmon or diploid ABT salmon escaping from the broodstock facility on PEI (itself a highly unlikely event) would be able to survive and disperse in the local PEI environment. The potential fate of post-smolt AquAdvantage Salmon or diploid ABT salmon is less clear if they were to escape; it is quite possible that they would not be able to survive in the marine/estuarine environment due to desmoltification and/or a failure to transition to wild prey diet. Because this cannot be concluded with absolute certainty, at this time the agency has made the conservative assumption that these fish would not undergo desmoltification and could be able to survive in such an event.

#### *7.3.1.2 Panama Grow-out Facility*

The Panama grow-out facility lies at an elevation of approximately 5,000 feet above sea level with fresh water supplied by a nearby spring. The temperature of the spring water is fairly constant throughout the year; at approximately 15 ºC, it is similar to that of the river that runs next to the facility and receives its water discharges. This temperature is near the optimum for Atlantic salmon growth and would not be an impediment to survival should any eggs or fish escape from the facility. Atlantic salmon are not found in the surroundings of the grow-out site in Panama; however, artificially introduced populations of rainbow trout are reported to exist in the area as a result of previous stocking efforts by Panamanian authorities. Rainbow trout, a salmonid species that is related to Atlantic salmon, also requires fairly low water temperatures and high dissolved oxygen concentrations. Although the presence of these rainbow trout indicates that the environment is suitable for salmonids, as will be discussed below, the average water temperature further downstream of the facility exceeds the lethal-maximum that Atlantic salmon can tolerate.

As shown in Table 7, the temperature of the nearby river increases substantially as the river drops in elevation, merges with another river downstream, and the combined flow approaches the Pacific Ocean. In the lower reaches of the watershed, the water temperature is in the range of 26 – 28ºC. This temperature is at or near the upper incipient lethal level[58] for Atlantic salmon, approximately 28ºC for acclimated juveniles (Elliott, 1991; see discussion in Appendix A, Section A.3). Feeding stops when the water temperature exceeds 22.5ºC; therefore, it is expected that long-term survival would be compromised due to starvation at locations even further upstream (where water temperatures are cooler) of those where the water temperatures are acutely lethal. As a result, it is extremely unlikely that AquAdvantage Salmon would be able to survive and migrate to the Pacific Ocean. In addition, because surface water temperatures in the Pacific Ocean along the Panamanian coast are in the range of 25-28ºC throughout the year (National Oceanic Data Center, online data for 2009)[59], survival of any salmon in the ocean in this locale is virtually impossible; there is no *a priori* reason to believe that the upper tolerance limit (i.e., upper incipient lethal limit) would be higher for AquAdvantage Salmon than for non-GE Atlantic salmon.

[58] The upper incipient lethal level is the highest temperature that can be survived up to seven days.

[59] Available HERE.

Salmon have a relatively high requirement for DO compared to many other fish species. GH-transgenic Atlantic salmon have been reported to have an increased requirement for DO compared to non-GE counterparts (see Appendix A, Section A.3), presumably due to their faster growth and increased metabolic rate. The physiological implication of this requirement is a reduced tolerance to higher water temperatures, as the DO content of water at saturation is inversely related to water temperature. Stevens *et al*. (1998) have shown that DO content of water starts to become limiting for GH-transgenic Atlantic salmon when DO concentrations drop to 6 g/L (ppm). Oxygen alone would not appear to be limiting for AquAdvantage Salmon if they were to reach the lower reaches of the watershed as the lowest levels of DO levels in the river basin are 7.0 to 7.2 mg/L based on water quality monitoring conducted over the years 2002-2008 (see Table 10).

In addition to high water temperatures, several other conditions of the aquatic habitat in the lower sections of the watershed are also not favorable for salmonid survival or establishment. First, salmonids have a requirement for clear water; the levels of solids in the water column and amounts of transported sediment are high in these areas (Table 10). Second, food sources may be limited, as the macroinvertebrate fauna, although diverse, are not abundant. Third, having been reared their entire lives on synthetic diets, escaped salmon are often recaptured with empty stomachs presumably due to their inability to switch from a pelleted diet to one of natural prey (see discussion in Section 7.3.1.1.3), a limitation that would be exacerbated by the low abundance of such prey in the environment at the grow-out site. This would increase the likelihood for starvation and early mortality. Finally, and more specifically for AquAdvantage Salmon, additional factors would further reduce the likelihood of their survival and dispersal, including a reduced swimming ability and predator avoidance that would likely increase their predation mortality.

The potential impact of predation is unclear. There are reports of a resident population of introduced rainbow trout in the area, but its prevalence and distribution in the watershed are unknown. Rainbow trout constitute a known and formidable predator of salmon fry, fingerlings, and juveniles. Adult rainbow trout present in the adjacent watershed would be expected to prey on smaller salmon that might manage to escape from the grow-out site.

Finally, a significant amount of the water volume in the downstream watershed is diverted for use in local hydroelectric power plants. These power plants and their associated water diversion dams appear to constitute significant, although not entirely complete, barriers to fish movement within the watershed, particularly with respect to potential downstream migration of AquAdvantage Salmon to lower parts of the watershed and the Pacific Ocean.

In summary, in the unlikely event that escape of AquAdvantage Salmon were to occur in Panama, survival would only be possible in the vicinity of the grow-out facility and upper watershed of the adjacent river, as conditions further downstream are highly unfavorable for the survival and dispersal of Atlantic salmon populations. High temperature conditions and water diversion projects downstream would limit the long-term survival of all life stages of AquAdvantage Salmon precluding long-range dispersal. Survival outside of freshwater conditions in the highlands of Panama (i.e., in the Pacific Ocean) is considered impossible due to high water temperature conditions. In addition, as was discussed in relation to the PEI facility, older AquAdvantage Salmon may undergo desmoltification as a result of being reared in freshwater and, if such is the case, would not be able to survive the high salinity conditions in the lower parts of the estuary and Pacific Ocean, even if for some reason, they could survive the lethal temperatures.

### 7.3.2 Conclusions for Question 2

The geographical and geophysical conditions present in the aquatic environments surrounding both the PEI broodstock and the Panama grow-out facilities are sufficiently inhospitable to limit the potential establishment and spread of AquAdvantage Salmon or diploid ABT salmon to other locations. In the unlikely event that an escape were to occur, the likelihood of survival of AquAdvantage Salmon or diploid ABT salmon would be a function of the life stage(s) of the animal escaping and the location into which it escapes. This is particularly true for the earliest life stages (eggs and embryos) in PEI, which would be unlikely to survive if exposed to high salinity and low temperature conditions in the nearby aquatic environment, and for all life stages of these salmon in Panama, which would be unlikely to survive the high temperature conditions in the lower reaches of the watershed.

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

In the extremely unlikely event that AquAdvantage Salmon or diploid ABT salmon escape, and could survive in the two environments surrounding the PEI broodstock and the Panama grow-out facilities, the likelihood that they would be able to reproduce and subsequently establish is largely a function of the extent and adequacy of biological containment in the fish that escape. Because conspecifics and closely related relatives of Atlantic salmon (i.e., brown trout) are not found in the local aquatic environments near either facility, the essential concern is over reproduction between escaped fish. The following discussions on biological containment relate to AquAdvantage Salmon only. Discussions related to potential establishment of escaped diploid ABT salmon are addressed later in Section 7.4.2.

Information the sponsor submitted to FDA regarding bioconfinement for AquAdvantage Salmon is summarized in Section 5.3.2 and important aspects are discussed further below. Under the conditions that would be established in the NADA, if approved, all AquAdvantage Salmon eggs produced for shipment to the Panama grow-out facility would be subjected to pressure treatment to induce triploidy, which will effectively sterilize the population.

### *7.4.1 Biological Containment (Bioconfinement)*

Biological confinement will be ensured in AquAdvantage Salmon through the use of triploidy and the production of all-female populations for grow-out. These techniques are not new and have been under study for many years for aquaculture purposes and have been used by fisheries biologists to reproductively isolate stocked game fish from their wild counterparts and protect species that may be threatened or endangered (Thorgaard, 1983; Benfey and Sutterlin, 1984; Benfey, 2001). As of 2005, officials in 10 different states were sterilizing (i.e., triploiding) hatchery salmonids as part of their stocking programs for hatchery-reared salmonids (Kozfkay *et al*., 2006). In addition, going back to the 1980s and 1990s, the use of sterile Atlantic salmon triploids has been proposed as a possible strategy to reduce interactions and interbreeding between escaped farmed and wild salmon (Heggeberget *et al*., 1993; McGeachy *et al*., 1995; McGinnity *et al*., 1997; Benfey, 2001; Benfey, 2015). The use of sterile triploids has been also proposed for biological containment of GE fish going as far back to the early 1990s (Devlin and Donaldson, 1992; Thorgaard *et al*., 1992). The usefulness of triploidy as a means of eliminating genetic interactions and reducing the general impact of escaped farmed fish on wild populations has been demonstrated in a large-scale field study (Cotter et al., 2000a).

#### *7.4.1.1 Validation of Triploidy Method*

As described earlier in Section 5.3.2.3, the sponsor has conducted a study to validate the method and conditions used for the production of triploid Atlantic salmon at its PEI broodstock facility. The primary objective of the study was to determine if the conditions for induction of triploidy using hydrostatic pressure treatment could be employed in a reproducible manner for the batch-wise production of triploid eggs during the commercial production of AquAdvantage Salmon.

During the study, one-to-one crosses were established with eggs from non-GE female Atlantic salmon fertilized with milt from ABT salmon males hemizygous for EO-1α. The fertilized eggs from each cross were apportioned volumetrically into five replicate groups: one diploid control group that was not pressure treated, and four treated replicates that were subjected to hydrostatic pressure shock (9500 psi for five minutes at 300°C-min post-fertilization).

After treatment, when fertilized eggs had developed to the 'eyed' stage (~325-400°C-day), 350 eyed-eggs were arbitrarily sub-sampled to estimate the proportion of triploid individuals in the aggregate population. Ploidy analysis was performed on sub-samples of homogenates of a pool of 10 eyed-eggs collected from each of the four treated replicates from the five different independent crosses (i.e., a total of 20 independent pressure shocked groups). Ploidy was determined using a flow cytometer with samples from the diploid control groups serving as a reference standard.

Based on the analysis of ploidy in all 20 batches, the average proportion of triploids produced from the five independent crosses was 99.8%. For individual treatment events, the proportion of triploidy ranged from 98.9% to 100%. Triploidization was very similar for each of the five independent crosses, on average ranging from only 99.7% to 99.9%. The lowest effectiveness observed for an individual batch of eggs was 98.9%; triploidization in 14 of the 20 batches was 100%.

More recently, the sponsor has conducted additional validation studies on triploidy using high-capacity pressure vessels. These studies have confirmed the initial results previously described. In the follow-up validation studies, the percentage of triploids for 10 independent crosses (n = 200 eggs per cross) also averaged 99.8%, with 100% triploidy in six crosses and 99.5% triploidy in the other four crosses.

#### *7.4.1.2 Triploidy and Triploidization*

All AquAdvantage Salmon eggs sold or distributed for grow-out will be subjected to pressure treatment shortly after fertilization to induce triploidy. As part of the Durability Plan to which the sponsor has committed, ploidy testing will continue to be conducted on all composite batches of fertilized eggs intended to be sold or distributed. Per the Durability Plan, if, based on testing, triploidization in these eggs does not exceed 95% (based on the statistical 95% lower confidence limit), the entire batch of eggs must be destroyed (We note again that during method validation testing, the lowest effectiveness observed for triploidization in an individual batch of eggs was 98.9% and the mean was 99.8%). Because the testing methodology used for verifying triploidy results in egg destruction, it would be impossible to ensure 100% triploidy in all of the eggs actually used for grow-out through testing.

#### *7.4.1.3 Sterility of AquAdvantage Salmon*

AquAdvantage Salmon have been described throughout this EA as being "sterile" or "effectively sterile" or "functionally sterile." The common characterization in the fisheries

and aquaculture scientific literature is that "triploidy" equals "sterility," in other words, the major consequence or outcome of triploidy is gonadal sterility (Piferrer *et al*., 2009). Because of this, the two words are often used interchangeably. Although adequate demonstration of triploidy has been provided to FDA, there are no specific data demonstrating that triploid AquAdvantage Salmon are indeed sterile, that is, incapable of producing viable offspring; however, as discussed below, there are several reasons why this is believed to be the case.

Triploidy is believed to effectively sterilize Atlantic salmon (and other fish) because it interferes with normal gametogenesis (the formation of cells that become eggs or sperm) when cells enter meiosis. This is believed to be due to mechanical problems associated with the pairing of homologous chromosomes in the presence of a third set of homologues (Benfey, 1999). Information discussed in Section 5.3.2.4 (*Effectiveness of triploidy in inducing sterility*) indicates that it is highly likely that triploid Atlantic salmon, particularly female salmon, will be effectively sterile due to failure of the gametes to mature normally. Most germ cells do not progress through the first meiotic prophase (an early stage in the formation of the sex cells) in triploids of either sex. Triploid females rarely produce eggs, but, if they do, the eggs usually are very few, undeveloped and unfertilizable (Piferrer *et al*., 2009). Most triploid oögonia fail to proceed to the oöcyte stage and, as a result, there are very few (if any) ovarian follicles that develop to a stage of functional steroid biosysnthesis (Benfey, 2015). Triploid females do not product sufficient vitellogenin for oöcytes to develop to a stage necessary for the production of viable eggs, and any oöcytes that do complete vitellogenesis will not be released due to the lack of endocrine signaling for final maturation and ovulation (Benfey, 2015).

Although there have been isolated reports of limited gonadal development in triploid fish of several different species, mostly in males (Benfey, 1999; Mair *et al.,* 2007), relevant research on triploids of Atlantic salmon and related species indicates functional sterility in females. In a study on triploid landlocked Atlantic salmon, Benfey and Sutterlin (1984) found the ovaries of triploid females had the external appearance of undeveloped gonads, but still produced a small number of oocytes (from 1 to 12, versus several hundred in each diploid female). The viability of these oocytes was never determined. Subsequently, Johnstone and colleagues (Johnstone *et al*., 1991; Johnstone, 1992) showed that approximately 0.1% of triploid Atlantic salmon females underwent sexual maturation after two years. When fertilized with normal sperm, eggs stripped from triploid females were markedly variable in size, and most underwent little obvious development (Johnstone, 1992). Approximately 10% of the fertilized eggs developed to the eyed-egg stage, but the embryos were clearly malformed and none survived beyond hatching, confirming that triploid females are functionally sterile. Similar results have been reported from a study on Arctic char (*Salvelinus alpinus*), a salmonid species related to Atlantic salmon, in which although a few of the triploid females developed ovaries, fecundity was low, and the fertilized eggs from the triploid females did not hatch (Gillet *et al*., 2001), also demonstrating that successful reproduction was functionally and effectively precluded through triploidization. Therefore, based on the available evidence, FDA has concluded that triploidy would ensure functional sterility and reproductive incompetence in the sponsor's all-female populations of AquAdvantage Salmon.

#### *7.4.1.4 Female, Mono-Sex Populations*

As described and illustrated in Section 5.3.1.1, the sponsor uses a complex production process involving gynogenesis and neomales (sex-reversed females)[60] to ensure that a monosex, all-female population of AquAdvantage Salmon would be produced for grow-out. Using gynogenesis[61] as part of the production process, rather than chemically-induced sex-reversal alone, not only eliminates the time and labor that would be needed to distinguish neomales from true males following androgen treatment, but also essentially ensures 100% effectiveness in producing a genetically all-female population with a full complement of maternal DNA. When combined with a sterilization technique such as triploidy, the production of all-female populations of fish ensures a highly effective form of biological containment, which is the reason that production of all-female triploids has often been discussed in relation to GE fish (NRC, 2004; Devlin *et al*., 2006; Mair *et al*., 2007).

To ensure the future validity of the production process in making all-female population, the NADA approval would require additional genotypic post-approval monitoring of the AquAdvantage Salmon neomales as part of its Durability Plan (see FDA Briefing Packet for further details; FDA, 2010). The Durability Plan involves periodic testing and annual reporting on this (and other) processes. Records kept by the sponsor on this and other processes are subject to validation by the sponsor and inspection by the agency.

#### *7.4.1.5 Residual spawning behavior*

In addition to reproductive containment, production of monosex populations has one other important advantage, particularly when all-female fish populations are produced. One concern with the production of all-male triploid populations is that if these fish should escape physical containment and reach the environment, while functionally sterile[62], they would still be capable of exhibiting spawning behavior with fertile, wild females, if females are present. This could potentially lead to decreased reproductive success for these wild-type females. This type of interaction and effect cannot occur if the fish populations are all-female, as is the case for AquAdvantage Salmon that would be produced for grow-out.

#### *7.4.1.6 Potential interactions with conspecifics and relatives*

Because AquAdvantage Salmon, as defined and specified in the NADA would only be produced as all-female triploids, it is important to consider the interactive effects of triploidy and sex on Atlantic salmon in their natural environment and how this might influence interactions between farm-raised fish that have escaped, including AquAdvantage Salmon, and wild salmon. Ocean migration studies in Ireland with tagged Atlantic salmon showed that male triploids return to their natal area in nearly the same proportions as diploids,

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

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

[62] Triploid males often produce small amounts of viable sperm that have aneuploid chromosome numbers and other abnormalities. Fertilization of eggs with viable sperm from triploid males produces progeny that die as embryos or larvae.

whereas female triploids mostly do not (Wilkins *et al*., 2001). In another Irish study, the return rates of female triploid Atlantic salmon, both to the coast and to freshwater environments, were substantially reduced (four- to six-fold lower) compared to those for their diploid counterparts (Cotter *et al*., 2000a). Of direct relevance to triploid AquAdvantage Salmon females, the triploid females in this study had severely immature ovarian development (Murphy *et al*., 2000) and abnormal gonadal steroid and gonadotropin hormone profiles (Cotter *et al*., 2000b). From the reduced rate-of-return and inability to produce viable offspring demonstrated in these studies and others (e.g., Johnstone *et al*., 1991; Johnstone, 1992), FDA can infer that triploidy combined with all-female populations can be effectively used as a means of eliminating reproduction and genetic interactions between cultured and wild populations.

