U.S. Food and Drug Administration

AquAdvantage® Salmon

Draft
Environmental Assessment

In support of a proposed agency action on
a New Animal Drug Application for
the integrated α-form of the *opAFP-GHc2* gene construct
at the α-locus in the EO-1α line of triploid, all-female genetically engineered
Atlantic salmon (AquAdvantage Salmon) to be produced as eyed-eggs and
grown-out only in the physically-contained freshwater culture facilities
specified in the sponsor's application

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Prepared by the
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

~	approximately
ABRAC	Agricultural Biotechnology Research Advisory Committee
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
NEPA	National Environmental Policy Act
NMFS	National Marine Fisheries Service, National Oceanic and Atmospheric Administration, Department of Commerce
NRC	National Research Council
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
SW	Sea Winter
USC	United States Code
USDA	U.S. Department of Agriculture
VMAC	Veterinary Medicine Advisory Committee

TECHNICAL TERMS*

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*.
Arctic char	A salmonid species related to Atlantic salmon, 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 structure composed of one very long molecule of DNA and associated proteins (e.g., histones) that carries hereditary information.
°C-day [min]	Compound unit of "time" (°C x days [min]) for relative determination of growth rate that accounts for effect of water temperature.
Conspecific	An organism of the same species as another organism.
Construct (gene construct)	A synthetic gene comprising regulatory and coding sequences constructed *in vitro* and usually incorporated into the genome of an organism with the intended purpose of modifying its phenotype. Often used interchangeably with "transgene".
Diploid	A cell, tissue, or organism having two complete sets of chromosomes, one from each parent.
EO-1	The mosaic, female founder of the AquAdvantage Salmon line created by microinjection of the *opAFP-GHc2* construct into a fertilized egg.
EO-1α	Functional, stably integrated form of *opAFP-GHc2* in the AquAdvantage Salmon genome.
Egg	Unfertilized haploid sex cells developed by females.
Expression (gene)	The process by which information from a gene is used in the synthesis of a functional gene product (e.g., cell structures or proteins).
Flow cytometry	Method used to confirm ploidy by determination of DNA content in a dye-labelled cell population via relative fluorescence intensity.
Gamete(s)	Haploid reproductive cells produced in sexually mature organisms. A mature reproductive cell capable of fusing with the cell of similar origin but of opposite sex to form a zygote from which a new organism can develop. Gametes normally have haploid chromosomal content. In animals, including fish, gametes are sperm and oocytes (eggs).
Genome	The entire set of genetic instructions found in a cell.
Genotype	An organism's collection of genes. The term also can refer to the two alleles inherited for a particular gene. The genotype is expressed when the information encoded in the genes' DNA is used to make protein and RNA molecules.

Haploid	A cell, tissue, or organism having a single set of chromosomes (as opposed to *diploid* or *triploid*). Haploid cells are generally found in gametes (sex cells) of higher organisms.
Hemizygous	Having one copy (or allele) of a given (trans)gene.
Homozygous	A genetic condition where 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. Analogous to semen in mammals.
Molecular Cloning	A process by which scientists amplify a desired DNA sequence. The target sequence is isolated, inserted into another DNA molecule (known as a *vector*), and introduced into a suitable host cell (usually bacteria). Then, each time the host cell divides, it replicates the foreign DNA sequence along with its own DNA.
Neomale	A genetically female fish converted to a phenotypic male by hormone treatment.
opAFP-GHc2	AquAdvantage recombinant DNA construct comprising regulatory sequences from an ocean pout AFP gene and growth hormone-coding sequences from chinook salmon.
Polymerase chain reaction	A standard technique to amplify DNA often used to confirm genotype.
Phenotype	An organism's actual observed properties, such as morphology, development, or behavior, which derive from predominantly from its genotype.
Passive integrated transponder	Implantable radio-beacon for fish identification.
Plasmid	A circular, self-replicating, non-chromosomal DNA molecule found in many bacteria, although many artificial ones have been made. Often used as vectors for genetic engineering.
Ploidy	The number of complete sets of chromosomes contained within each cell of a higher organism (see *haploid*, *diploid*, and *triploid*).
Promoter	A regulatory sequence of DNA needed to turn a gene on or off. The process of transcription (production of RNA from DNA) is initiated at the promoter. Usually found near the beginning of a gene, the promoter has a binding site for the enzyme used to make a messenger RNA (mRNA) molecule.
Protein-coding sequence	The DNA sequence of a gene that is transcribed into mRNA and subsequently translated into protein.
Raceway	A rectangular channel or tank with a continuous flow of water constructed or used for high-density fish production. 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)	A technology that, among other things, uses enzymes to cut and paste together DNA sequences of interest that are linked together. The recombined DNA sequences, or rDNA construct, can be placed into vehicles called vectors (see *plasmid*) that ferry the DNA into a suitable host cell where it may be copied or incorporated, and expressed.
Regulatory sequence	Non-protein coding DNA sequence of a gene controlling its expression.
Salmonid	A ray-finned finfish of the family Salmonidae, a taxonomic group that includes salmon, trout, chars, freshwater whitefish and graylings. The family includes fish of the following genera, among others: *Salmo*, *Salvelinus*, and *Onchorhynchus*.
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	Any cell of the body except sperm and egg cells. Somatic cells are diploid, meaning that they contain two sets of chromosomes, one inherited from each parent.
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 small DNA molecule (plasmid, virus, bacteriophage, artificial or cut DNA molecule) used to deliver DNA into a cell; it must be capable of being replicated and contain sites for the introduction of foreign DNA.

*The various sources used for these definitions include Wiley's *Dictionary of Microbiology and Molecular Biology*, Revised 2nd Ed., John Wiley and Sons, New York, 1994; *Animal Cloning: A Risk Assessment*, U.S. Food and Drug Administration (Center for Veterinary Medicine), 2008, final version found here; National Human Genome Research Institute, *Glossary of Genetic Terms*, accessed at www.genome.gov/Glossary; Human Genome Project, accessed at www.genomics.energy.gov.

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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 a genetically engineered (GE) Atlantic salmon[1] to be produced and grown under specified conditions. This fish, named AquAdvantage Salmon, is designed to exhibit a rapid-growth phenotype that allows it to reach smolt[2] size faster than non-GE farmed salmon.

The AquAdvantage Salmon founder animal was generated in 1989 by micro-injecting a recombinant deoxyribonucleic acid (rDNA) construct, *opAFP-GHc2* composed of a promoter from an ocean pout antifreeze protein (AFP) gene and a protein-coding sequence from a chinook salmon growth hormone (GH) gene 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 eight generations. Under the conditions proposed for the NADA, AquAdvantage Salmon would be produced as triploid, all-female populations with eyed-eggs as the product for commercial sale and distribution. These eggs would be produced in the sponsor's facility on Prince Edward Island (PEI) in Canada. After confirming the genetic integrity of the broodstock used for manufacture and effective induction of triploidy in the eyed-eggs, these eggs would be shipped to a grow-out facility in the highlands of Panama, where they would be reared to market size and harvested for processing.

Under the proposed action, AquAdvantage Salmon would not be produced or grown in the United States, or in net pens or cages, and no live fish would be imported for processing.

As a part of the NADA review and approval process, 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) regulations (21 CFR part 25), FDA's Center for Veterinary Medicine (CVM) has thoroughly evaluated the potential environmental impacts of approving an NADA for AquAdvantage Salmon, and has prepared this draft Environmental Assessment (EA).

FDA approvals for articles regulated under the new animal drug provisions of the Federal Food, Drug, and Cosmetic Act (FD&C Act) may be for a specific set of conditions where such conditions are proposed in the drug sponsor's NADA, or are required by FDA to mitigate potential risks, and are explicitly set forth in the conditions of approval. Any other uses are not approved, and 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. Approvals by FDA of NADAs related to GE animals are limited to a very specific set of conditions with the GE animal remaining under regulatory oversight as long as it is produced and marketed. FDA has determined that for the proposed action, conditions of use should include appropriate and redundant mitigation measures

[1] The NADA is for approval of the integrated α-form of the *opAFP-GHc2* gene construct at the α-locus in the EO-1α line of Atlantic salmon under the conditions of use specified in the application.For ease of reference, this document 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.

such as 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 proposed in the materials submitted by the sponsor in support of an NADA 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 fish fillets, steaks, etc.) in Panama prior to retail sale in the United States. The specific proposed limitations on the production and use (grow-out) of AquAdvantage Salmon, including the production of triploid, all-female fish populations, are designed to mitigate potential adverse environmental impacts.

The proposed action is limited to an NADA approval for a specific set of conditions. Any modifications that the sponsor may propose to the conditions of an approval would require notification of FDA. Major and moderate changes require the filing and review of a supplemental NADA. Approvals of such supplemental applications would constitute agency actions and trigger environmental analyses under NEPA.

As part of the NADA review process, but separate from the environmental analysis itself, CVM has evaluated both the direct and indirect food safety impacts of AquAdvantage Salmon and any potential impacts of the rDNA insertion on target animal safety. With respect to food safety, FDA has concluded that food from AquAdvantage Salmon is as safe as food from conventional Atlantic salmon, and that there is a reasonable certainty of no harm from consumption of food from triploid AquAdvantage Salmon. Further, FDA has concluded that no significant food safety hazards or risks have been identified with respect to the phenotype of the AquAdvantage Salmon (FDA, 2010).

As the proposed action would only allow 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, effects on the local environments of Canada and Panama have not been considered and evaluated in this draft 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.

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 draft EA preliminarily indicates that the proposed action will not significantly affect the physical environment of the United States. Courts have held that under NEPA, social and economic effects must be considered only once it is determined that the proposed agency action significantly affects the physical environment.

FDA's approach in this draft environmental assessment is one based on a characterization of hazards, an evaluation of potential exposure pathways, and the likelihood of any resulting risk. The environmental analysis of consequences in the draft EA incorporates the 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 draft EA center on the likelihood and consequences of AquAdvantage 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 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?

AquAdvantage Salmon would be produced and grown-out in secure facilities that have been verified and validated by FDA. As a result, the possibility that GE fish could escape from containment, enter the local environments of PEI or Panama, and survive to reproduce is extremely remote. In addition, because the production process for AquAdvantage Salmon would ensure that populations produced will be triploid (effectively sterile), all-female animals, the possibility of their reproducing in the wild is likewise extremely remote. Finally, the inhospitable environmental conditions around the egg production and grow-out facilities further reduce the possibility of establishment and spread. Based on the evidence collected and evaluated by FDA, FDA has made the preliminary determination that it is reasonable to believe that approval of the AquAdvantage Salmon NADA will not have any significant impacts on the quality of the human environment of the United States (including populations of endangered Atlantic salmon) when produced and grown under the conditions of use for the proposed action. FDA preliminarily concludes that the development, production, and grow-out of AquAdvantage Salmon under the conditions proposed in the materials submitted by the sponsor in support of an NADA, and as described in this draft EA, will not result in significant effects on the quality of the human environment in the United States.

FDA has considered the no action alternative for this action, that is, denial of the NADA for AquAdvantage Salmon. There are two general likely scenarios to consider as a result of the no action alternative: (1) cessation of production of AquAdvantage Salmon, and (2) production of AquAdvantage Salmon at suitable locations outside 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, an assessment of potential effects on the environment becomes highly uncertain. Because production of AquAdvantage Salmon would be possible at any number of locations worldwide, under different containment conditions, and potentially within areas where native Atlantic salmon are present, there are too many variables and unknowns to perform a comprehensive assessment and make any predictions with respect to potential environmental impacts on the

United States. NEPA does not require an analysis of effects in foreign sovereign countries. However, to the extent that production would occur with less restrictive containment conditions than those proposed (e.g., fish might not be triploid, might not be reared in land-based facilities, or might not be subjected to multiple and redundant forms of physical containment), it is expected that adverse environmental impacts to the United States might be more likely to occur than under the conditions of production and grow-out for the proposed action.

FDA, having reviewed the materials submitted in support of an NADA for AquAdvantage Salmon, has made a "no effect" determination under the Endangered Species Act (ESA), 16 USC § 1531 et seq., that approval of the AquAdvantage Salmon NADA 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, when produced and reared under the conditions described within this draft EA. The two federal agencies responsible for administering the ESA, the National Marine Fisheries Service (NMFS) of the National Oceanic and Atmospheric Administration (Department of Commerce) and the U.S. Fish and Wildlife Service (FWS) of the Department of Interior, have been provided with this "no effect" determination and underlying information in support of it, provided herein. 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 draft EA is being prepared as part of the regulatory considerations for a possible approval of an NADA for AquAdvantage Salmon, a GE Atlantic salmon produced by AquaBounty Technologies, Inc. AquAdvantage Salmon contain a 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. The commercial intent of AquAdvantage Salmon is to benefit commercial salmon farming by significantly reducing time-to-market and improving the economics of land-based production. The conditions of approval for this NADA 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. Any modifications that the sponsor may propose to the conditions of an approval would require notification of FDA. Major and moderate changes require the filing and review of a supplemental NADA. Approvals of such supplemental applications would constitute agency actions and trigger environmental analyses under NEPA.

FDA regulates animals containing rDNA constructs under the new animal drug provisions of the FD&C Act, and meets it obligations for environmental analysis under NEPA. An rDNA construct that is intended to affect the structure or function of a GE animal meets the statutory definition of a new animal drug (see CVM Guidance for Industry (GFI) 187), and 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 are required by FDA to mitigate potential risks, and are explicitly set forth in the conditions of approval. 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 and moderate changes require a supplemental application that must be approved by the agency prior to implementation. 21 CFR 514.8. In general, these would include any changes that could adversely affect the safety, effectiveness, or quality of the approved product. FDA would consider the addition of a new egg production or grow-out facility, changes to the security or containment at an existing facility, or alterations of the approved product definition (see Section 4.1) to be changes that require approval of a supplemental application.

FDA has determined that this application for AquAdvantage Salmon should include appropriate and redundant mitigation measures such as 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 proposed in the materials submitted in support of an NADA, 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 fish fillets, steaks, etc.) in Panama prior to retail sale in the United States. The proposed specific limitations on the production and use (grow-out) of AquAdvantage Salmon, including the production of triploid, all-female fish populations, are designed to mitigate potential adverse environmental impacts, and are described in detail in Section 5.3 of this draft 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 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 over ten years ago.[3]

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 different purposes, including to support game fisheries and to rebuild wild populations, most farmed fish are raised for human consumption.[4]

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[5] 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

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

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

[5] 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.

