# AquAdvantage® Salmon

---

# Environmental Assessment

Supplement to NADA 141-454 to allow the grow-out of AquAdvantage Salmon at AquaBounty Technologies, Inc.'s Indiana Facility

## 20 April 2018

**DATE:** **April 20, 2018**

**NAME OF APPLICANT/PETITIONER:** **AquaBounty Technologies, Inc.**

**ADDRESS:** **2 Mill and Main Place, Suite 395**
**Maynard, MA  01754**

# TABLE OF CONTENTS

# LIST OF FIGURES

# LIST OF TABLES

**LIST OF ACRONYMS AND CONVENTIONS EMPLOYED**

| | |
|---|---|
| AAS | AquAdvantage Salmon |
| ABRAC | Agricultural Biotechnology Research Advisory Committee |
| ABF | AquaBounty Farms |
| ABT | AquaBounty Technologies, Inc. (the Sponsor) |
| AFP | antifreeze protein |
| CCD | Charged Coupled Device |
| CWA | Clean Water Act |
| CVM | Center for Veterinary Medicine |
| DNA | deoxyribonucleic acid |
| DO | dissolved oxygen (concentration) |
| EA | environmental assessment |
| EO-1$\alpha$ | the integrated form of the AquAdvantage rDNA construct |
| EPA | U.S. Environmental Protection Agency |
| FDA | U.S. Food and Drug Administration |
| FONSI | finding of no significant impact |
| FWS | U.S. Fish and Wildlife Service, Department of Interior |
| GE | genetically engineered |
| GH | growth hormone |
| IBI | Index of Biotic Integrity |
| IDEM | Indiana Department of Environmental Management |
| IDNR | Indiana Department of Natural Resources |
| mRNA | messenger ribonucleic acid |
| NADA | New Animal Drug Application |
| NEPA | National Environmental Policy Act |
| NRC | National Research Council |
| ONADE | Office of New Animal Drug Evaluation |
| Op | ocean pout promoter regulatory region |
| OSC | Office of Surveillance and Compliance |
| PEI | Prince Edward Island, Canada |
| PVC | polyvinyl chloride |
| rDNA | recombinant deoxyribonucleic acid |
| RFS | Radial-Flow Settler |
| SOPs | Standard Operating Procedures |
| TSS | Total Suspended Solids |
| USDA | U.S. Department of Agriculture |
| USGS | U.S. Geological Survey |

**TECHNICAL TERMS\***

| | |
|---|---|
| Allele | Any alternative form of a gene that can occupy a particular chromosomal locus. |
| AquAdvantage construct | The recombinant DNA construct used to generate AquAdvantage Salmon, referred to as *opAFP-GHc2*. |
| Biological containment (bioconfinement) | Use of biological methods, such as induced sterilization (e.g., triploidy), to prevent gene flow and reproduction in the environment. |
| Chromosome | A physical structure consisting of DNA and supporting proteins called chromatin that carries hereditary information. |
| °C-day [min] | Compound unit of "time" (°C x days [min]) for relative determination of growth rate that accounts for effect of water temperature. |
| Conspecific | An organism (plant or animal) of the same species. Herein, the term typically refers to wild or native Atlantic salmon. |
| Construct (gene or DNA construct) | A synthetically-assembled nucleic acid that frequently contains regulatory and coding sequences usually incorporated into the genome of an organism with the intended purpose of modifying its phenotype. |
| Diploid | A cell, tissue, or organism having two complete sets of chromosomes, one from each parent. |
| EO-1 | The mosaic, female founder of the AquAdvantage Salmon line created by microinjection of the *opAFP-GHc2* construct into a fertilized egg. |
| EO-1$\alpha$ | Functional, stably integrated form of *opAFP-GHc2* in the AquAdvantage Salmon genome. |
| Egg | A mature female germ cell extruded from the ovary at ovulation |
| Expression (gene) | The process by which the information encoded in a gene is used to direct the assembly of a protein molecule. |
| Gamete(s) | Mature male or female reproductive cell (sperm or ovum) with a haploid set of chromosomes. In animals, including fish, gametes are sperm and oocytes (eggs). |
| Genome | The entire set of genetic instructions found in a cell. |

| | |
|---|---|
| Genotype | The genetic constitution of an organism or cell; it also refers to the specific set of alleles inherited at a particular locus. |
| Haploid | A cell, tissue, or organism having a single set of chromosomes (as opposed to *diploid* or *triploid*). Haploid cells are generally found in gametes (sex cells) of higher organisms. |
| Molecular Cloning | A process of making identical copies of a DNA sequence. The target sequence is isolated, inserted into another DNA molecule (known as a *vector*), and introduced into a suitable host cell (usually bacteria). Then, each time the host cell divides, it replicates the foreign DNA sequence along with its own DNA. |
| *opAFP-GHc2* | AquAdvantage recombinant DNA construct Chinook salmon growth hormone (GH) gene and gene product, ocean pout and Chinook salmon–derived regulatory sequences, and a short synthetic linker. |
| Phenotype | An organism's actual observed properties, such as morphology, development, or behavior, which derive predominantly from its genotype. |
| Plasmid | A small, often circular, DNA molecule found in bacteria and other cells. Plasmids are separate from the bacterial chromosome and replicate independently of it. Plasmids are often used to make multiple copies of a recombinant DNA construct. |
| Ploidy | The number of complete sets of chromosomes contained within each cell of an organism (See *haploid*, *diploid*, and *triploid*). |
| Promoter | A sequence of DNA needed to regulate the expression of gene, including whether the gene is transcribed or not. The process of transcription (production of RNA from DNA) is initiated at the promoter. Usually found near the beginning of a gene, the promoter has a binding site for the enzyme used to make a messenger RNA (mRNA) molecule. |
| Protein-coding sequence | The DNA sequence of a gene that is transcribed into mRNA and subsequently translated into protein. |
| Recombinant DNA (rDNA construct) | DNA artificially constructed by combining genes from different organisms or by cloning chemically altered DNA, usually for the purpose of genetic manipulation. The recombined DNA sequences, or rDNA construct, can be placed into vehicles called vectors (See *plasmid*) that ferry the DNA into a suitable host cell where it may be copied or incorporated, and expressed. |
| Regulatory sequence | A nucleic acid sequence involved in regulating the expression of genes. |

Salmonid — A ray-finned finfish of the family Salmonidae, a taxonomic group that includes salmon, trout, chars, freshwater whitefish, and graylings. The family includes fish of the following genera, among others: *Salmo*, *Salvelinus*, and *Onchorhynchus*.

Smolt — A freshwater juvenile Atlantic salmon that has undergone the physiological changes necessary to be able to survive in salt water.

SW — Sea winter: Number of winters spent at sea (e.g., 1SW, 2SW).

Transgene — A gene comprising regulatory and coding sequences constructed *in vitro* and usually incorporated into the genome of a different species/organism with the intended purpose of modifying its phenotype. Often used interchangeably with "rDNA construct."

Triploid — Having three complete sets of chromosomes per cell (See *haploid* and *diploid).*

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

*The various sources used for these definitions include: Wiley's *Dictionary of Microbiology and Molecular Biology*, Revised 2nd Ed., John Wiley and Sons, New York, 1994; *Animal Cloning: A Risk Assessment*, U.S. Food and Drug Administration (Center for Veterinary Medicine), 2008, final version found at http://www.fda.gov/AnimalVeterinary/SafetyHealth/AnimalCloning/ucm055489.htm (accessed 12/19/2017); National Human Genome Research Institute, *Talking Glossary of Genetic Terms*, accessed at www.genome.gov/Glossary (accessed 12/19/2017); Human Genome Project, accessed at www.genomics.energy.gov (accessed 12/19/2017).

# 1    SUMMARY

AquaBounty Technologies, Inc. (ABT or the sponsor) has provided data and information in support of a supplemental New Animal Drug Application (NADA) for a genetically engineered (GE) Atlantic salmon referred to as AquAdvantage® Salmon, which is designed to exhibit a rapid-growth phenotype that allows it to reach a size of ~100 g [1] faster than non-GE farm raised salmon. The NADA (NADA 141-454) was approved for the production of AquAdvantage Salmon[2] in Canada and grow-out of the fish in Panama. ABT now seeks approval for grow-out of the fish at an additional site, in Indiana. Other aspects of production remain unchanged.

This Environmental Assessment (EA) was prepared to support the supplemental NADA. As a part of the NADA review and approval process under the Federal Food, Drug, and Cosmetic Act (FD&C Act), 21 U.S.C. § 321 et seq., and consistent with the mandates in the National Environmental Policy Act of 1969 (NEPA), 42 USC § 4321 et seq., and the Food and Drug Administration's (FDA's) environmental impact considerations regulations (21 CFR Part 25), the approval of the NADA was supported by an EA prepared by the Center for Veterinary Medicine (CVM), U.S. Food and Drug Administration (FDA) dated November 15, 2015 (NADA EA). FDA issued a Finding of No Significant Impact (FONSI) on November 15, 2015, concluding that the action to approve the NADA for AquAdvantage Salmon (NADA 141-454), under the specific conditions established in the NADA, "would not individually or cumulatively have a significant impact on the quality of the human environment in the United States." The specific conditions were based on production of eyed-eggs at a single, specific facility in Prince Edward Island in Canada (PEI facility), with shipment of eyed-eggs to a single, specific land-based grow-out facility in the highlands of Panama (Panama facility), where they are reared to market size and harvested for processing.

ABT is seeking approval of a supplemental NADA to allow grow-out at a land-based facility in Albany, Indiana (Indiana facility). Under the supplemental NADA, eyed-eggs would be produced at the PEI facility, while grow-out could occur either at the Panama facility or the Indiana facility. Approval of such a supplemental application constitutes a major agency action and triggers environmental analysis under NEPA, unless otherwise excluded. Grow-out of AquAdvantage Salmon at the Indiana facility constitutes a major change in the conditions established in the approved NADA that requires FDA approval of a supplemental NADA and environmental analysis under NEPA. This EA constitutes part of that environmental analysis, and relies extensively on the previous EA prepared by FDA for the AquAdvantage Salmon NADA approved in November, 2015. In particular, this EA describes the physical, biological, and geographical/geophysical forms of containment at the Indiana facility and evaluates the potential environmental impacts of the action (approval of this specific supplemental NADA) and the no action alternative.  FDA's approval of the supplemental NADA would be for the

---

[1]    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.

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

specific set of conditions described in this EA and as enumerated in FDA's supplemental NADA approval letter. No other conditions of production and use of AquAdvantage Salmon would be permitted within the scope of the supplemental NADA approval, or have been evaluated in this EA.

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

The approach in this EA is one based on a characterization of hazards, an evaluation of potential exposure pathways, and a consideration of the likelihood of any resulting risk. The environmental analysis of consequences in the EA incorporates the principles described in the previous EA (see NADA EA at Section 1) as well as the U.S. Environmental Protection Agency's (EPA) approach to ecological risk assessment (EPA, 1992). The potential hazards and harms addressed in this EA center on the likelihood and consequences of AquAdvantage Salmon escaping, surviving, and becoming established in the environment, and then dispersing or migrating such that there might be an exposure pathway causing an adverse outcome (the risk) to the environment of the U.S. These hazards were previously addressed in the EA for NADA 141-454 for the production of eyed-eggs at the PEI facility and grow-out to market size at the Panama facility, within the framework of a conceptual risk assessment model and the following series of risk-related questions. In this EA, these hazards are addressed for grow-out to market size at the Indiana facility. The risk-related questions are:

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 should AquAdvantage Salmon escape the conditions of confinement?

The proposed land-based grow-out facility in Indiana has multiple and redundant forms of effective physical containment. Based on this analysis, the likelihood that AquAdvantage Salmon could escape from containment, survive, and become established in the local environment of the Indiana facility is very low. Should unintentional release from the Indiana facility occur, the environmental conditions in the geographic setting would afford additional means of containment of any escaped eggs or fish, given that the facility location is well outside of the natural range of Atlantic salmon, and the environmental conditions would be generally hostile to their long-term survival, reproduction, and establishment. In addition, because the

production process for AquAdvantage Salmon would ensure[3] that populations produced will be triploid (effectively sterile), all-female animals, the possibility of their reproducing in the wild is likewise extremely remote. No effects on populations of threatened and endangered species in Indiana are anticipated (see Section 7.6).

The addition of the Indiana grow-out facility theoretically adds to the cumulative risk discussed in the EA for NADA 141-454, which was based on the production facility in PEI and the grow-out facility in Panama, as more AquAdvantage Salmon can be produced and shipped to two, rather than just one, grow-out location if the Indiana facility is approved. However, because it is concluded that the likelihood of escape, survival, and establishment of AquAdvantage Salmon from the Indiana facility is negligible, the cumulative risk is negligible.

In summary, the evidence collected and evaluated in this EA indicates that the proposed action on the supplemental NADA for AquAdvantage Salmon, including grow-out of these GE salmon at the Indiana facility under the conditions of use and production described in this EA, would not result in a significant impact on the quality of the human environment in the U.S.

---

[3] See FN 22.

## 2   PURPOSE AND NEED

This EA was prepared as part of the regulatory considerations for approval of a supplement to NADA 141-454 for AquAdvantage Salmon, a GE Atlantic salmon produced by AquaBounty Technologies, Inc. (ABT or the sponsor), to allow for grow-out of these fish at the Indiana facility. Because the previous NADA approval was based upon specific conditions, which included grow-out of AquAdvantage Salmon only at a single facility in Panama, the addition of a new grow-out facility for rearing requires FDA approval of a supplemental NADA application.

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

This EA describes the use of physical, biological, and geographical/geophysical forms of containment at the Indiana facility, a new location where eyed-eggs from the PEI facility will be shipped for rearing to market size, followed by harvesting and processing (e.g., preparation of eviscerated fish, fish fillets, steaks, etc.) at off-site processing facilities before retail sale as food.

## 3    APPROACH TO ASSESSMENT

### 3.1    Introduction

The approach used in this EA follows that used in the NADA EA, and centers on the likelihood and consequences of AquAdvantage Salmon escaping from confinement, surviving, dispersing, reproducing, and establishing in the unconfined environment. These hazards have been previously addressed, and determined to support a conclusion that the likelihood of escape, survival, and establishment is very low, for the production of eggs at the PEI facility and grow-out of fish to market size at the Panama facility (see NADA EA, Section 7.8). In this EA, the hazards are addressed for grow-out to market size in Indiana. The framework is that of a conceptual risk assessment model and a series of risk-related questions (see Section 3.3). This analysis and its outcomes are discussed in the Environmental Consequences section of this EA (Section 7).

### 3.2    Use of Redundant Containment Measures to Mitigate Risks

The principal method of managing risks associated with the production and rearing of any fish in aquaculture is through the application of confinement or containment measures designed to minimize the likelihood of escape or release into the environment. Additional confinement measures may be implemented to reduce the subsequent likelihood of harm to the environment should escape or release 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):

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

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

- Biological confinement: limiting reproduction of the fish within the culture system, preventing reproduction of the fish once they enter the receiving environment, or preventing the expression of the genes of concern (e.g., the transgene) in the event of an escape.

The three primary aims of confinement as cited by Mair *et al.* (2007) are listed below along with a brief description of the containment measures that would be used for the grow-out and disposal of AquAdvantage Salmon. Section 5 of this EA describes confinement and containment measures and how they would specifically apply to AquAdvantage Salmon at the grow-out site in Indiana. These confinement measures have been incorporated as integral components of the supplemental NADA.

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

    The primary form of preventing AquAdvantage Salmon from entering the environment under the conditions established in the supplement to the NADA, if approved, is the

5

mandated use of redundant physical and physico-chemical barriers at the site. The salmon will further be prevented from surviving in the receiving environment because of naturally occurring geographic and geophysical conditions.

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

In the highly unlikely event of escape from the Indiana grow-out facility, gene flow from AquAdvantage Salmon would be prevented because the fish would be triploid females that are incapable of reproduction, either among themselves or with wild fish.

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

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

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

This EA describes conditions of use for approval of the supplemental NADA for AquAdvantage Salmon (NADA 141-454). Although each individual method has intrinsic strengths and weaknesses, by combining complementary measures based on different principles of containment, an extremely high level of effectiveness results. The reliability of these measures is further ensured by adherence to a strong management operations and emergency response plan that includes staff training, Standard Operating Procedures (SOPs), daily internal inspections of containment equipment, and routine audits, complemented by inspections by FDAand some oversight by state authorities.

As described in Section 5, multiple and redundant forms of containment are in effect at the Indiana facility to effectively prevent the escape and establishment of AquAdvantage Salmon. In addition to effective physical (mechanical) containment, effective biological containment would be present in the form of a population of salmon that is entirely female, triploid, and thus functionally sterile. The environment surrounding the grow-out facility in Indiana is also inhospitable to all life stages of Atlantic salmon due to periods of insufficient water quantity and impaired water quality that diminish the likelihood of survival, dispersal, and establishment in the receiving water body.

### 3.3    Risk-Related Questions

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

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 should AquAdvantage Salmon escape the conditions of confinement?

3.3.1    Likelihood of Escape from Confinement

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

Fish have life stages in which they are small, can be difficult to contain, and may be impossible to re-capture if they escape. They can be highly mobile if the aquatic environment is sufficiently hospitable. These factors generally oblige the use of redundant, multiple-level containment strategies. The U.S. Department of Agriculture's (USDA) Agricultural Biotechnology Research Advisory Committee (ABRAC) has prepared Performance Standards for safely conducting research with genetically modified fish and shellfish (ABRAC, 1995). These Performance Standards are conceptual in nature and neither require nor recommend specific types and/or numbers of containment measures. For risk management, the Performance Standards state that although the number of independent containment measures[4] is site- and project-specific, they should generally range from three to five.

---

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

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

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

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.[5]

The likelihood of establishment depends on all three parameters; however, the ability of the GE
animal to escape is considered the most important of these because without escape (or intentional
release) there can be no establishment in the environment and thus no resulting impacts. In other
words, if there is no environmental exposure, there is also no environmental risk.

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

A key factor affecting the fitness of a GE animal is the nature of the introduced trait, and its
effects on survival, reproduction, and establishment. For example, an introduced trait could

---

[5]   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).

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 offer suitable food, habitat, and environmental conditions (e.g., temperature and, for fish, salinity and water quality). Often the presence of conspecifics[6] or species closely related to the GE escapee in accessible ecosystems implies that a suitable environment exists (provided that the fitness of the escapee does not differ significantly from conspecifics or closely related species in that environment) (Kapuscinski *et al*., 2007).

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

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

3.3.3   Likely Consequences of Escape

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

---

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

of 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; Devlin *et al*., 2015). Although primarily hypothetical to date, general risks identified by one of these groups (Snow *et al*., 2005) include the following:

1. Creating new or more vigorous pests and pathogens;

2. Exacerbating the effects of existing pests through hybridization with related transgenic organisms;

3. Harm to non-target 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, for aquatic GE animals, the Snow *et al*. (2005) report cited the following possible effects in the event of an escape: heightened predation or competition, colonization of GE animals in ecosystems outside of their native range, and alteration of population or community dynamics due to activities of the GE animal. The report states that in extreme cases, these effects might endanger or eliminate non-GE conspecifics, competitors, prey, or predators. Further consideration of these effects in relation to AquAdvantage Salmon is presented in Section 7.5.

## 4    ALTERNATIVES INCLUDING THE PROPOSED ACTION

For major Federal actions, including an action to approve a supplemental NADA for grow-out of AquAdvantage Salmon at an additional facility that was not approved as part of NADA 141-454, 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 alternatives, which includes the action and one "no action" alternative.

The alternatives are approval of the supplemental NADA under the conditions of production and use described in this EA and that would be set forth in the FDA approval, if the supplemental NADA is approved and the "no action" alternative, which considers the environmental impacts of not approving the supplemental NADA.

### 4.1    Proposed Action – Approval of Supplemental NADA to Allow Grow-Out at ABT's Indiana Facility Under Specific Conditions

The action evaluated in this EA is the approval of the supplemental NADA, which would permit grow-out of AquAdvantage Salmon at ABT's Indiana facility. The only other conditions of production and use of AquAdvantage Salmon would be those that are permitted under the approval of NADA 141-454, which allows commercial production of eyed-eggs for AquAdvantage Salmon at the PEI facility and the grow-out of AquAdvantage Salmon at the Panama facility. No other conditions of production and use of AquAdvantage Salmon would be within the scope of the NADA or supplemental NADA approvals. Any changes and/or additions to the conditions of production and use for AquAdvantage Salmon would require notification of FDA. FDA indicated in the NADA EA, Section 4.1, that it would consider production in a new facility to be a major change that would require a supplemental NADA approval prior to implementation. FDA also indicated in the NADA EA that any supplemental approval would constitute a new agency action triggering additional environmental analysis under NEPA (see 21 CFR 25.20(m)) to address the potential and cumulative impacts of any proposed changes and/or additions.

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

The no action alternative would be the decision by FDA not to approve the supplemental NADA. Should FDA decide not to approve the supplemental NADA to allow grow-out of AquAdvantage Salmon at the Indiana facility, ABT could either continue to produce AquAdvantage Salmon at only the PEI and Panama facilities or it could seek approval to grow-out AquAdvantage Salmon at one or more alternative grow-out facilities. The first of these outcomes would maintain the status quo and would result in no environmental impacts other than those that were evaluated in the NADA EA, which resulted in an FDA Finding of No Significant Impact. Because this outcome would not result in a significant impact on the environment, this EA does not address it. The second of these outcomes, would require submission of one or more additional supplemental NADAs that would constitute an agency action(s) requiring separate analysis under NEPA (see Section 4.1 above). Because this outcome would require a separate NEPA analysis, this EA does not address this potential outcome.[7]

---

[7]    ABT could also seek to raise AquAdvantage Salmon at new suitable locations outside of the United States (and/or to sell the eggs, fish, or the technology to producers outside the United States) with no intent to market food from these fish in the United States, i.e., outside of FDA jurisdiction.

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

This section provides details on the phenotype of AquAdvantage Salmon and the specific conditions that would apply for production and use of these animals under the proposed action, including the applicable types of physical and biological containment. Information on the rDNA construct used in the genetic engineering of AquAdvantage Salmon and the genotype of this salmon is available in Appendix E of the NADA EA (https://www.fda.gov/downloads/AnimalVeterinary/DevelopmentApprovalProcess/GeneticEngin eering/GeneticallyEngineeredAnimals/UCM466218.pdf ) and is not discussed further herein. Background information on the life history and biology of Atlantic salmon is presented in Appendix A. Appendix A also contains information on salmon farming and the interactions between domesticated (farm-raised) salmon and wild salmon. This information provides a baseline for the 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

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

### 5.2    Phenotypic Characterization of AquAdvantage Salmon

This section discusses the phenotype of AquAdvantage Salmon relative to non-GE farm-raised Atlantic salmon to help characterize its fitness. Most of this information has been presented in the NADA EA, (see NADA EA § 5.2) but is presented again here, incorporating new information.

Any consideration of the fitness of Atlantic salmon, regardless of its status with respect to genetic engineering, requires understanding that in general, Atlantic salmon display a high degree of phenotypic plasticity and complex life history that enable them to adapt to variable conditions and rigorous environments. In addition, genotype-by-environment interactions will produce different phenotypes when animals with the same genetic background are exposed to different environmental conditions. Given the high degree of phenotypic plasticity of Atlantic salmon, and the impact of genotype-by-environment interactions, it is not surprising that the wide spectrum of traits observed in wild-type Atlantic salmon generally encompasses those of AquAdvantage Salmon.