***PEI***: There is no evidence to indicate that triploid AquAdvantage Salmon females or diploid ABT salmon could cause reproductive interference with native conspecifics, even if these conspecifics were present, which they are not. As discussed in Section 6.1.1.3, wild Atlantic salmon populations (or those resulting from stocking efforts on the island), although once prevalent in PEI waters and currently inhabiting 22 other rivers on PEI (Carins *et al*., 2010), no longer occur in the Fortune River basin/estuary where the PEI facility is located or in any of the other rivers in the area (Guignion *et. al*., 2010; Cairns *et al*., 2010). This strongly suggests that the local aquatic ecosystem is no longer suitable for reproduction and establishment of Atlantic salmon. Several serious threats to salmon populations on PEI have been identified, including stream sedimentation, pesticide runoff and associated kills, and blockage to fish passage, among others (Cairns *et al*., 2010). Most importantly, with no local populations of Atlantic salmon present, reproduction of all-female AquAdvantage Salmon would not be possible in the event of an escape. With no reproduction, long-term establishment of populations of these fish also would not be possible.

In addition, as mentioned earlier in Section 6.1.1.3, although widely occurring in many other parts of Canada and North America, there are no brown trout present on PEI (DFO, undated). Thus, although Atlantic salmon (*Salmo salar*), including diploid ABT salmon are able to interbreed with brown trout (*Salmo trutta*), which are of the same genus, to produce viable hybrids (see Section 5.2.2.7), interactions and genetic introgression with brown trout will not occur because the two species will not be present together in the same location. In addition, as shown by Galbreath and Thorgaard (1995), progeny resulting from backcrosses of Atlantic salmon X brown trout hybrids are either non-viable, or triploid and therefore effectively sterile. These results preclude the potential for any further introgression of the transgene into brown trout or Atlantic salmon genomes via backcrossing and indicate that any highly unlikely hybrid populations will die out after a single generation.

Aside from Atlantic salmon, two other salmonids species, brook trout (*Salvelinus fontinalis*) and non-native rainbow trout[63] (*O. mykiss*) are found in PEI streams (Guignon *et al*., 2010). Of these, only brook trout are found in the Fortune River where the PEI egg production facility is located. Laboratory crosses of male brook trout with female Atlantic salmon have been shown to produce small numbers of viable fry (1-5%), but the reciprocal crosses (female brook trout crosses with male Atlantic salmon) are unsuccessful (Sutterlin *et al*., 1977; Gray *et al*., 1993). More importantly, FDA is unaware of any reports of natural hybridization between these two species in the wild despite the fact that they have coevolved in North America and often coexist, at least as juveniles, within habitats where their native ranges overlap (Fausch, 1998).

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

***Panama***: Even if they were not sterile, mature female AquAdvantage Salmon escaping into the watershed near the grow-out site in Panama would not encounter conspecifics or even closely-related species with which to spawn or interbreed. Atlantic salmon, wild or otherwise, do not occur in accessible environments anywhere near the grow-out site. Non-native rainbow trout (*Oncorhynchus mykiss*) are reported to inhabit the general area where the Panama grow-out facility is located as a result of previous stocking efforts, but no other species of salmonids are known to live locally. As previously discussed, Atlantic salmon (*Salmo salar*) are able to interbreed with brown trout (*Salmo trutta*), which are of the same genus, to produce viable hybrids, but they do not successfully interbreed with rainbow trout (Teufel *et al.*, 2002; Hindar, 1993), which are of a different genus (*Oncorhynchus*); see Sections 5.2.2.7 and 6.1.2.3 for additional information and references.

The presence of rainbow trout locally indicates that the immediate environment is suitable for the establishment of salmonids, potentially including Atlantic salmon; however, any long-term establishment of AquAdvantage Salmon would require reproduction, which would not be possible because of the lack of conspecifics. Reproduction amongst just AquAdvantage Salmon would not be possible because the population for grow-out would be entirely female. 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, which is not a realistic possibility for either the egg-production or grow-out sites for AquAdvantage Salmon due to the small population sizes relative to grow-out in net pens, as well as the highly redundant containment and security measures that are employed at both sites.

Any significant downstream movements of escaped AquAdvantage Salmon would be greatly limited by physical structures (i.e., hydroelectric dams and water diversion canals) and water temperatures. As discussed previously, the water temperatures in sections of the lower watershed are at or above the lethal maximum that Atlantic salmon can tolerate for an extended period of time. In addition, high water temperatures in the lower reaches of the watershed would preclude the spread of any escapees into the eastern tropical Pacific Ocean, which also does not have indigenous populations of Atlantic salmon, or any populations of Pacific salmon species (i.e., chinook, chum, coho, sockeye, or pink salmon), or steelhead trout within several thousand miles of Panama[64].

Even if interactions with wild Pacific salmonids were somehow possible, the weight of evidence indicates that it is highly unlikely that there would be successful hybridization of Atlantic salmon (or AquAdvantage Salmon specifically) with Pacific salmon, which are of a different genus, *Oncorhynchus*. The potential for hybridization and genetic introgression between Pacific salmon species and Atlantic salmon, which are widely cultured in net pens on the west coast of Canada, and to some extent in the coastal waters of Washington State, has been one of concern in both countries for many years. As a result, the issue has undergone extensive research and examination by NOAA's National Marine Fisheries

[64] Inshore populations of steelhead trout (an anadromous form of rainbow trout, *Onchorhynchus mykiss*) exist as far south as southern California. Populations of the Pacific salmon species have historically been found as far south as the areas north of Monterey Bay, California. Some distinct population segments of these species are currently classified as threatened or endangered in California, Oregon, and Washington.

Service, DFO, and the State of Washington (see for example Alverson and Ruggerone, 1997; PCHB, 1998; Nash, 2001; Nash, 2003; and Waknitz *et al*., 2003). These groups have examined the available scientific literature and determined that hybrids between Atlantic salmon and the Pacific salmonids species can be produced *in vitro*, but with difficulty. These reports have noted that hybrids are not observed in nature, whether for introduced Atlantic salmon in North America, or for introduced North American salmonids to Europe and other countries. Taking this information into consideration, as well as behavioral and other ecological factors, a review board in Washington State concluded that there was no reasonable potential for hybridization between escaped Atlantic salmon and native Pacific salmon in Puget Sound (PCHB, 1998).

There are no native populations of Atlantic salmon in the Pacific Ocean; the native range of this species is limited to the northern Atlantic Ocean (see Appendix A). There has been concern over possible establishment of this species on the west coast of the United States and of Canada as a result of escapes from net pens in marine salmon farms in coastal Washington state and British Columbia. To date, there has been no compelling evidence of any colonization and establishment (i.e., self-sustaining populations) of Atlantic salmon in these areas[65], although many escapes have been documented and a few suspected wild juvenile fish have been recovered from two coastal streams in British Columbia (Piccolo and Orlikowska, 2012). The lack of establishment on the west coast is not surprising considering that many attempts have been made to introduce Atlantic salmon to geographic areas outside of its native range, all without success in producing self-sustaining populations of anadromous fish (Waknitz *et al*., 2003; NOAA, 2001; Dill and Cordone, 1997; Alverson and Ruggerone, 1997)[66], and that it is very difficult to reintroduce Atlantic salmon back into their native rivers after populations have failed. The implication of these observations is that it would be highly unlikely for AquAdvantage Salmon to interact with Atlantic salmon on the west coast of North America even in the extremely unlikely event that they were able to survive migration through the salmon-lethal equatorial ocean temperatures, and migrate north several thousand miles to locations where Atlantic salmon are currently farmed.

In summary, FDA has concluded, based on the available evidence, that any reproduction or long-term establishment of AquAdvantage Salmon in the watershed of the Panama grow-out facility, or further afield, as a result of an escape is essentially precluded.

#### *7.4.1.7 Potential for establishment due to escaped AquAdvantage broodstock in PEI*

The greatest potential risk to the environment of the United States would occur in the event of the escape of AquAdvantage broodstock from the PEI facility. These fish are reproductively competent and will be homozygous for the *opAFP-GHc2* gene (Figure 4). There are also non-GE Atlantic salmon in the PEI facility as these fish are needed in the

[65] Although there is one report that escaped Atlantic salmon have successfully reproduced in the Tsitika River drainage of British Columbia (Volpe *et al.*, 2000), the researchers did not document spawning behavior directly, and only inferred reproduction from multiple age classes of juveniles. There have been no subsequent reports of population establishment in this area, and there are no other known confirmed reports of spawning in Pacific Coast streams (Piccolo and Orlikowska, 2012).

[66] For example, between 1905 and 1934, the government of British Columbia released 7.5 million juvenile Atlantic salmon into local waters. None of the releases were successful in establishing Atlantic salmon populations, although some natural reproduction may have occurred (MacCrimmon and Gots, 1979; Carl et al. 1959).

production process. As a result, the potential for reproduction and establishment of these fish has been considered in the event of an escape, the likelihood of which is very low, although it cannot be totally eliminated. As discussed previously, Atlantic salmon have not been found in the Fortune River since before the year 2001 (see previous section and Section 6.1.1.3; Guignion, 2009; Carins *et al*., 2010; Guignion *et al*., 2010). They also have not been found in any of the other watersheds on the northeast coast of PEI in the most recent surveys conducted in 2007 and 2008 (Cairns et al., 2010). This indicates a very low potential for reproduction and establishment given that Atlantic salmon were once intentionally stocked in many of these rivers and the Fortune River was once well known for its salmon run (Guignion *et al*., 2010). 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; Moreau *et al*., 2011a; Moreau and Fleming, 2011 ), it is expected that a significant number of fish would need to escape in order for there to be any potential chance of reproduction and establishment. As previously discussed in Section 7.2.1.1, there is a very low probability of that occurring at the PEI egg production facility due to the many levels of containment there, which has been confirmed by Failure Mode Analysis conducted by Canadian authorities.

### 7.4.2 Conclusions for Question 3

The conditions of use specify that, based on testing, a minimum of 95% of the AquAdvantage Salmon eggs sold for commercial production use would be triploid and 100% are expected to be female. Based on the results of multiple method validation studies, the actual average percentage of triploidy is consistently at 99.8%. The fertility of triploid females is negligible compared to normal diploid females. The combination of triploidy and an all-female population is expected to render AquAdvantage Salmon effectively and functionally sterile resulting in complete reproductive containment.

These characteristics essentially preclude establishment of a population of these fish in the accessible environments in the highly unlikely event that an escape occurs. The only potential means for establishment (or pseudo-establishment) would be through the escape of reproductively competent broodstock at the PEI facility or through a continual series of escapes at the Panama facility. Neither of these scenarios is likely given the physical containment measures in place at both facilities. Both would require the escape of a significant number of animals, a condition that is even less likely. Given the difficulty in reintroducing Atlantic salmon into rivers in which they once occurred in PEI, and the lack of any self-sustaining populations on the west coasts of the U.S. and Canada where significant numbers of Atlantic salmon escape each year from net pen salmon farms, these scenarios are considered even more unlikely. Therefore, given the available information, FDA concludes that there is a negligibly small likelihood that AquAdvantage Salmon or diploid ABT salmon would reproduce and establish self-sustaining populations if they escaped from facilities either in PEI or Panama. Although slightly greater, the hypothetical risk that reproductively competent AquAdvantage broodstock that have escaped from the PEI egg production facility could establish self-sustaining populations is still considered very low and within an acceptable range given the current lack of Atlantic salmon in the Fortune River and surrounding watersheds.

## 7.5 Question 4: What are the likely consequences to, or effects on, the environment of the United States should AquAdvantage Salmon 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 transgenic Atlantic and coho salmon. Among his observations were that variations in phenotype and characteristics within a species were not only dependent 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 highly unlikely event of an escape, AquAdvantage Salmon or diploid ABT salmon are expected to occupy the same ecological niche as wild and domestic Atlantic salmon, competing for food, shelter, and other resources. Although AquAdvantage Salmon or diploid ABT salmon would have one key increased fitness attribute (i.e., more rapid growth to smolt stage) relative to their wild and domesticated counterparts, in many other respects, their fitness would be reduced (e.g., increased need for food, increased dissolved oxygen utilization, etc.). Natural selection would act on these fitness attributes in the environment, but there is considerable uncertainty associated with predicting or quantifying any particular outcome, as we are 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 previously documented that physical/mechanical containment is very stringent for both the egg production and grow-out facilities, and escapes from either of these facilities is considered to be highly unlikely. In the event, however unlikely, that escapes should occur, biological containment would be imposed on the population of AquAdvantage Salmon that would be numerically most prevalent -- the production animals located in Panama. Geographical and geophysical containment present in the environment would also provide significant hurdles to long-term survival, establishment, and persistence of AquAdvantage Salmon in Panama (See Section 7.3). These elements are part of the exposure pathway that could potentially result in effects on the United States. Because AquAdvantage Salmon would be produced as triploid (sterile) females, they would be unable to reproduce or contribute their genes to conspecifics in the environment. As discussed subsequently in this section, the numbers of AquAdvantage broodstock are quite small, and the surrounding environment so inhospitable that long-term establishment would be highly unlikely.

We further note 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, any escapes of AquAdvantage Salmon or diploid ABT salmon, if they should occur, would likely be of an extremely low magnitude due to the small scale of production and the limited conditions of grow-out.

### 7.5.1 Proposed Action (Preferred Alternative)

As previously noted, the proposed agency action for which this EA has been prepared is the approval of the AquAdvantage Salmon NADA under specific conditions of use. We have considered the potential outcomes within the constraints established by NEPA and FDA's implementing regulations, as previously described (see Section 2.3.2). As noted earlier, NEPA does not require analysis of effects on the environment in foreign sovereign countries. In this EA, we have considered the potential for survival, dispersal, reproduction and establishment in Canada and Panama in the context that these events are involved in the

exposure pathways that could potentially result in effects on the environment in the United States. Approval of the AquAdvantage Salmon NADA by the United States would not preclude any evaluation of effects/impacts, or regulation of AquAdvantage Salmon or diploid ABT salmon, by authorities in Canada and Panama.[67]

#### *7.5.1.1 Effects on the United States as a Result of Escape in PEI*

##### 7.5.1.1.1 Exposure Pathways for Effects on the United States

We consider two scenarios that could potentially lead to introductions of AquAdvantage Salmon to the local environment in PEI, and subsequently could potentially result in effects on the environment in the United States. The first is the accidental escape of a large number of reproductively competent broodstock[68] from the PEI egg production facility as a result of a catastrophic event (e.g., hurricane, tornado, tsunami) causing simultaneous and complete failure of all of the physical containment systems in the facility. As previously discussed, this situation is extremely unlikely due to redundancies in the containment measures and the very infrequent occurrence of these types of events in the vicinity of PEI (see Sections 2.6 and 6.1.1.2). The second and more likely scenario is an act of vandalism resulting in the intentional malicious release of a large number of AquAdvantage broodstock. This scenario is also considered improbable due to surveillance and redundant security measures in place at the facility (see Section 5.4.2). Regardless of the scenario, the number of adult broodstock in the PEI facility will be limited to several thousand at any one time[69]; therefore, the potential for the mass release of many thousands to hundreds of thousands of post-smolt fish, as sometimes occurs during net-pen farming of Atlantic salmon, will not occur.

Depending on the time of year that escape/release was to occur, escaped or released juveniles or adult salmon broodstock could potentially survive in the local PEI environment. As previously discussed in Section 7.3.1.1.3, non-GE Atlantic salmon reared under fresh-water conditions typically undergo desmoltification and lose their ability to tolerate high salinity conditions if they are not moved to seawater soon after they smolt. We have, however, made the conservative assumption herein that AquAdvantage broodstock would be able to survive in estuarine or marine salinity conditions should they escape or be released.

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[67] Canada and Panama regulate AquAdvantage Salmon facilities in their countries under their own authorities. See CSAS Summary at 18 ("[T]he risk to the Canadian environment associated with the manufacture and production of AAS is low with reasonable certainty."); Appendix G.

[68] As discussed in Section 5.3.1.1, broodstock are diploid GE Atlantic salmon, homozygous for the EO-1α gene construct, and either true females or neomales (sex reversed genotypic females). Some diploid, hemizygous true males are also used for research and development purposes and for broodstock development.

[69] Larger numbers of non-triploid eggs, fry, and pre-smolt parr could be present in the PEI facility for research and development purposes and to produce broodstock for future production of AquAdvantage Salmon. Although potentially reproductively competent at maturation, these eggs and fish would not survive the salinity conditions in the nearby estuarine environment if they were released.

Although native to PEI, as a result of habitat loss and overexploitation, significant runs of natural Atlantic salmon are no longer found in many of the rivers on the island. Prior to European settlement, it is believed that approximately 70 rivers on PEI contained Atlantic salmon runs; by 1960, that number had dropped to a possible 55 rivers (Guignion, 2009). In a comprehensive survey conducted in 2000-2002, some salmon remained in only 33 large streams. Six years later, salmon runs were lost from 11 additional rivers, and in seven others the populations were precariously low. The river system located adjacent to the sponsor's PEI egg production facility, the Fortune River, is one of those reported to not have a salmon population since sometime before 2002 (Guignion, 2009). In addition, all of the other river systems in the general vicinity of the PEI facility no longer have populations of Atlantic salmon. Based on the river classification system described by Guignion (2009), none of the rivers in this area are classified as Class I (i.e., having sustainable annual salmon runs) or Class II rivers (i.e., rivers which should have sustainable runs if water quality conditions and beaver populations are managed properly). Future returns of Atlantic salmon in PEI rivers are expected to remain largely dependent on stocking of hatchery-reared fish (Cairns, 1998).

The disappearance and main impediments to wild Atlantic salmon prevalence on PEI are believed to be due to stream sedimentation (mainly through agricultural runoff) and barriers to migration such as beaver dams (see Section 6.1.1.3). Over-wintering habitat is lacking in many stream reaches, and blockages that may affect instream movement or migration patterns are common in most rivers (Guignion, 2009). In addition, water quality problems resulting from soil erosion and agricultural runoff are present in some watercourses. In upstream sections of the river that is adjacent to the sponsor's facility, there are man-made and beaver blockages that have caused summer water temperatures to exceed tolerable levels for salmonids; oxygen levels also fall well below minimum accepted concentrations (Guignion, 2009). Water quality is compromised in much of the main stem of the river down to the head of the tide.