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, gynogenesis, has been used successfully in the aquaculture industry for many years (Dunham, 2004; Luo *et al*., 2011).

In 2006, the world consumed approximately 110.4 million (*MM*) metric tons (*MT*) of fish, with almost half of that amount (around 51.7 MM MT) from commercial aquaculture. Total consumption has increased 16.8 MM MT over worldwide consumption just eight years earlier.[6] The Food and Agricultural Organization (FAO) of the United Nations has estimated that by 2030, annual commercial aquaculture production will need to increase by an additional 28.8 MM MT (i.e., 80.5 MM MT total) in order to maintain per capita fish consumption at current levels (FAO, 2008a).

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 MT of salmon annually, of which two-thirds were farmed rather than wild caught (Knapp *et al*., 2007). This is especially true for Atlantic salmon, 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 recently issued 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 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 (or transgenic[7]) animals in 1973 (Cohen *et al*., 1973). Although initial interest 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;

[6] See Food and Agriculture Organization of the United Nations, Depleted Fish Stocks Require Recovery Effort (Mar. 7, 2005) (available at http://www.fao.org/newsroom/en/news/2005/100095/index.html).

[7] In general, FDA uses the term "genetically engineered" to refer to organisms containing either heritable or non-heritable rDNA constructs; elsewhere, the term "transgenic" is used to refer to similar organisms, particularly those bearing heritable rDNA constructs. These terms are used interchangeably in this draft EA.

Guyomard *et al*., 1989; Chen *et al*., 1994). 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 most commercially advanced of those efforts to date. (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 draft EA is intended to provide material assistance to the FDA for making a decision to prepare either a finding of no significant impact (FONSI), or an environmental impact statement (EIS). The draft EA also addresses FDA's compliance with its obligations under the ESA.

2.3.1 Federal Food, Drug and Cosmetic Act

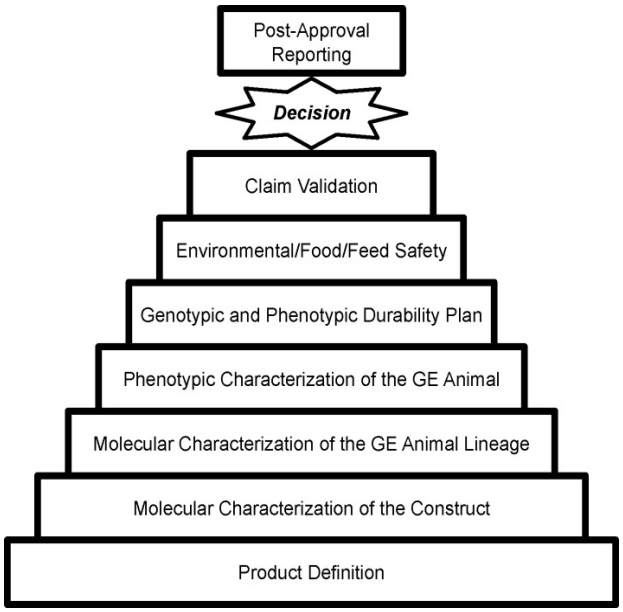
FDA's authority over new animal drugs comes from the FD&C Act (21 USC § 321 et seq.). The definition of a drug, in section 201(g) of the FD&C Act, includes "*articles (other than food) intended to affect the structure or any function of the body of man or other animals*" (21 USC § 321(g)). The definition 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)), or 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)).

In order to clarify the applicability of NADA requirements and procedures to GE animals, FDA has published 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 or feed derived from a GE animal is safe, and (2) whether the GE animal will significantly affect the quality of the human environment.

Figure 1. Regulatory Review Process for GE Animals



Under the FD&C Act, FDA must consider food[8] 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 are 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 food or feed. 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. The FDA assessment has concluded that food from these salmon "is as safe as food from conventional salmon, and there is a reasonable certainty of no harm from consumption of food" from these fish (FDA, 2010). The Agency's complete 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).

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

[8] In this draft Environmental Assessment, the term "food" encompasses food for humans and animals (feed).

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 when a proposed action does not normally require an EIS nor is it normally categorically excluded from the requirement to prepare an EIS or EA, 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 (see 40 CFR 1501.4(b), 1508.9(a)(1)). NEPA requires consideration of all potentially significant environmental impacts from the proposed action (see 40 CFR 1508.8). From time to time, CEQ has also issued additional guidance to Federal agencies that augment its NEPA regulations, including the recently issued (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 (see 21 CFR Part 25).

Social, economic, and cultural effects have not been analyzed and evaluated in this draft EA. Courts have held that 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. Our analysis in this draft EA preliminarily indicates that the proposed 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 will undertake an evaluation of interrelated social, economic and cultural effects, as appropriate.

As the proposed action would only allow 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). NEPA does not require an analysis of environmental effects in foreign sovereign countries. Effects on the local environments of Canada and Panama have not been considered and evaluated in this draft EA. In order to determine whether there would be significant effects on the environment of the United States, however, in this draft EA we have evaluated the exposure pathways that originate from the production and grow-out facilities in Canada and Panama.

2.3.4 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 draft 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[9]--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 described within this draft EA. In addition, FDA has determined that this action, in the event of a potential future approval of AquAdvantage Salmon, will not jeopardize the continued existence of a listed 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, provided herein. 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 proposed agency action.

2.3.5 North Atlantic Salmon Conservation Organization (NASCO) – Williamsburg Resolution

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[10], 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. These Guidelines (Williamsburg Resolution, Annex 5) state that, "*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 believes that the two facilities proposed to be used for production and grow-out of AquAdvantage Salmon as part of the proposed action comply with this stipulation in the NASCO

[9] 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.

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

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).

2.4 Foreign Regulatory Oversight

Under the proposed action, the production and use (grow-out) of AquAdvantage Salmon would occur only at locations outside of the United States. As a result, there would 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.

Canada

In Canada, regulation of fish that are the product of biotechnology takes place under the New Substances Notification Regulations (Organisms) of the Canadian Environmental Protection Act. The sponsor is currently preparing a New Substance Notification (Organisms) for submission to Canadian regulatory authorities for use of the PEI facility for production and commercial export of eyed-eggs of AquAdvantage Salmon to Panama. Review of this Notification dossier will involve Environment Canada (EC), Fisheries and Oceans Canada (DFO), and Health Canada, with the primary responsibility for scientific risk assessment falling to DFO for this particular submission. Acceptability of the New Substance Notification (Organisms) is necessary to proceed with commercial production of AquAdvantage Salmon in PEI.

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. Anyone who owns or works with aquatic animals and knows of or suspects a reportable disease is required by law to notify CFIA.

Panama

As authorized under Law 48 of August 2002, 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, fish are held in quarantine and subject to observation and diagnostic sampling. If cleared, they are released to commercial grow-out rearing facilities.

2.5 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 would be used for the production, grow-out, and disposal of AquAdvantage Salmon. Sections 5 and 7 of this draft EA describe confinement and containment measures and how they would specifically apply to AquAdvantage Salmon. These confinement measures have been incorporated as integral components of the proposed action.

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

   AquAdvantage Salmon would be prevented from entering the environment by the use of redundant physical and physico-chemical barriers at the sites of egg production and grow-out. They would be further prevented from surviving in the receiving environment because of geographic and geophysical issues. At the egg production facility at Prince Edward Island, escaped early-life stages that are adapted to fresh water would encounter salt water and, during part of the year, lethally cold temperatures. Either of these two conditions would prevent survival. In Panama, where grow-out would occur, escapees would encounter lethally warm temperatures, poor habitat, a series of water diversion

structures and hydro-electric facilities, and possibly predation by an established population of rainbow trout.

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

   Gene flow from AquAdvantage Salmon would be prevented because the fish would be triploid females incapable of reproduction, either among themselves or with wild fish, should they escape and survive.

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 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.

It is highly unlikely that any single containment measure will be completely effective at all times; therefore, optimum containment is best effected by implementing multiple independent and complementary measures in series. The National Research Council (NRC, 2002) has recommended the simultaneous use of multiple, redundant containment strategies for GE fish, and three to five separate measures have been recommended by a body of biotechnology risk experts (ABRAC, 1995). By combining containment measures with different stringencies, attributes, and modes-of-action, the compromise of aggregate containment by the failure of a single measure becomes increasingly unlikely.

FDA determined that this application for AquAdvantage Salmon should mitigate environmental risks by the appropriate use of biological, physical, and geographical/geophysical means of containment. 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 can result. 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), and routine audits, complemented by inspections by FDA, as well as local authorities.

As will be 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 and containment depends primarily on multiple, redundant physical and geo-physical containment measures. In addition, as will be described later in this document, 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, there effective biological containment is present in the form of a population of salmon that is entirely

female, almost completely triploid, and thus functionally sterile (see Section 5.3). 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, predation risk, and abundant physical barriers that diminish the likelihood of survival 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 genetically engineered 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 draft environmental assessment is one based on an evaluation of exposure pathways, hazards and risk. The environmental analysis of consequences in the draft EA conceptually incorporates the 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).

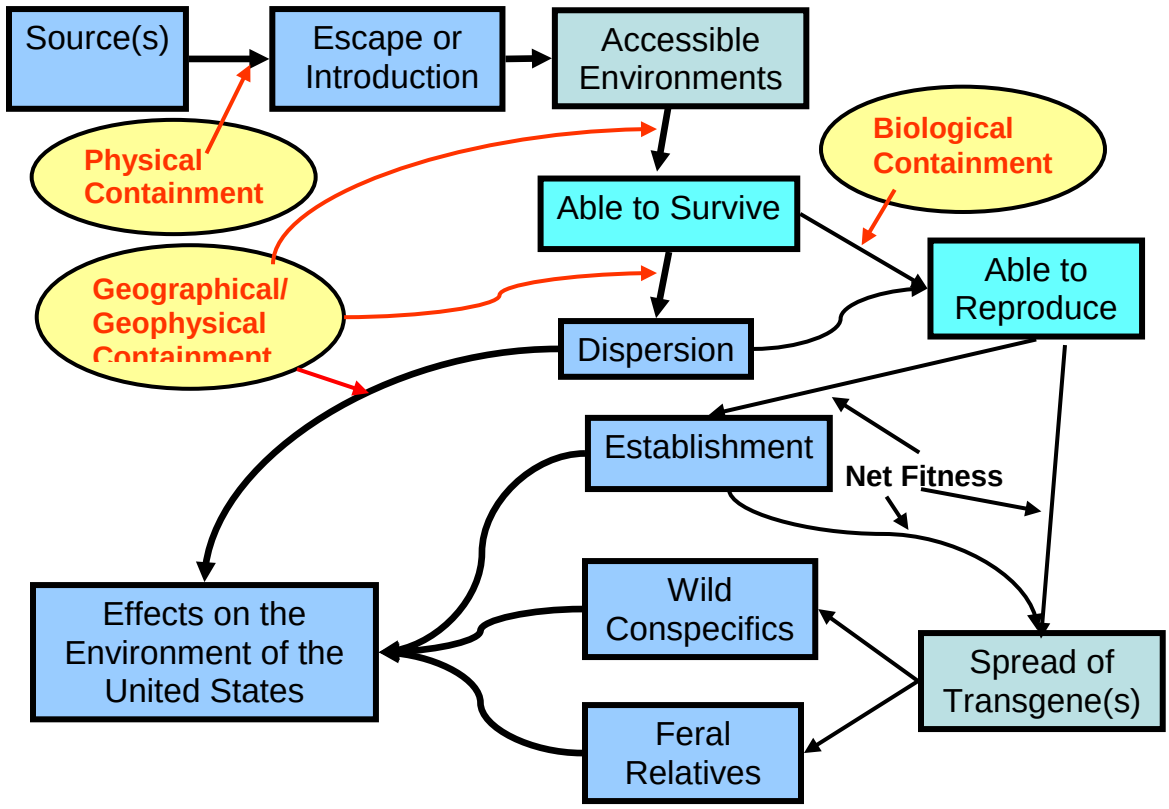
The potential hazards and harms addressed in this draft EA center on the likelihood and consequences of AquAdvantage Salmon escaping, surviving and becoming established in the

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 draft 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 draft EA around the following cascaded 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?

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[11] 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

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

generally needed in order for escape or release to present a hazard. Therefore, for the purposes of assessing risks of GE animals in the environment, exposure has been defined as the establishment of a GE organism in the community into which it is introduced or escaped (NRC, 2002). Three variables have been identified by NRC as important for determining the likelihood of establishment for a GE animal:

(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[12].

The likelihood of establishment is dependent on all three parameters, if the risk associated with any one of these three variables is negligible, the overall concern would be low, but not negligible (NRC, 2002).

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[13] or species closely related to the GE escapee in accessible ecosystems

[12] 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).

[13] A conspecific is an organism belonging to the same species as another. For example, farmed and wild Atlantic salmon are conspecifics.

implies that a suitable environment exists (provided that the fitness of the escapee does not differ significantly from conspecifics or closely related species in that environment) (Kapuscinski *et al*., 2007).

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

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

3.2.3 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 individuals 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 they were first developed, several groups of scientists have identified the general types of environmental concerns or possible risks associated with GE organisms in general, including GE animals (Snow *et al*., 2005; NRC, 2002; NRC, 2004; Devlin *et al*., 2006). General risks identified by one of these groups (Snow *et al*., 2005), though primarily hypothetical to date, 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*. (2005) report goes on to present several major environmental concerns associated with GE organisms, although not all of these are applicable to GE animals or to fish in particular. 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 the proposed action being considered 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 proposed 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, the FDA and the sponsor developed the proposed risk-mitigated conditions for production and grow-out of AquAdvantage Salmon that are an integral part of the proposed action. 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 proposed in the NADA.

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

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

The proposed action evaluated in this draft 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 are proposed in the NADA or considered in this draft EA, as no others are being considered for approval. It is the preferred alternative.