5.2.1    Comparative Studies

Multiple studies have been conducted by ABT comparing farm-raised Atlantic salmon to AquAdvantage Salmon. Data and information published in peer-reviewed journals, which may include comparisons to wild Atlantic salmon, are also considered. In a few instances, when potentially relevant, results have also been included from studies that have been conducted in other GE fish including diploid, mixed-sex GE GH Atlantic salmon, and other species of salmon,

most notably coho salmon.[8] The extent to which these results may be applicable to Atlantic salmon in general, and to AquAdvantage Salmon in particular, have not been demonstrated (see Veterinary Medicine Advisory Committee Meeting Briefing Packet, https://wayback.archive-it.org/7993/20170404230823/https://www.fda.gov/downloads/AdvisoryCommittees/Committees MeetingMaterials/VeterinaryMedicineAdvisoryCommittee/UCM224762.pdf).

### 5.2.1.1   *Nutritional and Hormonal Composition*

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

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

The gross anatomy, histopathology, and clinical chemistry of male and female, 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 triploid AquAdvantage Salmon and the non-GE comparators, with the number of abnormal findings being greater for triploid fish (both GE and non-GE) than for diploid fish, especially with regard to irregularities in gill structure. An examination of nine internal organs or structures, as well as relative organ weights, revealed no differences between GE and non-GE salmon or between diploid and triploid salmon. The pathology findings associated with the AquAdvantage construct were limited to an increased presence of minimal-to-mild focal inflammation of unknown cause in some tissues, especially among diploid fish, and a low occurrence of jaw erosions among both male and female diploids. Most of the other findings, which included gill and fin abnormalities, soft tissue mineralization, hepatic vacuolization, and cardiac shape abnormalities, affected the triploids of both groups. In the aggregate, these findings were generally of low magnitude, limited distribution, and non-debilitating nature; they were deemed unlikely to compromise the overall health of AquAdvantage Salmon in commercial production.

In the same comparator-controlled study, no severe malformations were noted among the AquAdvantage Salmon and diploid ABT salmon enrolled. Irregularities in the fins and gill structure of triploid AquAdvantage Salmon as well as triploid non-GE salmon were noted, while diploids in both groups had a low incidence of jaw erosion. The observed abnormalities are within the range of frequency and severity commonly noted in cultured salmonids, as described in the following paragraphs.

Morphologic irregularities occur in non-transgenic salmonids, most commonly affecting cartilaginous and boney structures (Brown and Núñez, 1998), and are often associated with the development of new commercial lines or husbandry techniques and culture conditions. Developmental malformations of cartilage and bone have been observed quite commonly in

---

[8]   Many of the comparisons have been made to GE GH coho salmon, which is a different species (*Onchorynchus kisutch*), and contains a different growth hormone construct (i.e., the sockeye salmon growth hormone under the control of the metallothionein-B promoter of the same species (Mori and Devlin, 1999)).

association with intensive commercial farming of salmon (*Salmo*) and trout (*Oncorhynchus*) species, including *S. salar* (Bæverfjord *et al*., 1996; Vägsholm and Djupvik, 1998; Silverstone and Hammell, 2002; Fjelldal *et al*., 2012) *S. trutta*, (Poynton, 1987), *O. mykiss* (Mbuthia, 1994 as cited by Silverstone and Hammell, 2002; Madsen & Dalsgaard, 1999), and *O. kuta* (Akiyama *et al*., 1986). They are also observed in salmonids in the wild (DeVore and Eaton, 1983). These malformations include irregularities of the head, jaw, and operculum, and twisting or compression of the spine. In farmed non-GE Atlantic salmon, vertebral deformities are now categorized into 20 different types, with those associated with fusions and compressions as the most common in harvest sized fish (Fjelldal *et al*., 2012). Although the incidence of these malformations has not been studied systematically, a background incidence of 3–5% is not uncommon in experimental control animals (Ørnsrud *et al*., 2004). Veterinary field studies have identified the periodic occurrence of spinal compression (humpback) in 70% of salmon in Norwegian farming operations (Kvellestad *et al*., 2000) and jaw malformation in 80% of salmon at commercial sites in Chile (Roberts *et al*., 2001). Nonetheless, aggregate data for the industry have not been reported, and the experience of individual commercial operations remains closely held. Such irregularities are not limited to salmonids, but have also been reported in the culture of other fish species.

Neither intensive selection for growth nor inbreeding depression are deemed responsible for these morphologic irregularities (Bæverfjord *et al*., 1996), which have been linked more commonly to suboptimal culture conditions (e.g., nutrition, water quality, and environmental stressors). In general, mild-to-moderate malformations of the head, jaw, operculum, or spine have limited impact on morbidity or mortality when other rearing conditions are optimized; rearing conditions that are otherwise deficient and present significant environmental stressors can lead to the increased mortality of these fish.

Triploidization induced by hydrostatic pressure has been shown to induce vertebral deformities in Atlantic salmon (Fjelldal and Hansen, 2010; Leclercq *et al*., 2011). The prevalence of deformities in young triploid Atlantic salmon as determined by palpation or visual observation has been reported to range from 1-3% (Fjelldal and Hansen, 2010) and 1.2–2.5% (Taylor *et al*., 2011), but were not always higher than in diploids. Using sensitive radiography, more triploids were found to have one or more deformed vertebrae than diploids (mean %, 22.0 vs 42.7 and 24.4 vs 48.9 in diploid and triploid, parr and post-smolts, respectively; Fraser *et al*., 2013). Increasing the level of dietary phosphorus in freshwater can counteract the problem (Fjelldal *et al*., 2012). Triploid Atlantic salmon post-smolts are also more prone to cataracts than diploids (Benfey, 2016).

Almost all of the values for hematology and serum chemistry parameters of AquAdvantage Salmon were consistent with published values that represent the normal range for Atlantic salmon. The statistically significant differences that were observed are believed to be related to the inherent difference in metabolic rates between AquAdvantage Salmon and comparator salmon, the effect of triploidy on red cell number and size, and unavoidable limitations in study design.

Tibbetts *et al*. (2013) have reported on the growth and nutrient utilization of GE GH Atlantic salmon (both diploid and triploid) fed a practical grower diet (see following section for a description of results related to growth). This study included a skeletal bone analysis, as well as

an appearance assessment conducted using a ranking system (1= no obvious skeletal disorder, marketable; 2 = minor skeletal disorder, marketable; and 3 = major marketable disorder, unmarketable). The overall occurrence of major skeletal disorders (rank = 3) was low (<4%) in all salmon regardless of ploidy or whether or not the fish contained the GH transgene. Triploid salmon had a slightly higher prevalence of major skeletal disorders (2.9% for nontransgenics; 3.7% for transgenics) than diploids (0.3% for nontransgenics; 0.9% for transgenics). These results are very similar to those presented by Fjelldal and Hansen (2010) for vertebral deformities in diploid and triploid non-GE Atlantic salmon underyearling smolts (triploids 1–3%; diploids 0–1%) and suggest that triploidization has a greater effect than transgenesis on the malformation rate, although neither had a substantial effect on producing skeletal disorders that would make the salmon unmarketable.

### 5.2.1.3 *Growth Rates*

The main difference between AquAdvantage Salmon and non-GE Atlantic salmon, and the basis for the value of the product, is the significant increase in growth rate of the former. Studies of early-generation GE salmon conducted in academic settings deriving from the program that led eventually to identification and development of the EO-1α line provided estimates of growth rate that were two- to six-fold greater than non-GE comparators during the first year of life (Du *et al*., 1992). A comparator-controlled study of growth performance in $F_6$-generation AquAdvantage Salmon has confirmed their significant growth advantage over a period of approximately 2,700°C-day in both average size (261.0 g *vs*. 72.6 g for diploid controls) and proportion of animals larger than 100 g (98.6% *vs*. 4.9% for diploid controls). Data from this study are summarized in the EA for NADA 141-454.

Tibbetts *et al*. (2013) have reported on the growth and nutrient utilization of GE GH Atlantic salmon (with a single copy of the EO-1α gene construct), both diploid and triploid, compared to full-sibling, size-matched non-GE Atlantic salmon, both diploid and triploid. GE salmon consumed a significantly higher amount of feed on a daily basis, resulting in a three-fold increase in target weight gain in 40% of the time of non-GE fish. GH genetically engineered Atlantic salmon also had enhanced specific growth rates (%/day), higher thermal growth coefficients ($g^{1/3}$/degree day), better feed conversion ratios, and higher nitrogen retention efficiencies. As a result, the overall total amount of feed required to produce the same fish biomass was reduced by 25% in GE fish. Feed intake was lower in triploid GE salmon compared to diploid GE salmon, but feed efficiency, digestibility and nutrient retention efficiencies were equal to those of GE diploids. In addition, without exception, GE triploids out-performed their related non-GE counterparts regardless of ploidy.

### 5.2.2 Other Phenotype and Fitness Characteristics

Rapid-growth phenotypes, including those produced in domesticated Atlantic salmon though selective breeding, appear to share several key physiological and behavioral attributes regardless of breeding methodology, including the following: the use of a common endocrine pathway to accelerate growth; elevated metabolism, feeding motivation, and efficiency; increased aggression and foraging activity; and reduced anti-predator response (in farm-raised Atlantic salmon, Fleming *et al*., 2002; in early-generation, GH transgenic Atlantic salmon, see Abrahams and Sutterlin, 1999 and Cook *et al*., 2000a; in growth-accelerated GE fishes, see Devlin *et al.*, 2015).

Differences appear to occur in the scale of trait expression rather than in the scope or character of the trait expressed.

The extent to which the "fitness" of AquAdvantage Salmon has been altered relative to comparator Atlantic salmon can be estimated by the evaluation of the following phenotypic changes, as suggested by Kapuscinski and Hallerman (1991):

- ♦ Metabolic rate;
- ♦ Range of tolerance values for physical factors;
- ♦ Behavior;
- ♦ Resource or substrate use; and,
- ♦ Resistance to disease, parasites, or predation.

If AquAdvantage Salmon were to escape into an uncontained environment, these factors could affect the fitness of the escaped AquAdvantage Salmon, their potential for survival and establishment, and their interactions with other organisms and the ecosystem.

### 5.2.2.1  *Metabolic Rates*

Metabolic rates influence the components of the overall energy budget for an individual; the components of the energy budget in turn influence an individual's impact on nutrient and energy flows, and other organisms. The distinguishing feature of AquAdvantage Salmon is rapid growth, which is an integrated composite of many physiological rates. AquAdvantage Salmon exhibit growth and behavioral traits that also appear in other fast-growing Atlantic salmon or in brown trout (*Salmo trutta*) that have been treated with time-release GH implants (Johnsson and Björnsson, 2001). Selection for faster growth in domesticated Atlantic salmon is generally associated with increases in pituitary and plasma GH levels (Fleming *et al*., 2002); however, such increases are also observed in wild salmon during winter famine, smoltification, and sexual maturation (Björnsson, 1997). The only unique attributes of GE fish appear to be an increase in the magnitude of trait expression associated with the increase in growth rate when food is available, and the allocation of energy to growth that occurs at the expense of stored reserves (Cook *et al*., 2000b).

The expression of growth hormone alters aggregate metabolic activity in several ways: lipid breakdown and mobilization are increased, and energy is deployed more readily for maintenance or growth; protein synthesis is increased, providing the raw material for additional body mass; mineral uptake is increased, promoting skeletal development and a longer, leaner morphology; and, feeding efficiency (i.e., feed conversion ratio) is improved (Björnsson, 1997). The cost to the animal is higher oxygen utilization due to increased digestive demand and protein synthesis. In comparison to non-GE comparators, GH transgenic Atlantic salmon had lower initial energy reserves, 2.1- to 2.6-fold greater feed consumption, and a propensity to deplete body protein, dry matter, lipids, and energy more quickly during starvation (Cook *et al*., 2000a & 2000b). Routine oxygen uptake in GH transgenic Atlantic salmon was 1.7 times that of controls (Stevens *et al*., 1998) and oxygen consumption during activity was 1.6-fold greater, further increasing with effort (Stevens and Sutterlin, 1999).

Although these GH transgenic Atlantic salmon have demonstrated an ability to reduce their metabolic rate in response to starvation, their enhanced metabolic profile and lower initial energy reserves would greatly reduce the likelihood of their growing rapidly, or even surviving, outside of the highly supportive conditions provided by commercial farming (Hallerman *et al*., 2007).

Polymeropoulos *et al*. (2014) studied the effects of both GH transgenesis and polyploidy in Atlantic salmon on metabolic, heart, and ventilation rates and heat shock protein response. The experiments were conducted on alevins of AquAdvantage Salmon reared at the PEI facility. Mass-specific metabolic rates were increased under both normal and hypoxic conditions as compared to diploid non-transgenic alevins. However, this was not reflected in improved oxygen uptake through heart or ventilation rate or in altered heat shock protein responses under normal oxygen conditions. Under severe hypoxic conditions, ventilation rate decreased in both diploid non-transgenics and triploid transgenics. The findings of this study show that cardiorespiratory functions under oxygen-limiting conditions are altered in early development of Atlantic salmon by the combination of GH transgenesis and induced triploidy. Hypoxia did not induce a cellular stress response, which may have a negative effect on the ability of the fish to deal with harsh environments.

### 5.2.2.2   *Tolerance of Physical Factors*

Tolerance of physical factors such as temperature, salinity, pH, etc., potentially can be altered in GE organisms. If an increased tolerance of these factors is sufficiently large, changes in lethal limits or optimum values could possibly shift or change preferred habitats, seasonal patterns, and/or the organism's geographic range.

Although specific information addressing these potential changes is limited for AquAdvantage Salmon specifically, studies have shown that oxygen consumption in adult GH transgenic Atlantic salmon is higher than in non-GE comparators (Abrahams and Sutterlin, 1999; Cook *et al*., 2000a; Cook *et al*., 2000b; Deitch *et al*., 2006). In contrast, oxygen consumption of eyed embryos, newly hatched larvae (alevin), and first-feeding juveniles (fry) is similar to that of non-GE salmon (Moreau, 2011; Moreau *et al*., 2014). The increased requirement for oxygen in adults would engender a reduced tolerance for diminished oxygen content in general, and a reduced capacity for survival when the dissolved oxygen (DO) concentration is critically low, which is more likely to occur when water temperatures are elevated,[9] compared to their non-GE counterparts in the wild. In experiments with GH transgenic Atlantic salmon, oxygen uptake was independent of oxygen concentration above 10 mg/L, but started to decrease at approximately 6 mg/L DO in GE fish versus 4 mg/L DO in control fish (Stevens *et al*., 1998). Although under conditions of high DO, GE salmon are not at a disadvantage compared to controls, as oxygen demand is readily satisfied,[10] escape into water with a DO level less than approximately 6 mg/L would place the GH transgenic Atlantic salmon at a physiological disadvantage.

---

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

[10]   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

Although the temperature tolerance of AquAdvantage Salmon has not been investigated, because AquAdvantage Salmon are triploid fish, triploidy itself, and not just the presence or expression of the rDNA construct, may also affect the tolerance limits of these fish. Data exist for a variety of species of fish to indicate that triploidy could be responsible for reduced survival of early-life stages and reduced survival and growth of later-life stages, particularly when environmental conditions are not optimal (Piferrer *et al*., 2009). Atkins and Benfey (2008) have shown that compared to diploid siblings, triploid salmonid fishes such as brown, brook, and rainbow trout exhibit reduced tolerance to chronically elevated rearing temperatures, resulting in high mortality of the triploids at temperatures that are sub-lethal for sibling diploids. In addition, triploid Atlantic salmon also were observed to have higher metabolic rates than diploids at lower temperatures, and lower metabolic rates than diploids at higher temperatures, suggesting that triploids have lower thermal optima than diploids. The authors postulate that given a lower optimum temperature for metabolic processes, triploids may not be able to sustain a high metabolic demand, resulting in increased cardiac output and, ultimately, cardiac failure, at high temperatures that are not lethal to diploids. Hansen *et al*. (2015) found that triploid Atlantic salmon had reduced feed intake, condition factor, and growth, compared to diploids, at high seawater temperatures (19°C) and this was further exacerbated by reductions in DO from 100% to 70% of saturation. The authors suggest that this indicates triploid Atlantic salmon have a lower aerobic scope at 19°C and that they were approaching their upper thermal tolerance limit. Sambraus *et al.* (2017) monitored triploid and diploid Atlantic salmon post-smolts at different temperatures and oxygen saturation. Triploids progressively reduced feed intake with increasing temperature after peak feeding at 15 to 18°C. Triploids were also more sensitive to hypoxia (60% oxygen saturation), with lower feed intake than diploids at 6°C and higher mortality at 18°C. Benfey (2016) concluded that triploid Atlantic salmon were less likely to survive in habitats that are relatively warm or low in DO than their diploid counterparts.

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

### 5.2.2.3  *Behavior*

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

---

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.

In nature, swimming performance is important in foraging and predator avoidance. GH transgenic salmon did not differ from wild counterparts in critical swimming speed (Stevens *et al.*, 1998); however, they did demonstrate twice the movement rate of wild-type fish (Abrahams and Sutterlin, 1999). Crossin and Devlin (2017) reported that GH transgenic rainbow trout displayed a greater capacity for burst-swimming than did their wild-type siblings, both in predator and predator-free semi-natural stream mesocosms. They also found that the rearing environment is important, as all fish reared in a static hatchery environment, free from predators and with abundant food, had much lower capacity for burst-swimming.

GH also increases appetite in various species of salmonids (Abrahams and Sutterlin, 1999; Devlin *et al.*, 1999; Raven *et al.*, 2006), which influences behavioral traits associated with feeding, foraging, and social competition. The availability of food also influences behavior. Abrahams and Sutterlin (1999) have demonstrated that GH transgenic salmon would spend significantly more time feeding in the presence of a predator than non-GE salmon, indicating that they possess a higher tolerance for predation risk. Crossin *et al.* (2015) found that transgenic rainbow trout fry reared in a naturalized stream mesocosm environment were more susceptible to predation than wild-type rainbow trout fry but they suffered higher mortality even in the absence of predators, likely reflecting their inability to satiate their greater metabolic needs when reared in a food-limited environment.

The differences between GE and other fast-growing Atlantic salmon are less quantifiable for behavioral traits and further confounded by the effects of hatchery culture, particularly in acclimation to high rates of social interaction. Salmon form dominance hierarchies around foraging opportunities, and hatchery fish have more opportunities to reinforce their social status in confinement. In nature, social dominance is dampened by a resident advantage that generally deters other fish from evicting territory holders from home ground; based on experimental studies, a 25% difference in size has been suggested as necessary to overcome the resident advantage in Atlantic salmon (Metcalfe *et al.*, 2003).

The effect of triploidy on wild-type phenotype is also important to consider as AquAdvantage *S*almon are triploid. Ocean migration studies in Ireland revealed that male triploids returned to their natal area in nearly the same proportions as diploids, whereas female triploids mostly did not (Wilkins *et al.*, 2001). In another Irish study, the return rates of female triploid Atlantic salmon, both to the coast and to fresh water, were substantially reduced (four- to six-fold lower) compared to those for their diploid counterparts (Cotter *et al.*, 2000), inferring that triploidy could be used as a means both for eliminating genetic interactions between cultured and wild populations and for reducing the ecological impact of escaped farmed fish. Triploid Atlantic salmon demonstrated ram ventilation behavior under both normal and hypoxic conditions, which was not seen in diploid Atlantic salmon in experiments conducted by Hansen *et al.* (2015). However, Benfey (2016) concluded that results from laboratory studies on behavior and cognitive ability and from field trials suggest that triploid Atlantic salmon, if free from obvious deformities, would not differ from diploids in their abilities to forage, escape predation, and disperse in the wild in freshwater environments.

Under laboratory conditions, GH-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. Leggatt *et al*. (2017a) found that GH-transgenic coho salmon had decreased swimming performance and efficiency, in contrast to GH-transgenic Atlantic salmon, which had similar performance but decreased efficiency relative to non-transgenic counterparts (Stevens et al., 1998). Although these effects would have the potential to influence wild relatives both directly and indirectly, such observations were demonstrably muted when the GE fish were reared under simulated natural conditions (Sundström *et al*., 2007), indicating the complexity of gene-environment interactions. Sundstrom *et al*. (2017) further noted that the feeding and risk-taking behavior of GE coho salmon was strongly affected by rearing conditions which, to a large extent, had a greater effect than transgenesis. Leggatt *et al. (*2017b) found that, in addition to gene-environment interactions, the strain of the coho salmon influenced fitness. Moreau *et al*. (2014) also found family of origin to be an important factor influencing fitness in Atlantic salmon. In fact, Moreau et al. (2011b) found no differences in the competitive ability or survival of first-feeding GH transgenic or non-transgenic Atlantic salmon fry reared in low-feed, near natural stream conditions.

### 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 and Hallerman, 1990). As previously mentioned, GH increases appetite; however, Cook *et al*. (2000c) and Tibbets *et al*. (2013) have also found that feed conversion efficiency was improved by 10% in GH transgenic Atlantic salmon, suggesting some potential offset in the need for food.

### 5.2.2.5   *Impact of Disease and Parasites*

If a GE organism were to have improved resistance to disease or parasites, in theory it could out-compete its non-GE counterparts. Based on an evaluation of general health records, tank records, fish necropsies, and study data, no evidence has been found that AquAdvantage Salmon have any altered resistance to disease or parasites.

An outbreak of infectious salmon anemia (ISA) occurred in the PEI facility during the third quarter of 2009 (see NADA EA Section 5.4.2 for additional details). During this outbreak, no consistent difference in disease occurrence was noted between GE and non-GE Atlantic salmon for different year classes of fish. For the 2007 year class, the incidence of mortality during the ISA outbreak was much higher for non-GE salmon (21.7%) than for GE salmon (both AquAdvantage and diploid ABT salmon) (6.3%), while for the 2006 year class the rates were very similar (6.9% versus 6.1%). For the 2008 year class, in which the highest numbers of fish were potentially exposed to the ISA virus (ISAV), the mortality rates were almost identical for both GE (both AquAdvantage and diploid ABT salmon) and comparator fish (0.88% versus 0.83%) for animals that were held in the Early Rearing Area (ERA) of the PEI facility.

Pilot challenge studies conducted with ISAV strain HPR4 in 2009 indicated similar survival profiles for diploid and triploid AquAdvantage Salmon exposed via injection (ABT unpublished studies). No data were generated on non-GE comparators before the studies were discontinued.

No currently notifiable diseases or disease agents for finfish per Canadian or international (World Organisation for Animal Health (OIE)) requirements have been detected in recent years at the PEI facility as a result of periodic inspections by the DFO Fish Health Unit for the period from 2010 through 2014 and by CFIA from 2102 thru October 2017.  Pathogens encompassed by these inspections included several viruses and filterable replicating agents, such as ISAV, plus other common fish pathogens (see NADA EA Section 5.4.2 for additional details). FDA examined the facility's records related to the ISA outbreak during an inspection in June 2012 (see NADA EA Appendix F), and found extensive documentation of the outbreak and diagnosis of ISAV as the causative agent. FDA found ABT's response to the outbreak to be appropriate, and all information collected during the inspection was found to be consistent with that previously described in ABT's submissions to the Agency (see NADA EA, Section 5.2.2.5).

Further discussion about diseases and parasites is provided in the NADA EA.