For these reasons, it is highly unlikely that escaped or released GE salmon from the sponsor's PEI facility would be able to reproduce and establish in the local environment (or farther afield) and cause any significant impacts on the United States. Given that there are relatively few "true" (genotypic) males in the broodstock population (approximately half of the "males" in the facility are sex-reversed females, i.e., neomales that are in fact genotypic females, see Section 5.3.1.1), the potential for reproduction between either AquAdvantage broodstock and wild Atlantic salmon, or between females and males (or neomales) of the AquAdvantage broodstock population is highly unlikely. The inability of neomales, in general, to release milt on their own would further preclude potential reproduction should an escape or release of broodstock occur. In salmonids, sexual development is usually disrupted in neomales such that they usually have less well developed testes, and most individuals characteristically lack functional sperm ducts (also known as gonopores or gonoducts) (Fitzpatrick *et al*., 2005; Geffen and Evans, 2000; Johnston *et al*., 1978; Tsumura *et al*., 1991). As a result, in the hatchery, spermatozoa (milt) must usually be collected directly from the testis by sacrificing the fish. (In order to produce crosses resulting in AquAdvantage Salmon, the sponsor sacrifices the neomales and manually removes their milt in order to fertilize eggs.)

The reproductive performance of populations of male GH transgenic Atlantic salmon that are relatives of AquAdvantage Salmon, but are not triploid or all-female, has recently been assessed by Moreau *et a*l. (2011a), Moreau and Fleming, 2011, and others (see Section 5.2.2.7 for further details). These investigators found that nontransgenic, wild anadromous (i.e., large, migratory) males outperformed captively reared transgenic counterparts in terms of nest fidelity, quivering frequency, and spawn participation. In addition, captively

reared nontransgenic mature parr were superior competitors to their transgenic counterparts in terms of nest fidelity and spawn participation despite displaying less aggression. Further, nontransgenic parr had higher overall fertilization success than transgenic parr, and their offspring were represented in more spawning trials. Collectively, these results suggest that in the event of an escape, AquAdvantage broodstock would have compromised reproductive performance, that is, reduced fitness compared to wild Atlantic salmon.

Similar reproductive studies on GH transgenic coho salmon, although not necessarily representative of the diploid ABT salmon also indicate they are out-competed by wild-reared coho salmon in semi-natural mating arenas within a contained facility (Fitzpatrick *et al*., 2011). In competitive spawning experiments, GH transgenic coho salmon performed fewer courtship and aggressive 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. In addition, during competition with hatchery males, transgenic males failed to spawn and displayed less courtship behavior and competitive behavior

In the unlikely event of an escape or release of GE fish from the PEI 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 transmission. Because there are no populations of wild or stocked Atlantic salmon in the adjacent Fortune River system or any of the other rivers in the area, interactions of AquAdvantage broodstock with wild Atlantic salmon would be highly unlikely. Depending on the time of year when escape or release occurred, interactions with wild Atlantic salmon would require either significant migrations along the PEI coastline to locations where populations of wild Atlantic salmon still occur, or migrations out into the Gulf of St. Lawrence or Northumberland Strait.

Given the very low probability of escape/release, the relatively small numbers of GE broodstock in the PEI facility, the multiple factors likely to preclude long-term survival and establishment in the nearby aquatic environment, and the lack of wild Atlantic salmon anywhere nearby, the possibility for interactions with wild Atlantic salmon is very remote. However, out of an abundance of caution, for the purposes of this analysis it will be assumed that an escape has occurred and some consideration will be given to these possible interactions, particularly the possibility for gene flow.

The potential for gene flow, that is, the ability for an rDNA construct (transgene) to spread, is determined by natural selection and has been described by a net fitness model (Muir and Howard, 1999; 2001; 2002a; 2002b). Net fitness components included in the Muir and Howard model include viability (survival) and reproductive success. Factors used to determine the potential for reproductive success include age at sexual maturity, mating success, female fecundity, and male fertility. Although specific data on these net fitness parameters for AquAdvantage Salmon or diploid ABT salmon had not yet been published in the scientific literature, at the VMAC meeting on September 20, 2010, Professor William Muir (Department of Animal Sciences, Purdue University) reported that diploid ABT salmon although potentially larger than their age-matched wild counterparts, would not have a mating advantage. They are behaviorally out-competed by control males as determined by

nest fidelity, quivering frequency, and spawn participation. Dr. Muir also concluded that male GE salmon displayed reduced reproductive performance relative to control males[70]. Given that both survival and reproductive success of AquAdvantage Salmon and AquAdvantage broodstock are likely compromised to a significant extent (see Sections 7.3.1.1.3 and 5.2.2), the potential for gene flow of the AquAdvantage construct to wild salmon is considered very low.

In addition to pioneering the use of a net fitness model for assessing the environmental risk of genetically engineered fish (Muir and Howard, 2001; 2002b; 2004), Dr. Muir is one of the originators of the Trojan gene hypothesis, which explored possible extinction of populations through the flow of a gene that confers a reproductive advantage while also rendering offspring less able to survive in the natural environment (Muir and Howard, 1999; Howard *et al*., 2004). This hypothesis was generated for, and addresses data derived from, mating and growth behaviors of a laboratory model fish, medaka.

In comments presented to the VMAC in September 2010, Dr. Muir addressed the Trojan gene hypothesis and his data in relation to AquAdvantage Salmon [*sic* diploid ABT salmon] as follows:

> *I want to clearly state that this only occurs as a result of a conflict between mating success and viability fitness. And the data conclusively shows that there is no Trojan Gene effect as expected. The data in fact suggest that the transgene will be purged by natural selection. In other words, the risk of harm here is low.*

More recently, Dr. Muir has stated in a commentary by Van Eenennaam and Muir (2011) that he has reviewed actual AquAdvantage Salmon [*sic* GH transgenic Atlantic salmon] data collected by Moreau and colleagues quantifying critical life history characteristics, such as relative viability and mating success of these fish in multiple environments (these data were discussed in Section 5.2.2, primarily in Section 5.2.2.7). Dr. Muir states that,

> "Analysis of the data showed that none of the net fitness components of AquAdvantage salmon [*sic* GH transgenic Atlantic salmon] were enhanced by expression of the transgene. As a result, the Trojan gene effect would not be predicted to occur in the unlikely event AquAdvantage salmon [*sic* diploid ABT salmon] did escape from confinement. Rather, selection over time would be expected to simply purge the transgene from any established population, suggesting a low probability of harm resulting from exposure to AquAdvantage salmon [*sic* diploid ABT salmon]."

In another recent publication, Dr. Muir stated that, "[b]ased on their data, the long-term risk of GE salmon is close to zero as no fitness advantages in any component were demonstrated, resulting in a purge scenario for the transgene" (Van Eenennaam *et al*. 2013).

Disease transmission to wild populations in the event of escape is another theoretical outcome to be considered in relation to the PEI facility. Although disease transmission is often a concern for aquaculture facilities, it is not an issue at the sponsor's PEI facility for two reasons. First, there are no data to suggest that AquAdvantage Salmon or ABT diploid Atlantic salmon are more susceptible to disease than non-GE salmon and thus more likely to

[70] Transcript available HERE.

be affected by disease (see Section 5.2.2.2). Second, and more importantly, there have been no positive findings of any Canadian or OIE notifiable diseases or disease agents[71] 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. The facility contains state-of-the-art equipment for water treatment and will continue to be inspected annually by Canadian authorities. It will also undergo periodic FDA inspections to verify that this remains the case. Therefore, disease transmission from the PEI 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 PEI 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*., 2000c); however, the availability of food and specific environmental conditions also influence behavior and competition for resources. The recent study of Moreau *et al*. (2011b) on GH transgenic Atlantic salmon indicates 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 (see Table 12). 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 12; 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 diploid ABT salmon. No significant risks associated with production of AquAdvantage Salmon in PEI have been identified.

[71] Canadian reportable diseases include ISA, Viral Haemorrhagic Septicemia (VHS), Infectious Hematopoietic Necrosis (IHN), Infectious Pancreatic Necrosis (IPN), whirling disease (*Myxobolus cerebralis*), and ceratomyxosis (*Ceratomyxa shasta*). OIE notifiable diseases and infections include Epizootic haematopoietic necrosis, infection *with Aphanomyces invadans* (epizootic ulcerative syndrome), infection with *Gyrodactylus salaris*, infection with HPR-deleted or HPR0 ISAV, infection with salmonid alphavirus, Infectious haematopoietic necrosis (IHN), and Viral haemorrhagic septicaemia (VHS).

**Table 12. Potential Environmental Concerns/Impacts for GE Organisms***

| Process | Potential Ecological Consequence | Risk Associated with AquAdvantage Salmon or diploid ABT Atlantic salmon 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<br>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<br>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.<br>The integrated rDNA 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.<br>The rDNA 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 Consequence information derives from Snow *et al*. (2005).

In order to migrate to waters of the United States, any surviving AquAdvantage Salmon or diploid ABT salmon that have escaped from the PEI facility would have to complete a significant long-distance migration. There is no reason to expect any escaped/released AquAdvantage Salmon or diploid ABT salmon to undertake a migration to waters of the United States 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, AquAdvantage adults and diploid ABT salmon should remain in the general vicinity of the PEI broodstock facility in the event of an escape or release, while as previously discussed, pre-smolt life stages would not be expected to survive the local high salinity conditions.

Even if AquAdvantage Salmon or diploid ABT salmon were to undertake such a migration, it is unlikely that any significant numbers would survive the journey. Based on recent return rate data for United States 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 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.

7.5.1.1.2 Effects on Populations of Endangered Atlantic Salmon in Maine

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 diploid ABT salmon would affect those populations for the reasons previously discussed: physical containment at the PEI 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 hostile to survival, as evidenced by the lack of self-sustaining salmon populations in an environment that used to possess plentiful salmon runs. In addition, the fitness of AquAdvantage Salmon and diploid ABT salmon appears to be low in the wild; AquAdvantage Salmon would likely be reproductively incompetent; and they would not carry disease from the broodstock facility. 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 (as well as other areas of the north Atlantic Ocean where the Maine Atlantic salmon populations might migrate to as part of their life cycle), distances which are greater than several hundred miles by sea.

7.5.1.1.3 Conclusions with Respect to Egg Production

FDA has performed an analysis to address the potential environmental impacts of escape or release of AquAdvantage broodstock on the United States, including stocks of endangered wild Atlantic salmon in Maine. Adequate data and information exist to perform this analysis, and none indicates that escape or release of GE salmon (including AquAdvantage broodstock) from the egg production facility would result in significant effects on the environment of the United States. FDA also notes that the containment conditions for AquAdvantage Salmon and diploid ABT salmon in PEI are consistent with guidelines from NASCO in its "Williamsburg Resolution" (see Section 2.3.4). These guidelines call for rearing of transgenic (i.e., GE) salmon in secure, self-contained, land-based facilities.

*7.5.1.2 Effects on the United States as a Result of Escape in Panama*

7.5.1.2.1 Exposure Pathway for Effects on the United States

As described above for the PEI facility, the probability of escape from the Panama grow-out facility is very low due to multiple and redundant physical containment measures. The only likely scenarios for escape or release of any life stage of AquAdvantage Salmon to the local environment in Panama are the same as those previously described for the PEI egg production facility: (1) accidental escape to the adjacent river through complete failure of all physical containment systems at the facility due to a catastrophic event (e.g., major flood or earthquake), and (2) malicious intentional release through a break-in and act of

vandalism or eco-terrorism. Again, because of redundancies in security and containment measures (see Section 5.4.2) at the facility, neither scenario is likely to occur.

Under either scenario, escaped or released life stages of AquAdvantage Salmon could potentially survive, at least for a short time, in the local river near the grow-out facility; however, long-term survival at locations further downstream would be essentially precluded because of high water temperatures and other environmental conditions hostile to Atlantic salmon (see Sections 7.2 and 6.1.2 for additional discussion). Reproduction and permanent establishment in the local environment would also be precluded because all AquAdvantage Salmon will be females and approximately 99.8% will be triploid and effectively sterile (Section 5.3.2). In addition, there are no wild conspecifics or feral relatives with which they could interbreed (see Sections 7.3 and 7.4.1.5).

Because reproduction between females is not possible, establishment of a population of AquAdvantage Salmon could not occur. There are no populations of wild Atlantic salmon in the watershed (or within many thousand miles for that matter) and no populations of closely-related salmonid species with which reproduction is possible[72]; therefore, gene flow to related species will not be a possibility. As previously discussed at length, survival beyond the immediate local environment will not be possible due to hostile environmental conditions of temperature, water quality, and physical barriers further downstream.

No effects on the United States are reasonably foreseeable as a result of escape or release of AquAdvantage Salmon from the sponsor's grow-out facility in the highlands of Panama because there is no possible exposure pathway through which these fish could reach the United States. The grow-out facility is located many miles upstream from the Pacific Ocean. As discussed in Sections 6.1.2.1 and 7.3.1.2, high water temperatures and other forms of geographic/geophysical containment apply to the local watershed to ensure with a high degree of probability that AquAdvantage Salmon would not reach the Pacific Ocean and could not migrate to water of the United States.

#### 7.5.1.2.2 Effects on Populations of Wild Atlantic or Pacific Salmon in the United States

No effects on any populations of wild Atlantic salmon or any of the species of Pacific salmon in waters of the United Sates are reasonably foreseeable as a result of escape or release of AquAdvantage Salmon from the sponsor's grow-out facility in the highlands of Panama. The nearest populations of Atlantic salmon are thousands of miles away in the north Atlantic Ocean in and near the Gulf of Maine. Similarly, the nearest populations of related, but non-interbreeding species of Pacific salmon (e.g., coho, chinook) are also located thousands of miles north of Panama in the Pacific Ocean (i.e., off the central California coast and northward). As discussed in the previous section, no complete exposure pathway exists from the grow-out site in Panama to marine waters in the United States where populations of Atlantic and Pacific salmon live. High water temperatures and other forms of geographic/geophysical containment apply to the local watershed in Panama to ensure with a high degree of probability that AquAdvantage Salmon would not survive to reach coastal areas of the Pacific Ocean near Panama, let alone the north Atlantic Ocean (which would require a migration through the Panama Canal and/or around Cape Horn) and north Pacific Ocean..

[72] Rainbow trout are reported to occur in the watershed; however, Atlantic salmon cannot successfully breed with this species.

#### 7.5.1.2.3 Conclusions with Respect to Grow-out

There is adequate information to address the potential consequences of escape of AquAdvantage Salmon on the environment of the United States including stocks of wild Atlantic salmon. None of this information suggests that escape or release of AquAdvantage Salmon as a result of grow-out would result in significant effects on the environment of the United States.

### 7.5.2 Effects on the United States Due to Escape/Release During Transportation

As discussed above in Section 7.2.1.3, escape of AquAdvantage Salmon eggs during transport from PEI to Panama 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), 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 of the United States are anticipated.

### 7.5.3 Conclusions for Question 4

There is adequate information to address the potential consequences of escape of AquAdvantage Salmon or diploid ABT salmon on the United States, including stocks of wild Atlantic salmon. None of this information suggests that escape of AquAdvantage Salmon or diploid ABT salmon would result in significant effects.

## 7.6 Consequences for the No Action Alternative (Decision Not to Approve the NADA)

As described earlier, there are two general likely scenarios to consider as a result of the no action alternative, that is, an FDA decision not to approve the NADA for AquAdvantage Salmon: (1) cessation of production of AquAdvantage Salmon, and (2) continued production of AquAdvantage Salmon at the existing sites in Canada and Panama and/or at new suitable locations outside the United States (and/or sale of the fish or the technology to producers outside the United States) with no intent to market food from these fish in the United States. There are no consequences or potential environmental impacts arising from the first general scenario--with no production of AquAdvantage Salmon there would be no production sites and no potential for escape or release of these fish to the environment.

For the second general scenario, production of AquAdvantage Salmon at suitable locations outside the United States with no intent to market food from the fish in the United States, i.e., outside of FDA jurisdiction, an assessment of potential effects on the environment becomes highly uncertain as the conditions and effects are not reasonably foreseeable. Because production of AquAdvantage Salmon would be possible at any number of locations worldwide, under different containment conditions and levels of regulatory oversight, and potentially within areas where native Atlantic salmon are present (see Appendix E), there are too many variables and unknowns to define specific scenarios and perform a comprehensive risk assessment for them. A further set of unknowns includes the extent and nature of regulatory decisions in sovereign foreign countries with the authority to regulate either the technology or the products thereof. Thus, it is impracticable to make any accurate predictions with respect to potential environmental impacts on the United States other than to state that should production occur with less restrictive physical or biological containment conditions than those in the NADA, adverse environmental impacts to the United States could be more likely to occur because escape, reproduction, establishment and migration of

the GE salmon would be more likely. The same would be expected if production were to occur in locations where there would be less regulatory oversight than would occur under an FDA NADA approval.

## 7.7 Cumulative Impacts

CEQ regulations define cumulative impact as "the impact on the environment which results from the incremental impact of the present action when added to other past, present and reasonably foreseeable future actions . . . ." 40 CFR 1508.7. There would be no "incremental impact" because this would be the first NADA approval for AquAdvantage Salmon, and FDA is not aware of any specific, reasonably foreseeable future actions on NADAs for GE fish at this time. As a result, there would be no cumulative impacts on the environment of the United States for the action to approve this NADA for AquAdvantage Salmon.