Any changes and/or additions to the proposed 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 environmental analyses under NEPA 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, a GE animal containing an rDNA construct is "defined" in terms of its identity, the claim made for it (i.e., its effectiveness), and any limitations and/or conditions placed on its use. The following is the current product definition for AquAdvantage Salmon. Although no modifications are expected for the product identity or claim, depending on

the outcome of the agency's final determination, the limitations of use may be modified if there is an approval.

Product Identity

Triploid hemizygous, all-female Atlantic salmon (*Salmo salar*) bearing a single copy of the α-form of the *opAFP-GHc2* rDNA construct at the α-locus in the EO-1α lineage.

Claim

Significantly more of these Atlantic salmon grow to at least 100 g within 2700 ^{0}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.

In the event of an approval, the following warnings would also apply to AquAdvantage Salmon and would be prominent on product labeling accompanying all life stages of the 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 would also contain a statement that eggs and fry 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 this NADA for AquAdvantage Salmon, the outcomes that could result fall into one of two broad scenarios: (1) the sponsor ceases production or maintenance of AquAdvantage Salmon; or (2) the sponsor continues to raise AquAdvantage Salmon at locations outside the United States (or to sell the fish or the technology to producers outside the United States) with no intent to directly market food from these fish (or food/feed derived from them) in the United States.

Under the second scenario, production and grow-out of AquAdvantage Salmon could occur almost anywhere (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-though tanks and/or raceways, or in recirculating systems.

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 at locations outside the United States.

4.3 Alternatives Considered But Rejected For Further Evaluation

The proposed action is strictly limited to the set of conditions of use proposed in the application. This set of conditions is based on multiply redundant containment measures that are intended to mitigate potential risks. These include 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 fish eggs are produced and the fish grown to market size (i.e., screening, filters, netting etc.). The level of containment proposed by the sponsor for AquAdvantage Salmon as part of the NADA goes well beyond that which is normally applied to salmon and other fish grown for either commercial food production or stock enhancement purposes.

Currently, the great majority of Atlantic salmon that are 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, 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 draft EA: (1) ocean or open water net pens/cages; (2) land-based, closed recirculation systems; and (3) water-based, closed containment systems such as floating fiberglass tanks. Although all three of these are potentially viable alternatives, they were ultimately excluded from further evaluation in this draft EA. Ocean net pens or cages deployed in coastal marine locations have not proved to be consistently effective in preventing farmed salmon escapes, and likely would not insure sufficient primary physical/mechanical containment of AquAdvantage Salmon without further technological development, or significant decreases in the uncertainty associated with possible outcomes should AquAdvantage Salmon escape from ocean net pens in significant numbers. We therefore did not consider the use of net pen/cage technologies to be an appropriate risk mitigation option at this time. We reached a similar conclusion for water-based, closed containment systems such as floating fiberglass tanks.

These systems are still quite new and the history of their performance under commercial operating conditions is relatively short.

Land-based recirculation systems, although believed to be highly effective in insuring adequate containment of fish under commercial rearing conditions, were not evaluated further in this draft EA. These systems do not provide any significant advantage over flow-through systems (the type of systems that would be used in the preferred alternative) 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 recirculation 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 proposed action, including the applicable types of physical and biological containment. Information on the rDNA construct used in the genetic engineering of AquAdvantage Salmon and the genotype of this salmon is 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 (farmed) 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). An empirical confirmation that an AquAdvantage Salmon is, in fact, an Atlantic salmon can be accomplished 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[14].

"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 Transgenic Fish Atlantic salmon Salmo salar Edible Tissue with the FDA/CFSAN RFE Standard for Non Transgenic Fish* (FDA Report 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. The finding of these IEF banding patterns confirmed that AquAdvantage Salmon meets the standard of identity for Atlantic salmon under the criteria developed for the RFE, 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 relative to non-genetically engineered Atlantic salmon to help characterize its fitness. Any consideration of the fitness of

[14] Available HERE.

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 that of AquAdvantage Salmon.

5.2.1 Comparative Studies

We have evaluated multiple studies conducted by the sponsor comparing farmed 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 in other GE fish including diploid, mixed-sex relatives of AquAdvantage Salmon and in 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, are unclear (see Briefing Packet, Weight of Evidence determination).

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 farmed 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, diploid and triploid, AquAdvantage 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 AquAdvantage Salmon and the non-GE comparators, with the number of abnormal findings being greater for triploid fish of both treatments, 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 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.[15]

[15] Morphologic irregularities do occur in non-transgenic salmonids, most commonly affecting cartilaginous and boney structures (Brown & Nuñez, 1998), and are often associated with the development of new commercial lines

In the same comparator-controlled study, no severe malformations were noted among the AquAdvantage Salmon enrolled. Irregularities in the fins and gill structure of triploid genetically engineered 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.

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.

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 2- to 6-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 ~2700°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.

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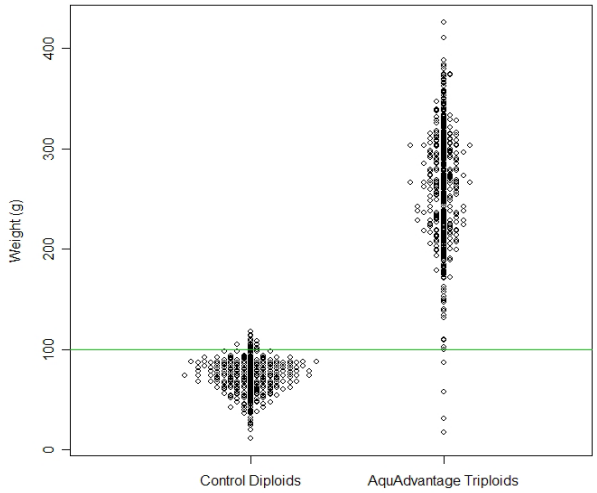
or husbandry techniques and culture conditions. Developmental malformations of cartilage and bone have been observed quite commonly in association with intensive commercial farming of salmon (*Salmo*) and trout (*Oncorhynchus*) species, including *S. salar* (Bæverfjord *et al*., 1996; Vägsholm & Djupvik, 1998; Silverstone & Hammell, 2002) S. *trutta*, (Poynton, 1987), *O. mykiss* (Mbuthia, 1994; Madsen & Dalsgaard, 1999), and *O. kuta* (Akiyama *et al*., 1986), as well as salmonids in the wild (DeVore & Eaton, 1983). These malformations include irregularities of the head, jaw, and operculum, and twisting or compression of the spine. 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). However, 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; however, rearing conditions that are otherwise deficient and present significant environmental stressors can lead to the increased mortality of these fish.

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



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, AquAdvantage-related transgenics, Abrahams & Sutterlin, 1999 and Cook *et al*., 2000a). 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 that have been 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, early-generation relatives of AquAdvantage Salmon (hereafter referred to as "AquAdvantage relatives") 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 AquAdvantage relatives 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 AquAdvantage relatives 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. can be altered in GE organisms. Changes in lethal limits or optimum values can shift or change preferred habitats, seasonal patterns, or the organism's geographic range.

The increased requirement for oxygen exhibited by AquAdvantage relatives (Abrahams & Sutterlin, 1999; Cook *et al*., 2000a; Cook *et al*., 2000b; Deitch *et al*., 2006) would engender a reduced tolerance for diminished oxygen content in general, and a reduced capacity for survival when the DO concentration is critically low, compared to their non-GE counterparts in the wild. In experiments with AquAdvantage relatives, oxygen uptake was independent of oxygen concentration above 10 mg/L, but started to decrease at about 6 mg/L DO in GE fish versus 4 mg/L DO in control fish (Stevens *et al*., 1998). Under conditions of high dissolved oxygen, GE salmon are not at a disadvantage compared to controls, as oxygen demand is readily satisfied,[16] however, escape into water with a DO level less than approximately 6 mg/L would place the AquAdvantage relatives at a physiological disadvantage.

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. AquAdvantage relatives 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 AquAdvantage relatives 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

[16] 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.

Because AquAdvantage Salmon are triploid fish, triploidy itself, and not just the presence or expression of the rDNA construct, may affect tolerance limits. Atkins and Benfey (2008) reported that triploids of Atlantic salmon had lower thermal optima than diploids, which could explain prior observations of mortality of other triploid salmonids (brown trout, brook trout & rainbow trout) at chronically elevated, but sub-lethal rearing temperatures. 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).

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 AquAdvantage relatives, 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 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 and non-GE Atlantic salmon at the AquaBounty PEI facility over the period from 2007 through 2011 shows similar rates of mortality between the two groups for five year classes of fish, indicating that AquAdvantage Salmon do not have an altered susceptibility to disease. With respect to an outbreak of infectious salmon anemia (ISA) that occurred in this facility during the third quarter of 2009, 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 AquAdvantage 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 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. No data were generated on non-GE comparators before the studies were discontinued.

As of November 22, 2011, no Schedule II diseases or disease agents, as described in Canada's Fish Health Protection Regulations (C.R.C. c812), had been detected in fish or eggs from either the ERA or the Grow Out Area (GOA) of the PEI facility as a result of four sequential inspections of each area over a period in excess of 18 months. Pathogens encompassed by the inspections included filterable replicating agents such as ISAV. See Section 5.4.2 for details.

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 AquAdvantage relatives (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 AquAdvantage relatives 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. AquAdvantage relatives 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 AquAdvantage 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 might be perceived as having an advantage over their wild counterparts. The relevance of these findings to the proposed agency action are unclear, as domesticated salmon in general have reduced spawning performance relative to wild fish, and only triploid (functionally sterile), female AquAdvantage Salmon would be raised for commercial use.

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 AquAdvantage relatives reached smolt size sooner than normal and the smoltification process was not inhibited by high temperatures (19ºC) or constant light.

5.3 Proposed 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 that are not location-specific, 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-1α (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-1α (i.e., it has only one copy of the genetic construct).

In order to produce AquAdvantage broodstock, individual AquAdvantage females homozygous for EO-1α 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.

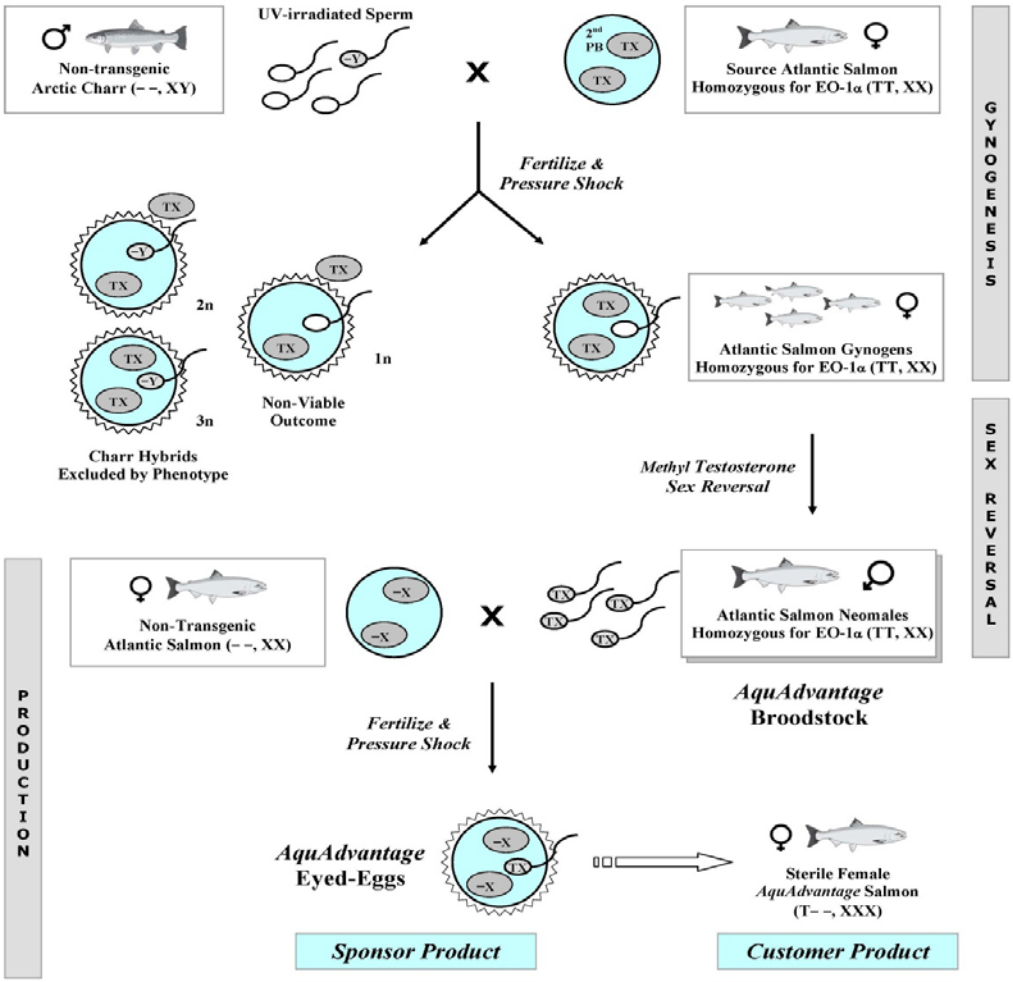
Atlantic salmon have an XY system of sexual determination, such that females are homogametic (XX) and males are heterogametic (XY). Many fish species experience a labile period after hatch when intentional exposure to sufficient levels of androgen or estrogen can influence phenotypic sexual maturity (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, 17α-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 17α-

methyltestosterone treatment of a mixed-sex population. Consequently, gynogenesis is used to produce an all-female population of salmon homozygous for EO-1α, which will generate *only* the homozygous GE neomales required for eyed-egg production when they are treated subsequently with 17α-methyltestosterone.[17]

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.

Figure 4. Reproductive Biology of AquAdvantage Broodstock & Eyed-Egg Production *



[17] 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).

* For AquAdvantage broodstock development, eggs from a female salmon homozygous for EO-1α 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-1α 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.