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

Changes in the morphology of the organism (e.g., size, shape, and color) could alter species interactions (ABRAC, 1995); however, it should be noted that accelerated growth, or increased body size, is not an assured outcome for GE salmon in nature. The rapid-growth phenotype is expressed only if supported by sufficient food, as has been shown in both genetically engineered coho salmon (Devlin *et al*., 2004b; Sundström *et al*., 2007) and GH transgenic Atlantic salmon (Cook *et al*., 2000b; Moreau *et al*., 2011b).  This is a function of both the productivity of the habitat and the density and behavior of competitors for the resource. In the experiments of Moreau *et al*. (2011b) on GH transgenic Atlantic salmon in food-limited stream microcosms, the GH transgene did not influence the growth in mass or survival of fry at either high or low fry densities. In addition, in this study transgenic and non-transgenic individuals were equally likely to be dominant in competitions for foraging territory. In the previous investigations of Abrahams and Sutterlin (1999), it was found that GH-transgenesis influences the genotype-by-environment interaction via powerful stimulation of appetite in the presence of food and a larger capacity for food consumption given the opportunity. GH transgenic Atlantic salmon consumed approximately five times more food than same-age controls that were also size-matched by delaying hatch time of the genetically engineered salmon: this consumption differential appears to derive from the increased feeding motivation of the GE salmon, which were 60% more likely than controls to be observed at both safe and risky foraging sites, and the increased willingness of the transgenic salmon to feed in the presence of a predator (Abrahams and 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. However, when reared under naturalized stream conditions, they exhibited more modest predation activity and were only 20% longer than controls (Sundström *et al*., 2007). Sundström *et al.* (2016) suggest that ecological impacts of GH-transgenic coho salmon in natural environments may be weaker than those observed using hatchery-reared animals.

5.2.2.7  *Reproduction*

Changes in the age at maturation, fecundity, and sterility could alter population and community dynamics and interfere with the reproduction of related organisms (ABRAC, 1995). Due to their enhanced growth rate, diploid ABT 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.

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

Oke *et al*., 2013 have reported on the hybridization of diploid GH-transgenic Atlantic salmon with closely related wild diploid brown trout (*Salmo trutta*). Experimental crosses produced in the laboratory using gametes from diploid fish resulted in transgenic hybrids (i.e., hybrids with the GH EO-1α transgene) that were viable[11] and grew more rapidly than GE salmon and other non-transgenic crosses in hatchery-like conditions. In stream mesocosms designed to emulate natural conditions, transgenic hybrids appeared to express competitive dominance and suppressed growth of transgenic and non-transgenic salmon. The researchers did not investigate the fertility of the transgenic hybrids or the viability of any progeny resulting from hybrid backcrosses[12] to either Atlantic salmon or brown trout. However, they did identify and discuss

---

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

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

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

In terms of hybridization and reproduction in general, the potential relevance of the findings discussed above to the proposed action regarding the Indiana grow-out site are limited as they are only relevant to the PEI egg production site where broodstock are located. At the Indiana facility, only triploid (functionally sterile), female AquAdvantage Salmon would be raised for commercial grow-out and, as will be discussed later in this document, there are no male Atlantic salmon or brown trout present at this location (Section 6), so reproduction there is precluded.

### 5.2.2.8  *Life history*

Changes in embryonic and larval development, metamorphosis, and life span could alter life-history patterns as well as population and community dynamics (ABRAC, 1995). GH constructs in salmonids have been shown to influence larval developmental rate (in coho salmon, Devlin *et al*., 1995b & 2004a) and smoltification (in Atlantic salmon, Saunders *et al*., 1998; in four species of Pacific salmon, Devlin *et al*., 1995a). Saunders *et al*. (1998) found that diploid GH transgenic Atlantic salmon reached smolt size sooner than normal and the smoltification process was not inhibited by high temperatures (19ºC) or constant light. Moreau *et al*. (2014) reported that GH-transgenic Atlantic salmon hatched less than one day earlier than their non-transgenic counterparts but were somewhat developmentally delayed, having more unused yolk and being slightly smaller; however, differences in family of origin were more significant than transgenesis. Somewhat unexpectedly, Moreau and Fleming (2011) found that enhanced growth through GH-transgenesis actually reduces precocial male maturation in Atlantic salmon. The authors concluded that the evidence suggests that the physiological mechanisms promoting growth do not play a causative role in precocial male maturation in fishes.

### 5.2.2.9  *Acute Stress Response*

Physiological responses to stress could be altered by GH transgene expression potentially resulting in changes in fitness and phenotype. Cnaani *et al*. (2013) have investigated the effects of stress on diploid GH transgenic Atlantic salmon, non-GE triploid Atlantic salmon, and what the authors refer to as wild-type Atlantic salmon. Groups of fish were subjected to either no stress (control), one-week of fasting, or low DO (1.5–2.0 ppm). Nine markers of primary and secondary stress response were quantified from blood samples taken from these fish. In general, the GH-transgenic salmon showed greater responses to stress than the two other genotypes, with the triploid fish producing intermediate responses. Wild-type fish maintained homeostasis more effectively than transgenic or triploid fish, exhibiting smaller changes in all measured stress-response parameters. The researchers concluded that poor stress response may reduce the fitness of GH-transgenic and non-GE triploid Atlantic salmon in the wild.

**5.3     Conditions of Production and Use**

Under the conditions of approved NADA 141-454, the commercial production of eyed-eggs of AquAdvantage Salmon may occur only at a single facility on PEI where broodstock are currently held. A detailed description of the PEI production facility, and the containment and security measures employed there can be found in the NADA EA, Sections 5.3 and 5.4. Under the conditions of approved NADA 141-454, commercial rearing and grow-out of eyed-eggs of AquAdvantage Salmon may occur at only one site: ABT's land-based, freshwater aquaculture facility in the highlands of Panama. The description, containment and security measures for the Panama facility can be found in the NADA EA, Section 5.5.

This EA was prepared to support a supplement to NADA 141-454, which proposes to allow commercial rearing and grow-out of eyed eggs of AquAdvantage Salmon to occur at a second facility, the sponsor's land-based, freshwater aquaculture site in Indiana. Section 5.4 describes the Indiana site and facility, for which approval is being sought.

**5.4     Grow-out at Indiana Facility: Facility Description, Containment, and Security**

If approved, under the conditions that would be established in the approval of the supplement to NADA 141-454, commercial rearing and grow-out of eyed eggs of AquAdvantage Salmon will also occur at a land-based, freshwater aquaculture facility in Albany, Indiana. This facility is operated by AquaBounty Farms (ABF), a subsidiary of AquaBounty Technologies, Inc.  The Indiana facility will also be subject to regulatory oversight by the Indiana Department of Environmental Management (IDEM) and the Indiana Department of Natural Resources (IDNR). As required by the NPDES permit issued to ABF (see Section 6.1), water quality will be monitored and reported to IDEM on a monthly basis. IDNR is responsible for issuing aquaculture permits and is primarily concerned about health and disease status. ABT will provide periodic reports on the health status of broodstock housed at the PEI facility and will work with IDNR authorities as requested or required by Indiana law.

5.4.1   Location and Operations

The AquaBounty Farms Indiana (ABF-IN) facility was built in 2011 and purchased by ABT from Bell Aquaculture in June, 2017. The facility was designed for a production capacity of approximately 1,000 tons of fish per year. Bell Aquaculture produced yellow perch and steelhead trout at different times in the life of the company. ABF has upgraded the facility to be capable of producing ~1,200 tons of AquAdvantage Salmon per year. There are currently no fish at the Indiana facility. Table 5-1 describes the buildings and key facilities in the Indiana facility.

**Table 5-1.    Buildings and Key Facilities in the Indiana Facility**

| Building or Unit | Purpose |
|---|---|
| Office | Administration |
| Hatchery | Produce alevin from eyed eggs |
| Nursery | Grow from alevin to fish of 35 to 50 g size |
| Grow-Out | Grow fish to market size of 5kg |
| Purge – Harvest | Removal of off flavor by fresh water exposure and harvest of fish |
| Feed Storage | Storage capacity of minimum 60 tons of feed |
| Water Filtration | Water pretreatment (degassing and iron removal) |
| Maintenance | Maintenance and repairs of equipment |
| Wells | 2 Groundwater wells - East and West Well |
| Effluent Treatment | Solids removal and composting |
| Wetlands ponds | 12 ponds for effluent polishing |
| Wetlands discharge valve | Controls outfall of effluent from wetland ponds to Riley Stafford Ditch |
| Outfall station | Collection point for samples for water quality determination prior to entering Riley Stafford Ditch |
| General Storage | Heavy materials and equipment |

Aquaculture activities will take place in four production units: Hatchery; Nursery; Grow-Out; and Purge-Harvest. In summary, the production process begins with receiving AquAdvantage Salmon eyed eggs produced at the PEI facility by ABT. Alevin at first feeding stage (approximately 0.2 g) are moved from the Hatchery Unit to the Nursery Unit where they are grown to 30–50 g. Upon reaching this size, fish are transferred from the Nursery Unit to the Grow-Out Unit where they will grow to a finished market weight of approximately 5 kg. Market-weight fish will be transferred to the Purge-Harvest Unit where they will be held for 10 to 15 days before harvesting. Harvesting will be done on site and slaughtered (dead) AquAdvantage Salmon will be delivered from the Indiana facility to off-site processors. At various times during the production process, the AquAdvantage Salmon will be graded for size, selected, counted and weighed. Inside the facility the fish will be kept separated in populations of similar sizes. The number and volume of tanks and sizes of fish in each unit are presented in Table 5-2.

**Table 5-2.    Production Units in the Indiana Facility**

| Production Units | Number of Egg Trays | Number of Tanks | Volume/tank (m$^3$) | Size of Fish on Entry | Size of Fish on Exit |
|---|---|---|---|---|---|
| Hatchery | 32 | NA | NA | Eyed eggs | 0.2 g |
| Nursery – Small Tanks | NA | 12 | 2.8 | 0.2 g | 3 – 5 g |
| Nursery – Large Tanks | NA | 24 | 4.7 | 3 – 5 g | 30 – 50 g |
| Grow Out – Small Tanks | NA | 6 | 90 | 30 – 50 g | 200 g |
| Grow Out – Large Tanks | NA | 24 | 265 | 200 g | ca. 5000 g |
| Purge-Harvest | NA | 6 | 65 | ca. 5000 g | ca. 5000 g |

Further detail on the production process is presented below.

5.4.2    Description of Process

Eyed AquAdvantage Salmon eggs will be shipped from AquaBounty Canada (ABC, a subsidiary of ABT) broodstock facilities located on PEI. Egg production and shipping protocols are defined in multiple SOPs previously reviewed and accepted by FDA pursuant to the approval of NADA 141-454. Additional information on shipping is provided in Section 5.5.

Each batch of eggs received at the Indiana Grow-out facility will contain approximately 115,000 eyed eggs. The Hatchery Unit is comprised of four Heath stacks with eight trays per stack. Incoming water is passed through a UV filter and then chilled to 6 - 8°C before entering the incubators.

After eggs hatch, the fry begin absorbing the yolk. After they have absorbed approximately 90% of the yolk, they will weigh about 0.20 g and begin consuming food. At that point, the alevin are transferred to the small tanks in the Nursery Unit, located adjacent to the Hatchery Unit. The time from receipt of the eyed eggs until they are transferred to the Nursery Unit is about one month. As the fish reach between 3 to 5 g in size they are sorted into size-matched cohorts and transferred into the larger nursery tanks. Once they reach between 30 and 50 g (about 3 months) they are again graded, sized and separated into size-based cohorts. They are then transferred to the Grow-Out Unit.

The Grow–Out Unit includes six 90 $m^3$ tanks and 24 tanks with a volume of 265 $m^3$. Fish are delivered from the Nursery to the smaller tanks in the Grow-Out Unit where they are kept until they reach approximately 200 g in size, at which point they are transferred to the larger (265 $m^3$) tanks. The large tanks house the fish from introduction at 200 g to a final weight of approximately 5 kg. Once the fish reach a size of 4 to 5 kg, feed is withheld for two- to five-days to eliminate feed and feces from the gut. They are then weighed, sized, and transferred to the Purge–Harvest unit.

The Purge-Harvest Unit consists of six 65 $m^3$ tanks where the fish are maintained for a period of 10 to 15 days prior to harvest.  In this final stage of production, the fish are exposed primarily to fresh water (pumped from the Water Filtration building, also referred to as the Water Filtration Facility). Keeping the fish in fresh water during this time rids the fish of secondary metabolites (Geosmin (GSM) and 2-Methylisoborneol (2-MIB)), that cause off-flavor in farmed salmon. These secondary metabolites are released by microorganisms such as cyanobacteria and actinomycetes that normally exist in recirculating aquaculture systems such as the ones in place in the Indiana facility.

A small proportion, generally no more than 10%, of the water in the Purge-Harvest Unit will be recirculated within the tanks. The remainder will be discharged to the effluent pipe that sends it to Lift Station 1 and on to the Effluent Treatment building (also referred to as the Effluent Treatment Facility).

Market-ready AquAdvantage Salmon are harvested by introducing the fish into a machine that stuns and renders them unconscious before cutting the gills and bleeding the fish. After the fish are dead, fish and water are placed into iced transport bins for delivery to off-site processing

facilities. There are no processing activities or facilities at the Indiana facility. Only dead, intact whole AquAdvantage Salmon will leave the facility.

At full capacity, the facility will harvest approximately 100 tons of AquAdvantage Salmon per month.

5.4.3   Water and Waste Management

Water for the facility is obtained from two wells, each of which can produce twice the amount of water required for facility operations. (When fully operational, the facility will require water in-flows of approximately 300 to 350 gpm). The groundwater used for the facility contains high levels (1.6–1.9 mg/L) of iron which is not suitable for production of salmon. Incoming well water is degassed and passed through 20 filtration tanks arranged in four banks of five units where the total iron content of the water is reduced to levels that are acceptable for salmon farming (between 0.02 and 0.07 mg/L). The degassing and filtration takes place in the Water Filtration Facility.

Upon leaving the Water Filtration Facility, water enters a set of independent pipes that deliver water to the Hatchery, Nursery, Grow Out, and Purge-Harvest Units. The Hatchery, Nursery, and Grow-Out Units operate as a Recirculating Aquaculture System (RAS). The Indiana RAS will operate at 95 to 97% efficiency, i.e. 95 to 97% of the water in the system at any given time has been recirculated.

In the Nursery and Grow-Out Unit recirculation systems, water exits the fish tanks and passes through drumfilters located within each Unit that separate large solids (mainly feces and uneaten feed) from the water. After leaving the drumfilters, the water then passes into sumps. Fresh make-up water is added to the system and mixed within the sumps. The water is then pumped to a biofiltration process using Moving Bed Biofilm Reactor (MBBR) technology. The MBBR technology utilizes thousands of polyethylene biofilm carriers operating in mixed motion within an aerated wastewater treatment tower. After exiting the biofilter, water passes through a degassing tower where $CO_2$ is extracted and vented to the outside. Oxygen is injected into the mixed water before it re-enters the fish tanks.

The small amount of water that is not recirculated is discharged from the Nursery Unit to Lift Station 1 and then pumped onwards to the Effluent Treatment Facility. The effluent from the Grow-Out Unit, except the effluent captured in floor drains, flows to Lift Station 2 and then is pumped to the Effluent Treatment Facility. Effluent captured in the Grow-Out floor drains flows to Lift Station 1 and from there to treatment.

No feed would be used in the Hatchery and Purge-Harvest Units and therefore no feces would be produced. Consequently, the recirculating systems in these units do not require drumfilters, biofilters, or degassing stacks. For the hatchery operations, incoming water from the Water Filtration Facility enters the Hatchery Unit, where it is disinfected via UV and chilled before flowing through the Heath stacks. After passing through the egg trays and two types of strainers ("Y" strainer and a cyclone strainer), recirculated water passes through the chiller before returning to the Heath stacks. The small amount of hatchery effluent generated eventually goes to Lift Station 1, which discharges the water to the Effluent Treatment Facility.

The Purge-Harvest Unit is primarily a flow-through system. However, a variable amount of the water will be recirculated within the tanks. It is anticipated that approximately 10% of the water in this Unit will be recirculated. Effluent from the Purge-Harvest Unit will flow to Lift Station 1 where it is pumped to the Effluent Treatment Facility.

Effluent leaving the systems passes through one of two lift stations, as described previously, and is ultimately delivered to the Effluent Treatment Facility. The Effluent Treatment Facility contains four parallel 100 m$^3$ settling cones where particulate matter (measured as Total Suspended Solids, i.e., TSS) is decanted and collected as sludge. Solids remaining in suspension after decanting are removed with mechanical filtration by passing the effluent through a drumfilter.

The final sludge product will contain about 20% solids, comprised of uneaten feed and fecal matter that equates to approximately 20 to 25% of the feed supplied daily. At full operational capacity this will be approximately one ton of sludge produced per day. Sludge will be disposed of by removal offsite for land application. Any dead fish will be removed by an approved vendor for landfilling, consistent with local regulations.

After solids are removed, the waste water leaving the Effluent Treatment Facility drumfilter is discharged into the adjacent wetlands ponds. The effluent passes sequentially, in a serpentine pattern, through 12 ponds lined with rock before being discharged into a small seasonal creek (Riley Stafford Ditch) on the property. The ponds are approximately 2 feet deep and will be intentionally seeded with designated wetland plants such as cattails that further clean the water as it passes through. The discharge is controlled through a valve before entering the Riley Stafford Ditch at an outfall. The outfall serves as a collection point for samples for water quality determination prior to entering the Riley Stafford Ditch which ultimately drains to the Mississinewa River, a tributary to the Wabash River.

A schematic of the flow of water and solids through the aquaculture system is provided in Figure 5-1.

The system for handling potable water and resultant wastewater is separate from the aquaculture system. Potable water is obtained from the same wells but is provided through a different plumbing system. Wastewater from restrooms goes to a septic tank on site.

**Figure 5-1.    Flow of water and solids through the Indiana facility**
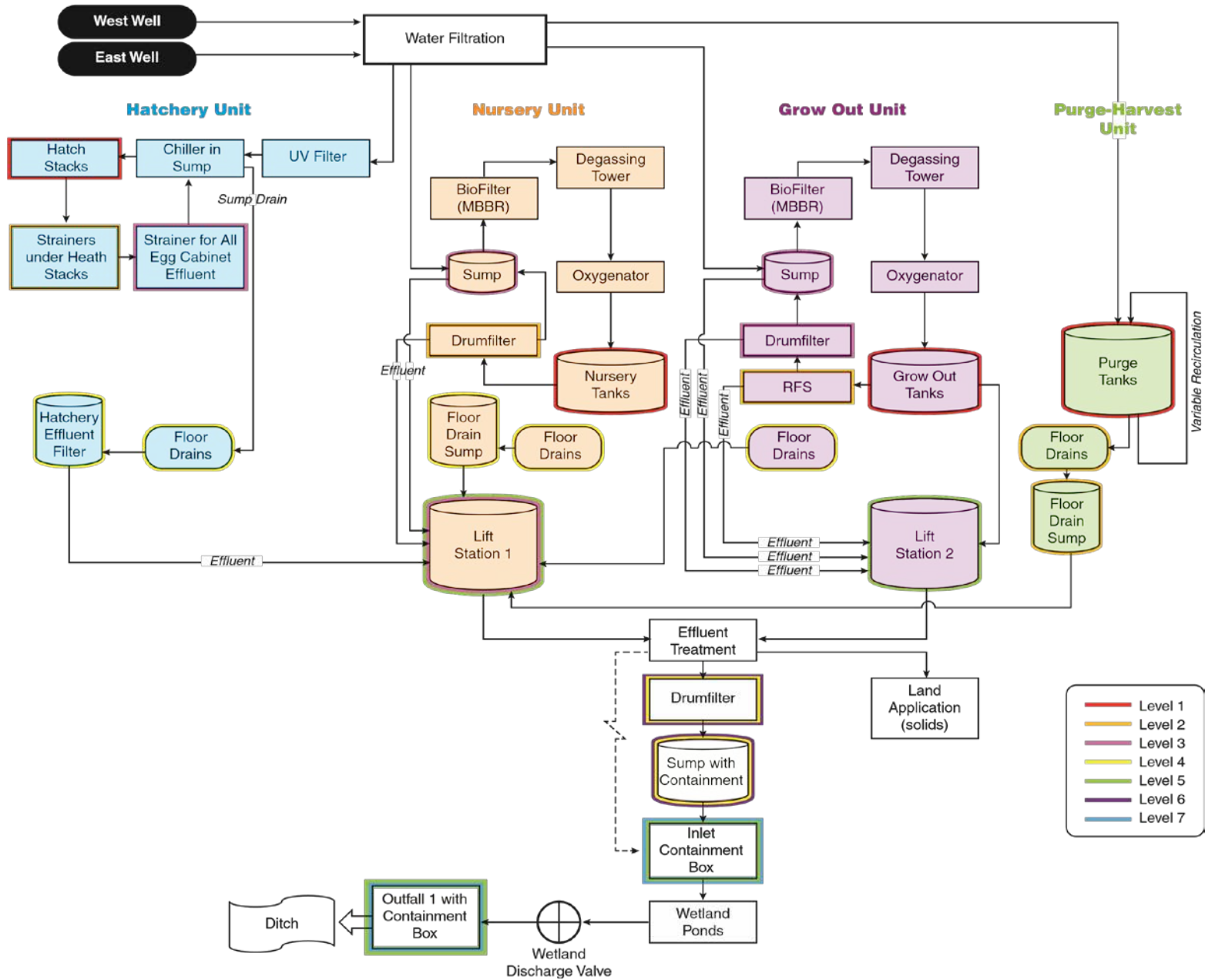
The containment barriers used in the Indiana facility are indicated in Figure 5-1 as color-coded boxes/figures with the color code based on the level of containment. There are at least five levels of containment in place throughout the facility, and in some units as many as seven levels. Boxes or figures that are outlined in more than one color, e.g., Lift Station 1, indicate the barrier is a different level of containment depending upon the specific aquaculture unit. Containment barrier details, including the level each barrier represents within a unit, are shown in Table 5-3 and described in detail in Section 5.4.3.