With regard to AquaAdvantage Salmon, at the present time, FDA has no other applications or proposals from ABT to develop and grow AquAdvantage Salmon anywhere but in the Canadian and Panamanian facilities covered by the current NADA, and FDA is not aware of any specific reasonably foreseeable future actions on NADAs for AquAdvantage Salmon. As discussed previously in Section 4.2 on the no action alternative, ABT has many potential options for production of AquAdvantage Salmon in the event that the NADA described herein is not approved by FDA. For example, the sponsor could continue to rear AquAdvantage Salmon at the existing locations outside the United States, and/or at new suitable locations outside of the United States, and could decide to sell the eggs, fish, or the technology to producers outside of the United States, with no intent to directly market food from the fish in the United States (i.e., outside the jurisdiction of the FDA). Many of these same options would also be possible with an FDA NADA approval. In addition, ABT could request to begin production within the United States at one or more locations under a supplemental NADA approval. Because production of AquAdvantage Salmon would be possible at any number of locations worldwide or within the United States, potentially under different containment conditions and within areas where native Atlantic salmon or other salmonid species are present, there are far too many variables and unknowns in relation to the conditions of use and potentially affected environment(s) for the agency to determine at the present time what might be a reasonable foreseeable action(s) in terms of a future NADA(s) for AquAdvantage Salmon. Thus, it is not possible to perform an accurate, comprehensive cumulative impacts assessment taking into account these potential future actions.

As previously stated, this EA pertains to only one specific set of production and use conditions for AquAdvantage Salmon. Should the sponsor at a later time seek to open, or ship to, any additional egg production or grow-out facilities, or to significantly expand existing facilities, a supplemental NADA would need to be submitted, reviewed, and approved prior to using, or shipping to, such a facility. Action by FDA on such an application would be considered a major federal action under NEPA and FDA regulations, and, as such, would require the preparation of an Environmental Assessment and potentially an Environmental Impact Statement, both of which would consider the cumulative impact of the addition of another facility or other proposed changes. Such a supplemental application would also require FDA to consult with NMFS and FWS regarding any potential effects on endangered species.

This EA considers only this one specific action—an approval of a new animal drug application relevant to AquAdvantage Salmon under a specific set of conditions. The agency does not

speculate about any future business expansion by the sponsor because any such speculation would be hypothetical, and the agency would have no particular conditions to evaluate. If such an expansion is proposed at a later time, FDA will have the obligation to consider the concrete specifics of the supplemental application at that time.

## 7.8 Summary

Using a risk-based approach, FDA has performed a rigorous environmental assessment and found no evidence that approval of an NADA for AquAdvantage Salmon would result in significant impacts on the environment of the United States. The agency's findings are summarized by the following list of questions and answers:

- What is the likelihood that AquAdvantage Salmon will escape the conditions of confinement?
    - Due to the presence of multiple, redundant and effective physical containment measures at the sites of both egg production and grow-out (and which would continue to be present under the conditions that would be specified in the NADA), the likelihood of AquAdvantage Salmon (or diploid ABT salmon) escaping into the environment is very low in both Canada and Panama.
- What is the likelihood that AquAdvantage Salmon will survive and disperse if they escape the conditions of confinement?
    - In the unlikely event of an escape or release, environmental conditions at both the egg production and grow-out sites are sufficiently inhospitable to limit long-term survival and spread of AquAdvantage Salmon or diploid ABT salmon to other locations. This is particularly true for the earliest life stages (eggs and embryos) in PEI, which because of their small size, would also be the mostly likely to escape. These life stages would be unlikely to survive if exposed to high salinity and low temperature conditions in the nearby aquatic environment. It is also true for all life stages of these salmon in Panama, which would be unlikely to survive the high temperature conditions in the lower reaches of the watershed in which the grow-out facility is located, or farther afield, such as in the tropical Pacific Ocean.
- What is the likelihood that AquAdvantage Salmon will reproduce and establish if they escape the conditions of confinement?
    - Under the conditions specified in the NADA, AquAdvantage Salmon must be produced as all-female, triploid fish. As such they would be effectively sterile. Such conditions would require that, based on testing, a minimum of 95% of the AquAdvantage Salmon eggs sold for commercial production use would be triploid; the actual average percentage of triploidy has been shown to be approximately 99.8% based on results of the method validation studies required by FDA. All of the fish are expected to be female based on the method of production. The fertility of triploid females is negligible compared to normal diploid females. The combination of triploidy and an all-female population is expected to render AquAdvantage Salmon effectively and functionally sterile resulting in complete reproductive containment. As a result, establishment of a population of these fish in the accessible environments of PEI and Panama would be essentially precluded in the highly

unlikely event that an escape occurs. The only realistic potential means for establishment (or pseudo-establishment) would be through the escape of reproductively competent diploid ABT salmon at the PEI facility or through a continual series of escapes of AquAdvantage Salmon at the Panama facility. Neither of these scenarios is likely given the physical containment measures in place at both facilities. Both would require the escape of a significant number of animals, a condition that is even less likely. Therefore, given the available information, FDA concludes that it is extremely unlikely that AquAdvantage Salmon or diploid ABT salmon would establish and reproduce if they escape from either facility.

- What are the likely consequences to, or effects on, the environment of the United States should AquAdvantage Salmon escape the conditions of confinement?

    - The collective information on the potential for survival, dispersal, reproduction and establishment indicates that exposure pathways for AquAdvantage Salmon or diploid ABT salmon to reach the United States are incomplete; therefore, no effects are expected on the environment of the United States (including populations of endangered wild Atlantic salmon in Maine).

In summary, the evidence collected and evaluated by FDA indicates that the development, production, grow-out and human consumption of AquAdvantage Salmon under the conditions that would be established in the NADA, if approved, and as described in this EA, would not result in significant effects on quality of the human environment in the United States.

## 8. PUBLIC AND AGENCY COORDINATION (Persons and Agencies Consulted)

### 8.1 Interagency Coordination

This Environmental Assessment is the culmination of many individual steps that have either been generated, prepared, or peer-reviewed under the direction or request of CVM at FDA. The following listing outlines some of the more significant steps during this 15 year process.

- In 1995, the sponsor requests an investigational exemption for AquAdvantage Salmon under 21 CFR Part 511.
- FDA issues an EA and FONSI for the investigational phase of the AquAdvantage Salmon New Animal Drug Application in 2001.
- Pivotal studies in support of an eventual New Animal Drug Application, including studies that support this EA, begin in 2001 once the sponsor establishes genetic stability of AquAdvantage Salmon over four generations.
- FDA conducts an inspection of the Prince Edward Island, Canada, broodstock facility in October 2008. Participants include subject matter experts from CVM as well as an inspector from FDA's Office of Regulatory Affairs.
- CVM issues Draft Guidance for Industry 187 for public comment in 2008. The guidance clarifies FDA's continuing authority to regulate GE animals and details the overall process for review of data submitted in support of an eventual New Animal Drug Application with CVM; the Guidance is issued in final form in early 2009.
- CVM experts in aquaculture, biotechnology, and environmental risk assessment conduct a site visit to the Panamanian grow-out facility in November 2009, accompanied by a fisheries expert from the National Marine Fisheries Service to provide additional expertise and consultation.
- In October 2010, FDA sends FWS and NMFS letters stating that FDA has made a "no effect" determination under the ESA. FDA clarifies the proposed conditions of use (PEI and Panama) and reaffirms that any additional facilities would require a supplemental application, a new environmental analysis, and a new ESA determination.
- In December 2010, FWS issues a concurrence letter to FDA regarding FDA's "no effects" determination with regard to AquAdvantage Salmon and populations of endangered Atlantic salmon. A copy of the FWS letter is provided in Appendix D.
- In April 2011, FDA hosts an Intergovernmental Workshop on FDA's review of AquAdvantage Salmon with authorities from the United States, Canada and Panama in attendance. In addition to staff from FDA, representatives of several other U.S. Federal agencies, including the National Marine Fisheries Service, Fish and Wildlife Service, and the U.S. Department of Agriculture are present at this workshop.

- In July 2011, NMFS issues a letter to FDA on the subject of FDA's "no effects" determination with regard to AquAdvantage Salmon and populations of endangered Atlantic salmon. A copy of the NMFS letter is provided in Appendix D.

These steps represent only some of the many conversations both within FDA and between FDA and other Federal agencies over more than the last 15 years.

In addition, in November 2013, the Canadian government issued a Significant New Activity (SNAc) for AquAdvantage Salmon based on risk assessments (including a qualitative Failure Mode Analysis) conducted by DFO that concluded that these salmon were not "CEPA Toxic" (see Section 2.5.1).

## 8.2 VMAC Public Meeting

On September 19-20, 2010, FDA's VMAC held a meeting to address science-based issues associated with the material submitted by the sponsor in support of the NADA for AquAdvantage Salmon.

As a part of that meeting, CVM released a great volume of data and analysis to the committee and the public. The September 19 session was an orientation for VMAC members on the technology of producing genetically engineered animals and the agency's regulatory process for evaluating these animals. During the September 20 session, CVM presented information on animal health, food safety, environmental concerns, and data supporting the safety and effectiveness of AquAdvantage Salmon. Both days of the VMAC meeting were open to the public. Interested members of the public were invited to present data, information, or views to the committee, orally or in writing. Materials presented at the meeting as well as the VMAC Chair's final report are available on FDA's website[73].

This EA differs from the EA released for the VMAC meeting.The draft EA released to the VMAC was prepared by the sponsor under the agency's overall direction. This EA has been prepared by the agency, and has taken into account comments that were submitted during the open public comment period.

### 8.2.1 Public Comment Period

FDA published notice of the release of a draft EA and the accompanying preliminary FONSI in the Federal Register on December 26, 2012. The public was initially given 60 days to submit comments on the document, but the comment period was later extended for an additional 60 days closing on April 26, 2013. In accordance with 21 CFR 25.51, after reviewing and considering the public comments, FDA has revised the draft EA and issued this final EA.

[73] Available HERE.

## 9. PREPARATION OF EA

This EA has been prepared by the Center for Veterinary Medicine at FDA, and includes changes made in response to substantive public comments. The initial draft EA was submitted by AquaBounty Technologies, Inc. and was included in the Briefing Packet prepared for the VMAC (FDA, 2010).

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## 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 non-GE 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 Geographic Range: Historical and Current

Atlantic salmon have historically inhabited the North Atlantic Ocean and associated coastal drainages. In North America, the species was distributed in river systems and marine waters from the Hudson River in New York state northward. In Canada, Atlantic salmon were found in the Bay of Fundy, throughout the Gulf of St. Lawrence and along the whole coast of Newfoundland and Labrador to the Fraser River. Self-sustaining populations no longer exist in many historical rivers at the southern distributional limits in the eastern United States and the adjacent Maritime Provinces of Canada (Webb *et al*., 2007). Native populations have also become extinct in the upper St. Lawrence River, including Lake Ontario. Where stocks of Atlantic salmon remain, populations are generally depressed and frequently supported by supplemental stocking programs.

Populations of Atlantic salmon in the Eastern Atlantic historically ranged from northern Portugal at the southern end to the tributaries of the Barents Sea and White Sea (Russia) in the northeast, including most rivers draining into the Baltic and North Seas. Native, wild stocks are no longer found in the Elbe and Rhine Rivers or in many of the rivers draining into the Baltic Sea (Webb *et al*., 2007). The species is also severely depressed or extinct in the rivers of France, Spain, and Portugal at the species' southern limit.

### 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, 1963; Scott & Crossman, 1973 as cited by Teufel et al., 2002). The ecomorphological demands of the spawning grounds are stringent and include the following: *water descent* of 0.2-3%; *water depth* of 50 to 90 cm; *running speed* of 0.3 to 0.7 m/s; *gravel size* of 3 to 5 cm; and, *nest size* of 1 to 2 m (MUNLV, 2001).

The eggs are buried in gravel at a depth of about 12-25 cm (Bigelow, 1963; Scott & 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 males or females salmon survive to spawn three or more times.

Only about 9-20% of the fertilized eggs in the redds survive to develop over the winter, and depending on temperature and water conditions will usually hatch in April. The hatchlings, often referred to as "**alevin,**" are mostly transparent, and have large yolk sacs. These alevin remain in the gravel feeding on their yolk sacs until they are absorbed, after which the young fish emerge from the redd and begin foraging for food in the water column. This typically occurs in May or June. Once "swim up" has occurred, these small fish are referred to as **fry** (as in "small fry") or swim-up fry. Hungry, they swim freely, and begin to eat—insect larvae, other small organisms called zooplankton, and fish eggs, including those of their own species.

As the fry mature, and become more fish-like in appearance, they develop a series of spots along their sides, from which dark vertical stripes descend. These markings, referred to as **parr marks**, aid in camouflaging the young fish, which are preyed on by other fish, mammals, and birds that live along rivers and streams. At this stage, the juveniles are referred to as "**parr.**" They remain in their **natal** (birth) **streams**, feeding on the larvae of insects, worms, and shellfish, and sometimes each other or related species (such as trout).

If there is plenty of food, and other environmental conditions are good (the water is clean and there is enough oxygen), those parr not consumed by other fish, birds, or other animals, grow rapidly during their first summer. Parr can be very territorial, and aggressively protect their space from other parr. As the parr become larger, their territories expand, probably to ensure a reliable source of food.

Parr may spend between one and six years (usually two to three years) in their natal streams; at some point, if they are not in land-locked lakes, they begin their downstream migration and prepare for life in the sea. They are usually about six inches long at this point in their development (as depicted here.). The seaward migration involves a change in physiology which allows the young salmon to adapt to salt water conditions. This transformation in physiology is referred to as "smoltification" and the young fish that migrate to the sea are called "**smolts**". In general, smolts tend to live for a while in brackish (part salt) water, such as bays and estuaries while they complete their adaptation to salt water. It is thought that the "imprinting" of the natal river occurs during smoltification (http://www.nmfs.noaa.gov/fishwatch). At this stage, the fish lose their parr marks and take on silver color. They also become more elongated than they were as parr and have darker fins.

At the end of the spring during which they have adapted to living in salt water, the smolt generally swim to sea. For example, Atlantic salmon leave Maine rivers some time in April or May and can be found in the waters off Labrador and Newfoundland by mid-summer. They then migrate to take advantage of available food supplies and generally spend their first winter at sea off the coast of Greenland. While at sea, salmon are sometimes referred to as "opportunistic pelagic feeders." That means they eat whatever is edible in the open sea: other 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 8-15 pounds, and be up to 30 inches long.

During their time in the open sea, which can last from one to several winters, the fish become sexually mature. Upon first entering the sea, the salmon keep the silver hue and darker fins of the smolts, and gain some black spots on their backs. Their bodies become even more elongated, and they become strong and elegant swimmers.

Post-smolt salmon age is counted in units of "winters at sea." In general, a salmon that spends one winter at sea prior to becoming sexually mature and returning to its natal stream to spawn is called a "**grilse**." A salmon that spends two years at sea is referred to as a "**2SW**" (sea winter) fish. In general, the longer a salmon spends at sea feeding, the larger it becomes, although Atlantic salmon rarely get bigger than about 25 pounds.

Salmon typically form schools after they enter the sea and may travel with or be mistaken for herring, mackerel or other pelagic fish, since post-smolts occur as by-catch in these fisheries according to the North Atlantic Salmon Conservation Organization (NASCO, 2007). Post-smolts follow ocean currents, feeding as they migrate, and adding fish to their diet of marine invertebrates at a size of about 27 cm (fork length) after a few months at sea. Survival in fresh water from egg to smolt varies from 0.3-2.6%. Survival in the sea from smolt to return as grilse varies from 1.3-17.4% (Hutchings & Jones, 1998). Most Atlantic salmon (70-80%) survive spawning and migrate to sea a second time as kelt; only about 10% of them return to spawn a second time (Fleming, 1998).

Regardless of their age, as Atlantic salmon migrate back to their natal rivers and streams, the fish become sexually mature, and their shape and coloration begin to change, with pigment changes more prominent in the males. In general, males become redder on their bellies, or red with purple spots; females tend to be blue-black in color. They become less elongated and thicker in the body; the females, in particular, become swollen with eggs. The males also develop teeth and an exaggerated hooked lower jaw referred to as a "**kype.**" These are useful in fending off the unwanted attentions of other males to their selected females during spawning.

A few salmon never make the transition to 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 before the sexually mature large males can. Because they are small, the precocious males are not recognized as threats by the larger mature males, and are generally not the object of their aggression. Precocious parr make up approximately 1% of the male population, but may end up fertilizing up to 20% of the total eggs that are released by females.

The size of the adult fish is more dependent on time spent feeding at sea than on age. Sea-run Atlantic salmon usually attain a larger size than do landlocked salmon (i.e., those living entirely in fresh water). Sea-run salmon range from 2.3 to 9.1 kg and commercially-raised fish average 4.5 to 5.4 kg. (Teufel *et al*., 2002). Many aspects of Atlantic salmon behavior are affected by size. Investigations of growth in parr have shown that they may segregate into two or more groups at the end of the first growth season. Parr in the upper modal group may smoltify at 1+ years versus the lower modal groups, which may smoltify later (Metcalfe *et al*., 1988). Within populations, therefore, the onset of the parr-smolt transition is dependent on growth rate. Smolt size can also vary widely among populations (Klemetsen *et al*., 2003). 1-SW salmon spawn usually every year, while older sea-age salmon are primarily biennial spawners; within populations, the proportion of biennial spawners increases with the size of fish at first maturity. The proportion of repeat spawners decreases with size of fish. This may be related to energy expenditure due to spawning: 1SW salmon may allocate 50% of their energy (Jonsson *et al.*, 1991) for spawning compared to 70% for older salmon (Jonsson *et al.*, 1997).

Fecundity, or potential reproductive capacity, is another trait that varies considerably both within and among salmon stocks. Fecundity is typically expressed in terms of numbers of eggs (gametes). Egg number and egg size increase with body size (Thorpe *et al.*, 1984; Jonsson *et al.*, 1996). Although absolute fecundity varies greatly among individuals, as expected owing to high variability in adult body size, relative fecundity (eggs/kg total egg mass) as a measure of reproductive effort varies much less. The faster that parr grow in fresh water before smoltification, the smaller their relative egg size becomes when they attain maturity. This phenotypic response has been explained as an adaptation to the potential growth opportunities in their nursery river. Usually, both egg size and fecundity increase with size of fish (Klemetsen *et al.*, 2003).