5.3.1.2 Technical Details and Logistics of Commercial Production

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

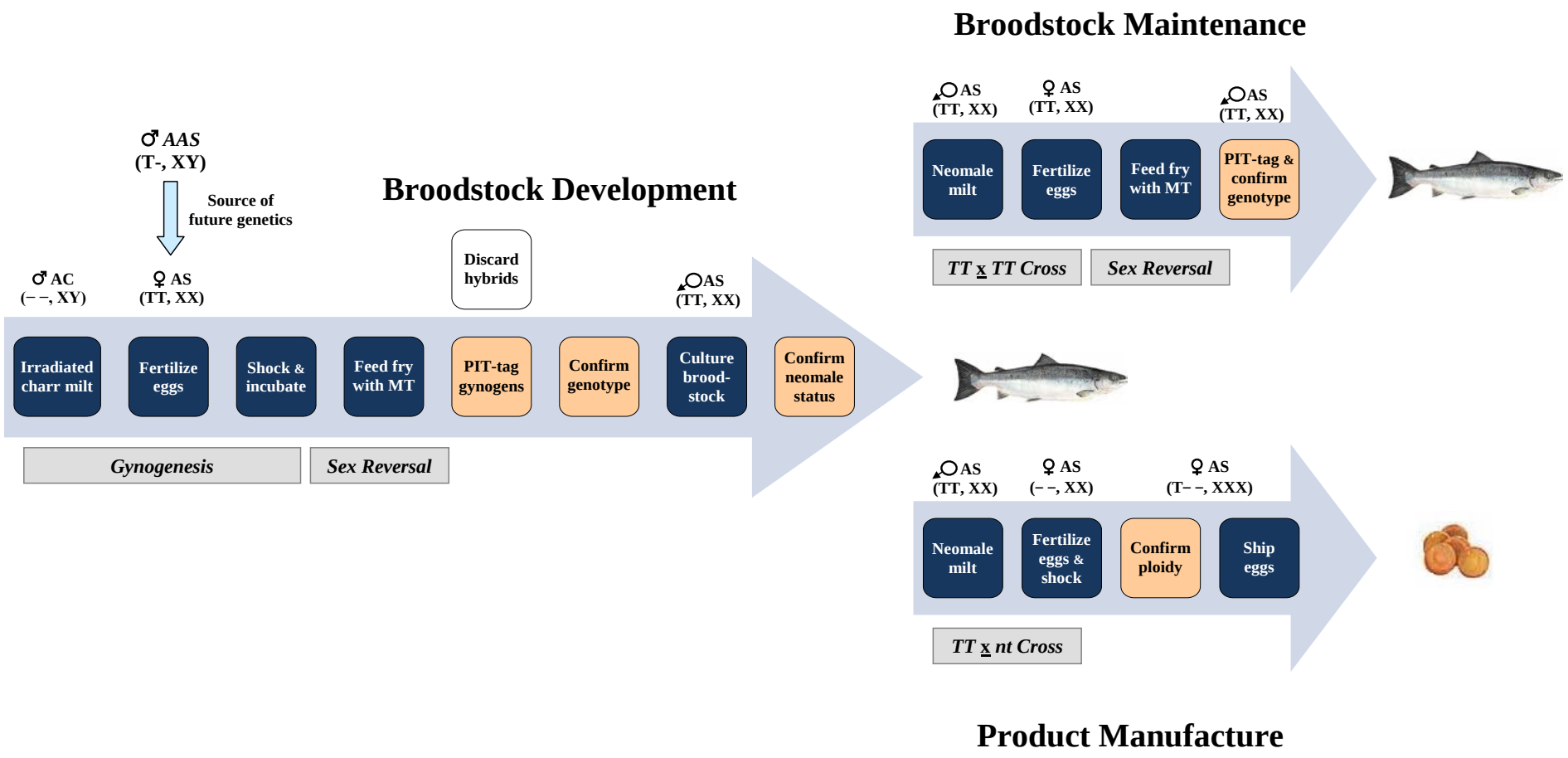
Development of AquAdvantage broodstock for post-approval egg production: Eggs collected from sexually mature, genetic-female salmon homozygous for EO-1α (TT, XX), in which the identity and integrity of the AquAdvantage gene construct has been confirmed using diagnostic methods, would be fertilized with irradiated milt from Arctic char, pressure shocked, and incubated until hatch. The fry (TT, XX) would be 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 AquAdvantage (neomale) broodstock (TT, XX) will be 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 AquAdvantage 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 would 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) would be fertilized with the milt from AquAdvantage (neomale) broodstock (TT, XX), and the fertilized eggs (T-, XX) would be pressure shocked to induce triploidy (T--, XXX). The eyed-eggs would 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 would be done to confirm the successful induction of triploidy via flow cytometry prior to release for commercial sale.[18] Confirmation of triploidy is discussed further in Section 5.3.2.3 below.

[18] ***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 absolutely 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.

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. In the case of AquAdvantage Salmon, the sponsor has incorporated two forms of complementary biological containment into the fish population through the egg production process: an all-female (monosex) genotype and triploidy (effective sterility). As discussed further below in Section 5.3.2.3, while 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 has also been included in AquAdvantage Salmon by ensuring that they are all females through the design of a production process using gynogenesis[19], which in this case is 100% effective (i.e., production of males is not possible). Although the production method could have been designed to produce an all-male fish population, the production of females is preferred since 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 would 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 used in 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.

[19] 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.

Triploidy is generally induced by either thermal or hydrostatic pressure shock of the eggs within the first hour after fertilization. Hydrostatic pressure shock is more easily controlled and therefore preferred (Benfey, 2001); this is the method that is used to generate triploid 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).

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 AquAdvantage Salmon males hemizygous for EO-1α. 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 (9500 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 indicated 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.

Should approval be granted, effectiveness of triploid induction for post-approval quality control in a statistically-appropriate sample of eyed-eggs from the production stream would be confirmed using established methods and procedures that require strict performance of controls and interpretability of analysis. Composite sampling of individual upwelling chambers, which comprise multiple batches of pressure-shocked eggs, would be conducted routinely. The

acceptance criterion for releasing a batch of eyed-eggs for grow-out would be 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.[20]

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). Triploid females rarely produce eggs, and if they do, the eggs usually are very few, undeveloped and unfertilizable (Piferrer *et al*., 2009). 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) cites several reports of the occasional production of mature oocytes by triploid females, 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 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.

In order to insure 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 proposed 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

[20] 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 made worse by the fact that the analysis requires destruction of the egg itself.

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).

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 on the northeast side of PEI, which is currently licensed to conduct research on GE fish under applicable Canadian regulations[21]. 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 AquaBounty 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. In terms of containment, DFO inspections characterized the facility as being "*as escape-proof as one can reasonably expect*"[22].

An FDA inspection of the facility found it to be as described by the sponsor and in compliance with applicable manufacturing establishment requirements. See Appendix F, Inspection and Site Visit Summaries.

5.4.1 Location and Operations

The PEI facility sits close to 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 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 screening used on these tanks varies with fish size (Table 1).

[21] The PEI facility is owned and operated by AquaBounty Canada, a wholly-owned subsidiary of AquaBounty Technologies, Inc. Some services in support 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.

[22] 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*.

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

Culture Tank		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 Effluent	*na*	5 mm (egg diameter)	Top & Bottom Sock filter		Standard Mesh 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 a 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 above) has conducted an on-site inspection 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 infectious salmon anemia (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 infectious salmon anemia virus (ISAV) was confirmed, AquaBounty notified DFO. CFIA was notified shortly thereafter.

AquaBounty Technologies 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 broodstock that had potentially been exposed to ISAV. The

ERA and GOA were also 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 November 22, 2011, no Schedule II diseases or disease agents, as described in Canada's Fish Health Protection Regulations (see below), had been detected in fish or eggs from either the ERA or the GOA of the PEI facility for over 18 months. Negative results had been found in four sequential inspections of each area. It is important to note that Canada requires four consecutive negative tests to grant "disease free" status to a facility once a positive finding has been made.[23] Pathogens encompassed by the inspections included filterable replicating agents such as ISAV. The most recent Fish Health Certificates issued by DFO for the ERA and GOA (see copies in Appendix G) specifically list the following pathogens as "not detected":

- Viral hemorrhagic septicemia virus
- Infectious hematopoietic necrosis virus
- Infectious pancreatic necrosis virus
- Other filterable replicating agent
- *Aeromonas salmonicida*
- *Yersinia ruckeri*
- *Myxobolus cerebralis*
- *Ceratomyxa shasta*

23 In order for the Fish Health Certificate to show a "disease free" status, Canadian Fish Health Protection Regulations require the facility to (1) implement a program to eliminate the specific diseases or disease agents that previously have been found, and (2) have four consecutive negative results in inspections over a minimum period of 18 months.

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 SOPs that govern physical containment as well as every other significant activity that occurs at the site.

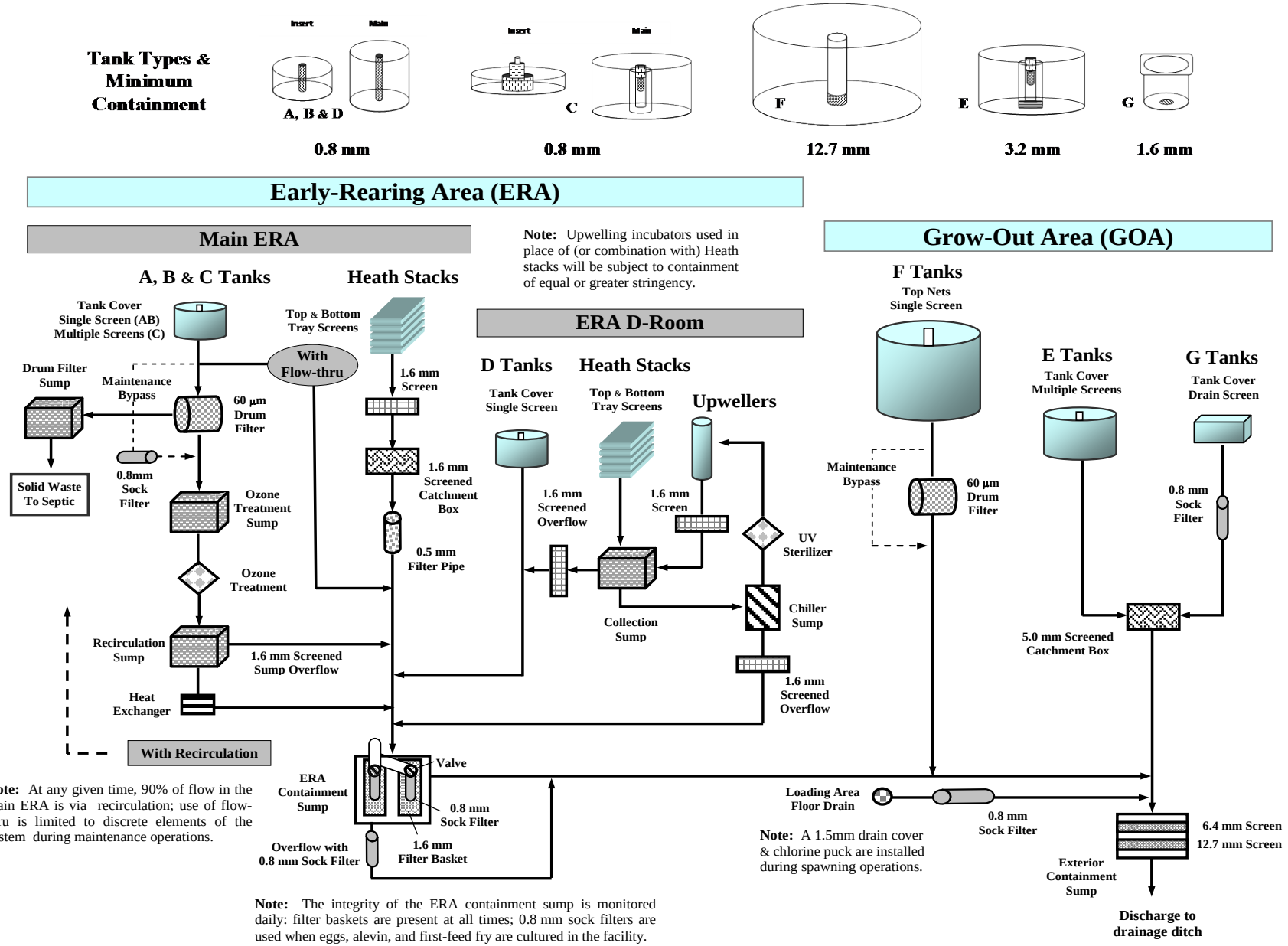
The potential for accidental escape could derive from any of the following components of the water system: influent water and makeup water; effluent and draw-down water; and waste slurries collected when filters are backwashed, screens scrubbed, or rearing units cleaned by siphoning (ABRAC, 1995). At 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 insure 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 into which fish could 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 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). All areas of the facility have at least three independent forms of mechanical containment, while many areas, including the egg incubation units and their discharges, have four or more. 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
To prevent escape over the side of a tank or egg incubator	Screened tank overflows Cover nets Jump fences Tank covers Incubator tray 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	Stainless steel barrier screens within drains of various sizes & locations
	Double screens within the exterior containment sump
	Mesh filter on drum filter gray water
	Heat exchanger
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



5.4.4 Security at the PEI Egg Production Facility

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

- ***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, which is 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

In the event of approval, commercial rearing and grow-out of eyed-eggs of AquAdvantage Salmon would only occur at one site: the sponsor's land-based, freshwater aquaculture facility in the 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 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 fry-tank housing, quarantine, feed storage and office space, and four outdoor culture 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, and acclimated to ambient water temperature and pH. After the eggs 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.

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 would be transferred from the fry tanks to the grow-out tanks, and would initially be stocked into two of the four grow-out tanks. Subsequently, as they grow and biomass approaches 35 kg/m^3, the fish would be distributed among all four grow-out tanks, where they would 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 would 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 ~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 could be harvested at different weights to test different markets and product presentations. The fish would 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 would leave the grow-out facility. The fish would be marketed in different presentations (e.g., whole on ice, whole-dressed on ice, fillet on ice & frozen or smoked fillet). Fish exported to the United States would be shipped in refrigerated containers by established wholesalers subject to Panamanian law, and subject to applicable U.S. regulations.