**Table 5-3.    Key Components and Levels of Containment at the Indiana Grow-Out Facility**

| Unit | Containment Level | Location | Barrier Type | Barrier Material(s) | Perforation Size (mm) | Fish Size (g) in Unit |
|---|---|---|---|---|---|---|
| Hatchery | 1 | Egg Trays | Screen | Molded plastic inserts with Polyester screen | 1.50 | ≤ 0.2 |
| Hatchery | 2 | Hatch stack drains | Strainer | PVC | 0.80 | ≤ 0.2 |
| Hatchery | 3 | Recirculation line | Strainer | Reinforced thermoplastic | 1.00 | ≤ 0.2 |
| Hatchery | 4 | Floor drain | In-Drain Screens | Stainless Steel (SS) | 1.50 | ≤ 0.2 |
| Hatchery | 4 | Hatchery effluent filter | Screen | SS | 1.50 | ≤ 0.2 |
| Hatchery | 5 | Lift Station 1 | Containment box screen | SS | 3.00 | ≤ 0.2 |
| Hatchery | 6 | Effluent Treatment Facility | Drumfilter screen | SS frame with polyester micromesh | 0.09 | ≤ 0.2 |
| **Nursery Small Tanks - First Feeder Alevins to Fingerling 0.2 to 7.0 g** | | | | | | |
| Nursery | 1 | Small tanks | Tank cover nets | Polyethylene | 4.00 | 0.2 - 7.0 |
| Nursery | 1 | Small tanks | Tank drain screens | PVC | 2.0 - 4.0 | 0.2 - 7.0 |
| Nursery | 2 | Drumfilters | Drumfilter screen | SS frame with polypropylene micromesh | 0.04 | 0.2 - 7.0 |
| Nursery | 3 | Small tanks sump (inlet) | Sump box screen | SS | 1.50 | 0.2 - 7.0 |
| Nursery | 3 | Small tanks sump (drain) | Standpipe cover | SS | 1.50 | 0.2 - 7.0 |
| Nursery | 4 | Floor drain | In-drain screens | SS | 3.00 | 0.2 - 7.0 |
| Nursery | 4 | Floor drain sump | Screens | SS mesh | 1.50 | 0.2 - 7.0 |
| Nursery | 5 | Lift Station 1 | Containment box screen | SS | 3.00 | 0.2 - 7.0 |
| Nursery | 6 | Effluent Treatment Facility | Drumfilter screen | SS frame with polyester micromesh | 0.09 | 0.2 - 7.0 |
| **Nursery Large Tanks - Fingerling to Juvenile, 7.0 g to 35 g** | | | | | | |
| Nursery | 1 | Large tanks | Tank cover nets | Polyethylene | 4.00 | 7.0 - 35.0 |
| Nursery | 1 | Large tanks | Tank drain cover | SS | 12.00 | 7.0 - 35.0 |
| Nursery | 2 | Drumfilters | Drumfilter screen | SS frame with polypropylene micromesh | 0.06 | 7.0 - 35.0 |
| Nursery | 3 | Large tanks sumps (inlet) | Sump box | SS | 3.00 | 7.0 - 35.0 |
| Nursery | 3 | Large tank sumps (drain) | Standpipe cover | SS | 3.00 | 7.0 - 35.0 |
| Nursery | 4 | Floor drain | Covers | Coated steel | 12.00 | 7.0 - 35.0 |
| Nursery | 4 | Floor drain | In-drain screens | SS | 3.00 | 7.0 - 35.0 |
| Nursery | 4 | Floor drain sump | Screens | SS mesh | 1.50 | 7.0 - 35.0 |
| Nursery | 5 | Lift Station 1 | Containment box screen | SS | 3.00 | 7.0 - 35.0 |
| Nursery | 6 | Effluent Treatment Facility | Drumfilter screen | SS frame with polyester micromesh | 0.09 | 7.0 - 35.0 |
| Nursery | 6 | Effluent Treatment Facility | Effluent discharge sump screen | SS | 12.00 | 7.0 - 35.0 |
| Nursery | 7 | Wetland pond inlet | Screened containment box | SS | 12.00 | 7.0 - 35.0 |
| Nursery | 7 | Outfall 1 | Outlet box screen | SS | 12.00 | 7.0 - 35.0 |
| **Grow-out Small Tanks - Juveniles** | | | | | | |
| Grow-out | 1 | Small Tanks | Tank cover net | Polyethylene | 4.00 | 35 - 200 |
| Grow-out | 1 | Small Tanks | Drain plate | SS | 4.50 | 35 - 200 |
| Grow-out | 1 | Small Tanks | Sidebox gate & screen | SS gate and HDPE mesh | 4.5 & 6.0, respectively | 35 - 200 |
| Grow-out | 1 | Small Tanks | Sidebox floor grate | Plastic | 10.00 | 35 - 200 |
| Grow-out | 1 | Small Tanks | Sidebox standpipe cover | Polyolefin | 12.00 | 35 - 200 |
| Grow-out | 2 | Radial Flow Settlers (RFS) | RFS still well cover | SS | 12.00 | 35 - 200 |
| Grow-out | 2 | RFS | RFS side-drain cover | SS | 12.00 | 35 - 200 |
| Grow-out | 3 | Drumfilter | Drumfilter screen | SS frame with plastic micromesh | 0.09 | 35 - 200 |
| Grow-out | 3 | Drumfilter sump | Overflow drain screen & cap | SS | 12.00 | 35 - 200 |
| Grow-out | 4 | Floor drains | Drain covers | Coated steel | 12.00 | 35 - 200 |
| Grow-out | 4 | Floor drains | Floor drain outlet screens | Polyolefin | 6.00 | 35 - 200 |
| Grow-out | 5 | Lift Station 1 (floor drain capture) | Containment box screen | SS | 3.00 | 35 - 200 |
| Grow-out | 5 | Lift Station 2 (system drains capture) | Screen | SS | 12.00 | 35 - 200 |
| Grow-out | 6 | Effluent Treatment Facility | Drumfilter screen | SS frame with polyester micromesh | 0.09 | 35 - 200 |
| Grow-out | 6 | Effluent Treatment Facility | Effluent discharge sump screen | SS | 12.00 | 35 - 200 |
| Grow-out | 7 | Wetland pond inlet | Screened containment box | SS | 12.00 | 35 - 200 |
| Grow-out | 7 | Outfall 1 | Outlet box screen | SS | 12.00 | 35 - 200 |
| **Grow-out Large Tanks - to Market Weight** | | | | | | |
| Grow-out | 1 | Large Tanks | Tank cover net | Polyethylene | 50.00 | 200 - 5000 |
| Grow-out | 1 | Large Tanks | Drain plate | SS | 7.00 | 200 - 5000 |
| Grow-out | 1 | Large Tanks | Sidebox gate & screen | SS gate and HDPE mesh | 9.0 & 6.0, respectively | 200 - 5000 |
| Grow-out | 1 | Large Tanks | Sidebox floor grate | Plastic | 10.00 | 200 - 5000 |
| Grow-out | 1 | Large Tanks | Sidebox standpipe cover | Polyolefin | 12.00 | 200 - 5000 |
| Grow-out | 2 | RFS | RFS still well cover | SS | 12.00 | 200 - 5000 |
| Grow-out | 2 | RFS | RFS side-drain cover | SS | 12.00 | 200 - 5000 |
| Grow-out | 3 | Drumfilters | Drumfilter Screen | SS Frame with plastic micromesh | 0.09 | 200 - 5000 |
| Grow-out | 3 | Drumfilter Sumps | Overflow drain screen & cap | SS | 12.00 | 200 - 5000 |
| Grow-out | 4 | Floor drains | Drain covers | Coated steel | 12.00 | 200 - 5000 |
| Grow-out | 4 | Floor drains | Floor drain outlet screens | Polyolefin | 6.00 | 200 - 5000 |
| Grow-out | 5 | Lift Station 1 (floor drain capture) | Containment box screen | SS | 3.00 | 200 - 5000 |
| Grow-out | 5 | Lift Station 2 (system drains capture) | Screen | SS | 12.00 | 200 - 5000 |
| Grow-out | 6 | Effluent Treatment Facility | Drumfilter screen | SS Frame with polyester micromesh | 0.09 | 200 - 5000 |
| Grow-out | 6 | Effluent Treatment Facility | Effluent discharge sump screen | SS | 12.00 | 200 - 5000 |
| Grow-out | 7 | Wetland pond inlet | Screened containment box | SS | 12.00 | 200 - 5000 |
| Grow-out | 7 | Outfall 1 | Outlet box screen | SS | 12.00 | 200 - 5000 |
| **Purge/Harvest** | | | | | | |
| Purge/Harvest | 1 | Tanks | Tank cover net | Polyethylene | 50.00 | ≥ 5000 |
| Purge/Harvest | 1 | Tanks | Recirculating pipe screen | Fibreglass | 6.00 | ≥ 5000 |
| Purge/Harvest | 1 | Tanks | Outlet grates (2/tank) | Polyolefin | 13.00 | ≥ 5000 |
| Purge/Harvest | 2 | Floor & effluent drain | Drain covers | Coated steel | 12.00 | ≥ 5000 |
| Purge/Harvest | 2 | Floor & effluent drain | Floor sump outlet grates | Polyolefin | 6.00 | ≥ 5000 |
| Purge/Harvest | 3 | Lift Station 1 | Containment box screen | SS | 3.00 | ≥ 5000 |
| Purge/Harvest | 4 | Effluent Treatment Facility | Drumfilter screen | SS frame with polyester micromesh | 0.09 | ≥ 5000 |
| Purge/Harvest | 4 | Effluent Treatment Facility | Effluent discharge sump screen | SS | 12.00 | ≥ 5000 |
| Purge/Harvest | 5 | Wetland pond inlet | Screened containment box | SS | 12.00 | ≥ 5000 |
| Purge/Harvest | 5 | Outfall 1 | Outlet box screen | SS | 12.00 | ≥ 5000 |

5.4.4    Physical Containment

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. 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. ABT has developed and employs an extensive number of SOPs that govern physical containment as well as every other significant activity that occurs at the Indiana facility.

A number of redundant measures have been implemented at the Indiana facility to provide physical containment of AquAdvantage Salmon. In general, the physical containment measures or barriers ensure entrapment of fish (i.e. via tank covers or nets), and redundancy in screening and filtration of the water flow paths (e.g., pipes and floor drains) into which fish could potentially gain access.

The four main units containing fish at the Indiana facility are the Hatchery Unit, the Nursery Unit, the Grow-Out Unit, and the Purge-Harvest Unit. All four units are inside buildings, so there is no risk of predation of the fish by wildlife. The key components of physical containment are summarized in Table 5-3 (above), as are all sequential measures, including effluent treatment and wetlands polishing. This shows that there are from five to seven levels of containment from the beginning of each Unit until final discharge into the Riley Stafford Ditch. The containment features particular to each unit are discussed below, followed by a discussion of the additional containment afforded by the effluent treatment process. The water- and waste-flow diagram presented in Figure 5-1 (above) provides an overview of the location of the containment barriers described below.

All containment equipment is inspected by facility staff on a daily basis and a form is completed documenting the results of this inspection. These records are subsequently reviewed by facility management. FDA's inspections have included review of the SOPs, the SOP for physical containment in particular, and verification of the processes described therein. No deviations were found by FDA.

5.4.4.1    *Hatchery Unit*

The production cycle at the ABF Indiana facility begins with eyed eggs received from the PEI facility. Eggs are received in the Hatchery unit and incubated in Heath stacks (vertical egg cabinets). The Hatchery Unit contains four Heath stacks with eight egg trays stacked vertically in each. Eyed eggs are approximately 5 mm in size and are incubated until they have hatched and absorbed most of their yolk sac. At that point the juvenile fish, termed alevin, are about 3.5 x 15 mm in size and weigh approximately 0.2 g. Alevin are ready to begin feeding and are transferred to the Nursery Unit for the next stage of growth.

The Hatchery Unit uses a recirculating water system that keeps 95 to 97% of water in the system. Inflow water is pumped from the Water Filtration Facility to the Hatchery Unit where it is passes through a UV filter and is chilled to 6 - 8 °C before flowing through the Heath stacks. The chiller sits in a sump which also acts as a containment point. Recirculated water flows out of the egg cabinets

by gravity and through two types of strainers. It is then returned to the chiller before being pumped back through the Heath stacks. The chiller sump discharges to the floor drains. The floor drains contain screens to prevent passage of any eggs or alevins and furthermore, all effluent from the floor drains passes through a filter fitted with a 1.5 mm stainless steel screen, before being directed to Lift Station 1.

**Transfer of fish from the Hatchery Unit to the Nursery Unit:** The Hatchery and Nursery units are in the same building. Alevin will be transferred in buckets from the Hatchery Unit to the Nursery Unit without leaving the building.

Five levels of containment are in place for the Hatchery Unit:

- **Primary containment:** 1.5 mm polyester screens in molded plastic frames sit on top of, and below, each incubator tray. Neither eggs nor alevin can pass through the screens.

- **Secondary containment:** Water from the Heath stacks passes through strainers fitted with 0.8 mm perforated PVC screens. Neither eggs nor alevin can pass through the screens.

- **Tertiary containment:** Water from the Heath stacks next passes through a reinforced thermoplastic strainer, fitted with a 1.0 mm screen, before re-entering the chiller unit and being recirculated through the Heath stacks.

- **Quaternary containment:** Floor drains contain numerous 1.5 mm perforated stainless steel screens, placed at regular intervals along the drains, to prevent eggs or alevin from passing along the floor drains. The floor drains collect any spilt water and the small quantities of water discharged from the recirculating incubation system. Effluent captured in floor drains (i.e. all water discharged from the Hatchery) is gravity fed to a 1.5 mm stainless steel filter screen system, contained within a concrete vault. The filter system is fitted with a debris collection gutter and purging outlet for cleaning.

- **Quinary containment:** Hatchery effluent flows to Lift Station 1 for transfer to the Effluent Treatment facility. Effluent entering the lift station passes through a containment box constructed of 3 mm stainless steel screen, with an access hatch to allow servicing. The inlet pipe to the lift station, to which the box is directly fastened, is fitted with a sluice gate which allows water discharge to be temporarily shut off. In turn, this allows removal of the containment box for routine maintenance (e.g., cleaning). Alternatively, a temporary screen of comparable specification may be installed, if the box is removed, to preserve water flow as well as containment. The facility's operational procedures detail inspection and servicing of the containment box, ensuring that there is no reduction in containment if it is removed.

- **Additional containment:** Further containment is afforded subsequent to the Hatchery Unit. All effluent from the aquaculture units flows or is pumped to and through the Effluent Treatment Facility and passes through the 0.09 mm polyester mesh drumfilter screen which acts as an effective barrier to eggs, alevin, and fish of all sizes found in the aquaculture units. The drumfilter effluent passes into a discharge sump and through a 12 mm stainless steel screen before exiting the Effluent Treatment Facility.

Outfall from the Effluent Treatment Facility passes through a series of wetland ponds that will be planted with appropriate plant species (when weather permits) to further clean the effluent. From the last pond in the system, effluent passes through a discharge valve into Outfall 1. Water entering the pond system from the Effluent Treatment Facility passes through a containment box fitted with a 12 mm stainless steel screen. Water exiting the system and into the Riley Stafford Ditch passes through a screened (12 mm stainless steel) outlet box in Outfall 1. This is the point where water samples are collected for quality analysis and is the last containment point in the system.

### 5.4.4.2  *Nursery Unit*

Alevin are transferred from the Hatchery Unit to the Nursery Unit. They will remain in the Nursery Unit until the fry have reached a size of approximately 30 x 150 mm and weigh between 35 and 50 g. Periodically fish will be sized and sorted into cohorts of similar size. They are moved between tanks as they grow so that feed can be matched to the size of the fish. The grading/sizing activity also takes place in the Nursery Unit.

The Nursery Unit contains 36 tanks arranged in three sets of 12 tanks. Twelve tanks are 2.85 m$^3$ in size and the other 24 tanks are 4.7 m$^3$. As described previously, the Nursery Unit operates on a recirculating water system that includes a drumfilter, a biofilter, a degassing tower, and an oxygenator. Each set of 12 tanks is equipped with a complete recirculation system.

**Transfer of fish from the Nursery Unit to the Grow-Out Unit:** The Nursery and Grow-Out units are in different buildings and fish will be moved from the Nursery Unit to the Grow-Out Unit using either plastic bins or a fish pump with 6 -8" flexible hoses. To ensure the fish are contained during transfer, if the fish pump method is used, the flexible hose used with the fish pump will be encased in a 12" PVC pipe, which provides redundant containment, for the transfer between buildings.

Five levels of containment are in place in the Nursery Unit:

- **Primary containment:**

  o **Tank Nets**: All tanks are covered with 4.0 mm polyethylene mesh nets.

  o **Tank drains and standpipe**: Water drains from the small tanks via 2.0 or 4.0 mm PVC drain screens, dependent on fish size. Water drains from the large tanks via 12.0 mm perforated stainless steel drain plates.

- **Secondary containment:**

  o **Drumfilter**: Water from each set of tanks passes through a drumfilter fitted with 0.04 mm or 0.06 mm screens in the small and large tank systems, respectively. In the event a fish did make it through primary containment they would be captured in the drumfilter.

- **Tertiary containment:**

  o **Drumfilter Sump**: After leaving the drumfilter, water is discharged into an adjacent sump. Water entering the sump passes through a screen fitted with either a 1.5 mm stainless steel screen (small tanks) or a 3.0 mm stainless steel screen (large tanks).

- o **Drumfilter sump standpipes**: Standpipes in the drumfilter sumps are fitted with either a 1.5 mm stainless steel cover (small tanks) or a 3.0 mm stainless steel cover (large tanks).

- ▪ **Quaternary containment:**

  - o **Floor drain covers and screens:** Floors in the Nursery Unit are guttered to collect water that is splashed from the tanks. These gutters are covered with 12.0 mm coated steel grates and inside the floor drains are numerous 3.0 mm perforated stainless steel screens.

  - o **Floor drain sump:** Effluent captured in the floor drains flows to the floor drain sumps, effluent leaving these sumps must pass through 1.5 mm stainless steel mesh screens as it flows to Lift Station 1.

- ▪ **Quinary containment:**

  - o **Lift Station 1:** All Nursery Unit effluent drains to Lift Station 1 where it is pumped to the Effluent Treatment Facility. All effluent entering the lift station passes through a containment box with 3.0 mm stainless steel screen panels.

**Additional containment:** Further containment is afforded subsequent to the Nursery Unit. All effluent from the aquaculture units flows or is pumped to the Effluent Treatment Facility where it passes through a 0.09 mm drumfilter which acts as an effective barrier to eggs, alevin, and fish of all sizes found in the aquaculture units. The drumfilter effluent passes into a discharge sump and through a 12 mm stainless steel screen before exiting the Effluent Treatment Facility. Effluent entering the wetland ponds must pass through a perforated stainless steel containment box with 12 mm perforations located at the entrance to the first wetland pond. Effluent exiting the wetland ponds must also pass through perforated stainless steel containment box with 12 mm perforations located at Outfall 1 before discharge to the Riley-Stafford Ditch.

5.4.4.3   *Grow-Out Unit*

The Grow–Out Unit will receive fish from the Nursery Unit of approximately 35 to 50 g in size and will rear them until they reach harvest size of approximately 5 kg. The Grow-Out Unit contains six 90-m$^3$ tanks and twenty-four 265-m$^3$ tanks. As previously described, the Grow-Out Unit operates on recirculated water. The six small (90 m$^3$) tanks have their own recirculation system and the 24 large tanks are organized in eight sets of 3 tanks. Each set of large tanks is on a separate recirculation system. As in the Nursery Unit, recirculated water passes through a drum filter, biofilters, degassing towers for $CO_2$ extraction, and oxygenators. Water re-enters the tanks after being oxygenated.

**Transfer of fish from the Grow-Out Unit to the Purge-Harvest Unit:** The Grow-Out and Purge-Harvest units are in different buildings and fish will be moved from the Grow-Out Unit to the Purge-Harvest Unit using either plastic bins or a fish pump with 8 - 12" flexible hoses. To ensure the fish are contained during transfer, if the fish pump method is used, the flexible hose used with the fish pump will be encased in a 14 -16" PVC pipe, which provides redundant containment, for the transfer between buildings.

Four levels of containment are in place in the Grow-Out Unit:

▪ **Primary containment:**

  o **Tank Nets**: All tanks are covered with polyethylene mesh nets to prevent fish from jumping out of the tanks. The small grow-out tanks are covered with 4.0 mm mesh and the large tanks are covered with 50 mm mesh.

  o **Tank Drains**:  Most water exits the tanks and into the recirculation system via underground tank drains that are covered with 4.5 mm or 7.0 mm stainless steel grates in the small and large tanks, respectively.

  o **Side Box**: Each tank has a side box that was previously used to collect fish and was plumbed for moving fish out of the tank to a grading station. ABF will not use the side boxes in the Grow-Out Unit and has disconnected and capped the PVC pipes that connect to the fish grader. The side box also drains into the recirculation system through a standpipe. Water passing out of the tanks and into the sidebox passes through a perforated stainless steel gate or a high density polyethylene (HDPE) mesh screen that ranges in size from 4.5 to 9 mm depending on the size of the fish in the tanks. Small tanks have gates and screens sized between 4.5 and 6.0 mm and on the large tanks the gate and screen perforations are 6.0 to 9.0 mm. Side boxes have floor drains covered with 10 mm plastic grates. Standpipes in the side boxes are covered with 12 mm polyolefin screens.

▪ **Secondary containment:**

  o **Radial-Flow Settler:** Water that leaves the tanks through the tank floor drain passes through a Radial-Flow Settler (RFS) that is used to collect suspended solids. Each RFS is fitted with a 12 mm stainless steel cover on the portion of the RFS where solids are collected ("still well") and 12 mm stainless steel screens over the RFS side drains through which water flows to the drumfilters.

37

- **Tertiary containment:**

    o **Drumfilter**: Water from each set of tanks passes directly through a drumfilter fitted with a 0.09 mm mesh screen to trap solids. In the event a fish did make it through primary containment or the RFS containment points, they would be captured in the drumfilter.

    o **Drumfilter Sump**: Standpipes in the drumfilter sump are fitted with 12 mm stainless steel covers.

- **Quaternary containment**

    o **Floor drain covers**: Floors in the Grow-Out Unit are guttered to collect water that is splashed from the tanks. These gutters are covered with 12.0 mm coated steel grates sized to exclude fish ≥35 g in size.

    o **Floor drain outlet**: Effluent captured in the many floor drains passes through 6.35 mm polyolefin screens as it flows to Lift Station 1.

- **Quinary containment:**

    o **Lift Station 1:** Effluent captured in the floor drains flows to Lift Station 1 where it is pumped to the Effluent Treatment Facility. All effluent entering the lift station passes through the containment box (3.0 mm stainless steel screen).

    o **Lift Station 2**: All other effluent from the Grow-Out Unit (except floor drains) flows to Lift Station 2 after exiting the Grow-Out Unit and from there is pumped to the Effluent Treatment Facility. Effluent entering Lift Station 2 passes through a 12 mm stainless steel screen. The Lift Station 2 screen is supported on a steel service platform and covers the entire diameter of the lift station. At the center of the screen platform is a debris collection basket, also constructed of 12 mm perforated stainless steel, which can be removed for routine maintenance (e.g. cleaning). This removable basket fits inside of a permanently fixed basket. Therefore, temporary removal of the debris collection basket does not compromise the lift station's level of containment.

**Additional containment:** Further containment is afforded subsequent to the Grow-Out Unit. All effluent from the aquaculture units flows or is pumped to the Effluent Treatment Facility where it passes through a 0.09 mm drumfilter which acts as an effective barrier to eggs, alevin, and fish of all sizes found in the aquaculture units. The drumfilter effluent passes into a discharge sump and through a 12 mm stainless steel screen before exiting the Effluent Treatment Facility. Effluent entering the wetland ponds must pass through a perforated stainless steel containment box with 12 mm perforations located at the entrance to the first wetland pond. Effluent exiting the wetland ponds must also pass through perforated stainless steel containment box with 12 mm perforations located at Outfall 1 before discharge to the Riley-Stafford Ditch.

### 5.4.4.4 *Purge-Harvest Unit*

Upon reaching mature size of approximately 5 kg, fish are moved from the Grow-Out Unit to the Purge–Harvest Unit where they are maintained in (primarily) fresh water without feed for 10 to 15

days before harvesting. The Purge–Harvest Unit is equipped with six 65 m$^3$ rectangular purge tanks. Approximately 10% of the water in the purge tanks recirculates within each tank. However, there is no recirculation system *per se*, i.e. no drumfilter, biofilter, degassing tower, or oxygenator, in the Purge–Harvest Unit. Water is simply pumped from one end of the tank back to the other end.