Atlantic salmon compete for food and space in fresh water (Chapman, 1966) where they may be "keystone species" like Pacific salmon (steelhead, *Oncorhynchus mykiss*), which along with California roach (*Hesperoleucas symmetricus*) were found to influence the entire food web in a Northern California river (Power, 1990). In marine waters, however, even at their highest levels of historical abundance, Atlantic salmon are rare relative to the available

space and few in proportion to total biomass of fish populations, and are thus expected to play a more minor role in the food web (Hindar, 2001).

## 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). Stead and Laird (2002) have cited the upper lethal temperature for salmon as being 23ºC. In a study examining the tolerance and resistance to thermal stress in juvenile Atlantic salmon, Elliot (1991) acclimated the fish for two weeks to various temperatures (5, 10, 15, 20, 25 & 27ºC) then raised or lowered the temperature by 1ºC per hour. The incipient lethal levels defined the tolerance zone within which salmon lived for a considerable time (i.e., survival over seven days). Salmon acclimated to 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 (Elliot, 1991).

This research collectively indicates that although fish acclimated to relatively high temperatures may be able to survive more than seven days at these high temperatures, they do not feed at temperatures above ~23ºC and would eventually starve. Willoughby (1999) presents the feeding and activity range for smaller Atlantic salmon (i.e., < 100 g) in fresh water as favorable up to ~23ºC, with mortality occurring at ~26ºC. For larger Atlantic salmon, the available data for sea water show the feeding and activity range as favorable up to ~20ºC, with mortality occurring at ~22ºC. Elliott (1991) noted that little is known about the upper temperature limits for survival of Atlantic salmon in the field, and reported studies showing tolerances similar to those observed in his laboratory. Other experimental studies summarized by Elliott (1981, 1991) indicate that the optimum temperatures for growth of young Atlantic salmon are in the range 16-19ºC.

The minimum ***pH tolerance*** is between pH 5.0-5.4 depending on other river variables (e.g., aluminum levels), with eggs being the developmental stage least sensitive to acidity, followed by parr, and then smolt and fry, which are the most sensitive (Amiro, 2006).

Salmonids are known for requiring more ***dissolved oxygen*** than "warm-water fish." Shepherd and Bromage (1995) state that the DO content of water in a salmonid farm

should never drop below 6 mg/L and that carbon dioxide (which influences the pH of the water) starts to be a problem for salmonids above 15 mg/L. Similarly, Stead and Laird (2002) suggest that DO levels should never fall below 5 mg/L; for good growth, a minimum of 7 mg/L is essential.

Other challenges to survival come from ***obstructions and siltation***. Passage of salmon upstream can be blocked by natural and man-made obstructions (e.g., dams), as most vertical obstructions in excess of 3.4 m will block the upstream passage of salmon. In addition, high concentrations of fine sediments in the spawning gravel may decrease embryo survival and fry emergence through a reduction in the intragravel flow necessary for adequate water oxygenation. For example, the presence of as little as 0.02% silt (<0.063 mm) during incubation has been shown to decrease embryo survival (Julien and Bergeron, 2006).

Atlantic salmon have the capacity to cope with a wide variety of ***flow conditions***, and juvenile salmon have been known to prefer pools at lower discharges and move from pool to riffle habitats at higher discharges. Their ability to adapt to changes in flow and tolerance of relatively high water temperatures enables juvenile salmon to occupy extensive sections of streams that experience variations in flow outside the range of useful habitat of some competitive sympatric species (Amiro, 2006).

## 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 19$^{th}$ 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 Administration's (NOAA) Fisheries Services and 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, approximately 1.5 million salmon returned to their natal rivers in Eastern Canada; by 2004, that number had dropped to approximately 350,000 (http://www.traffic.org).

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[74]. 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 5.3 of the EA. This section of the appendix discusses information about the interaction of domestic salmon with their wild counterparts to provide context for predicting how AquAdvantage Salmon might fare in the unlikely event that they would be released into the wild (Sections 7.4 and 7.5).

### A.6.1. Salmon Farming

Atlantic salmon farming can occur at locations throughout the world where there is access to clean, cold water. The greatest production currently occurs in Norway, Chile, Scotland and Canada where smolts are typically grown to market size (generally 2 - 5 kg) in ocean net pens or cages. Other countries with significant production of Atlantic salmon include Australia, China, New Zealand, the Faroe Islands, and the United States.

Salmon farming industries rely on domesticated breeding lines selected for commercially important phenotypic traits, most importantly, faster growth and delayed sexual maturation

[74] Available at http://www.nero.noaa.gov/nero/hotnews/salmon/FinalATSRPlan.pdf

(Gjedrem *et al*., 1991). The oldest of these lines, developed in Norway and incorporated into virtually all commercial breeding programs (except those in eastern Canada which are based on a local line), achieved a growth rate improvement of about 10% per generation over the first seven generations of development (Gjøen & Bentsen, 1997).

Although Atlantic salmon can complete their entire life cycle in fresh water, most commercial Atlantic salmon farming involves both fresh and saltwater phases. In the freshwater phase, eggs are provided with a continuous flow of oxygenated water until they hatch. Typically, the alevin are transferred to small fiberglass tanks while they absorb the yolk sac prior to first-feeding. Once established on feed, the fry are transferred to larger tanks and grown to the parr stage, when they are sorted by size, segregated by growth rate, and transferred to separate tanks. In some locations, the parr may be transferred to lakes for the final phase of freshwater rearing. When the parr reach 60-120 g and begin to take on the silver coloration of smolt, they are typically transferred to saltwater production units called net pens or sea cages.

Under ambient light and temperature conditions, the freshwater phase typically takes 14-16 months, but is often shortened to eight months by increasing the early-rearing temperature and introducing a short period of darkness after the summer solstice to trigger smoltification at the next equinox (fall rather than spring) (McCormick *et al*., 1987). Virtually all commercial smolt are vaccinated against pathogens of local concern to reduce the risk of disease, pathogen amplification, and the need for antibiotic treatment before transfer to sea water. The saltwater grow-out phase begins when the smolt are transferred to sea water and lasts for 12-26 months, depending on ambient sea temperature and the contingencies of harvest-to-order marketing. Feeding usually occurs twice a day, with feed generally moved by compressed air through tubes from a central hopper to each individual sea cage. The fish are fed until uneaten feed is detected by an underwater sensor.

### A.6.2. Interactions between Non-GE Domesticated and Wild Salmon

Four general areas of potential interaction between natural salmonid populations and escaped, farm-reared, non-genetically engineered fish that could conceivably lead to environmental impacts:

- Transfer of exotic pathogens or amplification of endemic pathogen loads (Saunders, 1991; McVicar, 1997);
- Genetic disturbance caused by transmission of fitness-reducing alleles (Ryman & Utter, 1987; Frankham, 1995), disruption of locally-evolved allelic combinations (Templeton, 1986; Ryman *et al*., 1995; McGinnity *et al*., 2003), or "swamping" of the native gene pool (Sægrov *et al*., 1997);
- Direct competition for environmental resources, such as habitat, food, or mating opportunities (McGinnity *et al*., 1997; Fleming *et al*., 2000); and
- Ecological disturbance through interference competition or disruption of local equilibria in complex systems, such as food webs, predator-prey relationships, or migration patterns (Lacroix & Fleming, 1998).

To provide additional context for potential application to AquAdvantage Salmon, each of these potential interactions is discussed in more detail below.

#### *A.6.2.1 Pathogen Transfer*

Documented examples of pathogen transmission between artificially-propagated and wild fish are not common, but have been known to occur through stock enhancement programs involving transfer of live fish and eggs (Brackett, 1991). For example, several incidents in the late 1980s suggest circumstantial involvement of farmed salmon in the movement of an endemic bacterium, *Aeromonas salmonicida*, which causes furunculosis, from Scotland to Norway (Johnsen & Jensen, 1994; Inglis *et al*., 1991). There is little direct evidence of bacterial disease transmission from commercial to wild salmon. None of the reviews that have evaluated the available scientific literature on the potential for disease interchange between wild and farmed salmon has found irrevocable evidence that fish farming has contributed to detectable adverse changes in wild fish populations (McVicar *et al*., 2006).

When wild fish are exposed to pathogens shed from farmed fish, it is not inevitable that infection or disease will occur in the wild fish population (Oliver, 2002). Critical factors affecting the spread of disease include:

- The occurrence and persistence of the infection in the source population;
- The availability of susceptible potential new hosts;
- The viability and concentration of the infectious organism in the environment; and
- The ability of the infection to affect the recipient population from individual fish infections.

The initial risk level of infection in wild fish associated with escaped farmed fish depends on the length of survival, behavior of the escaped fish after leaving the farm, and the reduced disease transmission opportunity in the lower fish densities outside of the farm (McVicar *et al*., 2006). In general, farmed fish are considered less fit or maladapted for survival in the wild (Fleming *et al*., 2002). In the event of escape, the presence of disease, if it occurs, would be expected to lead to the early disappearance of the most seriously affected fish, thus rapidly limiting the spread of disease transmission.

In contrast to disease transfer, the transmission of parasites by cultured fish is less subject to debate (McVicar *et al*., 2006). The introduction of *Gyrodactylus salaris* (the salmon fluke) to Norwegian waters in 1975 has been clearly linked to resource management activities (Johnsen and Jensen, 1991), but the role of farmed salmon in the subsequent epidemiology remains under investigation (Bakke & Harris, 1998). Salmon lice, *Lepeophtheirus salmonis*, are endemic throughout the native range of Atlantic salmon, making a direct link to salmon aquaculture difficult to establish. White (1940) associated the occurrence of "white spot" and salmon mortalities with sea lice infections in wild Atlantic salmon populations in eastern Canada as early as 1940, well before the advent of commercial salmon farming. Natural populations of parasites may be amplified in areas associated with salmon farming (Bakke & Harris, 1998), but sea lice abundance may be associated with rising marine temperatures as much as with the availability of hosts.

#### *A.6.2.2 Genetic Disturbance*

Atlantic salmon have been subject to significant selection pressure, both intentional and inadvertent, as a result of human activity for more than a century. The former include, but are not limited to, size-selective harvesting, stock-enhancement efforts, transplantation across drainages and ecosystems, and increasing importance of commercial and recreational objectives; the latter derive (in part) from hydro-electric dams, acid rain, agricultural (and other) run-off, increased sedimentation and water temperature due to deforestation, and stocking of native (striped bass) and non-native (rainbow & brown trout) salmonid predators. Despite these challenges, evidence of genetically-differentiated

population structuring is still evident for salmon at local, regional, and continental scale based on allozyme, mitochondrial, and nuclear DNA analyses (Ståhl, 1987; Bourke *et al*., 1997; Bermingham *et al*., 1991; McConnell *et al*., 1995; Taggart *et al*., 1995; King *et al*., 2001). The temporal stability of this structure has been traced over decades through the analysis of genetic material contained in archived scales (Nielsen *et al*., 1997; Tessier & 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.6.2.3 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 & Sægrov, 1991; Webb *et al*., 1991), only a small proportion of the number that escape from farms actually breed (Webb *et al*., 1993; Clifford *et al*., 1998), and then at a fraction of the spawning rate of wild Atlantic salmon (Fleming *et al*., 1996; Clifford *et al*., 1998). There are two primary reasons for this:

- ***Although socially dominant in culture environments, farmed Atlantic salmon are subordinate in nature:*** salmon form dominance hierarchies around foraging opportunities; farmed salmon establish their social status in confinement where foraging opportunities differ significantly from those in the wild. In nature, despite the imposition of dominance by large fish, there is a residual "resident advantage" held by the wild fish that deters even the largest fish from evicting territory holders from home ground; and

- ***Farmed salmon compete poorly for mates and spawning locations:*** males are particularly disadvantaged in both access to mating opportunities and breeding success (Fleming *et al*., 2000); farmed females enter rivers out-of-phase with wild salmon, make fewer, poorly-covered nests, breed for a shorter period of time, and retain more eggs that remain unfertilized (Jonsson *et al*., 1997; Webb *et al*., 1991).

Consequently, even when they are within their "home range", the reproductive success of escaped, domesticated Atlantic salmon from spawning to $F_1$-adult return ranges only from 2-19% (Clifford, 1998; Fleming *et al*., 2000; McGinnity *et al*., 2003) of that achieved by wild Atlantic salmon; the additional loss of 68% of eggs in the $F_2$-generation 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.6.2.4 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 & 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[75] 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 & Ruggerone, 1997).

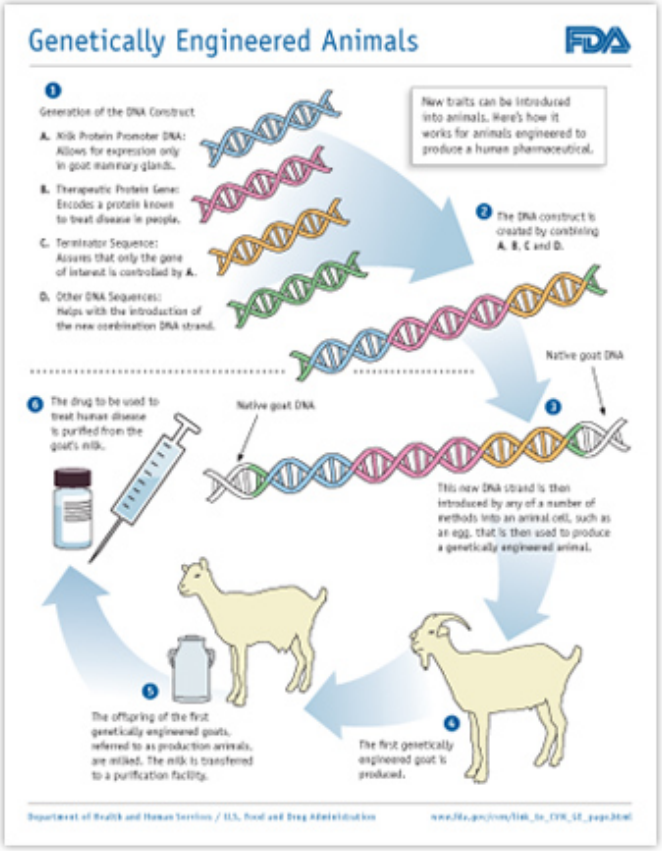
One key risk parameter, the number of animals escaping containment, is difficult to establish with certainty due to inconsistencies in reporting, lack of long time-series, decomposition of small fish that die in sea cages, and limited data collection on escapees at sea. One generally accepted estimate of escapees from sea cages in the North Atlantic is approximately 2,000,000 Atlantic salmon (McGinnity *et al*., 2003). This number represents an escape rate of about one percent. Less than two percent of wild Atlantic salmon currently return to spawn at their natal streams. Escaped farmed salmon survive marine conditions and migration at one-third to one-half of the rate for wild Atlantic salmon and return to fresh water at about 1% of the numbers that are estimated to escape (Butler e*t al*., 2005).

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

## Appendix B. Genetically Engineered Animals

Genetically engineered animals are produced when genes are introduced into the animals by the processes of modern biotechnology—sometimes these animals are referred to "bioengineered," or "genetically modified." In the U.S. regulatory system, they are referred to as "genetically engineered" or "GE" (See Figure A).

**Figure A: Overview of Genetic Engineering**

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Genetic engineering has been widely used to alter the characteristics (traits) of organisms so that they produce various products. Bacteria and other microorganisms have been produced that make enzymes used in food processing. They have also been engineered to produce pharmaceuticals for human use. For example, most of the insulin sold in the United States is produced in a genetically engineered strain of *Escherichia coli* that contains a gene for the human form of insulin (Humulin). Many of our staple crops such as corn and soy have been genetically engineered to be resistant to certain herbicides or to contain a protein that is toxic to the caterpillar phase of common pests such as the corn borer. Genetically engineered papaya is resistant to ring-spot virus, which nearly wiped out papaya crops.

FDA has approved one application related to a genetically engineered animal. This is for a goat engineered to produce a human pharmaceutical in its milk. That pharmaceutical has also been approved by the European Medicines Agency (EMEA). In the United States, FDA's Center for Veterinary Medicine approved the recombinant DNA construct in the goat, and the Center for Biologics Evaluation and Research approved the pharmaceutical (recombinant human antithrombin III) for use in individuals with clotting disorders.

Genetic engineering to introduce new traits (characteristics) is generally accomplished by selecting a gene of interest (a gene is a stretch of DNA that contains the information to code for a protein). In general, scientists join that piece of DNA to what are referred to as "regulatory signals" in a process sometimes referred to as "gene splicing" or "producing recombinant DNA."

The DNA in almost any cell in an organism contains all of the information required to direct the function of that organism—that is kidney cells from a cow contain all the information to allow a cow to be a cow, as do cells from the ear, or liver, or udder.

If we think of DNA as a roadmap of information that has instructions for producing substances necessary for life, then it's easy to see that without additional bits of information that serve as "traffic signals," cells wouldn't "differentiate" or take on specific functions. Those "traffic signals" generally tell the cell's machinery what genes to express and what genes to leave silent. Some of these traffic signals are referred to as "promoters." These sequences of DNA are usually found in front of genes and tell the cell's machinery when and where to start processing the information in the gene of interest.

"Expressing" a gene means that the DNA is "transcribed" into a chemical form (RNA) that can then be "translated" into a protein that actually does something. There are different kinds of promoters. Some are tissue-specific; that is, they only turn on those genes that are supposed to be expressed in a particular cell or organ. Mammary specific promoters, for example, tell the cells of mammary glands to make those proteins and other substances that make milk. Some promoters tell the cell to make some substances all the time—for example, those proteins that are responsible for the day-to-day functioning of the cell. Some promoters tell the cell to make certain substances at specific times during the organism's life, such as those responsible for sexual maturation. Scientists have attempted to isolate and use the promoters that are best suited for expressing the genes of interest that are being introduced to alter the characteristics of the organism that is being engineered.

In Figure A, scientists spliced a mammary-specific promoter to the gene of interest—one that contains instructions to make a human protein called antithrombin III, and introduced it into goat embryos. They then check the resulting goats to see which ones express that protein in their milk, and breed a line of goats that can pass that gene on to their offspring. The females of that herd are used to produce the human pharmaceutical in their milk (More information can be found here).