5.5.1 Physical Containment at the Panama Grow-Out Site

Physical containment to prevent the escape of fish at the grow-out facility is provided by the use of screens wherever water flows out of 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). These measures are summarized in Table 3; a schematic of the containment system is provided in 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 pass through a 500 μm (0.5 mm) sock filter that is inspected daily and empties into an excavated earthen drainage canal. 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. Distal to the sump, the water flows into a sequential series of four settling ponds, each of which is equipped with a 12 mm rigid-metallic outlet screen on which a secondary, variable-gauge screen is placed to facilitate flow, while maintaining exclusion of fish as they increase in size from fry to market size. From these ponds, the water is recycled 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 insure that “jumpers” (i.e., fish attempting to escape their tanks by jumping clear of the water) would not be

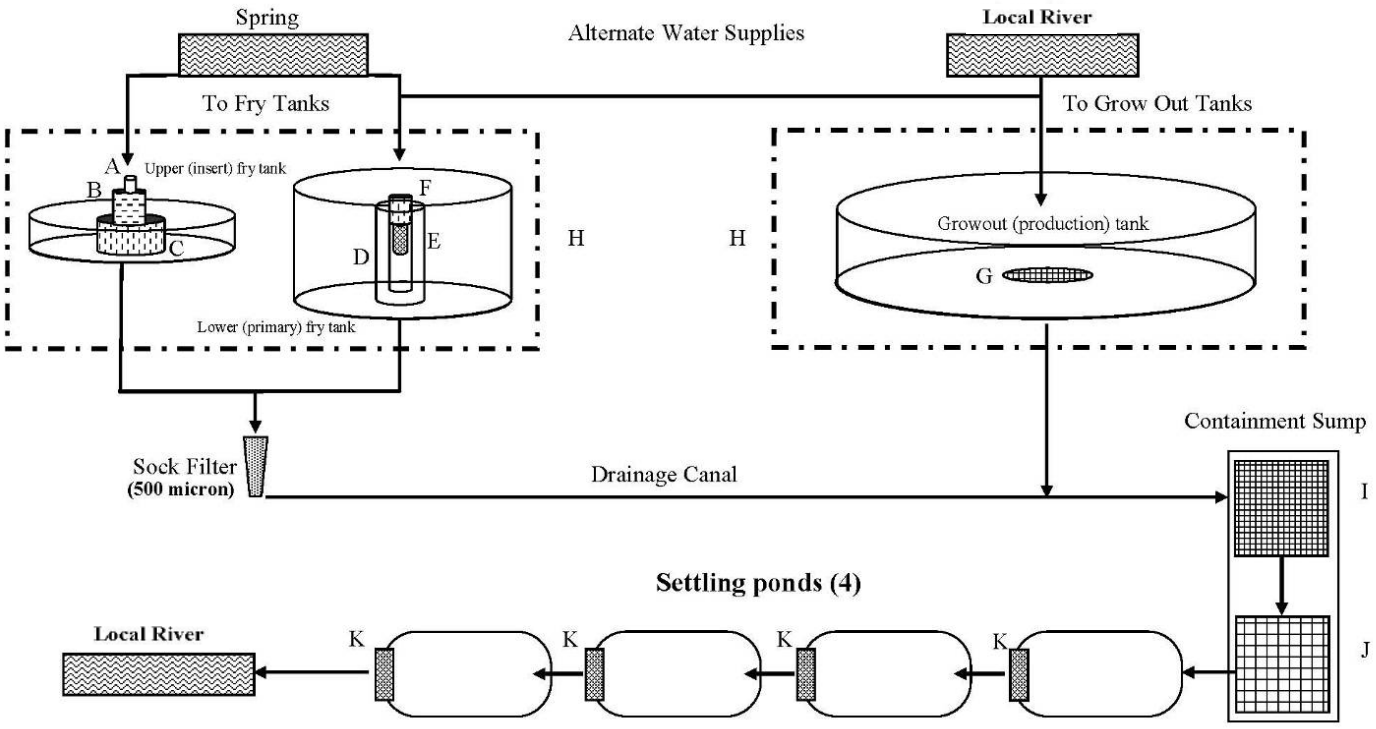
successful. In particular, the grow-out tanks are sealed horizontally and vertically inside a cage comprised of netting supported by a rigid structure. Escape from the tanks by jumping, or removal of fish by avian predators, would therefore be essentially impossible.

In total, a minimum of 11 sequential physical barriers are in place between the fry tanks and the local river, which would confine AquAdvantage Salmon to the site; seven of these barriers are installed following outflow from the grow-out tanks. In addition, netting on all tanks would 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 3. Key Components of Physical Containment at the Panama Grow-Out Facility

Purpose	Feature or Component
Primary Containment	
To prevent escape from the fry tanks via water	Center standpipe cut below tank rim to ensure water level is always below rim 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 from fry tanks into drains	Sock filter (500 μm) on the terminal end of the only drain pipe receiving effluent from the fry tanks
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 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
To prevent escaped fish from entering the river from the drain canal	Four sedimentation ponds in series, each with its own outlet screen

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



A	Interior Stand Pipe	I	Screen Basket (6 mm)
B	Interior Stand Pipe Screen (1.0, 1.5 mm)	J	Screen Plate (12 mm)
C	Exterior Stand Pipe Screen (1.5 mm)	K	Outlet Screen (12 mm)
D	Exterior Stand Pipe		
E	Basket Screen (3, 6 mm)		
F	Top Screen (3, 6, 12 mm)		
G	Slotted Drainage Plate (0.9 cm)		
H	Security Fence		

5.5.2 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 unintentional access. 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

The product being shipped from the production site on PEI would be limited (as a condition of approval) 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 Product Label printed on tear- and water-resistant paper would be affixed to both the egg crate and shipping container; this label would show the product name and provide 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 would also be included. The shipment would be identified as "Eggs & Fry" that is "Not for Resale." The following additional warnings (or facsimile thereof) would also likely appear on the Product Label: [24]

- 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 car (or truck) 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 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 $\geq$ 7 mg/L DO.

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

[24] The Product Label and Package Insert remain under FDA review at the time of this writing. In the event of an NADA approval, alternative and/or additional warnings or use limitations may be incorporated in the product labeling.

5.7 Operational Plans

Both the manufacturing and production sites are managed according to established SOPs that cover day-to-day operations; in addition, a specific written plan addressing responses to loss of operational capacity, breach of security, or catastrophic incidental occurrence has been formulated for the egg production facility on PEI. This *Overview of Facility Operating Systems and Emergency Procedures* 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 systems-supplies.

Similar planning and documentation thereof would be pursued for the grow-out facility in Panama following U.S. product approval, if this should occur.

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 Physical Site Characteristics of the Proposed Action (Preferred Alternative)

The accessible environments discussed in this draft EA are those surrounding the two facilities where AquAdvantage Salmon would be produced as eggs and grown to market size. Although effects on the local environments of Canada and Panama are not being evaluated in this draft 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 to reach and impact the environment of the United States.

6.1.1 PEI Egg Production Site

Production of AquAdvantage Salmon eyed-eggs would occur at a land-based, freshwater aquaculture facility located on the northeast side of Prince Edward Island. 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 4. 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 4. Weather Data for the Egg Production Site Environment *

Month	**Avg Daily Temp** (ºC)		**Avg Rainfall**	
	Min	**Max**	**Amt** (cm)	**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, including both rain and 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 local 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 estuary/river 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

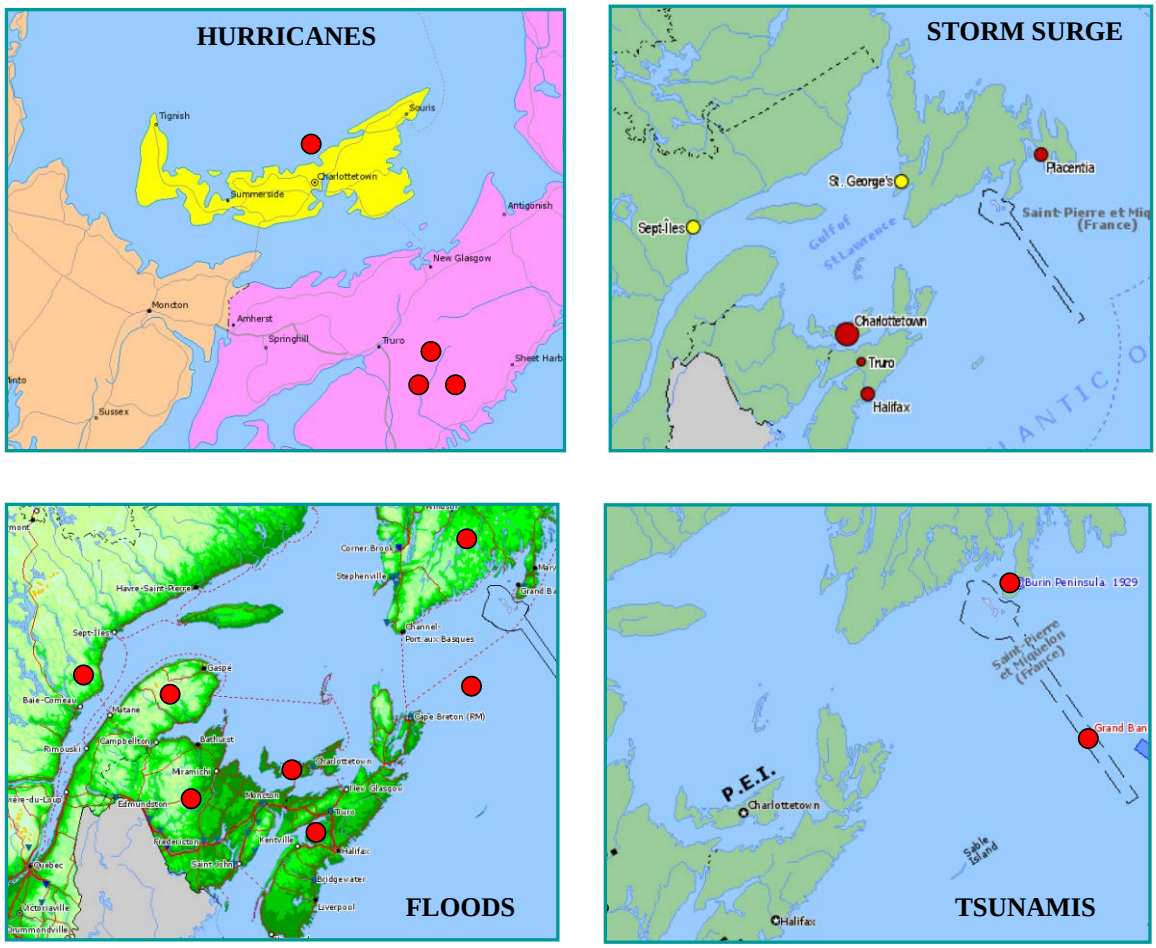
Although Prince Edward Island is frequently affected by outcomes such as power outages, rain and snow storms from December until April, it has rarely been subject to significant weather-related damage. As shown in Figure 8, Natural Resources Canada has reported that only four major hurricanes are reported to have occurred in the vicinity of PEI prior to 2000[25]. 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 C$200 million 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.

Flooding and severe storm surges, on the other hand, occur with regularity in the vicinity of Charlottetown on the south side of PEI (the ABT 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.

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

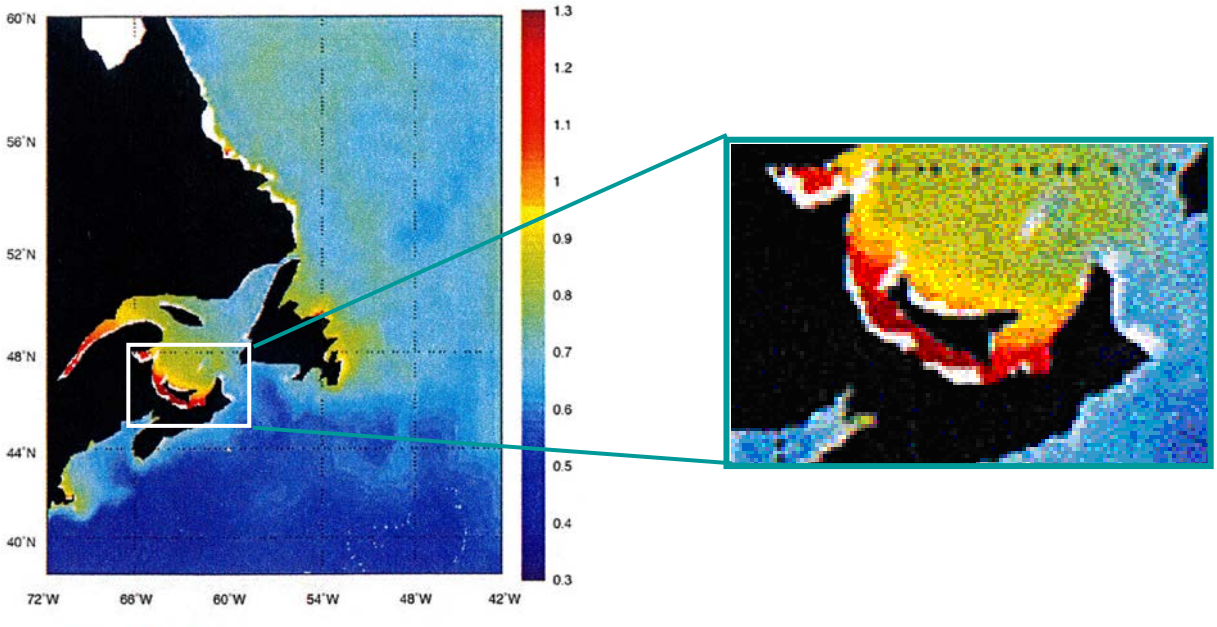
Figure 8. Occurrence of Natural Hazards in Proximity to PEI *



* 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). 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. Fish common to the area include the following: mackerel; herring; eel; gaspereau (e.g., alewife & blueback herring); silverside; smelt; and, salmonids. The salmonid group comprises the following: Atlantic salmon (*Salmo salar*); brook trout (*Salvelinus fontinalis*), which is native to the region; and, rainbow trout (*Oncorhynchus mykiss*), which was introduced into the region in 1925. Commercially important crustaceans include lobster and snow crab; bivalves (e.g., mussels, oysters, soft-shelled & bar clams, quahogs) are also fished commercially.