The three levels of containment in place for the Purge–Harvest Unit include:

- **Primary containment:**

  o **Tank Net**: Each purge tank is covered with 50 mm polyethylene mesh nets to prevent fish from jumping out of the tank

  o **Effluent Pipe Cover:** Purge tanks have two 3-inch effluent pipes located near the top of the end wall. These outlets are covered with 13 mm polyolefin grates. 5 kg fish will not fit through the pipe in any event, however the grate covers provide additional assurance of containment.

  o **Re-circulation Pipe:** Water that is recirculated within the tank passes through a 2-inch PVC pipe located on the outside of the tank near the floor. Although 5 kg fish cannot pass into this pipe due to its diameter, the opening is also covered with 6 mm perforated fiberglass screen.

- **Secondary containment:**

  o **Floor drains**: Floors in the Purge-Harvest Unit are guttered to collect water that is splashed from the tanks and wash water used to clean the Unit. These gutters are covered with 12.0 mm coated steel grates.

  o **Floor drain sump**: Effluent from floor drains and Purge tanks passes through a sump on the way to Lift Station 1. Water from the tanks is piped directly to this sump, where it must first pass through a 13 mm coated steel grate. The sump outlet is also fitted with a 6 mm polyolefin grate through which effluent must pass before exiting the facility on the way to Lift Station 1.

- **Tertiary containment:**

  o **Lift Station 1**: Effluent from the Purge–Harvest Unit flows to Lift Station 1 and from there is pumped to the Effluent Treatment Facility. Effluent entering Lift Station 1 passes through a 3 mm stainless steel screen.

**Additional containment**: Further containment is afforded subsequent to the Purge-Harvest Unit. All effluent from the aquaculture units flows or is pumped to the Effluent Treatment Facility where it passes through a 0.09 mm drumfilter which acts as an effective barrier to eggs, alevin, and fish of all sizes found in the aquaculture units. The drumfilter effluent passes into a discharge sump and through a 12 mm stainless steel screen before exiting the Effluent Treatment Facility. Effluent entering the wetland ponds must pass through a perforated stainless steel containment box with 12 mm perforations located at the entrance to the first wetland pond. Effluent exiting the wetland ponds must also pass through perforated stainless steel containment box with 12 mm perforations located at Outfall 1 before discharge to the Riley-Stafford Ditch.
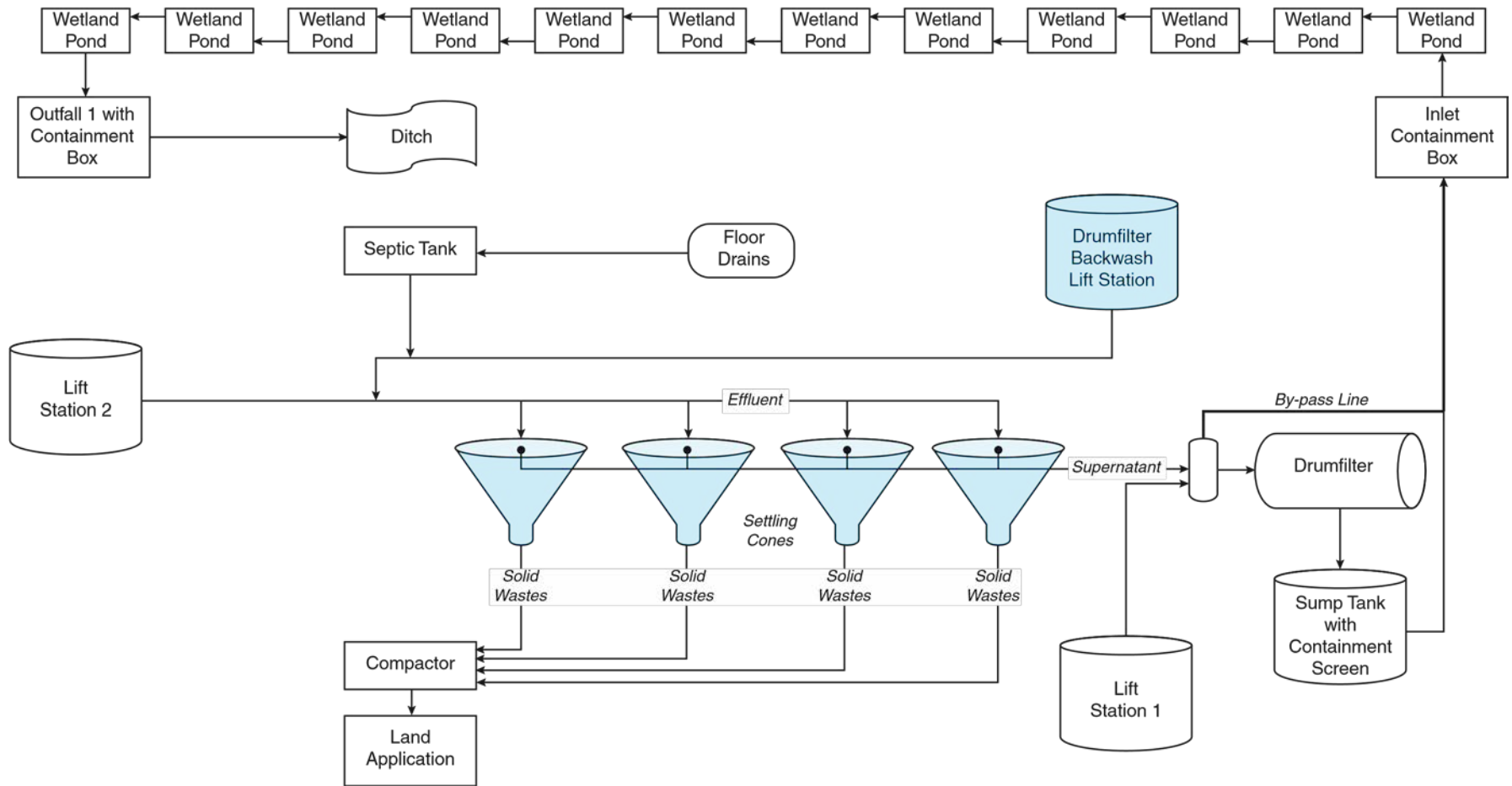
5.4.4.5  *Effluent Treatment Facility*

All effluent from aquaculture operations flows to and through the Effluent Treatment Facility (Figure 5-2). Effluent from all units passes through Lift Station 1 and/or Lift Station 2 (as described above) and then to the Effluent Treatment Facility. Effluent from Lift Station 2 is delivered to a set of four 100 m$^3$ settling cones that are connected in parallel. Particulates and suspended solids settle to the bottom of the cones and are periodically removed for use as land applied fertilizer. Supernatant leaves the cones and is gravity fed to a drumfilter containing a 0.09 mm filter to collect any solids not removed in the settling cones. Effluent is gravity-fed from the drumfilter to a sump tank (i.e., an enclosed sump) fitted with a 12 mm containment screen, and then outside to the wetland pond system adjacent to the Effluent Treatment Facility.

Effluent from Lift Station 1 enters the Effluent Treatment Facility via the supernatant pipe and goes directly into the drumfilter. Water used to backwash the drumfilter flows from the drumfilter to the drumfilter backwash lift station. From there it is pumped back into the effluent line that feeds the settling cones. In the rare event that the drumfilter is taken out service for repair or replacement, supernatant from the settling cones would be routed directly through a bypass pipe to the facility effluent line. Because this bypass procedure would result in a reduction of one level of containment for the entire facility, operational procedures are in place to ensure that no other 'communal' containment barriers (e.g., lift station screens, wetland boxes) are removed while the effluent drum filter is bypassed to prevent any further reduction in containment.

Additionally, certain physical activities would not be conducted inside the rearing units during a drumfilter bypass event. Notably, husbandry/fish management activities where livestock are handled outside of their primary containment (e.g., grading and egg handling). If it becomes necessary to bypass the Effluent Treatment Facility's drumfilter, details of the bypass will be documented in accordance with the company's standard operating procedures. Recorded information will include the reason for the bypass, mitigation measures in force during the bypass, and the time and duration of the bypass. In the rare event that it became necessary to bypass the drumfilters inside the Nursery or Grow-Out units, water would be diverted around the equipment and directly into the confines of the rearing system sump, where containment barriers are also present (i.e., the physical structure of the sumps and overflow screens). The balance of the water recirculation loop would continue to operate normally while the drumfilter was bypassed.

Effluent captured in the floor drains of the Effluent Treatment Facility flows into an external septic tank where it is held until the septic tank is pumped and effluent taken to a waste disposal facility. Environmental conditions inside the septic tank will not support any life-stage of salmon.

**Figure 5-2.    Effluent treatment at the Indiana facility**

### 5.4.4.6   *Wetland Ponds*

The Indiana facility includes a small area of wetlands that have been organized into 12 ponds. Effluent from the Effluent Treatment Facility passes through a containment screen inside the facility and through a screened containment box, with 12 mm perforations, on the inlet pipe of the first pond. The water travels in a serpentine fashion through remaining 11 ponds. The ponds are approximately 2 feet deep and will be intentionally seeded with designated wetland plants such as cattails (when weather permits) to further clean the water as it passes through the pond. Water from the pond system flows out of the last pond through Outfall 1 and  into a small drainage ditch identified as the Riley Stafford Ditch. All effluent from the wetland ponds passes through the final containment box and a 12 mm stainless steel screen in Outfall 1. The Riley Stafford Ditch ultimately drains into the Mississinewa River which is part of the Upper Wabash River Basin.

### 5.4.5   Security

Multiple and redundant forms of security are present at the Indiana facility to prevent malicious activities and unauthorized access to operational structures and AquAdvantage Salmon. Site security includes:

- ▪ **Aquaculture Units**

  - ○ **Perimeter security:**  A six-foot-high, heavy-gauge, galvanized chain-link fence of commercial quality and topped with three strands of barbwire, encloses the aquaculture units, back-up generators, liquid oxygen containment, and feed storage area.

    Primary access to the aquaculture area is through the Main Gate adjacent to Gregory Road. A Secondary Gate on the west side of the property also provides access to/from Gregory Road. Two gates located on the north side of the fenced area (East Gate; West Gate), provide access to the balance of the property, including well heads, Water Filtration Facility, Effluent Treatment Facility, wetland ponds, and several storage areas.

    Two additional gates, a 30-foot swinging gate and a 4-foot personnel gate, are located inside the perimeter and control exterior access to the Nursery, Grow-Out, and Purge-Harvest Units. These gates are located on the east side of the property just past the main entrance to the facility.

    All gates will remain locked and require authorized personnel to be opened.

  - ○ **Outside entries:** Steel exterior doors to the aquaculture units will be closed and locked at all times. When the Indiana Facility is in operation it will be staffed 24-hours per day and 365 days per year. On-site staff will use keys to enter locked areas.

  - ○ **Exterior Lighting:** Exterior lighting is in place to light the exteriors of the aquaculture units during overnight periods.

- o *Security Monitoring:* Eighteen exterior Closed Charged Device (CCD) cameras provide 24-hour surveillance and recording of the aquaculture units and the immediate surrounding areas. These cameras are in continuous operation and automatically capture digital images that are stored for later retrieval. Although there is not active monitoring of the video feed, it is continually broadcast to monitors in the Facility Manager's office, to a large television screen in the main facility office, and can be accessed on mobile devices. The system notes movement and recorded images can be quickly reviewed using motion tags.

  - ▪ *Wells, Water Filtration Facility, and Effluent Treatment Facility*

    - o *Well heads:* The two well heads are fenced, exterior doors always locked, and exterior lighting is in place to illuminate the buildings during overnight hours.

    - o *Water Filtration and Effluent Treatment Facilities:* Exterior entrances to the Water Filtration Facility and Effluent Treatment Facility are always locked and illuminated during overnight hours.

- ▪ *Security Monitoring:* Four exterior CCD cameras provide 24-hour surveillance of well heads, perimeter of the Water Filtration Facility, and the surrounding area, including Outfall 1. Two exterior CCD cameras are in place on the Effluent Treatment Facility to monitor the facility entrance and the portions of the surrounding area, including the Wetland pond inlet box. These cameras are in continuous operation and automatically capture digital images that are stored for later retrieval. Although there is not active monitoring of the video feed, it is continually broadcast to monitors in the Facility Manager's office, to a large television screen in the main facility office, and can be accessed on mobile devices. The system notes movement and recorded images can be quickly reviewed using motion tags.

- ▪ *Remote notification of status:* Environmental alarms are present to indicate emergent change in operational conditions (e.g., water level, dissolved oxygen levels) and are conveyed to on-site staff by means of audible alarms. Alarms are also sent to senior staff by text and phone messages. Because the Indiana facility will be manned 24 hours per day and 365 days per year, internal security alarms have not been put in place.

- ▪ *Additional security:* As conditions warrant, the sponsor may employ professional security personnel to remain on-site during overnight hours. If the situation warrants, professional security personnel will be present on-site during daytime working hours.

## 5.5    Labeling, Packaging, and Shipping

The product to be shipped from the PEI facility is limited (as a condition of approval) to eyed-eggs, which are the life stage most efficiently, effectively, and safely transported.

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

A bilingual (English and Spanish) Product Label printed on tear- and water-resistant paper is affixed to both the egg crate and shipping container; this label shows the product name and provides information on the product identity, claim, limitations, warnings, and handling instructions of immediate importance to the end-user. A bilingual Package Insert comprising detailed handling recommendations and important information regarding performance, animal safety, and environmental considerations is also included. Shipments are identified as "Eggs & Fry[13]" that is "Not for Resale." The following additional warnings (or facsimile thereof) also appear on the Product Label:

- Rear only in a physically-contained freshwater culture facility as specified in an FDA-approved application;

- Must not be reared in conventional sea cages or net-pens;

- Dispose of morbid or dead fish in a manner consistent with local regulations.

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

During handling, transport and opening, the container is maintained in an upright position; and upon receipt, egg temperature is determined to assess the need for equilibration to the receiving temperature if the difference between the two exceeds 2ºC.[14] The equilibrated eggs are held in fresh water at 6–8ºC[15] and $\geq$ 7 mg/L DO.

All tanks holding AquAdvantage Salmon at the Indiana facility will be marked with the product label.

## 5.6    Operational Plans and Procedures

The most important element of the containment system is well trained, knowledgeable staff who completely understand the operating systems and procedures, and who fully recognize the

---

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

[14] Package insert language does say 4°C difference. In practice ABT and ABF do not allow more than a 2°C difference.

[15] Conditions specified in the NADA EA were 2 – 8°C. The low end of the range is used at PEI facility to slow egg maturation time for shipping purposes. Equilibration of incoming eggs at the Indiana Facility will be in the range specified here as there is no need to delay egg maturation. The same temperature range is specified in the process description, Section 5.4.2 of this EA.

importance of following designated work procedures. The ABF and Indiana facility management team are highly experienced with over 70 years of collective experience in commercial aquaculture. ABT has a full-time Director of Regulatory Compliance who ensures all aquaculture employees are fully trained and that Standard Operating Procedures (SOPs) are in place for all operations. The Sponsor has operated its facilities in PE and Panama for over 20 years without a single serious breach of containment.

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

SOPs currently used at the PEI broodstock facility and at Panama Grow-out facility have been adapted and modified based on experience to date to address the site-specific operational conditions and equipment present at the Indiana facility. More specifically, these SOPs will describe the operational procedures regarding the following:

- Bio-security within the facility;

- Containment, including requirements for daily checks of critical containment barriers and procedures to follow in the unlikely event of a fish escape;

- Water quality maintenance and testing;

- Housing and management of fish populations;

- Handling, removal and disposal of mortalities and waste;

- Actions to take in the event fish are found at a particular containment point;

- Procedures to follow when collection of waste requires bypassing or removing any given containment barrier;

- All routine fish handling and maintenance operations; and,

- Emergency response procedures for unanticipated events.

Because of the redundant layers of containment that will be in place there will never be a time when eggs, fry, or fish could go directly from a tank to the effluent discharge point, i.e., when one layer of containment is being cleaned, there are always several more in place. If large modifications are required to a containment system, water will be re-directed or water flow will be shut off to the area being serviced so that no effluent is generated from that area. The production site in Indiana will be managed according to established SOPs that cover day-to-day operations.

# 6 ACCESSIBLE ENVIRONMENT

To assess exposure pathways that could potentially lead to impacts on the environment, this section discusses the physical environment in the vicinity of the Indiana facility. The NADA EA discusses the physical environment in the vicinity of the PEI egg production facility and the Panama grow-out facility (NADA EA, Section 6).

## 6.1 Physical Site Characteristics of the Indiana Grow-Out Facility

The Indiana facility is located on 43.45 acres of land approximately three miles from the town of Albany, Indiana, in Delaware County (Figure 6-1). The location is not in a flood zone (FEMA Map 18035C016tD), and is classified as Zone F (Farming) by Delaware County. Albany is the closest town; in 2010, it had a total population of approximately 2,100. It is part of the Muncie, Indiana, Metropolitan Statistical Area. The nearest larger cities are Muncie and Indianapolis (approximately 10 and 57 miles southwest, respectively) and Dayton, Ohio, 66 miles to the southeast. Regional transportation routes include I-69 to the west and I-70 to the south of Delaware County.

**Figure 6-1. Neighborhood view of Albany, IN with the ABF facility outlined in red**
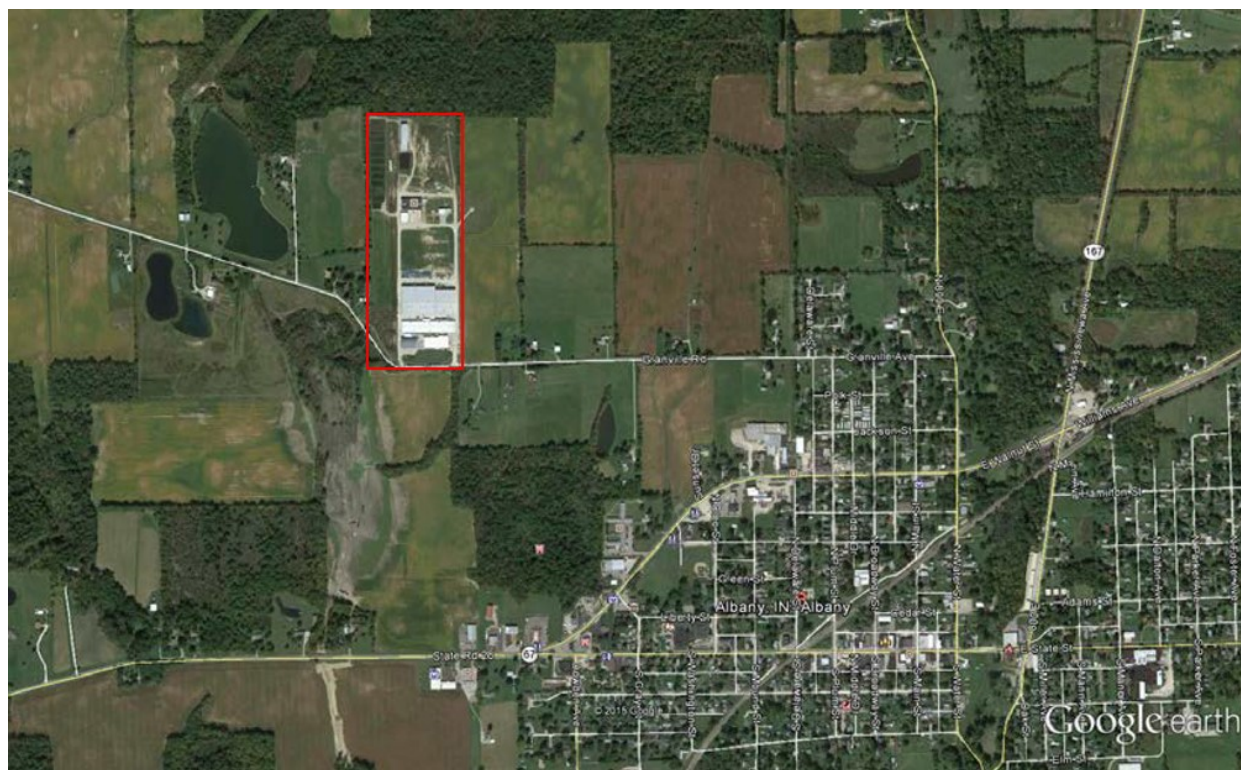


The property includes a series of wetland ponds that drain to a small drainage ditch known as the Riley Stafford Ditch, which in turn drains into the Mississinewa River (Figure 6-2 and Figure 6-3). The outfall from the last wetland pond is a permitted discharge (National Pollutant Discharge Elimination System [NPDES] Permit No IN0062669) by the Indiana Department of Environmental Management; this permit was transferred from Bell Aquaculture to ABF on

March 23, 2018. The Riley Stafford Ditch is a drainage ditch that is generally dry except during periods of wet weather. To this will be added contributions of effluent from the facility. It runs adjacent to the facility and then south for another approximately 4,000 feet through farm fields and small woodlots before emptying into the Mississinewa River. When fully operational, the Indiana facility discharge of approximately 350 gallons/minute is expected to raise water levels in the Riley Stafford Ditch to a total depth of 1–2 inches in the immediate vicinity of the facility. The Riley Stafford Ditch has been known to disappear underground in several locations, particularly during periods of low rainwater input. Figure 6-2 shows a view of the outfall (white box on left) taken on October 20, 2017; note that there is no water present in Riley Stafford Ditch. Figure 6-3, taken the same day, also shows no water present in Riley Stafford Ditch.

**Figure 6-2.     A view of the outfall (white box on left) and the Riley Stafford Ditch**

**Figure 6-3.     The Riley Stafford Ditch, running south along the west side of the ABF property**



The Mississinewa River is part of the Upper Wabash River watershed (Figure 6-4). The Indiana facility site is shown on Figure 6-4 as a red star. The Mississinewa River drains into the Wabash River near Peru, Indiana. There is one dam on the Mississinewa River, the Mississinewa Lake Dam, approximately 75 miles downstream of Albany, shown as a red line on Figure 6-4. The Wabash River flows southwest through Indiana where it flows into the Ohio River near the southwest corner of Indiana. The Ohio River flows along the boundary of Illinois and Kentucky until it flows into the Mississippi River at Cairo, Illinois. The Mississippi River flows south from Cairo to the Gulf of Mexico.

**Figure 6-4.** **The Wabash River watershed**



Source: https://commons.wikimedia.org/wiki/File:Wabashrivermap.png

## 6.2 Climate and Local Conditions

The local climate is generally a humid, continental climate, with cold winters and hot, wet summers. Albany lies in the eastern central region of Indiana, which receives an average monthly rainfall of 3.5 inches (8.8 cm)[16]. The average temperature is -2.8°C in January and 22.9°C in July (NOAA 2018). Monthly weather data for the eastern central region of Indiana are shown in

---

[16] http://www.weather.gov/ind/localcli; accessed December 5, 2017.

Table 6-1.  Over the past 30 years, average daily minimum and maximum temperatures by month have ranged from -8.1°C to 26.0°C, respectively (NOAA, 2018).