## Appendix C. FDA's Regulation of Genetically Engineered Animals

### C.1 Why does FDA regulate GE animals?

The rDNA construct, which is a piece of DNA that is added to an animal in order to alter or change its characteristics or traits, for example to make fish grow faster, meets the definition of a "drug" under the FD&C Act since this rDNA is "[an] article[] (other than food) intended to affect the structure or any function of the body of man or other animals." 21 USC § 321(g). As shorthand in this document, we sometimes refer to regulation or approval of the rDNA construct as regulation or approval of GE animals. The agency clarified its legal authority to regulate GE animals in GFI 187, which is available here.

GFI 187 describes, at a fairly general level, the kinds of information FDA needs to evaluate in order to reach decisions regarding safety and effectiveness of GE animals.

### C.2 How does FDA evaluate GE animals?

In the overall process described in GFI 187, FDA examines (1) safety of the rDNA construct to the animal; (2) safety of the food from the animal; (3) environmental impact; and (4) the extent to which the producers of GE animals (referred to as "sponsors") have met the claims made for those GE animals (effectiveness). All of these are based on a thorough analysis of the rDNA construct, its integration into the animal's DNA, and its stability in the animal over multiple generations. GFI 187 describes this in seven steps that we summarize in the following discussion. Each step is dependent on the results of the analysis performed in the preceding steps, so that the review in effect "rolls up" conclusions as it progresses through the entire process.

First, FDA reviews data and information on how the construct is made, and whether it contains any pieces of DNA from viruses or other organisms that could pose adverse health risks to the animal or people or other animals eating food from the animal. We evaluate the rDNA construct to determine whether pieces of DNA came from viruses that could intermix with similar viruses (in that species or other species with which it has close contact) and perhaps create a new virus that could pose health risks, similar to the way that avian flu arose. The agency also looks to see if any pieces of the construct will make new proteins (except for the intended ones) that could possibly cause health concerns. GFI 187 refers to this analysis as the "Molecular Characterization of the Construct."

Second, FDA evaluates studies submitted by the producer to determine what happens when the rDNA construct is incorporated into the animal, and how it behaves over multiple generations in what GFI 187 refers to as the "Molecular Characterization of the GE Animal Lineage." This includes analyzing whether the construct remains in the same place over time, and whether animals continue to express the trait (characteristic) that the construct is supposed to introduce.

Third, FDA determines whether the rDNA construct is safe for the resulting line of GE animals by performing what GFI 187 refers to as the Phenotypic Characterization. The agency does so by reviewing studies that characterize the actual GE animals over several generations. Questions that the agency asks include whether the resulting GE animals look like their "regular" counterparts by comparing them to both closely related animals and to animals of the species in general. The agency asks whether the GE animals are healthy, including disease resistance, and whether they reach the same developmental milestones that comparison animals do. Another safety question that is evaluated is whether there are any abnormalities that would not be found in other relatives of the GE animal which might

express similar traits, but via conventional breeding. For example, if an rDNA construct were introduced to make the animal grow faster, would close relatives that had been selected to grow faster via other assisted reproductive technologies or natural breeding show any effects that could be due to fast growth? In addition, we evaluate the results of necropsies (examinations of the bodies and tissues of animals that have been sacrificed for that purpose) to make sure that cells, tissues, and organs look normal. We also assess the results of the kinds of tests that doctors might perform on people when they get a physical, such as blood cells, blood chemistries, etc., to determine whether the animals not only look healthy, but also that their bodies are functioning appropriately. The agency evaluates the actual chemical composition of edible animal tissues to make sure that there are no substances in the tissues that could harm the GE animal or people who eat it, if it is intended for food use.

Fourth, FDA performs what GFI 187 calls a Durability Assessment. This reviews the plan that the sponsor will agree to in order to ensure that the GE animals produced in the future will be equivalent to the GE animals that we evaluate as part of the pre-approval review. This involves returning to some of the data presented in the characterization of the lineage of GE animals described in the second step, to ensure that the rDNA construct remains stable in multiple generations of the GE animal, and reviewing the plan that the sponsor is proposing in order to monitor subsequent generations of the GE animals.

Fifth, FDA assesses whether GE food animals are safe to eat. This evaluation relies on information gathered in the parts of the application that look at the rDNA construct and the health of the animal. FDA experts in food safety look carefully at the composition of the edible tissues of the GE animal to determine whether its meat or milk or eggs differ in any way that affects safety or nutrition from the non-GE counterparts that we eat today. These experts evaluate whether the levels of key substances such as proteins, fats, minerals, and vitamins are in the same range as they are in the food we eat from non-GE animals. If there are any differences, FDA must determine that there is a reasonable certainty of no harm from any of those differences.

In addition, FDA's food safety experts evaluate data to determine whether the GE animal poses any more allergenicity risks than its non-GE counterparts currently on the market. There are eight food groups that cause about 90% of all of the food allergies that people have. These include peanuts, tree nuts (such as almonds, filberts, and Brazil nuts), milk, eggs, wheat (not to be confused with gluten intolerance), soy, finfish, and shellfish. If the GE animal is one to which people already tend to be allergic, it is likely that they would avoid that species in order to avoid an allergic reaction. For example, if people are allergic to shrimp, they would not likely eat GE shrimp.  Regardless, in this part of the agency's evaluation,FDA looks to see whether the GE animals are more allergenic, that is, pose more of an allergic risk, than their non-GE counterparts.

Sixth, the agency evaluates the potential for the GE animal to cause significant environmental impacts. We do this by evaluating the results of an EA for the specific proposed conditions of use for a particular application. If the agency finds, based on a review of the EA, that there is no significant impact on the environment under those conditions, a FONSI is prepared and published. On the other hand, if the agency does find that there is a significant impact, a considerably more extensive assessment is required—resulting in preparation of an EIS, in which the nature of the anticipated impact(s) are reviewed in detail.

In the seventh, and final, step of the process sponsors submit information and data in support of their claims for the GE animal. (For conventional articles regulated as drugs, this

is referred to as "effectiveness.") For example, for the GTC goat, FDA determined that the goat did indeed produce human antithrombin in its milk. For an animal that is intended to grow faster, the agency evaluates data to determine if the GE animals do indeed reach some size or weight more rapidly than their non-GE counterparts.

# Appendix D. Federal Agency Letters in Reference to the Endangered Species Act

## D.1 Letter From the Fish and Wildlife Service

United States Department of the Interior

FISH AND WILDLIFE SERVICE
Washington, D.C. 20240

In Reply Refer To
FWS/AES/DCHRS/046979

DEC 16 2010

Dr. Larisa Rudenko
Center for Veterinary Medicine
Food and Drug Administration
Rockville, Maryland 20857

Dear Dr. Rudenko,

We have reviewed your letter of October 22, 2010, which requested our response to a "no effect" determination with respect to the endangered Gulf of Maine distinct population segment (DPS) of the Atlantic salmon (*Salmo salar*) and your agency's potential approval of genetic modifications to Atlantic salmon as a new animal drug. The transgenic salmon are known as AquAdvantage Salmon. We have also reviewed your letter of September 2, 2010 and the material transmitted with it, including an Environmental Assessment and extensive briefing material. Your letters referred to two listed DPS of Atlantic salmon, referred to as the Gulf of Maine DPS and the Kennebec River DPS; the U.S. Fish and Wildlife Service now considers salmon that spawn in the Kennebec to belong to the Gulf of Maine DPS.

As described in your correspondence, approval of the DNA construct in AquAdvantage Salmon would apply only to its presence in fish spawned at a land-based facility on Prince Edward Island, Canada, and hatched and raised at a second land-based facility in Panama. Concern for effects on listed Atlantic salmon would arise if there were a detectable probability that the transgenic salmon could interbreed or compete with or consume the listed fish. Given the nature of the facilities described, any of these outcomes appears to be extremely unlikely, and your "no effect" determination seems well supported for this approval.

We understand that use of any other facilities to breed or raise AquAdvantage salmon for sale in the U.S. would require additional environmental review and consideration of the potential need for consultation under the Endangered Species Act. If you have further information on this project or questions related to this issue, please contact Dr. John J. Fay of this Division at (703)358-2353.

Sincerely,

Richard E. Sayers

Richard E. Sayers, Chief
Division of Conservation, HCPs,
Recovery and State Grants

TAKE PRIDE IN AMERICA

## D.2 Letter From the National Marine Fisheries Service

UNITED STATES DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
NATIONAL MARINE FISHERIES SERVICE
Silver Spring, MD 20910

JUL 6 2011

Larisa Rudenko
Center for Veterinary Medicine
Food and Drug Administration
5600 Fishers Lane, Pkln Bldg. (HFE-88)
Rockville, Maryland 20857

Dear Dr. Rudenko:

NOAA's National Marine Fisheries Service (NMFS) received your letter, dated October 22, 2010, regarding your determination that the Food and Drug Administration (FDA) Center for Veterinary Medicine's approval of an application for AquAdvantage Salmon will have no effect on the endangered Gulf of Maine (GOM) distinct population segment (DPS) of Atlantic salmon in accordance with section 7(a)(2) of the Endangered Species Act of 1973, as amended (16 USC 1536(a)(2)). The final determination of "no effect" in your recent letter amends the "may effect, not likely to adversely affect" determination made in your September 13, 2010, letter that included an Environmental Science Review, Briefing Packet, and Environmental Assessment.

Despite concluding this action will have no effect to GOM DPS Atlantic salmon, NMFS and FDA engaged in technical discussions on December 3, 2010, and March 30, April 13, April 19, May 2, May 6, May 25, and May 31, 2011. During those meetings, the FDA provided further clarification about the proposed action, the containment measures, and a more detailed analysis of the risks the genetically engineered broodstock of Atlantic salmon pose to the environment and listed GOM DPS Atlantic salmon or their critical habitat. Based on the discussions noted above and the October 22, 2010, FDA letter that concluded this action will have no effect to listed species or their critical habitats, NMFS better understands this particular action and looks forward to consulting with FDA on future actions they determine may affect listed species or their critical habitat.

Thank you for the opportunity to discuss this proposed action. If you have any questions or believe this or any similar, future actions may affect listed species or their critical habitat, please contact Jason Kahn or me at, 301-713-1401.

Sincerely,

James H. Lecky
Director,
Office of Protected Resources

# Appendix E. AquAdvantage Salmon Genotype

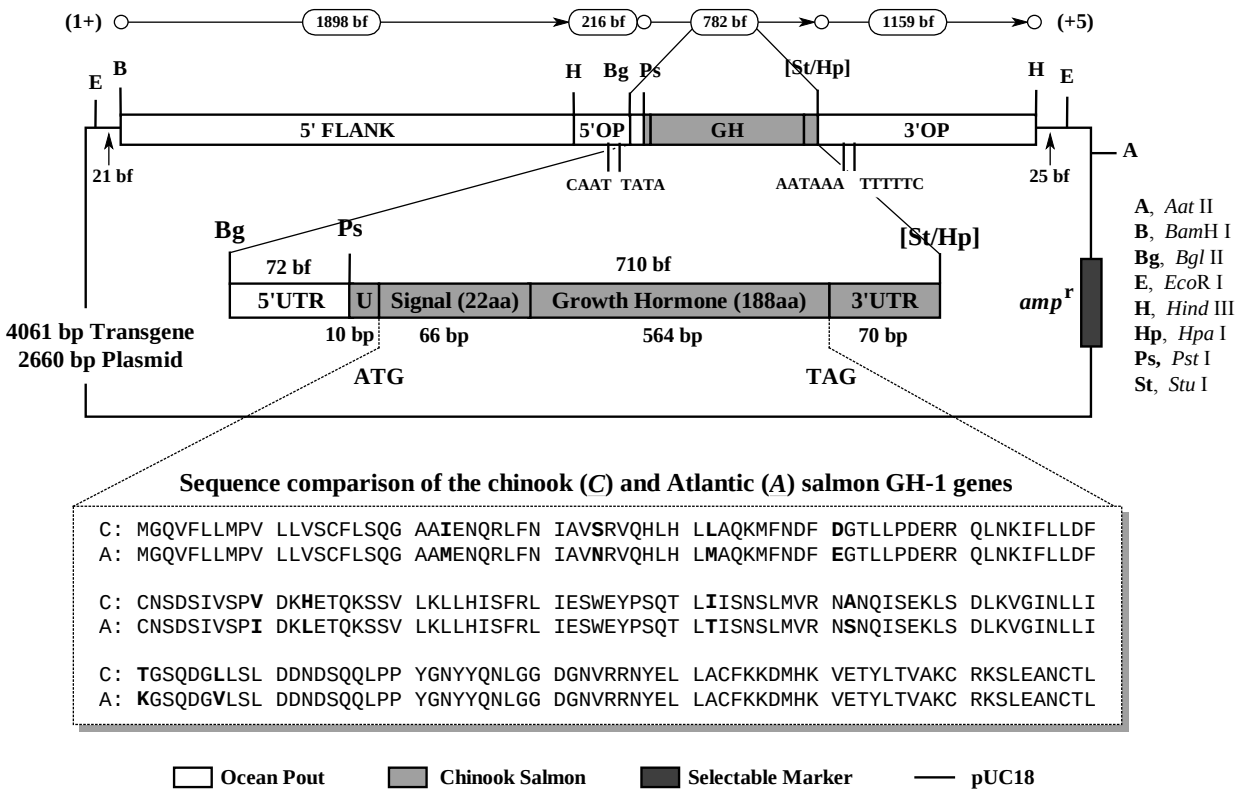
## E.1 Characterization of the rDNA Construct

### E.1.1 Characterization of the Plasmid Form, *opAFP-GHc2*

The plasmid form of the AquAdvantage rDNA construct, referred to by the sponsor as the *opAFP-GHc2* construct, comprises 5′- and 3′-regulatory sequences from an ocean pout AFP gene and the complementary deoxyribonucleic acid (cDNA) sequence of a chinook salmon GH gene as an integrated transcriptional unit, which has been shown to retain the molecular-genetic integrity required for GH expression in salmonid cells (sponsor submissions to CVM).

As illustrated in Figure E.1, the *opAFP-GHc2* construct (hereafter termed 'the construct' or 'the genetic construct') is a 6721 base-pair (bp) recombinant plasmid comprising 4061 bp of fish DNA and 2660 bp of vector backbone DNA derived primarily from pUC18. The characterization of the genetic construct has been the subject of several sponsor submissions providing a thoroughly detailed account of the following: source of fish DNA sequences used in construct development; molecular-genetic methods used to prepare the construct; *in vitro* expression studies confirming transcriptional capacity of the construct in fish cells; and, consensus nucleotide sequence of the construct, including a comparison of that sequence to the published sequences of the constituent fish DNAs. CVM has independently evaluated these submissions and found them to be acceptable for characterization of the plasmid construct.

**Figure E.1. Physical Description of the AquAdvantage Construct, opAFP-GHc2 ***




* ***bp*** length is used in the narrative and figures in reference to the physical size of a DNA in fully-duplexed form; base fragment (***bf***) length is used in reference to the number of bases between, and inclusive of, the 5′- and 3′-nucleotides comprising the restricted recognition sequences on the boundaries of the **+** strand. ***amp*$^{r}$**, *bla* gene providing ampicillin resistance.

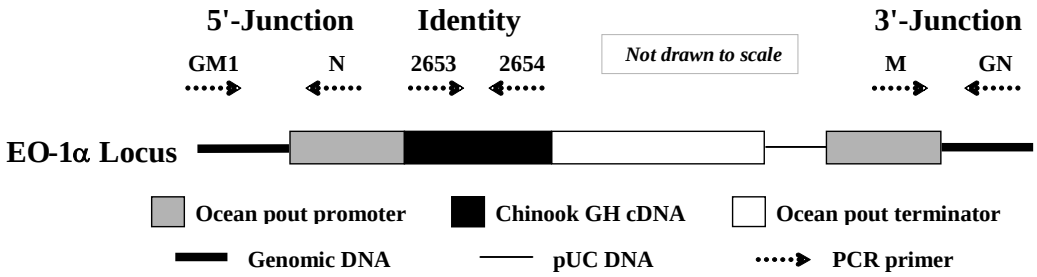
## E.2 Characterization of the Integrated Form, EO-1α

The founder animal from which the AquAdvantage Salmon line derives was a mosaic, genetically engineered female (EO-1) generated in 1989 by micro-injecting a linearized form of the genetic construct into the fertilized eggs of wild Atlantic salmon. Two rapidly-growing, genetically engineered $F_1$-progeny of EO-1 were selected for further development and found to harbor two independently segregating integrants: a functional α-form and a non-functional β-form. During the breeding of eight subsequent generations (i.e., $F_2$-$F_9$), an AquAdvantage Salmon line (EO-1α) was established that bears a single copy of the α-integrant, which has been the subject of several submissions to CVM providing a thorough account of the following: the development of the EO-1α line; diagnostic methods able to discriminate the α- and β-integrants; functional and molecular-genetic characterization of the EO-1α locus; multi-generational heritability and stability of the EO-1α locus; and, the consensus nucleotide sequence of the α-integrant in $F_2$- and $F_4$-generation AquAdvantage Salmon, including its comparison to the input-construct sequence.

As shown in Figure E.2, the α-form was subject to partial 5′→3′ rearrangement during its integration into the genome of EO-1. This particular integration event, the location thereof,

and the molecular-genetic form of the construct therein (collectively, the EO-1α□ locus) compose the defining characteristic of the AquAdvantage Salmon for which FDA approval is being sought.

**Figure E.2. Physical Description of the Integrated AquAdvantage Genetic Construct & Means of Diagnostic Assessment ***




| Diagnostic PCR Assessments | | | Differential Signals | |
|---|---|---|---|---|
| **Objective** | **Transgene Target** | **Primers** | **EO-1α** | **Endogenous GH** |
| Identity | GHc cDNA | 2653-2654 | 207 bp | 798 & 1150 bp |
| Stability | 5′-junction | GM1-N | 572 bp | *None* |
| | 3′-junction | GN-M | 350 bp | |

* Abbreviations: ***bp***, base-pair; ***cDNA***, complementary DNA; ***Endogenous***, native GH genes in the Atlantic salmon genome (i.e., GH-1 & GH-2); ***GH***, growth hormone; ***GHc***, chinook salmon GH; ***PCR***, polymerase chain reaction.