Between 1971 and 1985, the estimated abundance of 1-SW Atlantic salmon 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. The factors contributing directly to reduced marine survival of Atlantic salmon remain largely unknown, while significant factors affecting survival in fresh water include acid rain and poaching.

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). Restocking and habitat enhancement 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 5, vary little from month-to-month and are representative of year-round conditions.

Table 5. Air & Water Temperatures in the River Adjacent to the Grow-Out Facility

		Temp (°C)	
Point	**Elevation** (m)	**Air**	**Water**
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 6, 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 6. Weather Data in the Higher-Elevation Vicinity of the Grow-Out Facility *

Temp (ºC)	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**
Avg	18.9	18.9	19.2	19.6	19.2	18.9	16.8	18.2	18.5	18.2	18.1	19.0
Min	11.6	13.2	13.7	14.8	13.1	14.0	13.8	14.2	13.9	14.3	13.6	12.4
Max	23.3	27.9	27.7	28.8	28.1	26.5	26.1	27.7	27.4	28.2	27.0	26.0
Humidity (%)	56.5	59.6	60.0	64.1	80.0	78.5	77.6	83.2	84.0	85.3	82.8	58.9
Rain												
Days with	1	5	4	9	21	17	24	30	24	25	18	10
Days without	30	24	27	21	10	14	7	1	6	6	12	21
Total mo^{-1} (cm)	0.4	1.9	2.9	9.1	104.0	32.9	78.8	101.3	79.6	89.7	53.9	8.2
Total yr^{-1} (cm)	0.4	2.3	5.2	14.3	118.3	151.2	230.0	331.3	413.8	503.5	557.4	569.6

* 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 7, 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 7. Weather Data for the Near Sea-Level Locations *

Month	Avg-Daily Temp (ºC)		Avg Rainfall	
	Min	Max	Amt (cm)	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 8.

Table 8. 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 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 some bridges, roads, and buildings within the general watershed. Because the grow-out facility is sited at a higher elevation than the associated flood plain, no serious damage was incurred by this event, and no problems of significance to aquaculture operations occurred.

6.1.2.3 Biological/Ecological Properties

A diversity of macroinvertebrates exist 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, and a population of rainbow trout that were introduced in the upper basin could prey on salmon. These rainbow trout were intentionally stocked beginning in 1925, and are reported to constitute an established, naturally reproducing population (Welcomme, 1988); however, their abundance has not been well documented. In the upper-basin, vegetation on the river banks is scarce, and the substrate tends to consist of medium to very large round stones.

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 site could potentially support the earlier life stages of salmonids, physical barriers, sub-optimal habitat, and lethally-high water temperatures would be likely to prevent the long-term survival and establishment of Atlantic salmon in the river downstream.

6.2 Physical 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) production of AquAdvantage Salmon at suitable locations outside 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, 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 posses any combination of these characteristics. They could range from ocean net pens to highly contained systems identical to the ones proposed in this application. 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 these conditions were controlled to be within a suitable range. 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 inland 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.

It is important to note that the FDA proposed action is limited to an NADA approval for a specific set of conditions. As previously stated, any modifications that the sponsor may propose to the conditions of an approval would require notification of 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 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) are, or would be, in place at the facilities proposed 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) is 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 FDA inspection 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 proposed 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; Wong and Van Eenennaam, 2008). Confinement of GE fish in closed, land-based facilities is considered optimal in order to insure 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 preliminarily concludes that the likelihood of escape of AquAdvantage Salmon is extremely low. The following discussion provides the reasoning for this preliminary conclusion.

Physical containment for egg production and grow-out is described in Sections 5.4.2 and 5.5.1 of this draft EA, respectively. Key components of physical containment for the PEI facility are identified and illustrated in Table 2 and Figure 6, and in Table 3 and Figure 7 for the Panama facility. In addition, Section 2.5 describes the redundant, multi-level strategy used to insure containment at both facilities. Several SOPs are in place at the PEI facility to help insure containment, including an SOP that addresses physical containment of GE salmonids. There are also SOPs in place to address biosecurity, disinfection, decontamination, and other disease-related issues.

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 commitment to 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 ensure 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

The adequacy of physical containment at the sponsor's PEI facility was addressed in FDA's evaluation of the sponsor's 2001 EA (prepared in support of investigational studies on AquAdvantage Salmon), and in the FDA facility site inspection conducted in October 2008 (see description below). All areas of the PEI facility have at least three 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 four to five separate, independent forms of physical containment.

Currently, eggs at the PEI facility are being incubated using Heath Stack incubators. If future production is scaled up, egg incubation is expected to occur 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.

7.2.1.1.2 FDA 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's Office of New Animal Drug Evaluation. The facility was found to be in compliance with FDA regulations; no Form FDA 483[26] was issued at the conclusion of the inspection.

[26] Form FDA 483 is used to communicate investigational observations that may need correction.

Background

An EA was submitted in December 2001 by the sponsor to address potential environmental effects as a result of investigations on 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. All areas of the facility were found to have at least two independent levels of containment and some have three or four[27]. Components shown and described in the SOP that provide containment include the following:

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

[27] 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.

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 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 DFO is no longer inspecting the facility with respect to containment of GE fish, and that regulatory oversight in this area is now under the oversight of Environment Canada.

As a result of the inspection, no concerns or issues for follow-up or correction were identified. Since the 2008 FDA inspection, additional containment and isolation measures have been implemented at the PEI facility, and are reflected in Figure 6.

7.2.1.1.3 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 Prince Edward Island, 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 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 PEI facility inspection 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 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.

7.2.1.1.4 Preliminary Conclusions for the PEI Facility

The probability that AquAdvantage Salmon will 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 verified through inspections by FDA and Canadian authorities. Physical security and containment is sufficient to insure that it is highly unlikely there will be any unintentional releases of AquAdvantage Salmon due to equipment

failures, natural disasters or malicious activities. 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

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). 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 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 are 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.

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 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. Each of the four ponds has a 12 mm rigid metal screen on its outlet. 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, and counting the series of four settling ponds with screens as only a single form, 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), egg incubation units would be added within the Panama facility if the NADA for AquAdvantage Salmon is approved by the FDA, the necessary Panamanian approvals are received, and commercial-scale production commences. The sponsor has indicated that physical containment conditions for the incubation units would be similar to, and no less effective than, those currently in place for egg incubation at the PEI facility, offering a minimum of four levels of containment.

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

Information reported in the sponsor's EA for the Panama facility was verified during the site visit conducted by FDA and NMFS staff in mid-November, 2009 (see below). The facility appeared to be newly built and well-maintained with the multiple forms of physical (mechanical) containment present as described in the sponsor's submissions to FDA.

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 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 should they somehow escape 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 three or four levels of containment between both the fry tanks and grow-out tanks and the river. This includes a very conservative counting of the series of four downstream settling ponds (each with its own outlet screen) as only a single level of containment.

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 1925 (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 5), with much of it coming in the wet summer months. There was a significant flood of the river in the recent past 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. 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. In the time since this accident occurred, redundant intake piping has been added and many of the pipes have been moved underground to prevent such future occurrences. Considering that this 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.

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 SOPs in place at the Panama facility to address containment failure and security issues. A more formal written operational plan would be developed after an approval of AquAdvantage Salmon, if approval occurs. Employees at the Panama facility currently undergo training and the facility is subject to periodic audits by Panamanian authorities 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 insure that the probability of occurrence of such an event would be extremely low.

7.2.1.2.4 Preliminary Conclusions for the Panama Facility

The probability that AquAdvantage Salmon will 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 insure that it is highly unlikely there will 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 AquAdvantage 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.

In PEI, mortalities and culls requiring disposal will be stored frozen until they are incinerated. In Panama, fish mortalities will be deposited in 1-m deep, on-site burial pits. Individual fish will be separated by a layer of caustic lime and the pit will be filled with soil once it has reached a depth of 0.5 m with mortalities. Fish wastes from the PEI facility are subject to extensive treatment prior to discharge to the local estuary. In Panama, wastes from the grow-out tanks will be removed from the facility's effluent in a series of four sedimentation ponds prior to discharge 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. 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 has not been described by the sponsor, except to indicate that that disposal will be in accordance with applicable Panamanian laws. The lack of additional information on disposal is not a concern in that 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. As a result, the disposal exposure pathways originating in Canada and Panama are considered incomplete and are not expected to result in any effects on the environment of the United States.

For 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 Preliminary Conclusions for Question 1

For AquAdvantage Salmon, both the production of eyed-eggs and the grow-out of the fish is to be conducted *only* in land-based facilities with redundant physical containment measures and with point-to-point control of shipping and land-based materials transfer. There are multiple and redundant physical 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. 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 preliminarily considers the likelihood that AquAdvantage Salmon could escape from confinement at these sites to be very low. In addition, FDA has made the preliminary determination that physical security and containment to ensure unintentional releases of salmon due to natural disasters or malicious activities is 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, a strong operations management plan is in place at the PEI site (and would be implemented for the Panama facility in the event of an approval), comprising policies and procedures that meet the recommendations for an integrated confinement system for GE organisms as summarized in Table 9.

Any breakdown of these measures would be highly unlikely because of the following factors: the sponsor's commitment to 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.

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

Table 9. Implementation of an Integrated Confinement System for AquAdvantage Salmon *

	Egg Production & Grow-Out Sites	
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.
** Written plan to be prepared in the event of an approval

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 AquAdvantage Salmon escaped, the likelihood of survival and dispersal is a function of two complementary sets of parameters: AquAdvantage Salmon's 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 for AquAdvantage Salmon and is discussed separately below for both the PEI broodstock and Panama grow-out sites. As an overall statement, the spread of AquAdvantage 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 will greatly limit or preclude the possibility of a complete exposure pathway by which AquAdvantage Salmon could reach the United States. For the reasons discussed in the following sections, FDA preliminarily concludes that the geographical and geophysical settings of the AquAdvantage Salmon egg production and grow-out sites make the possibility of environmental impacts on the United States from survival and dispersal of AquAdvantage 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)[28]. 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

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

habitat for at least some life stages of AquAdvantage 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

Detailed analyses of the phenotype of AquAdvantage Salmon (see Section 5.2 of this draft 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 its 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 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 animal safety. However, reports on these fitness characteristics from peer-reviewed journals on GH transgenic Atlantic salmon (described in Section 5.2) 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 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 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 disease resistance, which appears not to be affected, the available information on the phenotype of adult AquAdvantage 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 low 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 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 to AquAdvantage 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 (>22‰). Nonetheless, because there are no specific data to indicate that AquAdvantage 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 Prince Edward Island). In fact, as noted by the Council on Environmental Quality 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 watershed in which the PEI facility is located (Guignion et al., 2010). Atlantic salmon are not currently present in the local watershed or any nearby watersheds, although they were once stocked in the area over the years from 1900-1949 (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.

Escaped farmed salmon, which are raised their entire lives on artificial feeds, tend to starve before they learn to seek natural prey (Muir, 2004). This would also be expected to be the case for AquAdvantage Salmon since they are raised on similar diets. 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 would not be particularly fit for the local PEI environment, even if they were to escape.

In conclusion, based on all of these factors, FDA preliminarily concludes that it is unlikely that early life stages of AquAdvantage 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 is less clear if they were to escape; therefore, we have made the conservative preliminary assumption that they would not undergo desmoltification and could be able to survive 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 5, 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[29] 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)[30], 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.

Salmon have a relatively high requirement for DO compared to many other fish species. GH-transgenic 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 salmon (AquAdvantage relatives) 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

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

[30] Available HERE.

river basin are 7.0 to 7.2 mg/L based on water quality monitoring conducted over the years 2002-2008 (see Table 8).

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. Salmonids have a requirement for clear water; the level of solids in the water column is high (Table 8). In addition, food sources may be limited, as the macroinvertebrate fauna, although diverse, are not abundant. Escaped salmon tend to starve before they learn to seek natural prey rather than feed pellets (Muir, 2004), a limitation that would be exacerbated by the low abundance of such prey in the environment at the grow-out site. For AquAdvantage Salmon more specifically, 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 is unknown. Rainbow trout constitute a known and formidable predator of salmon fry, fingerlings, and juveniles. Adult rainbow trout present in the adjacent watershed would 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 expected 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 could undergo desmoltification and, therefore, 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 Preliminary 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 to other locations. In the unlikely event that an escape were to occur, the likelihood of survival of AquAdvantage 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 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 AquAdvantage Salmon are not found in the local aquatic environments near either facility, the essential concern is over reproduction between escaped fish. (Note that by definition, the discussion on biological containment excludes fertile broodstock found at the PEI facility some of which are GE salmon[31], although technically not AquAdvantage Salmon, and some of which are wild type[32]. See Section 5.3 for details on the production process).

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. In the event of an approval, all AquAdvantage Salmon eggs produced for shipment to the Panama grow-out facility would be subjected to pressure treatment to induce triploidy, which would effectively sterilize the population.

7.4.1 Biological Containment (Bioconfinement)

Biological confinement would be insured 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). 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).

[31] The eggs retained within the facility for broodstock production and research and development purposes would not be triploid; therefore, mature fish reared from these eggs would be reproductively competent.

[32] The wild type fish would be expected to undergo desmoltification and thus would not be able to survive if they were to escape.

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 AquAdvantage 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%.

7.4.1.2 Triploidy and Triploidization

All AquAdvantage Salmon eggs sold or distributed for grow-out would 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 would continue to be conducted on all composite batches of fertilized eggs intended to be sold or distributed. Per the Durability Plan, if 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 draft 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).

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). However, the viability of these oocytes was never determined. In the subsequent work of Johnstone *et al*. (1992; Johnstone, 1991) on triploid Atlantic salmon females, it was shown that only approximately 0.1% of these fish underwent maturation after 2 years time. 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. Thus, Johnstone concluded that the expectation that triploid females are functionally sterile has therefore been confirmed. Similar results have been reported from a study on Arctic char (*Salvelinus alpinus*), a salmonid species related to Atlantic salmon. In the study on Arctic char, 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), demonstrating that successful reproduction was functionally and effectively precluded. Therefore, based on the available evidence, FDA preliminarily concludes that triploidy would insure functional sterility and reproductive incompetence in the sponsor's proposed 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)[33] to insure that a monosex, all-female population of AquAdvantage Salmon would be produced for grow-out. Using gynogenesis[34] 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 insures 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 insures 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 insure the future validity of the production process in making all-female population, the sponsor has made a commitment to conduct 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 would be 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[35], 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.