**Table 6-1.      Weather Data for eastern central Indiana\***

| Month | Avg Daily Temp (°C) | | | Avg Precip (cm) |
| --- | --- | --- | --- | --- |
| | Min | Avg | Max | |
| Jan | -7.7 | -2.8 | 3.6 | 6.9 |
| Feb | -8.1 | -1.1 | 4.9 | 5.6 |
| Mar | 0.6 | 4.5 | 12.1 | 8.0 |
| Apr | 7.9 | 10.8 | 13.7 | 10.3 |
| May | 12.9 | 16.3 | 19.8 | 11.7 |
| Jun | 18.4 | 21.3 | 22.8 | 11.8 |
| Jul | 20.1 | 22.9 | 26.0 | 10.9 |
| Aug | 19.4 | 22.0 | 24.9 | 9.0 |
| Sep | 16.1 | 18.2 | 20.7 | 8.0 |
| Oct | 7.7 | 11.8 | 15.4 | 7.4 |
| Nov | 1.4 | 5.6 | 9.1 | 8.3 |
| Dec | -8.2 | -0.5 | 5.8 | 7.7 |

\* source: NOAA (2018). All data are monthly averages over the period of 1987–2017. Min = Minimum, Avg = average, max = maximum.

Water quality data for nearby habitats in the Mississinewa River watershed are limited. Local U.S. Geological Survey (USGS) monitoring stations upstream (USGS station 03325500; Mississinewa River near Ridgeville, Indiana) and downstream (USGS station 03326500; Mississinewa River at Marion, Indiana) of the facility do not measure any chemical or physical characteristics of the river other than discharge. The average monthly discharge as reported at the Ridgeville monitoring station is shown in Table 6-2.

**Table 6-2.      Monthly discharge\* of the Mississinewa River near Ridgeville, Indiana, at USGS Station 03325500, 1986–2015**

| Month | Min | Avg | Max |
|-------|-----|-----|-----|
| Jan | 17.2 | 219.8 | 1160.0 |
| Feb | 37.9 | 212.5 | 547.6 |
| Mar | 43.7 | 264.8 | 840.3 |
| Apr | 24.7 | 239.6 | 634.2 |
| May | 15.3 | 158.0 | 424.2 |
| Jun | 6.5 | 190.5 | 779.8 |
| Jul | 2.9 | 137.0 | 649.6 |
| Aug | 2.4 | 27.3 | 199.3 |
| Sep | 0.9 | 48.1 | 383.5 |
| Oct | 1.0 | 58.2 | 314.1 |
| Nov | 6.4 | 120.1 | 729.1 |
| Dec | 13.4 | 204.1 | 872.3 |

\* all values are shown in $ft^3s^{-1}$

Average monthly water temperature is available for the Mississinewa River at the outfall of Mississinewa Lake (USGS station 03327000), immediately downstream of Mississinewa Lake Dam, approximately 75 miles downstream of Albany. Average monthly water temperatures measured at that station are shown in Table 6-3. This indicates annual fluctuations typical of temperate climates, with temperatures during the summer months above the lethal limit (approximately 23°C) that has been identified for Atlantic salmon (see Appendix A for additional information on their temperature tolerance).

**Table 6-3.      Monthly water temperatures (°C) at the outfall of Mississinewa Lake, near Peoria, Indiana**

| Month | Min | Avg | Max |
|-------|-----|-----|-----|
| Jan | 1.1 | 2.7 | 4.6 |
| Feb | 0.5 | 2.3 | 5.0 |
| Mar | 2.4 | 5.4 | 9.6 |
| Apr | 9.4 | 11.3 | 14.6 |
| May | 12.9 | 15.5 | 18.4 |
| Jun | 18.2 | 20.3 | 22.6 |
| Jul | 19.2 | 23.2 | 26.6 |
| Aug | 22.7 | 24.0 | 25.6 |
| Sep | 21.1 | 22.2 | 24.1 |
| Oct | 14.2 | 16.4 | 19.1 |
| Nov | 7.9 | 9.9 | 12.5 |
| Dec | 2.0 | 4.2 | 6.8 |

Additional local water quality data are available from a 2014 field survey performed by the Indiana Department of Environmental Management (IDEM). The survey was conducted in the

Upper Mississinewa watershed in support of developing total maximum daily loads (TMDLs) to address beneficial-use impairments noted in the watershed. Of the 35 locations monitored in 2014, three sampling stations on the Mississinewa River were within four miles of the ABF facility (Figure 6-5). Stations 117 and 119 are 0.4 and 1.2 miles upstream of the confluence of Riley Stafford Ditch and the Mississinewa River, respectively, while station 125 is approximately nine miles downstream.

**Figure 6-5.     Select locations associated with a 2014 IDEM field sampling program**
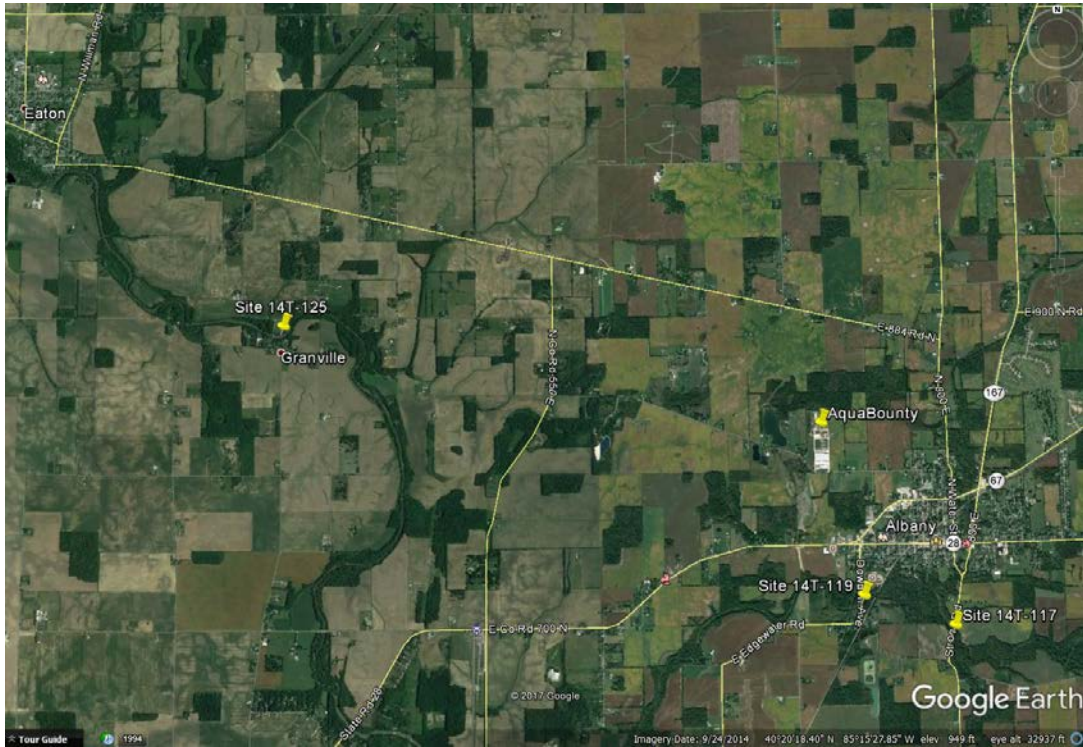


Based on this IDEM sampling, conducted every two to three weeks during the spring, summer and fall, average values for select water quality parameters included 8.57 mg/L dissolved oxygen (DO) (range 4.49–14.34), 642 µmho/cm specific conductance (range 426–813), and 7.98 pH (range 7.55–8.34) (IDEM, 2016). With the exception of low DO in the summer, these conditions are generally suitable for fish and other aquatic organisms. DO measurements (in mg/L) made at the three sampling stations shown in Figure 6-5 during the 2014 sampling event are presented in Table 6-4, along with the corresponding water temperatures. Values in bold indicate conditions under which Atlantic salmon would not be expected to survive for extended periods of time (see Section 5.2.2.2 and Appendix A.3).This is evidenced by the lack of cold-water fish species, including salmonids (e.g., rainbow trout, brook trout, brown trout), in the upper Mississinewa River drainage (see Section 6.4 and Table 6-5).

**Table 6-4.** **Dissolved oxygen at selected sampling locations in the Upper Mississinewa River during 2014**

| Date | Station and DO value, mg/L | | | Station and Temperature, °C | | |
|---|---|---|---|---|---|---|
| | 14T-117 | 14T-119 | 14T-125 | 14T-117 | 14T-119 | 14T-125 |
| 4/15/2014 | 9.65 | 9.66 | 10.11 | 9.45 | 9.22 | 9.5 |
| 4/22/2014 | 9.15 | 9.17 | 10.06 | 14.25 | 14.3 | 14.75 |
| 4/29/2014 | 8.63 | 8.73 | 9.33 | 12.18 | 12.26 | 13.96 |
| 5/6/2014 | 10.71 | 11.23 | 12.1 | 12.71 | 12.99 | 13.78 |
| 5/13/2014 | 6.71 | 6.61 | 7.28 | 19.44 | 19.63 | 21.31 |
| 6/10/2014 | 8.02 | 7.89 | 7.57 | 17.21 | 17.03 | 17.76 |
| 7/8/2014 | 8.16 | 7.76 | 8.8 | 20.93 | 20.9 | 20.99 |
| 8/6/2014 | n/a | 6.46 | n/a | n/a | **23.03** | n/a |
| 8/12/2014 | **4.49** | n/a | 6.19 | **23.57** | n/a | **22.64** |
| 8/19/2014 | **5.56** | **5.4** | 7.56 | 21.29 | 21.2 | 21.27 |
| 9/16/2014 | 8.04 | 7.8 | 8.61 | 14.79 | 15.09 | 14.91 |
| 10/28/2014 | **5.43** | 6.37 | 6.87 | 13.05 | 13.82 | 13.93 |
| 11/19/2014 | 14.34 | n/a | n/a | -0.19 | n/a | n/a |
| 12/15/2014 | 12.31 | n/a | n/a | 4.2 | n/a | n/a |

Values in bold are outside of the tolerance range of Atlantic salmon. DO = dissolved oxygen

## 6.3    Occurrence of Natural Disasters

The Indiana facility is not located in a flood plain (IDNR 2017). With limited impervious surfaces in the immediate vicinity, flooding is not expected to be an issue at this site. Any extreme cold weather experienced during the winter (e.g., blizzards) would not pose a risk for release of organisms, as they would be unable to survive in the cold temperatures and under heavy snowfall. There have been 11 tornadoes reported in Delaware County over the past 50 years (NOAA 2017). The last tornado to affect Albany was in 2003; no injuries or deaths resulted, and reported damages were minimal. Statewide, Indiana is subject to an average of 22 tornadoes per year. Tornadoes can result in damage to buildings, vehicles, and vegetation, and result in power outages. The most likely impact of tornadoes on the facility would be a sustained loss of electrical power, which could potentially cause loss of the ability to handle effluent flows. However, the facility has backup generating capabilities in place, and furthermore, the multiple levels of physical containment (e.g., nets, screens) that are in place do not require electricity to operate. Another potential impact of tornadoes could be physical damage to the buildings, tanks, pipes, or containment structures, which could allow the breach of containment measures. In the event of partial damage to the facility, the presence of multiple, redundant containment measures make it unlikely that fish would escape all the way through the treatment facility and serial wetland ponds to the Riley Stafford Ditch. In the event of a tornado severe enough to damage the entire facility, it is unlikely the fish would be able to survive due to the loss of water of appropriate quality from the tanks in which they are kept.

## 6.4 Biological/Ecological Properties

The Indiana grow-out facility is located in the Rees Ditch-Mississinewa River subwatershed (HUC level 12 address 051201030402), which was listed in 2016 (under Section 303(d) of the Clean Water Act [CWA]) as impaired for DO, *Escherichia coli* (*E. coli)*, and impaired biotic communities.[17] As a result of the 2014 survey discussed previously, the U.S. Environmental Protection Agency (EPA) determined that bacteria, nutrients, and sediment are occurring at levels that prevent the attainment of the designated uses of the watershed. These specific impairments are typical of areas where the land use is dominated by farming. Land cover in the Mississinewa River watershed is primarily agricultural as shown in Figure 6-6. The Indiana facility site location is indicated by a small red x in the figure..

**Figure 6-6.     General land cover in the Mississinewa River Watershed (IDEM 2016)**



---
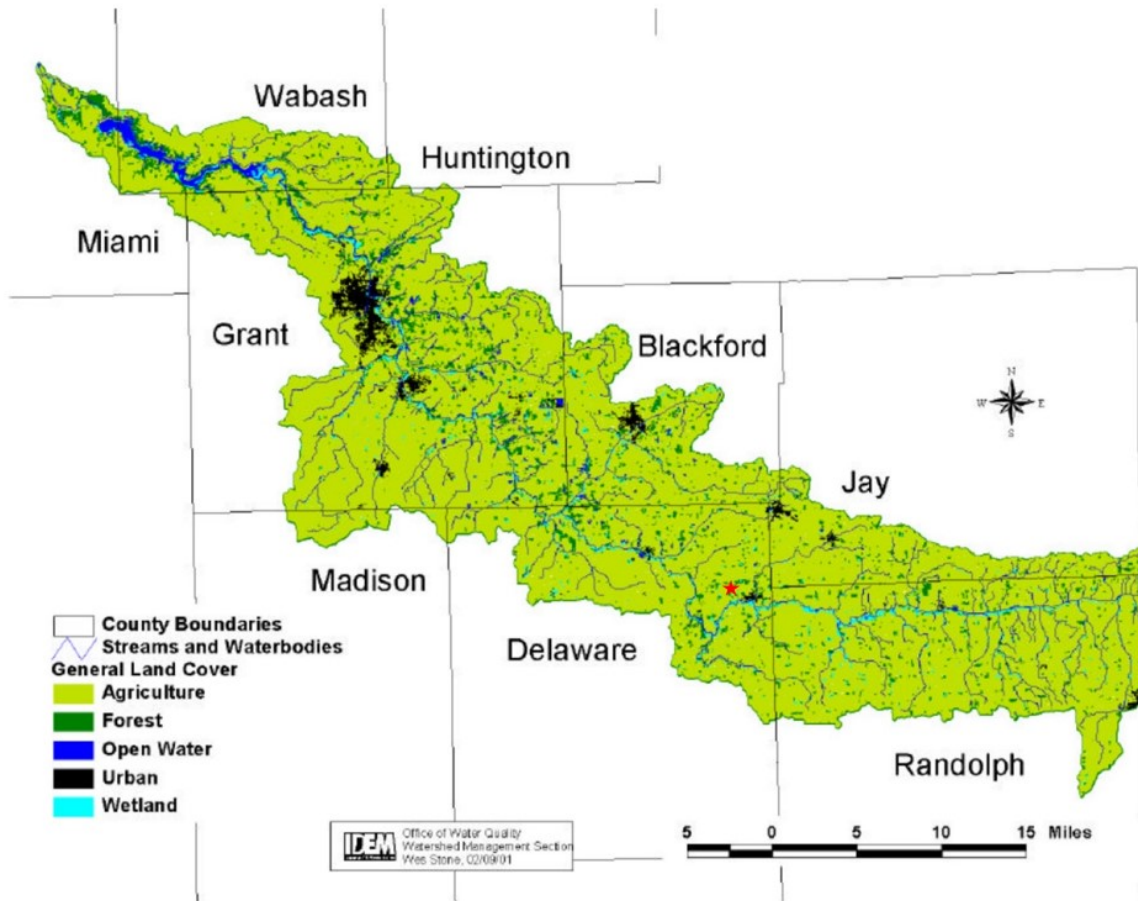
[17]  The IDEM Office of Water Quality (OWQ) develops Indiana's 303(d) List of Impaired Waters as part of the state's Integrated Water Monitoring and Assessment Report (IR), which is submitted to EPA every two years in accordance with Sections 305(b) and 303(d) of the CWA. CWA Section 305(b) requires states to make water quality assessments and provide water quality reports to EPA, and CWA Section 303(d) requires states to identify waters through their Section 305(b) water quality assessments that do not or are not expected to meet applicable state water quality standards with federal technology-based standards alone.

In addition to measuring physical and chemical variables, the 2014 survey also sampled fish and invertebrate communities throughout the Upper Mississinewa watershed. The full set of 35 sampling sites, including the three discussed previously, are shown in Figure 6-7. The red star on the figure indicates the location of the ABF Indiana facility.

**Figure 6-7.    Locations of sampling stations used in 2014 IDEM watershed survey**
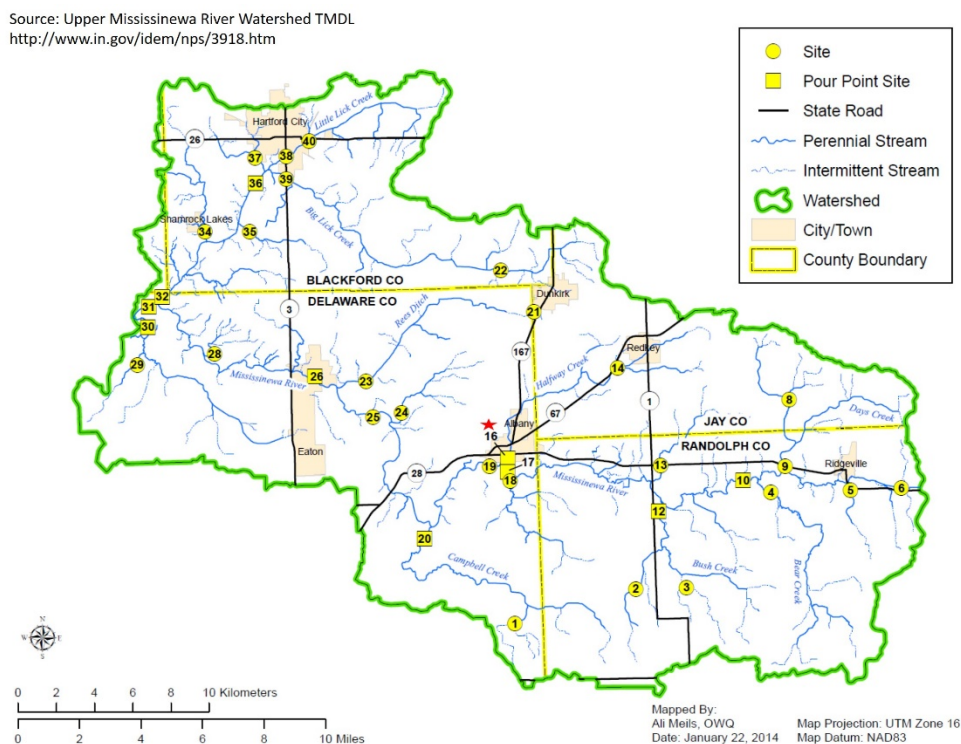


Results of the fish survey indicate that, with over 6,000 fish captured and identified, there are 57 fish taxa present in the Upper Mississinewa River watershed. The river can be described as a warm-water fishery, with no species of trout or other cold-water salmonids found at any of the 35 sampling sites. Fish communities at each sampling site were given Index of Biotic Integrity (IBI) scores, which take into account twelve metrics, including number of species counted, number of sensitive species counted, percent tolerant individuals, etc. Each metric is scored from 1–5, for a maximum total score for any given sampling site of 60. Fish IBI scores for the watershed ranged from 14 to 56; the three sampling stations closest to the Indiana facility (corresponding to points 17, 19, and 25 in Figure 6-7) had IBI scores of 52, 50, and 54, respectively, indicating generally healthy fish communities. Longear and green sunfish numerically dominated the local fish community, both at these three sites and in the Upper Mississinewa watershed as a whole. Of the 57 species found in Upper Mississinewa River, 44 were present at these three stations; these are listed in Table 6-5. Various warm-water species (minnows, darters, catfish, sunfish, etc.) were found, but no cold-water salmonid species such as rainbow trout or brook trout were collected.

**Table 6-5.      Fish species observed at selected sampling locations in the Upper Mississinewa River during 2014**

| | |
|---|---|
| Black Redhorse | Northern Hog Sucker |
| Blackside Darter | Orangespotted Sunfish |
| Blackspotted Topminnow | Orangethroat Darter |
| Bluegill | Rainbow Darter |
| Bluntnose Minnow | Redfin Shiner |
| Brindled Madtom | Rock Bass |
| Central Stoneroller | Sand Shiner |
| Common Carp | Silver Redhorse |
| Creek Chub | Silverjaw Minnow |
| Creek Chubsucker | Slenderhead Darter |
| Dusky Darter | Smallmouth Bass |
| Emerald Shiner | Spotfin Shiner |
| Freshwater Drum | Spotted Bass |
| Golden Redhorse | Spotted Sucker |
| Goldfish | Steelcolor Shiner |
| Grass Pickerel | Stonecat |
| Green Sunfish | Striped Shiner |
| Greenside Darter | Suckermouth Minnow |
| Johnny Darter | Tadpole Madtom |
| Logperch | White Crappie |
| Longear Sunfish | White Sucker |
| Mottled Sculpin | Yellow Bullhead |

Similar sampling was done for aquatic macroinvertebrates, and similar IBIs were developed based on metrics which included number of species counted, percent mayfly/stonefly/caddisfly (%EPT), and percent tolerant and intolerant taxa. Macroinvertebrate IBI scores for the watershed ranged from 24 to 44 (out of a maximum possible value of 60); the three sampling stations closest to the Indiana facility had IBI scores of 38, 34, and 38, indicating that the macroinvertebrate communities were more impacted than the fish communities. (A macroinvertebrate IBI score of <35 is indicative of "poor" or "very poor" conditions).

Although they were not seen in the 2014 sampling effort, the Indiana Department of Natural Resources has been stocking rainbow trout into streams across the state for over a decade, including a site on the Mississinewa River upstream of the Indiana facility in Ridgeville, IN since 2005 (IDNR 2014). The fact that no species of trout were detected in the extensive 2014 sampling effort indicates that the habitat may not be suitable for long-term trout survival and establishment. Natural reproduction of rainbow trout has not been observed in the watershed and is not expected, due to warmer summer water temperatures.[18]

---

[18]     Jeb Pearson, IDNR, personal communication with Michael Kierski of Exponent, 11/9/2017.

Sampling was also conducted at various points across the Mississinewa River watershed, including both up- and downstream of the facility, in 1998, 2003, 2008, and 2015, although these sampling efforts were less intensive than 2014. No rainbow trout, or salmonid species of any kind, were found in those surveys.[19]

In summary, while conditions may exist in the Upper Mississinewa watershed that may be amenable to Atlantic salmon survival during some times of the year, the connection from the outfall to the river downstream is a ditch with low or intermittent flow (even including the facility discharge) that is unlikely to provide conditions for survival and transit downstream. Seasonal temperature extremes, particularly warm water and low DO conditions in the summer, are detrimental to Atlantic salmon survival.[20] The overall watershed does not support salmonids, even though rainbow trout have been stocked for over a decade. This may be due to the seasonal temperature extremes as well as water quality that has been identified as impaired by elevated levels of bacteria, nutrients, and suspended solids. These factors, as well as a dam downstream of the facility, pose impediments to the long-term survival and establishment of Atlantic salmon in the river downstream of the facility.

---

[19]  Kevin A Gaston, Indiana Department of Environmental Management (IDEM), Assessment Information Management System (AIMS) Database, Indianapolis, Indiana, Personal communication with Konrad Kulacki of Exponent, April 10, 2018.

[20]  DO is inversely related to water temperature, so that low DO conditions occur when water temperatures are highest. Both conditions limit the long-term survival and establishment of Atlantic salmon.