**Note:** The pUC DNA sequence residing between the ocean pout terminator and downstream portion of the ocean pout promoter subject to 5′→3′ rearrangement during construct integration comprises 45 bp derived from the polycloning sites of the parent pUC vectors used in insert construction. These sequences are non-coding and beyond the open-reading frame of EO-1 □.

The molecular-genetic tools that were developed during investigation of this integrated form have provided diagnostic means of determining its presence and stability, which has been done across numerous generations of AquAdvantage Salmon through the $F_6$-generation, and which will continue to be done during commercial production as a matter of post-approval surveillance of product integrity and durability.

## Appendix F. Facility Inspections and Site Visit Summaries

The EA notes that FDA personnel have twice inspected the site in Canada where AquAdvantage Salmon eggs would be produced and have conducted a site visit to the facility in Panama where the fish would be grown out. This appendix provides more detail on those visits.

### F.1 Prince Edward Island Broodstock and Hatchery Facility

#### F.1.1. FDA 2008 Inspection

The PEI facility was described in an EA submitted by the sponsor in December 2001 in support of the investigational use of AquAdvantage Salmon. Analysis of information in the sponsor's 2001 EA resulted in FDA's preparation of a FONSI for investigational studies under the subsequent INAD. Section 4.0 of the 2001 EA described the various passive and active forms of containment present at the sponsor's Canada facility in PEI, Canada. Passive containment includes physical-biological containment afforded by the surrounding environment (e.g., temperature, salinity, predators), while active containment describes the presence of physical barriers in the facility design (e.g., screens, nets) to prevent the escape or accidental release of fish and fish eggs to the outside environment.

Appendix IV of the 2001 EA contained SOPs in place at the facility relating to secure containment. The most relevant of the SOPs addressed physical containment of GE salmonids (SOP/ABPEI/2400). A key part of the SOP was Figure 1, a schematic of the confinement equipment in place in the facility's early rearing annex and grow out area, and the associated key to the components shown in this figure. The containment level (i.e., primary, secondary, etc.) for each component was described. According to the figure and key, all areas of the sponsor's facility have at least three independent forms of mechanical containment, and some areas, including the egg incubation units and their discharges, have as many as four.

In connection with the materials submitted by the sponsor in support of an NADA for AquAdvantage Salmon, the FDA conducted an inspection of the sponsor's PEI broodstock and hatchery facility from October 7 to 9, 2008 as a limited directed inspection under CPGM 7368.001 (Preapproval inspections for NADAs). The FDA inspector was accompanied by three technical experts from CVM. The facility was found to be in compliance with FDA regulations. No Form FDA 483 was issued at the conclusion of the inspection[76].

During the site visit, the most recent version of SOP/ABPEI/2400 was requested. The sponsor provided a copy of version 2400.004, which was dated as effective on September 29, 2008. Figure 1 in this version of the SOP has been changed to reflect physical additions and modifications made to the facility several years prior to the inspection, including enlargement of the early rearing area and changes in the sizes, shapes, and arrangement of tanks in certain parts of the facility. All areas of the facility were found to have at least two

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

levels of containment and some have three or four[77]. Components shown and described in Figure 1 of the SOP provide that containment include the following:

EARLY REARING AREA

- Screened trays (egg incubators)
- PVC screening
- Catchment box & sock filters
- Containment sump with stainless steel perforated baskets (filters)
- Floor drain covers
- 60 micron drum filter and septic tank for solids removal
- Tank covers, slotted stand pipes, and overflow screens

GROW-OUT AREA

- External stand pipe screens
- Stand pipe covers
- Top nets or surround nets for each tank
- Floor drain covers (perforated steel plate; 1.5 or 7.0 mm)
- Chlorine puck in floor drain sump (during spawning of fish)
- Effluent containment sump with primary and secondary screening

The types and general locations of the containment components shown in Figure 1 of SOP 2400.004 were verified by visual inspection during a walk through of the PEI facility. Photographs were also taken of many of the key components. A detailed piping and instrument drawing was not available for the water/wastewater distribution system; therefore, it was not possible to verify the specific location and presence of each piece of equipment with a containment function. All components of the containment system that were observed appeared to be in good operational condition and functioning as designed.

Records the sponsor maintained relative to inspection of hatchery effluent screens and containment equipment indicated that these components were being inspected internally by the sponsor on a regular basis.

The Canadian governmental authorities charged with responsibility for the regulatory oversight of the research and development and the commercial deployment of transgenic aquatic organisms are Environment Canada and DFO. Inspections of the facility by DFO occurred in 1996 and 2001. Reports from both DFO inspections found the facility "*is as 'escape-proof' as one can reasonable expect*." During the current inspection, a more recent DFO inspection report was requested. The FDA inspector was informed that the facility is no longer being inspected by DFO with respect to containment of GE fish and that regulatory oversight in this area is now under the oversight of Environment Canada.

F.1.2. FDA 2012 Inspection

[77] The inspection report reported a minimum of two forms of mechanical containment, but counted the primary and secondary screens in the effluent containment sump as only one form. Here these two stainless steel screens are considered to be independent forms of containment as they are physically distinct.

FDA conducted a follow-up inspection of the PEI facility in June of 2012 with two primary goals:

(1) to examine facility records, SOPs, and responses in relation to a disease outbreak and detection of the ISAV that occurred in the fall of 2009 after the previous facility inspection was conducted in October 2008, and
(2) to examine and evaluate the effectiveness of physical containment equipment and operational procedures within the PEI facility to prevent the escape of AquAdvantage Salmon eggs, fry, juveniles and adults.

The FDA inspector was accompanied by experts in aquaculture and biotechnology from CVM. The facility was found to be in compliance with FDA regulations, and no Form FDA 483 was issued at the conclusion of the inspection.

A. Inspection Activites Related to the Disease Outbreak and ISAV

When FDA became aware of a disease outbreak and detection of ISAV at the PEI facility in December of 2011, FDA asked ABT to provide information on the outbreak and any resulting mitigation measures. ABT made several submissions with information relevant to this request and in response to further questions from CVM. Following up on these submissions, the goals of the inspection with respect to the disease outbreak and ISA were three-fold:

1) Determine that adequate records were kept on the increase in mortality;
2) Determine that a satisfactory diagnosis was made;
3) Determine that adequate remediation and mitigation steps were taken to:
   a. Depopulate and decontaminate potentially infected areas, and
   b. Protect broodstock from future exposures to infectious agents.

During the inspection, inspectors examined tank mortality and post-mortem records from a time prior to the appearance of increased mortality (early July 2009) until mortality rates returned to "background" levels found before the disease outbreak (early October 2009). Examination of the tank records indicated that in addition to the average daily mortality (averaging approximately a total of 1-3 fish per day in the facility), beginning in late July and early August, there was a sharp spike in the total number of dead or moribund fish in two tanks. By mid-August, mortality in one tank had reached 30-60 fish per day, and continued through the end of that month. By early September, the number of dead or moribund fish increased to approximately 80+ fish per day in one tank, with significantly increased mortalities observed in a few other tanks. In general, mortalities began to decrease in mid-September, and by early October 2009 were back at background levels observed in the facility prior to the disease outbreak.

Examination of the tank morality and post-mortem records indicated the appearance of symptoms consistent with infection with ISAV. ISAV is an orthomixovirus first noted in farmed Atlantic salmon in Norway in 1984. Symptoms include lethargy, severe anemia, ascites (fluid collecting in body cavities, usually the abdomen), bleeding from small blood vessels, and severe liver effects including hemorrhaging and necrosis. ISAV was recognized in North American in salmon farms off the coast of Canada and the US. Mortality in net pens may be slow and cumulative, or rapid and rampant, with losses approaching 90% of the salmon in a net pen (Rimstead and Mjaaland (2002)[78]. Mortality observations and post-

[78] Rimstad, Espen and Siri Mjaaland. 2002. An orthomyxovirus causing and emerging infection in Atlantic salmon (review article). *APMIS* 110: 273-82.

mortem results in the daily records at ABT were generally consistent with this constellation of symptoms (e.g., internal bleeding, fluid inside and inflammation of organs, liver off-color and spotted).

**Diagnosis of Potential Etiological Agent**

As the mortality increased, ABT attempted to determine its cause, assuming from gross anatomical observations that the cause might have been ISAV. ISAV was confirmed in samples sent to The Center for Aquaculture Technologies (CAT), a contract research organization formerly a part of ABT. CAT made an initial diagnosis of ISAV on 9/14/2009, using the correct primer size (211 bp vs 224 bp) per Dr. John Buchanan of CAT, using as reference Munir et al. 2004. FDA concurred that the results of these diagnostic evaluations are consistent with the detection of ISAV, that appropriate chain of custody of samples was maintained, and that the appropriate documentation substantiates both chain of custody and results.

**Disease Mitigation Steps**

As documented in an ABT submission, ABT instituted several mitigation measures subsequent to the outbreak of ISAV in 2009 to eliminate it from the facility and reduce the possibility of a reoccurrence of ISA or another infectious disease. ABT culled all fish displaying any characteristics of poor health or high viral load, most of the broodstock, and other non-essential fish from the facility. In the Grow Out Area (GOA), ABT retained only asymptomatic AquAdvantage broodstock and a few non-GE females, while ABT completely depopulated and decontaminated the Early Rearing Area (ERA). Subsequently, ABT constructed quarantine areas within the GOA to house and isolate important broodstock that had potentially been exposed to ISAV. Additional mitigation measures included (1) permanent, physical isolation of the ERA and GOA into two distinct, biosecure facilities; (2) installation of ultraviolet (UV) lights to disinfect both the incoming well water and the recirculated water within both the ERA and GOA; and (3) installation of ozone treatment to disinfect water recirculated within the ERA.

During the facility inspection, FDA confirmed that the ERA and GOA were physically isolated and biosecure. In addition, FDA inspectors verified the presence of the UV and ozone water treatment systems. Inspectors collected records to verify that fish were culled and properly disposed of during the ISAV outbreak. All information collected during the inspection is consistent with that described in ABT's submissions and confirms the goals of the inspection related to ISAV. An NADA approval would include post-approval requirements for reporting of any future outbreaks of illness.

B. Information Relevant to Physical and Procedural Containment

ABT has provided CVM with information on physical containment (e.g., screens, filters, netting, etc.) and procedures in place to ensure containment (e.g., SOPs) at the PEI facility in several submissions and in the draft EA documents that it prepared. FDA inspectors verified the presence and locations of the physical containment equipment in the previous facility inspection in October 2008; however, there were several significant changes and additions since that time, necessitating verification and documentation. ABT personnel escorted FDA inspection staff throughout the facility giving FDA complete access to all areas of the ERA and GOA, and to all records. Using the most recent version of the schematic diagram of the physical containment components at the PEI facility that was prepared by ABT, inspectors verified the presence of all containment equipment on the schematic.

During inspection, ABT staff informed FDA inspectors that an additional stainless steel filter screen was added in the exterior catchment sump (to supplement the two existing stainless steel screens) to ensure there are always at least two forms of physical containment in this sump in the event that one of the other filter screens needs to be removed for cleaning. In addition, all wood supporting structures were eliminated from the exterior catchment sump and the design of containment system was changed to eliminate the possibility of a failure of the supports. FDA verified the presence of the additional stainless steel screen and change in support structures during the inspection.

As a result of the inspection, FDA concluded that (1) the results of the diagnostic evaluations are consistent with the detection of ISAV; (2) ABT facililty staff took appropriate biosecurity measures in response to the outbreak, including installation of UV and ozone water treatment systems; and (3) effective and redundant physical containment equipment are present within the facility to prevent the escape of Atlantic salmon eggs, fry, juveniles, and adults. FDA also confirmed that ABT added an additional stainless steel filter screen in the exterior containment sump (to supplement the two existing stainless steel screens) to ensure there are always at least two forms of physical containment in this sump in the event that one of the other filter screens needs to be removed for cleaning.

## F.2 Panama Grow-out Facility

From November 10 to 12, 2009, a site visit of the sponsor's grow-out facility in Panama was conducted by two FDA experts in aquaculture, accompanied by a fisheries scientist from NMFS. This site visit was conducted primarily to verify that the conditions of rearing and containment at the grow-out facility were as described in the sponsor's submissions, and to evaluate any other factors which could influence the potential for escape. A secondary objective of the visit was to observe and gain information on the local environment, including portions of the river adjacent to and downstream of the grow-out facility, to help ascertain whether AquAdvantage Salmon would be likely to survive and establish should they somehow escape the grow-out facility.

Information provided by the sponsor with respect to the Panama facility was verified during the site visit conducted by FDA and NMFS staff. Multiple forms of physical (mechanical) containment were present and as described in materials submitted by the sponsor. In addition, the facility appeared to be newly built and well-maintained.

The Panama grow-out facility includes small sizes of tanks for rearing fry and juveniles, plus large tanks for growing fish to market size (see Figure 7 in the EA). The fry tanks contain either interior or exterior stand pipes, plus a series of two to three mechanical fine mesh screens (1 – 1.5 mm for small fry; 3 – 12 mm for larger fry and juveniles) made of metal to prevent fish from escaping. In addition, all water from these tanks must pass through a 500 micron (0.5 mm) sock filter prior to entering a drainage canal that collects all water from the facility and sends it to a series of four settling ponds (and from there to a nearby river). Thus, at a minimum, three levels of physical containment would be present for these early life stages of AquAdvantage Salmon.

Grow-out (production) tanks have external stand pipes (to control the water height) and drain water through a slotted (0.9 cm), rigid PVC drainage plate in the tank bottom. The drainage plate and slots serve as the primary form of physical containment for the fish in these tanks.

From the grow-out tanks, water is routed to the drainage canal that also collects water from the fry tanks and other facility operations. There are two additional mechanical (6 and 12

mm) screens within a concrete containment sump that filter water from the drainage canal prior to it entering the series of four settling ponds. There is also a 12 mm rigid metal screen on the outlet of each of the four ponds. These larger screens would act as effective barriers to larger fry, juveniles and adults, but would not be expected to preclude passage of small fry (or eggs). Taken as a whole, counting the series of settling ponds with screens as only a single form, there are four independent forms of physical containment that would be applicable to fish reared in the grow-out tanks.

Additional containment in the way of tank netting and chain link security fences is present to limit access by potential predators and unauthorized personnel.

Based on observations made and information gathered during the site visit, the descriptions and schematics provided by the sponsor on the Panama grow-out facility and the river and surrounding environment have been accurately represented. There are a minimum of three or four levels of containment between both the fry tanks and grow-out tanks and the river. This includes counting the series of four downstream settling ponds (each with its own outlet screen) as only one level of containment.

Visual observations of the river adjacent to the sponsor's grow-out facility indicated a very high gradient profile with high current velocity and substrate consisting predominately of large rocks and boulders. Except in terms of water temperature, the river habitat in the vicinity of the sponsor's facility does not appear to be favorable to Atlantic salmon, or most other fish species for that matter, although it would not necessarily preclude survival and possibly establishment (if salmon were reproductively competent). Populations of rainbow trout are reported to occur in the river as a result of intentional stocking by the Panamanian government as far back as 1925. The abundance of these trout, however, has not been well documented, and they were not observed by the visiting U.S. Government staff during the site visit.

## Appendix G: Panama Regulations and Oversight

The first portion of this Appendix comprises of a set of PowerPoint Slides presented by Panamanian attendees at the Joint Intergovermental Workshop of FDA's Review of AquAdvantage Salmon, held at FDA on April 13, 2011. The second part comprises correspondence between FDA and the Panamanian Autoridad Nacional del Ambiente regarding AquaBounty Technologie's compliance with Panamanian regulations particularly regarding the grow-out facility and water discharge permits

# Operation of the National Biosafety Committee for Genetically Modified Organisms

Interagencies Working Group on Biosafety
MIDA – ANAM – MINSA – MTI – AUPSA-
IDIAP-ARAP-SENACYT-INDICASAT
April 13th, 2011

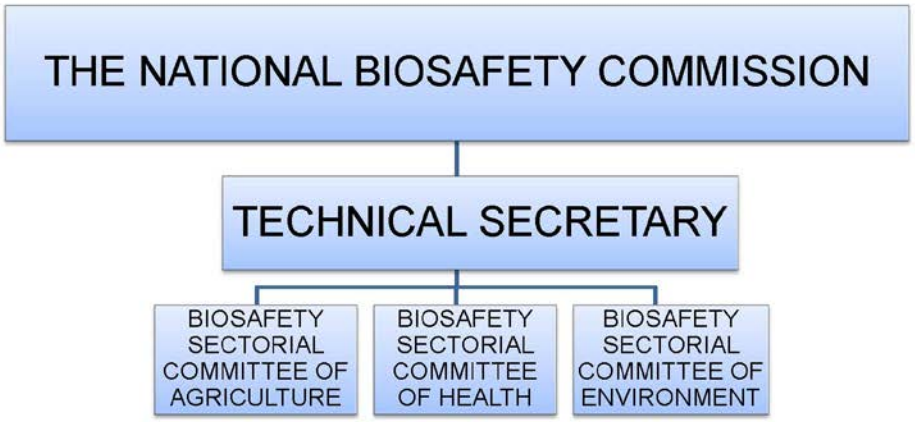
# National Biosafety Committee for Genetically Modified Organisms

- Panama signed the Convention on Biological Diversity in June 13, 1992, at the meeting of the United Nations Conference on Environment and Development held in Rio de Janeiro.

  With respect to the Protocol of Cartagena, Panama signed this agreement, in May 11, 2001, and the same is ratified in May 1, 2002, entered into force on September 11 of the same year.

The National Biosecurity Commission has its own internal regulation since August 27, 2001.

The resolution No. DAL-008 ADM-2011 create The Sectorial Agriculture Biosecurity Committee.