[33] 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.

[34] 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.

[35] 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.

7.4.1.6 Potential interactions with conspecifics

Because AquAdvantage Salmon are defined as, and in the event of approval would 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 farmed 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, 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, we 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 females could cause reproductive interference with native conspecifics, even if these conspecifics were present, which they are not. 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 particular river basin/estuary where the PEI facility is located or any of the other rivers in the area (Guignion 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.

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 salmonids are known to live locally. Atlantic salmon (*Salmo salar*) are able interbreed with brown trout (*Salmo trutta*), which are of the same genus, to produce hybrids, but they cannot interbreed with rainbow trout (Teufel *et al.*, 2002; Hindar, 1993), which are of a different genus.

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 from net pens, which is not a realistic possibility for either the egg-production or grow-out sites for AquAdvantage Salmon due to the redundant containment and security measures that are employed at both sites.

Adult rainbow trout are known formidable predators of salmon fry, fingerlings, and juveniles. The presence of large rainbow trout in the watershed in which the Panama grow-out facility resides further limits the possibility of prolonged survival of any escaped AquAdvantage Salmon.

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 tropical Pacific Ocean, which also does not have indigenous populations of Atlantic salmon, or any populations of Pacific salmon species (e.g., chinook, sockeye, pink) within several thousand miles. Further, Atlantic salmon are not able to breed with Pacific salmon, which are of a different genus. Thus, any reproduction or long-term establishment of AquAdvantage Salmon in the watershed of the Panama grow-out facility, or further afield, is essentially precluded.

7.4.2 Preliminary Conclusions for Question 3

The proposed conditions of use specify that 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 method validation study, the actual average percentage of triploidy is expected to be 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. Therefore, given the available information, FDA preliminarily concludes that there is a negligibly small likelihood that AquAdvantage Salmon would reproduce and establish if they escaped from facilities either in PEI or Panama.

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). In the very unlikely event of an escape, AquAdvantage 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 would have one key increased fitness attribute relative to their wild and domesticated counterparts (i.e., more rapid growth in their first year), 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 draft 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.

Biological containment of broodstock would be counterproductive to commercialization.

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, if they should occur, are likely to be of an extremely low magnitude due to the proposed small scale of production and the limited conditions of grow-out.

7.5.1 Proposed Action (Preferred Alternative)

The proposed action is the approval of the AquAdvantage Salmon NADA under specific proposed 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 in foreign sovereign countries. In this draft 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, by authorities in Canada and Panama.

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 AquAdvantage broodstock[36] 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 2.5 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 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 would be limited to several thousand at any one time[37]; 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, would be impossible.

Depending on the time of year that escape/release was to occur, escaped or released juveniles or adult AquAdvantage 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.

[36] AquAdvantage Salmon are defined as being triploid, female, and hemizygous for the AquAdvantage construct. As discussed in Section 5.3.1.1, AquAdvantage broodstock are diploid, 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 AquAdvantage broodstock development.

[37] 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 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 AquAdvantage 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 AquAdvantage 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 al. (2011a). 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, actively 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 significantly compromised reproductive performance.

Similar reproductive studies on GH transgenic coho salmon, although not necessarily representative for AquAdvantage 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."

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 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, and the factors likely to preclude long-term survival and establishment in the nearby aquatic environment, the possibility for interactions with wild Atlantic salmon is very remote. However, out of an abundance of caution, 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 have 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 AquAdvantage 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[38]. 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 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."*

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 are more susceptible to disease than non-GE salmon and thus more likely to be affected by disease (see Section 5.2.2.2). Second, and more importantly, all fish-holding areas of the PEI facility are currently certified as disease-free with respect to Schedule II diseases or disease agents[39] by Canadian Fish Health Officials (see Section 5.4.2 and Appendix G); there have been no positive findings resulting from a series of inspections over more than 18 months. The facility will continue to undergo regular inspections to verify that this remains the case. In addition, any new eggs and/or fish brought into the facility will be from certified disease-free sources. 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 might be more aggressive and thus

[38] Transcript available HERE.

[39] Schedule II diseases and disease agents include Viral Hemorrhagic Septicemia (VHS) Virus, Infectious Hematopoietic Necrosis Virus (IHNV), Infectious Pancreatic Necrosis Virus (IPNV), other filterable replicating agents including ISAV, furunculosis (*Aeromonas salmonicida*), enteric redmouth disease (*Yersinia ruckeri*), whirling disease (*Myxobolus cerebralis*), and ceratomyxosis (*Ceratomyxa shasta*).

outcompete their wild counterparts for resources. Research on AquAdvantage relatives 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 AquAdvantage relatives 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.

Snow *et al*. (2005) have presented six major environmental concerns or impacts may be associated with, or affected by, GE organisms (see Table 10). 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 10; 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. No significant risks associated with production of AquAdvantage Salmon in PEI have been identified.

Table 10. Potential Environmental Concerns/Impacts for GE Organisms*

Process	Potential Ecological Consequence	Risk Associated with AquAdvantage 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 See discussion in text.
Interbreeding with related taxa	Incorporation of transgenes could result in greater invasiveness or loss of biodiversity, depending on particular transgenic trait and gene flow from generation to generation.	NO SIGNIFICANT RISK See discussion in text.
Horizontal gene flow	Non-sexual gene transfer is common in some microbes but rare in plants & animals; ecological consequence would depend on particular transgenic trait and gene flow.	NO SIGNIFICANT RISK. The integrated rDNA construct (transgene) in AquAdvantage Salmon is incapable of being passed thru non-sexual means.
Change in viral disease	In virus-resistant transgenic organisms, genetic recombination could lead to increased virulence of viral disease and undesirable effects on natural hosts.	NO SIGNIFICANT RISK. The rDNA construct incorporated into AquAdvantage Salmon 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 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 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, escaped farmed Atlantic salmon of hatchery origin show a strong tendency to migrate into rivers in the vicinity of the site of escape as they mature (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 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 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 as 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 would impact 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 persisting salmon populations in an environment that used to possess plentiful salmon runs. In addition, the fitness of AquAdvantage 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 Preliminary Conclusions with Respect to Egg Production

We have 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 reproductively competent diploid AquAdvantage Salmon and AquAdvantage broodstock) from the egg production facility would result in significant effects on the environment of the United States. We also note that the proposed containment conditions for AquAdvantage Salmon in PEI are consistent with guidelines from NASCO in its "Williamsburg Resolution" (see Section 2.3.5). This resolution calls 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 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 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 would be females and approximately 99.8% would 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 thousands of miles for that matter) and no populations of closely-related salmonid species with which reproduction is possible[40]; therefore, gene flow to related species would not be a possibility. As previously discussed at length, survival beyond the immediate local environment would not be possible due to hostile environmental conditions of temperature, water quality, and physical barriers.
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 Untied 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 insure 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

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

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 many 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 insure 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 (which would require a migration through the Panama Canal or around Cape Horn) and north Pacific Oceans.

7.5.1.2.3 Preliminary 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 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 Preliminary Conclusions for Question 4

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

7.6 Consequences for the No Action Alternative (Denial of NADA Approval)

As described earlier, there are two general likely scenarios to consider as a result of the no action alternative: (1) cessation of production of AquAdvantage Salmon, and (2) production of AquAdvantage Salmon at suitable locations outside 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, an assessment of potential effects on the environment becomes highly uncertain. Because production of AquAdvantage Salmon would be possible at any number of locations worldwide, under different containment conditions, and potentially within areas where native Atlantic salmon are present (see Appendix E), there are too many variables and unknowns to perform a comprehensive risk assessment that would result in a characterization of the consequences. 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. Because production under this scenario would not be under FDA jurisdiction and the containment conditions could differ greatly from those in the proposed action (e.g., fish might not be triploid, might not be reared in land-based facilities, or might not be subjected to multiple and redundant forms of physical containment), it is very difficult to make any predictions with respect to potential environmental impacts except to predict that environmental impacts would be expected to be more likely to occur than under the proposed conditions of production and grow-out for the proposed action (preferred alternative). Under this scenario, the potential for mass escapes of AquAdvantage Salmon to occur could also be higher, thus the magnitude of effects on the environment could easily be expected to be higher than for the proposed action, depending on the location, containment, and regulatory oversight that could be imposed.

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. For all of the reasons discussed previously in Section 7, CVM has preliminarily concluded that approval of an NADA for AquAdvantage Salmon would not have any significant environmental impacts. The absence of environmental impacts means that there would be no "incremental impact"; because this is the first approval for AquAdvantage Salmon, there would be no cumulative impacts.

As previously stated, this draft EA pertains to only one specific set of proposed 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 application 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, 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. Such a supplemental application would also require FDA to consult with NMFS and FWS regarding any potential effect on endangered species.

This draft EA considers only the specific proposed action (including the specific proposed conditions) before FDA at this time. 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 preliminarily 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 preliminary 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 multiple, redundant containment measures at the sites of egg production and grow-out, the possibility of AquAdvantage Salmon (or the broodstock used to produce these fish) escaping into the environment is extremely remote.
- 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 to other locations. 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.
- What is the likelihood that AquAdvantage Salmon will reproduce and establish if they escape the conditions of confinement?
    - AquAdvantage Salmon would be produced as all-female, triploid fish. As such they would be effectively sterile. The proposed conditions of use specify that a minimum of 95% of the AquAdvantage Salmon eggs sold for commercial production use would be triploid; the actual average percentage of triploidy is expected to be approximately 99.8% based on results of the method validation study 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 AquAdvantage 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. Therefore, given the available information, FDA preliminarily concludes that it is extremely unlikely that AquAdvantage 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 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 proposed in the materials submitted by the sponsor in support of an NADA, and as described in this draft 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 draft 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.

- Throughout 2000, FDA participates in a cross-government working group coordinated by the Office of Science and Technology Policy formulating individual case studies evaluating regulatory issues pertaining to GE animals, and is the lead agency for what becomes the growth-enhanced salmon case study; these case studies are released to the public in early 2001.

- 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 draft EA, begin in 2001 once the sponsor establishes genetic stability of AquAdvantage Salmon over four generations.

- In 2002, FDA hosts a meeting to exchange information and discuss environmental risk assessment issues for AquAdvantage Salmon. The 45 meeting participants included representatives from the following:

    - FDA's Center for Veterinary Medicine
    - FDA's Center for Food Safety and Applied Nutrition
    - FDA's Office of the Commissioner
    - FDA's Office of International Programs
    - National Marine Fisheries Service
    - U.S. Fish and Wildlife Service
    - Canadian Food Inspection Agency
    - Environment Canada
    - Canada's Department of Fisheries and Oceans
    - Australia's Department of Primary Industries
    - Academics from Harvard University, Southampton University, and the University of New Brunswick
    - AquaBounty Technologies, Inc.

- In late 2003, the National Marine Fisheries Service issues a biological opinion to the U.S. Army Corps of Engineers discussing the use of off-shore net pens to farm fish off the coast of Maine. A part of this opinion considers the potential use of GE fish in such aquaculture systems. NMFS concludes that prohibition of net pens as a form of farming GE fish would "*eliminate the potentially adverse disease and ecological risks posed by the use of transgenic salmonids in aquaculture*." Subsequently, FDA and the sponsor agree that the conditions of use for AquAdvantage Salmon would be limited to highly-contained land-based facilities subject to routine FDA inspection and oversight.

- 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 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 staff brief the interagency Joint Subcommittee on Aquaculture on the details of the pending review of AquAdvantage Salmon. Attendees include representatives from USDA, NMFS, FWS, and EPA, as well as OSTP.

- 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 the last decade.

8.2 VMAC Public Meeting

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

As a part of that meeting, CVM released an unprecedented 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 adequacy of 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[41].

This draft EA differs from the EA released for the VMAC meeting in several ways. The EA released to the VMAC was prepared by the sponsor under the agency's overall direction. This draft EA has been prepared by the agency, and has taken into account recommendations from the VMAC and other governmental agencies, as well as comments that were submitted to the VMAC from the public.

8.2.1 Public Comment Period

FDA has published notice of the release of this draft EA and the accompanying preliminary FONSI in the Federal Register. The public will have 30 days to submit comments on the document. In accordance with 21 CFR 25.51, after reviewing the public comments, CVM will make a final determination whether to issue a final EA and FONSI, reissue a revised draft EA and preliminary FONSI, or to proceed with the preparation of an Environmental Impact Statement.

[41] Available HERE.

9. PREPARATION OF DRAFT EA

This draft EA has been prepared by the Center for Veterinary Medicine at FDA, under the direction of the Animal Biotechnology Interdisciplinary Group, with participation from other FDA, U.S. Government, and private sector experts. Portions of this draft EA have been adapted and derived from the EA prepared by AquaBounty Technologies, Inc. that 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). 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 19th century, these populations began to decline precipitously. In the 1800s, Atlantic salmon became extinct in the Connecticut (CT), Merrimack (MA), and Androscoggin (NH, ME), rivers mostly likely due to the results of dam building to harness the energy of the water. These dams blocked access of the fish to their natal streams (and thus their spawning areas). Industrial pollution, from paper mills and textile factories, also contributed to the decrease in populations,

as did commercial overfishing and climate changes that affect the temperature of the water in the ocean at the depths at which Atlantic salmon are found (2-10 meters below the surface). (Atlantic salmon need clear, sediment-free water and cold temperatures to survive). As an example, "**weirs**" (structures in rivers or estuaries that let water through while either directing fish to nets to be caught, or directly trapping fish) in Maine were reported as catching 90 metric tons of Atlantic salmon in the late 1800s and half that in the early 1900s.

Today, very few rivers in Maine support wild Atlantic salmon. In fact, Atlantic salmon are extinct in 84 percent of the rivers in New England that historically supported salmon. They are in "critical condition" in the remaining 16 percent. In 2004, only 60-113 individual fish were counted in the eight rivers in Maine that support Atlantic salmon. In 2000, the National Oceanic and Atmospheric 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[42]. 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.