# 7    ENVIRONMENTAL CONSEQUENCES

This section discusses the potential effects of the proposed action, including potential effects on populations of Atlantic salmon and populations of threatened and endangered species in Indiana.

## 7.1    Scope and Approach to the Analyses of Effects

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

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

As discussed in Section 3, the likelihood of escape would depend primarily on the extent and adequacy of physical (mechanical) containment at the facility. GE fish are considered to pose little risk to native populations if they are adequately contained (Mair *et al*., 2007; Wong and Van Eenennaam, 2008). Confinement of GE fish in closed, land-based facilities is considered optimal to ensure an acceptably low risk of escape (Mair *et al*., 2007). Such is the case for the AquAdvantage Salmon grow-out facility in Indiana. As a result of multiple and redundant forms of effective physical confinement, it can be concluded that the likelihood of escape of AquAdvantage Salmon is extremely low. The following discussion provides the reasoning for this conclusion.

The purpose of the supplemental NADA is to seek approval for grow-out of AquAdvantage Salmon at a land-based facility, in Albany, Indiana. To ensure containment, a redundant, multi-level strategy has been used. Physical containment for this grow-out facility is described in Section 5.4.2 and summarized in Table 5-3 of this EA. As described in Section 5.6, the Indiana facility will be managed according to established SOPs that include daily checks of critical containment barriers, procedures for emergency response to unanticipated events (such as an interruption of the water supply), and to address the unlikely possibility of a fish escape.

In addition, there is point-to-point control of shipping and land-based materials transfer. These measures have been described in detail in Section 5.5; additional information and discussion is provided below. As discussed in Sections 5.4, 5.6, and 6.3, additional measures in place at the Indiana facility would include the use of multiple types of containment; operational oversight by a management team with extensive experience in commercial aquaculture; trained staff operating under established plans and procedures; automated monitoring of operational conditions and unauthorized intrusion; passive and active measures to ensure physical security; backup power generation; and the historical absence of natural disasters of sufficient magnitude to render these measures ineffective. These measures are further discussed below.

7.2.1    Physical Containment at the Indiana facility

Physical containment at the Indiana facility is described in detail in Section 5.4.3, Table 5-3, and Figure 5-1 and Figure 5-2. The Indiana facility includes the following units for the production of AquAdvantage Salmon: Hatchery, Nursery, Grow-Out, and Purge-Harvest. The Hatchery, Nursery, and Grow-Out Units will be operated as recirculating aquaculture systems, while the Purge-Harvest Unit will be operated under partial recirculating conditions. These conditions mean that the discharge of water, and concomitant potential for fish escape, is minimal. The entire process is housed within buildings, so there is no risk of escape of fish through predation by wildlife.

Five levels of physical containment are in place at each Unit except the Purge-Harvest unit which has three primary levels of containment. This is followed by additional containment measures in the effluent treatment and discharge process, resulting in a total of five to seven independent levels of containment for all production Units[21] (Table 5-3).  Materials used for containment barriers are durable and designed for operations in a RAS aquaculture environment. Details of the materials used for each containment barrier are included in Table 5-3 and described in detail in Section 5.

In the Hatchery Unit, polyester screens under each egg tray and under each Heath stack prevent the passage of eggs or alevin out of the Unit. Water flowing from the Heath stacks passes through two additional containment points, a PVC strainer placed directly below the Heath trays and a thermoplastic strainer located outside the chiller. Multiple 1.5 mm stainless steel screens are in place in all floor drains that will capture eggs and alevin found in the Hatchery unit, and all water leaving the Hatchery Unit flows through the Hatchery Effluent filter with a 1.5 mm stainless steel screen before flowing to Lift Station 1 (LS1). Effluent entering LS1 flows through a stainless steel containment box fitted with a 3.0 mm stainless steel screen before flowing onwards to the Effluent Treatment facility.

In the Nursery Unit, all tanks are covered by polyethylene nets with mesh sizes appropriate to the life-stage of the fish housed in each tank. All outlets from tanks (drains, standpipes, and side boxes) are covered by durable mesh or stainless steel screens of a size that is appropriate to the life-stages housed in the Nursery unit.  Water exiting the tanks passes through drumfilters with either a 0.04 mm mesh (small Nursery tanks) or 0.06 mm mesh (large Nursery tanks) that would capture any fish that did escape the tanks. Water leaving the drumfilters passes through a 1.5 mm or 3.0 mm stainless steel screen, small and large tanks respectively, prior to entering a sump. Standpipes are covered with stainless steel screens sized appropriately for the size of fish in the tanks. Multiple 2.0 mm stainless steel screens are in place in all floor drains in the Nursery, and

---

[21]    Containment is reduced by one level for eggs and early-life stages in the facility in the event the drumfilter in the Effluent Treatment Facility is bypassed for servicing. But in such an event, no handling of eggs or early life-stage fish is undertaken until the Effluent Treatment drumfilter is back online. Additionally, SOPs dictate that no other containment equipment is taken offline until servicing is completed and the drumfilter is back online. See Section 5.4.2.5 for additional details.

water captured in the floor drains passes flows through a stainless steel screened sump on the way to LS1. Effluent entering LS1 flows through a stainless steel containment box fitted with a 3.0 mm stainless steel screen before flowing onwards to the Effluent Treatment facility.

The tanks in the Grow-Out unit are also covered with polyethylene nets with mesh sizes appropriate to the life-stage of the fish housed in each tank. Water exiting the tanks going to the recirculation system passes through drains in the tank floor that are either PVC (small tanks) or stainless steel and sized to exclude fish housed in the tank. Water then passes through the RFS with additional stainless steel screens before entering the drumfilter. Side boxes in these tanks are closed off, and standpipes are capped with mesh screen. Water that is not recirculated drains through a mesh screen before arriving at Lift Station 1 which is fitted with screened containment box. From there, water is pumped to the Effluent Treatment facility. Multiple stainless steel grates with perforations smaller than any life-stage of fish housed in the Grow-out unit are placed in all floor drains. Water in the floor drains then flows to Lift Station 1. Grow-Out Unit effluent from all other sources flows to Lift Station 2, which has permanent stainless steel mesh floor with 12.0 mm perforations. Water from both Lift Stations is pumped to the Effluent Treatment facility.

In the Purge-Harvest Unit, the tanks are covered with polyethylene nets to prevent escape. The pipes through which water leaves the tanks, either for discharge or recirculation, are too small to allow passage of even the smallest fish that would be in this unit, and are also covered with fiberglass or polyolefin mesh screens. Floor drains are covered with coated steel perforated grates, and collected water, as well as effluent leaving this unit, enters a sump fitted with a stainless steel screen and then travels to a lift station fitted with a stainless steel screened outlet on its way to the Effluent Treatment facility.

Subsequent to each unit, there is additional containment prior to the discharge of any effluent. All effluent at the Indiana facility flows to and through the Effluent Treatment facility. Effluent from Lift Station 2 passes through settling cones to remove particulates; the supernatant joins the effluent from Lift Station 1 and goes through a drumfilter containing a 0.09 mm filter to collect any remaining solids. From there, effluent flows to a sump tank through another mesh filter and into the wetland pond system. This system contains 12 ponds in series through which the effluent moves in a serpentine fashion. Effluent from the last pond in the series is gravity fed through a final (12 mm) perforated stainless steel containment box before it is discharged through the outfall into the on-site Riley Stafford Ditch.

These multiple and redundant barriers prevent the escape of any life stages of AquAdvantage Salmon from the facility. In the highly unlikely event of any escape, survival in the Riley Stafford Ditch is highly unlikely. The Riley Stafford Ditch has intermittent flow and is fed primarily by rainwater and effluent from the Indiana facility. When fully operational, the Indiana facility discharge of approximately 350 gallons/minute is expected to raise water levels in the ditch to a total depth of only 1–2 inches in the immediate vicinity of the facility. This amount of water is unlikely to be sufficient to support the larger life stages of AquAdvantage Salmon, which would be the most hardy and have the best chance of survival outside the facility.

7.2.2   Issues Affecting Containment and Security

7.2.2.1   *Natural Disasters*

As discussed in Section 6.3, flooding is not expected to be an issue at the Indiana site. Extreme cold weather and heavy snowfall would preclude the survival of AquAdvantage Salmon outside of the facility. The most likely natural disaster in east central Indiana is a tornado, which could result in damage to the physical structure of the Indiana facility and/or a sustained power outage. Backup electrical generating capacity will mitigate against power outage. In the event of partial damage to the facility, the presence of multiple, redundant containment measures make it unlikely that fish would escape all the way through the treatment facility and serial wetland ponds to the on-site ditch. In the event of a tornado severe enough to damage the entire facility, it is unlikely that the fish would be able to survive for very long due to a degradation in water quality (i.e., appropriate DO and/or temperature) in the tanks in which they are kept. For example, without supplemental oxygenation, DO levels will quickly deplete to lethal levels.

7.2.2.2   *Physical Security*

The ABRAC Performance Standards call for security measures to (a) control normal movement of authorized personnel, (b) prevent unauthorized access to the site, and (c) eliminate access of predators that could potentially carry fish off-site (for outdoor projects). The Performance Standards also mention the possible need for alarms, stand-by power, and an operational plan (including training, traffic control, record keeping, and an emergency response plan).

Information about physical security measures at the Indiana grow-out facility has been described in Section 5.4.4. Measures include restricted entry to the site, security fencing and lighting, enclosure of operations in buildings, security cameras and sensors, and, if the situation warrants, professional security personnel. Access by predators is eliminated because the fish are maintained throughout the entire grow-out process indoors. In addition to the physical security measures, there are SOPs in place to address containment failure and security issues. Employees have undergone training and the facility would be subject to continued inspections by FDA.

7.2.2.3   *Malicious Intentional Release*

Given the redundancy in physical containment measures and the low probability of occurrence of severe natural disasters in the area, the most likely event leading to introduction of AquAdvantage Salmon to the environment surrounding the Indiana facility would be an intentional malicious release. ABT is aware that unauthorized access to the site may represent a potential hazard and has taken appropriate steps to reduce the possibility this will occur. As described in Section 5.4.4 and above, there are extensive security measures, equipment, and plans in place to ensure that the probability of such an event would be extremely low.

7.2.3   Conclusions for the Indiana Facility

The probability that AquAdvantage Salmon would escape from the Indiana grow-out facility is extremely small due to the presence of multiple, independent forms of physical (mechanical)

containment. Backup systems are in place in the event of equipment failures or a natural disaster, and site security measures are in place to prevent malicious activities. Physical security and containment ensure that it is highly unlikely there would be any unintentional escapes or releases of AquAdvantage Salmon due to equipment failures, natural disasters, or malicious activities.

The Indiana facility will also be subject to regulatory oversight by the Indiana Department of Environmental Management (IDEM) and the Indiana Department of Natural Resources (IDNR). As required by the NPDES permit issued to ABF, water quality will be monitored and reported to IDEM on a monthly basis. IDNR is responsible for issuing aquaculture permits and is primarily concerned about health and disease status. ABT will provide periodic reports on the health status of broodstock housed at the PEI facility and will work with IDNR authorities as requested or required by Indiana law.

7.2.4    Transportation of Eggs from PEI to Indiana

Section 5.5 describes shipping from the PEI facility to the Indiana facility as occurring via air freight with subsequent ground-shipment to the Indiana grow-out facility. When shipped, multiple containment measures are in place for AquAdvantage Salmon eggs. Eggs are shipped in coolers, sealed with tape and bound with packing straps, which are then placed in a sealed heavy cardboard shipping container. Unintentional escape of AquAdvantage Salmon eggs is therefore particularly unlikely.

7.2.5    Disposal of Fish and Fish Wastes

As discussed in the NADA EA, disposal of AquAdvantage Salmon (including non-viable eggs, mortalities, and culls) and the non-viable waste material associated with the production, processing, and consumption of the fish (e.g., feces, fish pieces) would not require different handling from that used for wild or domesticated non-GE fish: the rDNA gene construct added to this fish is stably integrated into the genome; it is not infectious, communicable, or transmissible from these materials, and will degrade in the same manner (i.e., rapidly) as other DNA in the environment.

Fish wastes and uneaten feed (biosolids) will be removed from the effluent at the Indiana facility through settling using settling cones followed by mechanical filtration through a drumfilter. Biosolids collected in the settling cones will be removed from the facility for land application. Dead fish will be collected and removed by an approved vendor for disposal in a landfill, consistent with local regulations.

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

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

7.2.6    Conclusions for Question 1

For this supplement to NADA 141-454, grow-out of AquAdvantage Salmon is proposed to be conducted *only* in a second, land-based facility in Indiana 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 Indiana grow-out facility to prevent the accidental release of eggs and/or fish to nearby aquatic environments. These barriers have been designed specifically to prevent the escape of different life stages of AquAdvantage Salmon. The facility has a minimum of four 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 (ABRAC, 1995).

The likelihood that any life stages of AquAdvantage Salmon could escape from confinement at this site is considered to be very low. In addition, physical security and containment to prevent unintentional releases of salmon due to natural disasters or intentional releases due to malicious activities are in place.

ABT also employs SOPs that govern physical containment, as well as every other significant activity that occurs at the Indiana facility. Operations at the Indiana facility are led by a team highly experienced in commercial aquaculture and  a strong operations management plan is in place at the Indiana facility, comprising policies and procedures that meet the recommendations for an integrated confinement system for GE organisms as summarized in Table 7-1.

Any breakdown of these measures would be highly unlikely because of the following factors ABT's use of multiple types of containment; use of trained staff operating under established procedures; automated monitoring of culture conditions and unauthorized intrusion; redundant passive and active measures to ensure physical security, and continued inspections by local and U.S. officials.

The combination of all of these factors results in an extremely low likelihood that any AquAdvantage Salmon present at the Indiana facility could escape into the wild and cause effects on the environment.

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

| Recommended element | Presence |
|---|:---:|
| Commitment by top management | ✓ |
| SOPs dictating actions in the event of a catastrophic facility failure, including documentation, monitoring, and remediation | ✓ |
| Training of employees | ✓ |
| Dedication of permanent staff to maintain continuity | ✓ |
| Use of SOPs for implementing redundant confinement measures | ✓ |
| Periodic audits by an independent agency | ✓ |
| Periodic internal review and adjustment to allow adaptive modifications | ✓ |
| Reporting to an appropriate regulatory body | ✓ |

\*    After Kapuscinski, 2005.

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

In the very unlikely event that any life stages of AquAdvantage Salmon escaped, the likelihood of survival and dispersal is a function of two complementary sets of parameters: their phenotype and fitness (e.g., tolerance to physico-chemical parameters such as temperature and DO) and the specific geographical and geophysical containment in the accessible environment that is a function of the specific location and environmental conditions at the site of escape. Geographical and geophysical containment is defined as the presence of inhospitable conditions in the surrounding environment that would preclude or significantly reduce the probability of survival, dispersal, and/or long-term establishment should an animal escape confinement at its site of rearing. Furthermore, unless deemed to be 100% effective under all reasonably foreseeable circumstances, containment of this type would normally be considered secondary to other containment measures.

Geographical/geophysical containment would be present at the Indiana facility as discussed below. As an overall statement, the spread of the 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 described in the NADA EA and provided again in Section 5.2 of this EA, 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 geographical settings of the grow-out site 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. For the reasons discussed in the following sections, it can be concluded that the geographical and geophysical settings of the AquAdvantage Salmon grow-out site in Indiana make the possibility of environmental impacts from survival and dispersal of AquAdvantage Salmon extremely low.

7.3.1   Indiana Facility

The Indiana facility is surrounded by farmland in the east central part of the state. The effluent from the facility will be discharged into a small seasonal ditch (Riley Stafford Ditch), and even with the flow from the effluent, it will only contain 1–2 inches of water in the immediate vicinity of the facility. Thus, in the improbable event of any escaped fish, it is unlikely that they can survive and be transported to the Mississinewa River, which is almost a mile distant. In addition, seasonal temperature extremes, particularly high water temperatures in the summer months, are likely to be lethal to all Atlantic salmon life stages. As discussed in Appendix A, Section A.3, feeding and activity of Atlantic salmon does not occur at temperatures above approximately 23°C for younger fish, with mortality occurring at approximately 26°C (Willoughby, 1999). For older fish, a temperature of approximately 22°C is lethal, although the lethal temperature may increase with prior acclimation. During the months of June, July, August, and September, observed maximum water temperatures downstream of the site ranged from 22.6–26.6°C (Table 6-3). At locations close to the site, water temperatures have been observed to exceed 22°C in August (Table 6-4). During the summer months, DO concentrations in the watershed are likely to be insufficient to support the relatively high demands of salmon. Salmon have a relatively high requirement for DO compared to many other fish species. Furthermore, adult GH-transgenic salmon have been reported to have an increased requirement for DO compared to non-GE counterparts (see Appendix A, Section A.3, and Section 5.2.2.2), presumably due to their faster growth and increased metabolic rate. The physiological implication of this requirement is a reduced tolerance to higher water temperatures, because 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 when DO concentrations drop to 6 mg/L (ppm). Triploidy also has an adverse impact on the tolerance of Atlantic salmon for high temperatures and low DO (Hansen *et al.*, 2015; Sambraus *et al.*, 2017). During certain times (e.g., typically summer), DO concentrations in the Upper Mississinewa River at stations close to the Indiana facility have been found to be below this minimum level (Table 6-4).

In addition to periods of high water temperature and low DO, several other conditions of the habitat in the watershed are also unfavorable for survival or establishment. First, salmonids have a requirement for clear water. However, the Upper Mississinewa Watershed has high levels of turbidity that preclude attainment of beneficial uses of the waters (the watershed is considered impaired based on levels of TSS, bacteria, and nutrients). Second, food sources may be limited, as the condition of the macroinvertebrate fauna in the watershed is somewhat impaired. Third, having been reared their entire lives on synthetic diets, escaped salmon are often recaptured with empty stomachs presumably due to their inability to switch from a pelleted diet to one of natural

prey, a limitation that would be exacerbated by the low abundance of such prey in the environment at the Indiana facility. This would increase the likelihood for starvation and rapid mortality. Finally, and more specifically for AquAdvantage Salmon, additional factors would further reduce the likelihood of their survival and dispersal, including a reduced predator avoidance that would likely increase their predation mortality. The third and fourth factors are discussed further below.

Although not extensively studied to date, the survival of escaped and released farmed Atlantic salmon has been found to be low (Whoriskey *et al*., 2006; Hansen, 2006), supported by the fact that marine survival rates for hatchery origin Atlantic salmon are also very low, 0.04 to 0.5%, and well below those of wild salmon (ICES, 2009). This low survival may be due, at least in part, to the hypothesis that farmed fish fail to adapt to feeding on live prey after they have escaped from net pens in which they have adapted to being fed on artificial feeds and thus starve to death (Muir, 2004). In support of this hypothesis, Olsen and Skilbrei (2010) simulated salmon escape from net pens and found the stomachs of recaptured fish were generally empty in the first few weeks after release. Using lipid analysis, they also found that none of the fish recaptured many months later near the release site had switched to wild prey diets. The previous work by Hislop and Webb (1992) found that that 65% of the escaped farmed salmon on the west coast of Scotland had empty stomachs, while only 35% had switched to natural prey. Similarly, Soto et al. (2001) found that approximately 60% of recaptured escaped Atlantic salmon in southern Chile had empty stomachs. Because they are raised on pelleted synthetic diets similar to those fed to farmed Atlantic salmon in ocean net pens and cages, this collective information suggests that AquAdvantage Salmon may not transition to a wild prey diet in the unlikely event they were to escape the Indiana facility, and thus would be susceptible to starvation and early mortality.

As discussed in Section 5.2.2.3, GH transgenic salmon have a higher tolerance than non-GE salmon for predator risk (Abrahams and Sutterlin 1999) making them more susceptible to predation. The presence of predators in the Upper Mississinewa Watershed is unclear; however, there are no documented populations of rainbow trout, which are a significant predator of salmon fry, fingerlings, and juveniles. Avian and mammalian predators may be present. These attributes (difficulty transitioning to a wild prey diet and predation risk) suggest that AquAdvantage Salmon would not be particularly fit for the environment in east central Indiana, in the unlikely event they were to escape.

Atlantic salmon are not found in the watershed, nor are any species of salmonid fish. For over a decade, the state has stocked rainbow trout in streams within the state, including a site on the Mississinewa River upstream of the Indiana facility, but none were found in IDEM's 2014 survey of the Upper Mississinewa River, despite extensive sampling. Sampling was also conducted at various points across the Mississinewa River watershed, including both up- and downstream of the facility, in 1998, 2003, 2008, and 2015. Although these sampling efforts were less intensive than 2014, no rainbow trout, or salmonid species of any kind, were found in those surveys (Section 6.4). These findings, in conjunction with the previously reported DO and temperature data for the Upper Mississinewa River (Table 6-4), indicates that the local environment is not supportive of the establishment of cold-water salmonids.

Finally, the existence of a dam downstream in the watershed appears to constitute a significant, although not entirely complete, barrier to fish movement within the watershed, particularly with

respect to potential downstream migration of AquAdvantage Salmon to lower parts of the watershed. The watershed ultimately drains to the Gulf of Mexico, an area where Atlantic salmon have not historically been present and which is inhospitable to them due to high water temperatures that are at or above the lethality levels for salmonids.

In summary, in the unlikely event that escape of AquAdvantage Salmon from the Indiana facility were to occur, long-term survival is unlikely even in the immediate vicinity. Stream flow ranges from intermittent to low in the Riley Stafford Ditch, so the habitat is generally unsuitable for fish survival. During summer months, water temperatures would be too high, and DO levels too low, to support survival of any escaped salmon for more than a short period of time. Moreover, AquAdvantage Salmon are less likely to be fit for the environment than their non-GE counterparts. The absence of other cold-water salmonid species in the watershed, despite stocking efforts, further indicates the unsuitability of the habitat. A dam downstream would limit passage, and even if any life stages survived, the ultimate drainage system (down the Mississippi River to the Gulf of Mexico) would not be supportive of Atlantic salmon survival and dispersal.

7.3.2    Conclusions for Question 2

The geographical and geophysical conditions present in the aquatic environments surrounding the Indiana facility 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 long-term survival of AquAdvantage Salmon is limited by low water flow, precluding passage downstream; unfavorable and generally impaired water quality conditions (with seasonally high water temperatures and low DO, as well as the presence of turbidity); potentially insufficient food; feeding and predation responses of AquAdvantage Salmon; a dam downstream; and ultimate drainage into an area where Atlantic salmon have never historically occurred.

**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 environment surrounding the Indiana facility, the likelihood that they would be able to reproduce and subsequently establish is largely a function of the extent and adequacy of biological containment in the fish that escape. Because conspecifics and closely related relatives of Atlantic salmon (i.e., brown trout) are not found in the local aquatic environments near the Indiana facility, the essential concern is over reproduction between escaped fish. This has been addressed in the NADA EA. In summary, AquAdvantage Salmon eggs produced for shipment to the Indiana grow-out facility are triploid and functionally sterile.[22]

---

[22]    With reference to AquAdvantage Salmon, "triploid" means that, based on sampling, at least 95% of eyed-eggs released for shipping have three sets of complete chromosomes per cell with a probability of 0.95 (i.e., the probability that these eggs are not at least 95% triploid is less than 0.05 (see Section 7.4.1.2 of the NADA EA ). In triploidy validation studies, the percentage of triploids for 10 independent crosses (n = 200 eggs per cross) averaged 99.8%, with 100% triploidy in six crosses and 99.5% triploidy in the other four crosses.