THE NATIONAL BIOSAFETY COMMISSION
TECHNICAL SECRETARY
BIOSAFETY SECTORIAL COMMITTEE OF AGRICULTURE
BIOSAFETY SECTORIAL COMMITTEE OF HEALTH
BIOSAFETY SECTORIAL COMMITTEE OF ENVIRONMENT

# National Biosafety Committee for Genetically Modified Organisms

*Law 48*, *article 2*. formation of the National Biosafety Committee

MINISTRY OF AGRICULTURE – MIDA
MINISTRY OF HEALTH – MINSA
MINISTRY OF TRADE AND INDUSTRY – MICI
MINISTRY OF FOREIGN AFFAIRS – MIRE
NATIONAL ENVIRONMENTAL AUTHORITY – ANAM
NATIONAL DIRECTORATE OF SCIENCE AND TECHNOLOGY – SENACYT
PANAMANIAN FOOD SAFETY AUTHORITY – AUPSA
PANAMANIAN ACUATIC RESOURCE AUTHORITY – ARAP
CIVIL SOCIETY

# BIOSAFETY SECTORIAL COMMITTEE OF AGRICULTURE

INTEGRATED BY THE FOLLOWING MEMBERS:

- MINISTRY OF AGRICULTURE DEPARTMENTS
- AGRICULTURAL RESEARCH INSTITUTE OF PANAMA – IDIAP
- PANAMANIAN FOOD SAFETY AUTHORITY – AUPSA
- PANAMANIAN ACUATIC RESOURCE AUTHORITY – ARAP
- ACADEMIC SECTOR:
  - FACULTY OF AGRICULTURAL SCIENCES
  - SCHOOL OF VETERINARY MEDICINE

The enforcement of Law No. 48 which creates the National Biosafety for GMOs and issues other provisions

The Ministry of Agriculture is the competent agency that regulates, monitor and research all GMOs and the implementation of technological developments affecting the Agricultural system production in the Republic of Panama.

The Ministry of Health is the national agency that regulates, control and monitor research on GMOs and development technology that could affect human health.

The enforcement of the Law No. 48 which creates the National Biosafety for GMOs and issues other Provisions

The National Environmental Authority is the agency responsible for the environmental management of the Republic of Panama.

The following is a .jpg copy of correspondence between US FDA and the Panamanian Autoridad Nacional del Ambiente regarding AquaBounty Technologie's compliance with Panamanian regulations particularly regarding the grow-out facility and water discharge permits. CCI and personal information have been redacted.

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration
Silver Spring, MD 20993

29 de enero 2015

Sr. Yamil Sánchez
Director
Protección de la Calidad Ambiental
Autoridad Nacional del Ambiente
Edificio 804, Albrook
Calle Diego Domínguez
Balboa, Ancón
PANAMA

Estimado señor Sánchez:

La Administración de Alimentos y Medicamentos de los Estados Unidos de América (FDA), está en el proceso de revisión de una solicitud de aprobación de una nueva aplicación para un medicamento de uso en animales, relacionado con AquAdvantage Salmón, de AquaBounty Tecnology.

La FDA regula los animales genéticamente modificados bajo las nuevas disposiciones para los medicamentos de uso en animales, de la Ley Federal de Alimentos, Medicamentos y Cosméticos, porque la construcción de ADN que se inserta en el ADN de un animal genéticamente modificado con el fin de alterar su estructura o función, cumple con la definición de un medicamento.

Como parte del proceso de revisión, la FDA emitió un borrador de Evaluación Ambiental que fue sometido a extensos comentarios del público, llevó a cabo dos inspecciones a las instalaciones de reproducción de la AquaBounty en Isla del Príncipe Eduardo, Canadá, y, en conjunto con un experto en el cultivo de peces del Servicio Nacional de Pesca Marina de Estados Unidos de América (que es parte de la Administración Nacional Oceánica y Atmosférica), llevó a cabo una extensa visita al lugar en las instalaciones de cría en [redacted] Panamá.

Desde el momento de nuestra visita a las instalaciones de Panamá, nos dimos cuenta de que AquaBounty no estaba en pleno cumplimiento de las normas panameñas, tal y como se indica en la carta adjunta de fecha 21/11/13 "Denuncia con sellos de recepción" del señor Silvano Vergara, Administrador General de la Autoridad Nacional del Ambiente, en relación con algunos problemas ambientales de sus instalaciones.

Les estamos escribiendo para dar seguimiento, y para determinar si hay asuntos pendientes en relación con el cumplimiento de los requisitos legales y reglamentarios panameños asociados con la instalación de cría en Boquete.

Respetuosamente les solicitamos que nos informen del estado actual de las instalaciones de AquaBounty en [redacted] con respecto al cumplimiento de los requisitos panameños y locales. Su pronta respuesta sería muy apreciada. Si usted tiene documentación escrita que indique que AquaBounty ha cumplido con estos requisitos, le estaremos muy agradecidos si nos pudiera proporcionar una copia.

Atentamente,

Bernadette M. Dunham, D.V.M., Ph.D.
Directora, Centro de Medicina Veterinaria

cc: Laura Epstein
Larisa Rudenko
Michael Rogers

RECEIVED
FEB 02 2015
By

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration
Silver Spring, MD 20993

January 29, 2015

Mr. Yamil Sanchez
Director
Protección de la Calidad Ambiental
Autoridad Nacional del Ambiente
Edificio 804, Albrook
Calle Diego Dominguez
Balboa, Ancon
PANAMA

Dear Mr. Sanchez:

The United States Food and Drug Administration is in the process of reviewing an application for approval of a new animal drug application related to AquaBounty Technology's AquAdvantage Salmon. FDA regulates genetically engineered animals under the new animal drug provisions of the Federal Food, Drug, and Cosmetic Act because the DNA construct that is inserted into the DNA of a genetically engineered animal in order to alter its structure or function meets the definition of a drug.

As part of the review process, FDA issued a draft Environmental Assessment that underwent extensive public comment, conducted two inspections of the AquaBounty's broodstock facility in Prince Edward Island, Canada, and, in conjunction with an expert in fish farming from the United States National Marine Fisheries Service (a part of the National Oceanic and Atmospheric Administration) conducted an extensive site visit at the grow out facility in [redacted] Panama.

Since the time of our visit to the Panamanian facility, we learned that AquaBounty was not in full compliance with Panamanian regulations as outlined in the attached letter dated 21/11/13 "Denuncia con sellos de recepcion" from Senio Silvano Vergara, Adminsitrador General de la Autoridad Nacional del Ambiente regarding some environmental problems with the facility.

We are writing to follow up and determine whether there are any outstanding issues with respect to meeting Panamanian legal and regulatory requirements associated with the grow out facility in Boquette.

We respectfully request that you inform us of the current status of the AquaBounty facility in [redacted] with respect to meeting Panamanian and local requirements. Your prompt response would be most appreciated. If you have written documentation that AquaBounty has met such requirements, we would appreciate it if you could provide us with a copy.

Sincerely,

Bernadette M. Dunham, D.V.M., Ph.D.
Director, Center for Veterinary Medicine

cc: Laura Epstein
Larisa Rudenko
Michael Rogers

RECEIVED
FEB 02 2015
By

autoridad nacional del ambiente

**AUTORIDAD NACIONAL DEL AMBIENTE**
**DIRECCIÓN DE PROTECCIÓN DE LA CALIDAD AMBIENTAL**

Albrook, Edificio 804
Apartado C-0843 – Balboa, Ancón – Rep. de Panamá.
www.anam.gob.pa

Teléfono: 500-0837
Teléfono: 500-0847

autoridad nacional de ambiente
anam DIRECCION DE PROTECCIO DE LA CALIDAD AMBIENTAL

Panamá, 11 de Febrero de 2015
DIPROCA-107-15

Doctora
**BERNADETTE M. DUNHAM**
Directora de Centro de Medicina Veterinaria
Administración de Medicamentos y Alimentos de los Estados Unidos
Estados Unidos

Dra. Dunham:

Por este medio se le informa que la Autoridad Nacional del Ambiente por medio de la Dirección de Protección de la Calidad Ambiental realizó una inspección al Proyecto AquaBounty ubicado en la [redacted] como resultado de la inspección se obtuvo que el proyecto está cumpliendo con todos los compromisos ambientales establecidos. El único incumplimiento detectado, es que no cuentan con Permiso de Descarga de Aguas Residuales, basado en la normativa COPANIT DGNTI-35-2000.

Para cualquier consulta adicional favor establecer comunicación con la Ing. Malú Ramos al teléfono 500-0810 ó a la dirección electrónica mramosm@anam.gob.pa.

Atentamente:

**ING. YAMIL SÁNCHEZ**
Director de Protección de la Calidad Ambiental

YS/mr

**National Environment Authority of Panama**
**Directorate for Environmental Quality Protection**

Albrook, Building 804
P.O. Box C-0843 – Balboa, Ancón – Rep of Panamá
www.anam.gob.pa

Telephone +507-500-0837
Telephone +507-500-0847

Panamá, February 11, 2015
DIPROCA- 107-15

Doctor
Bernadette M. Duhham
Director, Center for Veterinary Medicine
United States, Food and Drug Administration

Dr. Dunham:

By this means, please be informed that the National Authority of the Environment (known by its Spanish Acronym ANAM) through the Directorate for Environmental Quality Protection made an inspection to the AquaBounty Project located in [redacted] As a result of the inspection we found out that the project is complying with all the environmental commitments established. The only unfulfillment detected, is that they do not have a Permit to Discharge Wastewater, based on the regulation COPANIT DGNTI-35-2000.

For additional questions, please establish communication with Eng. Malú Ramos to Telephone +507-500-0810 or to email mramosm@anam.gob.pa.

Sincerely;


Ing. Yamil Sánchez
Director, Environmental Quality Protection

YS/mr

Courtesy translation mlm

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration
Silver Spring, MD 20993

25 de febrero 2015

Ingeniera
Malú Ramos
Departamento de Protección de Calidad Ambiental, (ANAM)

Estimada Ingeniera Ramos:

Le agradecemos a usted y al señor Director, Ing. Yamil Sánchez por su pronta respuesta a nuestra consulta del 29 de enero de 2015, sobre el estado regulatorio de AquaBounty Technology, la información provista es de gran ayuda. En su respuesta, el Ing. Sánchez nos indicó que preferiría que le dirigiéramos las futuras correspondencias a usted; y yo en mi capacidad de Asesora Principal en temas de Biotecnología, también he recibido la misma solicitud de parte de la dirección.

De acuerdo con la carta del Ing. Sánchez, las instalaciones de la empresa AquaBounty fueron inspeccionadas y están en cumplimiento con todos los compromisos ambientales establecidos, con la excepción del Permiso de Descarga de Aguas Residuales, basado en la normativa COPANIT DGNTI-35-2000. ¿Podría usted aclararnos si el Permiso de Descarga de Aguas Residuales no fue otorgado, porque detectaron un problema o porque su agencia no ha completado el proceso para otorgar el permiso pero esperan hacerlo?

Nuevamente, estamos altamente agradecidos por su asistencia y respuesta tan pronto le sea posible.

Atentamente,

Larisa Rudenko, Ph.D., DABT
Senior Advisor for Biotechnology
Director, Animal Biotechnology Interdisciplinary Group
DHHS/FDA/CVM
[redacted]

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration
Silver Spring, MD 20993

February 25, 2015

Eng. Malú Ramos:

We wish to thank you and Director Sanchez for your very helpful and prompt reply to our request of January 29, 2015, inquiring about AquaBounty Technology's regulatory standing in Panama. In his reply, Director Sanchez indicated that he would prefer that we direct subsequent correspondence to you. And I, in my capacity as Senior Advisor for Biotechnology, also have been asked by my management to correspond with you.

According to Dir. Sanchez' letter, the AquaBounty facility has been inspected, and is in compliance with all of the environmental commitments established, except for a Permit to Discharge Wastewater, based on regulation COPANIT DGNTI-35-2000. Could you please clarify for us whether the Permit to Discharge Wastewater has not been issued because you detected a problem, or because your agency has not completed the process of issuing it but expects to do so.

Again, we greatly appreciate your assistance, and your response at your earliest convenience.

Sincerely,

Larisa Rudenko, Ph.D., DABT
Senior Advisor for Biotechnology
Director, Animal Biotechnology Interdisciplinary Group
DHHS/FDA/CVM
[redacted]

cc:
Director Sanchez
Mike Rogers
Bernadette M. Dunham

anam

**AUTORIDAD NACIONAL DEL AMBIENTE**
**DIRECCIÓN DE PROTECCIÓN DE LA CALIDAD AMBIENTAL**
**Laboratorio de Calidad Ambiental**

Albrook, Edificio 804
Apartado C-0843 - Balboa, Ancón - Rep. De Panamá.
www.anam.gob.pa

Telefax: 500-0859
Teléfono: 500-0837
Ext. Laboratorio: 6909

Panamá, 12 de marzo de 2015.
DIPROCA–LAB–031-2015

Señora
**ERIKA M. VILLARREAL Z.**
ANZOLA ROBLES & ASOCIADOS
E. S. D.

Señora Villarreal:

En respuesta al Impulso por Solicitud de Permiso de Descarga de la empresa **Aqua Bounty Panama S. de R.L.,** se le informa que el Laboratorio de Calidad Ambiental de la Autoridad Nacional del Ambiente (ANAM) realizo la verificación de descarga del Proyecto, el pasado 14 de Noviembre del 2014.

Como resultado de la verificación, el Proyecto cumplió con los Limites Máximos Permisibles en base a la normativa COPANIT-DGNTI 35-2000. siguiendo el procedimiento actual, se remitió Nota de Resolución de Aprobación al Departamento de Asesoría Legal de la ANAM, a fin de otorgar el respectivo Permiso de Descarga de Aguas Residuales.

Aprovecho la oportunidad para presentarle mi alta consideración.

Atentamente,

**YAMIL SANCHEZ**
Director de Protección de la Calidad Ambiental

autoridad nacional de ambiente
anam
DIRECCION DE PROTECCIOI
DE LA CALIDAD AMBIENTAI

YS/AT/tuñon

National Environmental Authority
Directorate of Environmental Quality Protection
Environmental Quality Laboratory

Albrook, Building 804
P.O. Box C-0843 - Balboa, Ancon - Republic of Panama
www.anam.gob.pa

Telefax: 500-0859
Telephone: 500-0837
Laboratory Extension: 6909

Panama, March 12, 2015.
DIPROCA-LAB-031-2015

Ms.
ERICKA M. VILLARREAL Z.
ANZOLA ROBLES & ASOCIADOS
E. S. D.

Ms. Villarreal:

In response to the Impulse for Permit Application for Download of the firm AquaBounty Panama S. de R.L., we inform you that the Environmental Quality Laboratory of the National Environmental Authority (ANAM) conducted the discharge verification of the Project last November 14, 2014.

As a result of verification, the Project met the Maximum Permissible Limits based on regulation COPANIT-DGNTI 35-2000. Following the current procedure, the Approval Resolution Note to the Legal Advice Department of ANAM was referred, in order to grant the respective Wastewater Discharge Permit.

I take this opportunity to give you my best regards.

Sincerely,

YAMIL SANCHEZ
Director of Environmental Quality Protection

YS/AT/Tuñon

autoridad nacional del ambiente

**AUTORIDAD NACIONAL DEL AMBIENTE**
**DIRECCIÓN DE PROTECCIÓN DE LA CALIDAD AMBIENTAL**

Albrook, Edificio 804
Apartado C-0843 - Balboa, Ancón - Rep. de Panamá.
www.anam.gob.pa

Telefax: 500-0859
Teléfono: 500-0837
Teléfono: 500-0816

Panamá, 03 de Marzo de 2015
DIPROCA-235- 2015

**LARISA RUDENKO; Ph.D., DABT**
Asesora Mayor en Biotecnología
Directora del Grupo Interdisciplinario de Biotecnología Animal
DHHS/FDA/CVM
Estados Unidos

Señora Rudenko:

Por este medio se le informa que el Laboratorio de Calidad Ambiental de la Autoridad Nacional del Ambiente (ANAM) realizó la verificación de descarga del Proyecto AquaBounty en [redacted] el pasado 14 de Noviembre del 2014, como resultado de la verificación el Proyecto cumplió con los estándares requeridos para su aprobación. Siguiendo el procedimiento actual de permisos de descarga, se remitió una Nota de Resolución de Aprobación al Departamento de Asesoría Legal de la ANAM.

Asesoría Legal se encargara de revisar la verificación de cumplimiento del proceso legal del expediente en base a la normativa COPANIT DGNTI-35-2000 a fin de otorgar el Permiso de Descarga de Aguas Residuales.

Para cualquier consulta adicional favor establecer comunicación con la Ing. Malú Ramos al teléfono 500-0810 o a la dirección electrónica mramosm@anam.gob.pa.

Atentamente,

**YAMIL SÁNCHEZ**
Director de Protección de la Calidad Ambiental

YS/mr

**National Environment Authority of Panama**
**Directorate for Environmental Quality Protection**

Albrook, Building 804
P.O. Box C-0843 – Balboa, Ancón – Rep of Panamá
www.anam.gob.pa

Telephone +507-500-0859
Telephone +507-500-0837
Telephone +507-500-0847

Panamá, March 3, 2015
DIPROCA- 235-2015

Larisa Rudenko, Ph.D., DABT
Senior Advisor for Biotechnology
Director, Animal Biotechnology, Interdisciplinary Group
Center for Veterinary Medicine
United States, Food and Drug Administration

Mrs. Rudenko:

By this means, please be informed that the Laboratory for Environmental Quality of the National Environment Authority of Panama (known by its Spanish Acronym ANAM) verified the discharge for the AquaBounty Project in [redacted] on November 2014, as a result of the verification; the project complied with the required standards for its approval. Following the current discharge permits procedures, a Note of Resolution of Approval was sent to ANAM's Legal Advisory Department.

Legal Advisory will be in-charge of reviewing the verification of compliance of the dossier's legal process, based on the regulation COPANIT DGNTI-35-2000 in order to issue the Wastewater Discharge Permit.

For additional questions, please establish communication with the Eng. Malú Ramos to the Telephone +507-500-0810 or to email mramosm@anam.gob.pa.

Sincerely;

Ing. Yamil Sánchez
Director, Environmental Quality Protection

YS/mr

Courtesy translation mlm