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

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 draft 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 (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 on the other hand is less subject to debate (McVicar *et al*., 2006). The introduction of *Gyrodactylus salaris* (the salmon fluke) to Norwegian waters in 1975 has been clearly linked to resource management activities (Johnsen and 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).

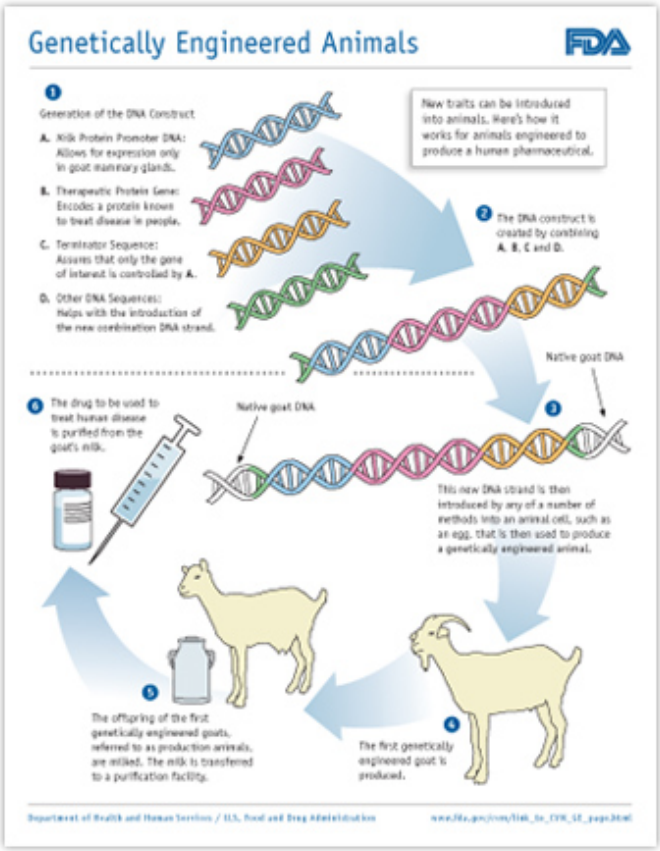
Although farmed salmon have been known to ener marine systems in large numbers by escape from containment nets, they can only become established by reproducing in adjacent freshwater ecosystems. Consequently, the fitness and behavior of feral[43] Atlantic salmon is of continuing interest as a matter 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).

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

Appendix B. Genetically Engineered Animals

Figure A: Overview of Genetic Engineering



16

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).

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, we review 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. We also look 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. We do 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. We evaluate 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, we perform 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 conventional 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 our evaluation, we look 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 we find, based on a review of the EA, that there is no significant impact on the environment under those conditions, we prepare and publish a FONSI. On the other hand, if we do 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 article 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 conventional 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 INAMERICA

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

Printed on Recycled Paper

Appendix E. AquAdvantage Salmon Genotype

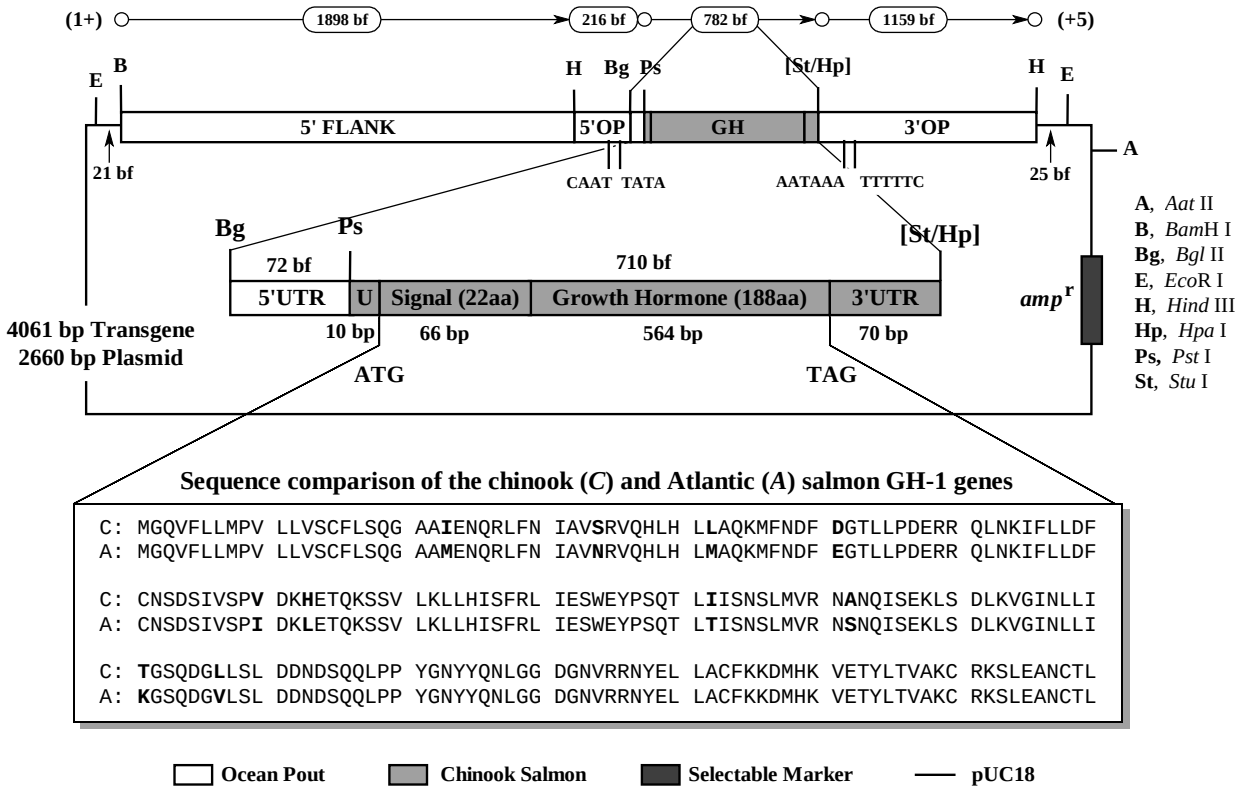
E.1 Characterization of the rDNA Construct

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.

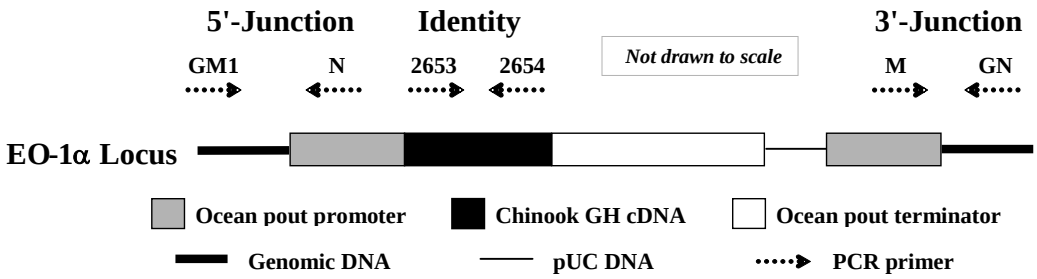
E.2 Characterization of the Integrated Form, EO-1α

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		
Objective	**Transgene Target**	**Primers**
Identity	GHc cDNA	2653-2654
Stability	5′-junction	GM1-N
	3′-junction	GN-M

Differential Signals	
EO-1α	**Endogenous GH**
207 bp	798 & 1150 bp
572 bp	*None*
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-1α.

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 Inspection and Site Visit Summaries

The draft EA notes that FDA personnel have visited the site in Canada where AquAdvantage Salmon eggs would be produced and the site in Panama where the fish would be grown out. This appendix provides more detail on those visits.

F.1 Prince Edward Island Facility

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 current 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[44].

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 levels of containment and

[44] Form FDA 483 is used to communicate investigational observations that may need correction.

some have three or four[45]. 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.

[45] 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.

F.2 Panama 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 draft 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 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 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. Fish Health Certificates for the PEI Facility

G.1 Early Rearing Area (Inspection of September 20, 2011)

Fisheries and Oceans Canada / Pêches et Océans Canada

Certificate No: RPC # 136
RPC Submission # 125739-FFA
RPC Job Number: FFA-J3307-1

FISH HEALTH CERTIFICATE

Eggs Only ☐ Fish and Eggs ☒

Name of facility/source: Aqua Bounty Canada Inc., Early Rearing Area Address: [redacted]

Telephone No.: [redacted] Fax No. [redacted] Email [redacted]

I, Rebecca Liston, as a Fish Health Official under the Canadian Fish Health Protection Regulations C.R.C., c812, certify that the source indicated above was inspected by the methods approved by the Minister of Fisheries and Oceans Canada, and that the following pathogen status was determined as required by those Regulations.

Pathogen	Detected	Not Detected	Not Tested
Viral Hemorrhagic Septicemia Virus	☐	☒	☐
Infectious Hematopoietic Necrosis Virus	☐	☒	☐
Infectious Pancreatic Necrosis Virus	☐	☒	☐
Other filterable replicating agent	☐	☒	☐
Aeromonas salmonicida	☐	☒	☐
Yersinia ruckeri	☐	☒	☐
Myxobolus cerebralis	☐	☒	☐
Ceratomyxa Shasta	☐	☒	☐

Notes: No clinical signs indicative of *Ceratomyxa shasta* were observed during necropsy.

Date of the last four previous inspections:
20/09/11 (D/M/Y); 30/03/11 (D/M/Y); 19/10/10 (D/M/Y); 17/03/10 (D/M/Y)

31/10/11 Date of Issue — Rebecca Liston — Signature and Address of Fish Health Official — 921 College Hill Road, Fredericton, NB, E3B 6Z9 — (506) 452-1379/ 452-1395 Telephone No./ FAX No.

This certificate expires on the date the pathogen status changes or 20/06/12 (D/M/Y) whichever is the earlier.

EXPORTER'S DECLARATION

I, _____ ☐owner ☐manager of the above noted facility which was last inspected on _____(D/M/Y) declare that, to my knowledge, no disease agent(s) listed in Schedule II of the Fish Health Protection Regulations (FHPR) that would alter the above described pathogen status have been detected, in this facility, according to the procedures outlined in the FHPR Manual of Compliance since the last FHPR inspection, that no introduction of fish or fish eggs from any source that would alter the above pathogen status has been made into the facility, that the shipment described below will be derived solely from this facility, and that eggs in the shipment will be surface disinfected prior to leaving the source.

I, _____ consignor of eggs taken from wild spawners declare that these eggs will be surface disinfected and that they derive solely from the above inspected source.

This shipment consists of:

_____ kg ☐ Live ☐ Eggs Species: _____

_____ Number ☐ Dead ☐ Fish Species: _____

Date — Signature and Address of Owner, Manager or Consignor — Telephone No. / Fax No.

IMPORTING INFORMATION

Departing city and country _____ Carrier _____

Bill of lading No. _____ Date _____

Anticipated port of arrival in Canada (City and Province): _____ Date _____

Date — Signature and Address of Importer — Telephone No.

rpc

G.2 Grow Out Area (Inspection of November 22, 2011)

Fisheries and Oceans Canada / Pêches et Océans Canada

Certificate No: RPC # 140
RPC Submission # 129070-FFA
RPC Job Number: FFA-J3316-1

FISH HEALTH CERTIFICATE

Eggs Only ☐ Fish and Eggs ☒

Name of facility/source: Aqua Bounty Canada, Grow Out Area Address: [redacted]

Telephone No.: [redacted] Fax No. [redacted] Email [redacted]

I, Rebecca Liston, as a Fish Health Official under the Canadian Fish Health Protection Regulations C.R.C., c812, certify that the source indicated above was inspected by the methods approved by the Minister of Fisheries and Oceans Canada, and that the following pathogen status was determined as required by those Regulations.

Pathogen	Detected	Not Detected	Not Tested
Viral Hemorrhagic Septicemia Virus	☐	☒	☐
Infectious Hematopoietic Necrosis Virus	☐	☒	☐
Infectious Pancreatic Necrosis Virus	☐	☒	☐
Other filterable replicating agent	☐	☒	☐
Aeromonas salmonicida	☐	☒	☐
Yersinia ruckeri	☐	☒	☐
Myxobolus cerebralis	☐	☒	☐
Ceratomyxa shasta	☐	☒	☐

Note: No clinical signs indicative of *Ceratomyxa shasta* were observed during necropsy.

Date of the last four previous inspections:
22/11/11 (RPC) (D/M/Y); 14/06/11 (RPC) (D/M/Y); 19/10/10 (RPC) (D/M/Y); 18/05/10 (RPC) (D/M/Y)

22/12/11 (Date of Issue) — Rebecca Liston — 921 College Hill Road, Fredericton, NB, E3B 6Z9 (Signature and Address of Fish Health Official) — (506) 452-1379/ 452-1395 (Telephone No./ FAX No.)

This certificate expires on the date the pathogen status changes or 22/09/12 (D/M/Y), whichever is the earlier.

EXPORTER'S DECLARATION

I, _____ ☐owner ☐manager of the above noted facility which was last inspected on _____ (D/M/Y) declare that, to my knowledge, no disease agent(s) listed in Schedule II of the Fish Health Protection Regulations (FHPR) that would alter the above described pathogen status have been detected, in this facility, according to the procedures outlined in the FHPR Manual of Compliance since the last FHPR inspection, that no introduction of fish or fish eggs from any source that would alter the above pathogen status has been made into the facility, that the shipment described below will be derived solely from this facility, and that eggs in the shipment will be surface disinfected prior to leaving the source.

I, _____ consignor of eggs taken from wild spawners declare that these eggs will be surface disinfected and that they derive solely from the above inspected source.
This shipment consists of:

_____ Kg ☐ Live ☐ Eggs ☐ Species: _____

_____ Number ☐ Dead ☐ Fish ☐ Species: _____

Date _____ Signature and Address of Owner, Manager or Consignor _____ Telephone No. / Fax No. _____

IMPORTING INFORMATION

Departing city and country _____ Carrier _____

Bill of lading No. _____ Date _____

Anticipated port of arrival in Canada (City and Province): _____ Date _____

Date _____ Signature and Address of Importer _____ Telephone No. _____

rpc