Biological confinement will be ensured in AquAdvantage Salmon through the use of triploidy and the production of all-female populations for grow-out. The methodologies for these processes and their effectiveness have been discussed at length in the NADA EA (see Section 7.4).

ABT has established SOPs governing the methods used to induce triploidy in AAS which were initially implemented in 2001. The method was validated using the current equipment in 2012. The procedures to qualify production lots of AAS eggs have been in use since 2012 and five commercial production lots of AAS eggs have been produced for grow-out in Panama since the SOP was finalized. In the five shipments made since 2015, the percentage of triploid eggs has been ≥99% in four lots and never dropped below 98.5%.

**Table 7-2.     Quality Control data collected on triploid conversion of AAS eggs**

| Production Lot | Date of Analysis | Flowcytometry Record # | Estimated % Diploid | Estimated % Non-viable | Estimated % Inconclusive | Estimated % Triploid |
|---|---|---|---|---|---|---|
| AAS-120815-005 | 25-Jan-16 | 904-907 | 0.5% | 0.0% | 0.0% | 99.5% |
| AAS-121615-008 | 02-Feb-16 | 920-923 | 0.0% | 0%** | 1%** | 99.0% |
| AAS-111016-001 | 27-Dec-16 | 993-1000 | 0.0% | 0.0% | 1.5% | 98.5% |
| AAS-112216-003 | 10-Jan-17 | 1012-1014 | 0.0% | 0.0% | 0.0% | 100.0% |
| AAS-112817-001 | 24-Jan-18 | 1142-1146 | 0.0% | 0.0% | 0.0% | 100.0% |

** Scored inconclusive prior to the implementation of aneuploid/non-viable scoring method. Peaks are in the aneuploid/non-viable range.

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 might escape physical containment and reach the environment, while functionally sterile, they would still be capable of exhibiting spawning behavior with fertile, wild females, if wild-type 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 in the Indiana facility. See NADA EA Section 7.4.1.4 for information on the process for production of an all-female population.

Because AquAdvantage Salmon, as defined and specified in NADA 141-454, and in the event of approval of this supplement to the NADA, would only be produced as all-female triploids, it is important to consider the interactive effects of triploidy and sex on Atlantic salmon in their natural environment and how this might influence interactions between farm-raised fish that have escaped, including AquAdvantage Salmon, and wild salmon. As discussed in the NADA EA, Section 7.4.1.6, it can be inferred 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.

Even if they were not sterile, mature female AquAdvantage Salmon escaping into the watershed near the Indiana facility would not encounter conspecifics (i.e., fish of the same species) 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 Indiana facility. No salmonid species have been found in multiple surveys of the Upper Mississinewa Watershed, including extensive sampling conducted by state officials in 2014 and less intensive surveys in 1998, 2003, 2008, and 2015 (see Section 6.4). In fact, despite repeated attempts to stock this watershed with rainbow trout, they have not been found in the watershed and are not expected to occur there due to habitat and water quality conditions, including high summer water temperatures. Any long-term establishment of AquAdvantage Salmon would require reproduction, which would not be possible because of the lack of conspecifics. Furthermore, reproduction amongst AquAdvantage Salmon would not be possible because the population at the Indiana facility would be entirely female.

A type of pseudo-establishment could potentially occur if successive waves of large numbers of salmon escaped confinement and entered the local environment, with each wave replacing or supplementing the former as fish die off or disperse. This scenario would require the periodic escape or release of large numbers of fish, such as sometimes occurs from net pens in the marine environment, which is not a realistic possibility for the Indiana facility due to the small population sizes relative to grow-out in net pens, as well as the highly redundant containment and security measures employed at the site.

Any significant downstream movements of escaped AquAdvantage Salmon would be greatly limited by physical structures (i.e., the Lake Mississinewa dam) and the effects of water quality. As discussed previously, the water temperatures during summer months can approach and exceed the lethal maximum that Atlantic salmon can tolerate for an extended period, approximately 22°C, while the DO concentrations are sometimes below the minimum suitable level for salmonids, approximately 6 mg/L. It is highly unlikely that escapees would manage to survive downstream all the way into the waters of the Gulf of Mexico, which also does not have indigenous populations of Atlantic salmon (see Appendix A) or any populations of Pacific salmon species (i.e., chinook, chum, coho, sockeye, or pink salmon) or steelhead trout.

Even if interactions with wild Pacific salmonids were somehow possible, the weight of evidence indicates that it is highly unlikely that there would be successful hybridization of Atlantic salmon (or AquAdvantage Salmon specifically) with Pacific salmon, which are of a different genus, *Oncorhynchus*. The potential for hybridization and genetic introgression between Pacific salmon species and Atlantic salmon, which are widely cultured in net pens on the west coast of Canada, and to some extent in the coastal waters of Washington State, has been a concern in both countries for many years. As discussed in the NADA EA, Section 7.4.1.6, a review board in Washington state concluded that there was no reasonable potential for hybridization between escaped Atlantic salmon and native Pacific salmon in Puget Sound (PCHB, 1998). Furthermore, despite numerous attempts to introduce Atlantic salmon to geographic areas outside of its native range, none have been successful at producing self-sustaining populations of anadromous fish (Waknitz et al. 2003; Dill and Cordone, 1997; Alverson and Ruggerone, 1997).

In summary, it can be concluded, based on the available evidence, that any reproduction or long-term establishment of AquAdvantage Salmon in the watershed of the Indiana facility or farther afield as a result of an escape is essentially precluded.

7.4.1    Conclusions for Question 3

The conditions of use for NADA 141-454 specify that, based on testing, a minimum of 95% of the AquAdvantage Salmon eggs sold for commercial production use would be triploid and 100% are expected to be female. Based on the results of multiple method validation studies, the actual average percentage of triploidy is consistently above 99% (see Table 7-2). 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 environment in the highly unlikely event of an escape. The only potential means for establishment (or pseudo-establishment) would be through a continual series of escapes at the Indiana facility, which is unlikely given the physical containment measures in place. Given the lack of any self-sustaining populations of Atlantic salmon on the west coasts of the U.S. and Canada where significant numbers of Atlantic salmon escape each year from net pen salmon farms, these scenarios are considered even more unlikely. Therefore, given the available information, and weight of evidence, it can be concluded that there is a negligibly small likelihood that AquAdvantage Salmon would reproduce and establish self-sustaining populations if they escaped from the Indiana facility.

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

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

In the 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 to smolt stage), in many other respects, their fitness would be reduced (e.g., increased need for food, increased DO utilization, etc.). Natural selection would act on these fitness attributes in the environment, but there is considerable uncertainty associated with predicting or quantifying any particular outcome, as ABT is not aware that any growth enhanced GE animal has ever been released into the wild. These potential outcomes, and their likelihoods, are discussed below.

This EA has documented that physical/mechanical containment is very stringent for the Indiana facility, and escapes from the facility are considered to be highly unlikely (see Section 7.2). In the event, however unlikely, that escapes should occur, biological containment would be

imposed on the population. Geographical and geophysical containment present in the environment would also provide significant hurdles to long-term survival, establishment, and persistence of AquAdvantage Salmon in Indiana (see Section 7.3). 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 (see Section 7.4).

It should also be noted that the scale and frequency of introductions of GE fish into a particular environment would have a large influence on the potential ecological risk. Any introductions would have to involve a critical mass that could offset natural mortality and be of sufficient frequency and in proper season to allow for long-term survival and establishment. If the scale and frequency of the escapes (i.e., introductions to the environment) are small, the chances of becoming established in the natural setting are extremely low (Kapuscinski and Hallerman, 1991). As previously discussed, the probability of escape from the Indiana 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 Indiana are (1) accidental escape to the adjacent seasonal Riley Stafford Ditch through complete failure of all physical containment systems at the facility due to a catastrophic event (e.g., tornado) 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) 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 Riley Stafford Ditch near the grow-out facility. However, this is highly dependent upon the size of the escaped life stages and the available flow in the Riley Stafford Ditch, which is often minimal to non-existent. Long-term survival at locations farther downstream would be essentially precluded because of water quality conditions inhospitable to Atlantic salmon (see Section 6 and Section 7.3.1 for additional discussion). Reproduction and permanent establishment in the local environment would also be precluded because all AquAdvantage Salmon would be females and overwhelmingly triploid (effectively sterile). In addition, there are no wild conspecifics or feral relatives with which they could interbreed (see Section 7.4).

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 of the Indiana facility) and no populations of closely related salmonid species with which reproduction is possible; 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 farther downstream.

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

No effects on any populations of wild Atlantic salmon or any of the species of Pacific salmon in waters of the United States are reasonably foreseeable as a result of escape or release of AquAdvantage Salmon from the Indiana facility. The nearest populations of Atlantic salmon are many thousands of miles away in the northwest 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, etc.) are also located thousands of miles away 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 Indiana to marine waters in the United States where populations of Atlantic and Pacific salmon live. Poor water quality and other forms of geographic/geophysical containment apply to the local watershed in Indiana to ensure with a high degree of probability that AquAdvantage Salmon would not survive to reach the Gulf of Mexico and, from there, the Atlantic or Pacific Oceans.

7.5.2   Effects Due to Escape/Release During Transportation

As discussed above in Section 7.2, escape of AquAdvantage eggs during transport from PEI to Indiana is not reasonably foreseeable. Any release of eggs during shipment would be the result of accidental release due to a major incident during transport. Due to the fragile nature of salmonid eggs and the unlikelihood of the eggs ending up in a suitable habitat for survival (i.e., cold freshwater with sufficient DO), survival of eggs through and after a significant shipping incident, such as a trucking accident or plane crash, is remote. As a result, no effects on the environment are anticipated.

7.5.3   Conclusions for Question 4

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

**7.6      Effects on Endangered Species**

Effects of the proposed action on endangered species are also considered. 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 Endangered Species Act (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). The following information is provided to support an FDA determination that the approval of the supplemental NADA will have no effect on any endangered species when produced and reared under the conditions that would be established in an approved supplemental application, and that are described in this EA. In addition, the following information is provided to support an FDA determination that approval of the supplemental NADA would not jeopardize the continued existence of a listed species or destroy or adversely modify designated critical habitat.

As discussed above (see Section 7.5.1), no effects on populations of wild Atlantic salmon are reasonably foreseeable as a result of escape or release of AquAdvantage Salmon from the grow-out facility in Indiana.

Federally listed threatened or endangered species in Delaware County, Indiana, include four species of mollusks (the northern riffleshell, the clubshell, the rabbitsfoot, and the rayed bean), one mammal (the Indiana bat), and one species of vascular plant (running buffalo clover). These

species are also on the Indiana list of endangered species. An additional five reptiles, five birds, and three vascular plants are state listed as endangered species.[23] No effects on any of these species are reasonably foreseeable as a result of escape or release of AquAdvantage Salmon from the grow-out facility in Indiana. None of these species serves as prey items for Atlantic salmon. Larval stages of many freshwater mussels use fish species as hosts. For the four species listed, none are solely dependent on salmonid species as a host because they do not have a sole species host requirement.[24] In addition, the historical absence of salmonid species from this area of Indiana precluded them from ever being a host for these freshwater mussels. Moreover, the likelihood of escape and survival of AquAdvantage Salmon in the watershed to an extent that they could serve as hosts for these larvae is remote.

## 7.7    Cumulative Impacts

As previously stated, this EA pertains to a supplement to NADA 141-454 to allow grow-out at the Indiana facility. All of the other specific production and use conditions for AquAdvantage Salmon approved under NADA 141-454 remain in effect.

Council on Environmental Quality regulations define cumulative impact as "the impact on the environment which results from the incremental impact of the present action when added to other past, present and reasonably foreseeable future actions" 40 CFR 1508.7.  The NADA EA concluded that the production and grow-out of AquAdvantage Salmon under the specified conditions, including production at ABT's PEI facility and grow-out at ABT's Panama facility, would not result in significant impacts on the environment of the United States. The current EA presents evidence that grow-out at the Indiana facility will not result in significant impacts on the environment of the United States. Therefore, the cumulative impact on the environment of the previous action to approve NADA 141-454 and the present action to approve the supplemental NADA for the additional grow-out facility in Indiana is negligible.

This EA does not examine any potential incremental impact for additional production facilities because, if ABT at a later time seeks to open or ship to any additional facilities or to significantly expand existing facilities, another supplemental NADA would need to be submitted, reviewed, and approved prior to using, or shipping to, such a facility. Action by FDA on such an application would be considered a major federal action under NEPA and FDA regulations, and, as such, would require the preparation of an EA which would consider the cumulative impact of the addition of another facility or other proposed changes.

---

[23]  http://www.in.gov/dnr/naturepreserve/files/np_delaware.pdf, Accessed 11/30.2017.

[24]  Host species for northern riffleshell include banded darter, bluebreast darter, brown trout, and banded sculpin (https://www.nrc.gov/docs/ML1126/ML112650644.pdf, accessed 12/6/2017). Host species for the rabbitsfoot include whitetail shiner, spotfish shiner, and bigeye chub (Yeager and Neves, 1986). Host species for the clubshell include blackside darter, central stoneroller, logperch, and striped shiner (https://mnfi.anr.msu.edu/abstracts/zoology/Pleurobema_clava.pdf; accessed 12/6/2017).  Host species for the rayed bean include Tippecanoe darter, greenside darter, mottled sculpin, and largemouth bass (Butler, 2002).

## 8    SUMMARY AND CONCLUSIONS

Using the risk-based approach outlined above, this environmental assessment has found no evidence that approval of a supplemental NADA for AquAdvantage Salmon to allow grow-out at the ABT Indiana facility, in conjunction with the existing grow-out in the Panama facility and egg production in the PEI facility, would result in significant impacts on the environment. The findings are summarized by the following list of questions and answers, specifically addressing the proposed grow-out at the Indiana facility:

♦    What is the likelihood that AquAdvantage Salmon will escape the conditions of confinement?

  o   Due to the presence of multiple, redundant, and effective physical containment measures at the site (which would be required under the conditions of the supplemental approval), the likelihood of AquAdvantage Salmon escaping into the environment is very low.

♦    What is the likelihood that AquAdvantage Salmon will survive and disperse if they escape the conditions of confinement?

  o   In the unlikely event of an escape or release, environmental conditions at the Indiana grow-out site are sufficiently inhospitable to limit long-term survival and spread of AquAdvantage Salmon to other locations. Limited flow in the immediate receiving water is likely to preclude survival to downstream reaches. In addition, unfavorable and degraded water quality conditions, including seasonal high temperatures and low DO concentrations, as well as physical barriers, will adversely impact survival and dispersal. This is evidenced by the lack of any salmonid species in the area of the Indiana facility. Furthermore, the fitness traits of any escaped fish are likely to make them particularly unsuitable for survival and dispersal.

♦    What is the likelihood that AquAdvantage Salmon will reproduce and establish if they escape the conditions of confinement?

  o   Under the conditions specified in NADA 141-454, AquAdvantage Salmon must be produced as all-female, triploid fish. As such they are effectively sterile. 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 environment of Indiana 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 a continual series of escapes at the Indiana facility. This is unlikely given the physical containment measures in place. This scenario would require the escape of a significant number of animals, a condition that is even less likely. Therefore, given the available information, it can be concluded that it is extremely unlikely that AquAdvantage Salmon would establish and reproduce if they escape from the Indiana facility.

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

o The collective information on the potential for survival, dispersal, reproduction, and establishment indicates that no effects are expected on the environment (including populations of endangered wild Atlantic salmon in Maine or populations of threatened and endangered species in Indiana) from grow-out at the Indiana facility.

In summary, the evidence presented indicates that the grow-out of AquAdvantage Salmon at the ABT Indiana facility under the conditions that would be established in the supplemental NADA, if approved, and as described in this EA, would not result in significant effects on the quality of the human environment in the United States, including populations of endangered Atlantic salmon.

## 9  MITIGATION MEASURES

Because the proposed action, approval of the supplemental NADA for grow-out of AquAdvantage Salmon at the Indiana facility under the conditions of the supplemental approval, would not have a significant effect on the environment, no mitigation measures would be necessary.

## 10  AGENCIES AND PERSONS CONSULTED

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

## 11  LIST OF PREPARERS

This document was prepared by Exponent, Inc., under the direction of Jane P. Staveley (with contributions from other Exponent staff) and Aqua Bounty Technologies, Inc. (Mark Walton).

## 12  CERTIFICATION

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

 

April 20, 2018

Mark Walton, Ph.D.                                    Date

Global Director, Regulatory Affairs

AquaBounty Technologies, Inc.

## 13  REFERENCES

ABRAC [Agricultural Biotechnology Research Advisory Committee] (1995). Performance standards for safely conducting research with genetically modified fish and shellfish. Document No. 95-04, Office of Agricultural Biotechnology, U.S. Department of Agriculture, 156 pp.

Abrahams, M.V., and A. Sutterlin (1999). The foraging and antipredator behaviour of growth-enhanced transgenic Atlantic salmon. *Anim. Behav.* **58**: 933–942.

Akiyama, T., T. Murai, and K. Mori (1986). Role of tryptophan metabolites in inhibition of spinal deformity of chum salmon caused by tryptophan deficiency. *Bull. Jap. Soc. Sci. Fish.* **52**(7): 1255–1259.

Alverson, D.L., and G.T. Ruggerone (1997). Escaped farm salmon: environmental and ecological concerns. In: Salmon Aquaculture Review, Discussion Paper B(3). Environmental Assessment Office, Government of British Columbia, Victoria, BC, 108 pp.

Amiro, P.G. (2006). A synthesis of fresh water habitat requirements and status for Atlantic salmon (*Salmo salar*) in Canada. Canadian Science Advisory Secretariat, Department of Fisheries and Oceans, Res. Doc. 2006/017, 39 pp.

Atkins, M.E., and T.J. Benfey (2008). Effect of acclimation temperature on routine metabolic rate in triploid salmonids. *Comp. Biochem. Physiol. A* **149**: 157–161.

Bæverfjord, G., T. Åsgård, B. Gjerde, *et al*. (1996). [Spinal deformities in Atlantic salmon are neither caused by inbreeding nor a side effect of breeding]. *Norsk Fiskeoppdrett* **10**: 34–35.

Bakke, T.A., and P.D. Harris (1998). Diseases and parasites in wild Atlantic salmon (*Salmo salar*) populations. *Can. J. Fish. Aquat. Sci.* **55**(Suppl. 1): 247–266.

Benfey, T.J. (2016). Effectiveness of triploidy as a management tool for reproductive containment of farmed fish: Atlantic salmon (*Salmo salar*) as a case study. *Reviews in Aquaculture* **8**: 264–282.

Bermingham, E., S.H. Forbes, K. Friedland, *et al*. (1991). Discrimination between Atlantic salmon (*Salmo salar*) of North American and European origin using restriction analyses of mitochondrial DNA. *Can. J. Fish. Aquat. Sci.* **48**: 884–893.

Bigelow, H.B. (1963). Fish of the Western North Atlantic. Sears Foundation for Marine Research, Denmark; as cited in Teufel *et al*., 2002.

Björnsson, B.Th. (1997). The biology of salmon growth hormone: from daylight to dominance. *Fish Physiol. Biochem.* **17**: 9–24.

Bourke, E.A., J. Coughlan, H. Jansson, *et al*. (1997). Allozyme variation in populations of Atlantic salmon located throughout Europe: diversity that could be compromised by introductions of reared fish. *ICES J. Mar. Sci*. **54**: 974–98.

Brackett, J. (1991). Potential disease interactions of wild and farmed fish. *Bull. Aquacul. Assoc. Canada* **91**(3): 79–80.

Brown, C.L., and J.M. Núñez (1998). Disorders of development. In: Fish Diseases and Disorders, Volume 2: Non-Infectious Disorders, J.F. Leatherland & P.T.K. Woo (eds.), pp. 1–17, CAB International, Cambridge, MA.

Butler, R.J. (2002). Status assessment report for the rayed bean, *Villosa fabalis*, occurring in the Mississippi River and Great Lakes systems (U.S. Fish and Wildlife Service Regions 3,4, 5 and Canada). Ohio River Valley Ecosystem Team, Mollusk Subgroup, Robert S. Butler, leader. U.S. Fish and Wildlife Service, Asheville, NC.

Butler, J.R.A., P.D. Cunningham, and K. Starr (2005). The prevalence of escaped farmed salmon, *Salmo salar* L., in the River Ewe, western Scotland, with notes on their ages, weights and spawning distribution. *Fish. Manage. Ecol.* **12**(2): 149–159.

Chapman, D.W. (1966). Food and space as regulators of salmonid populations in streams. *Am. Nat.* **100**: 345–357.

Clifford, S.L., P. McGinnity, and A. Ferguson (1998). Genetic changes in an Atlantic salmon population resulting from escaped juvenile farm salmon. *J. Fish Biol.* **52**: 118–127.

Cnaani, A., E. McLean, and E.M. Hallerman. 2013. Effects of growth hormone transgene expression and triploidy on acute stress indicators in Atlantic salmon (*Salmo salar* L.). *Aquaculture* **412-413**: 107–116.

Crossin, G.T., and R.A. Devlin (2017). Predation, metabolic priming and early life-history rearing environment affect the swimming abilities of growth hormone transgenic rainbow trout. *Biol. Lett*. **13**:20170279.

Crossin, G.T., L.F. Sundström, W.E. Vandersteen, and R.H. Devlin (2015). Early life-history consequences of growth-hormone transgenesis in rainbow trout reared in stream ecosystem mesocosms. PLoS ONE **10(3)**: e0120173.

Cook, J.T., M.A. McNiven, and A.M. Sutterlin (2000a). Metabolic rate of pre-smolt growth-enhanced transgenic Atlantic salmon (*Salmo salar*). *Aquaculture* **188**: 33–45.

Cook, J.T., A.M. Sutterlin, and M.A. McNiven (2000b). Effect of food deprivation on oxygen consumption and body composition of growth-enhanced transgenic Atlantic salmon (*Salmo salar*). *Aquaculture* **188**: 47–63.

Cook, J.T., M.A. McNiven, G.F. Richardson, *et al.* (2000c). Growth rate, body composition, and feed digestibility/conversion of growth-enhanced transgenic Atlantic salmon. *Aquaculture* **188:** 15–32.

Cotter, D., V. O'Donovan, N. O'Maoiléidigh, *et al.* (2000). An evaluation of the use of triploid Atlantic salmon (*Salmo salar* L.) in minimizing the impact of escaped farmed salmon on wild populations. *Aquaculture* **186**: 61–75.

Deitch, E.J., G.L. Fletcher, L.H. Petersen, *et al.* (2006). Cardiorespiratory modifications, and limitations, in post-smolt growth hormone transgenic Atlantic salmon *Salmo salar*. *J. Exp. Biol.* 209: 1310–1325.

Devlin, R.H., C.A. Biagi, and T.Y. Yesaki (2004a). Growth, viability and genetic characteristics of GH transgenic coho salmon strains. *Aquaculture* **236**: 607–632.

Devlin, R.H., M. D'Andrade, M. Uh, *et al.* (2004b). Population effects of growth hormone transgenic coho salmon depend on food availability and genotype by environment interactions. *Proc. Natl. Acad. Sci. USA* **101**: 9303–9308.

Devlin, R.H., J.I. Johnsson, D.E. Smailus, *et al.* (1999). Increased ability to compete for food by growth hormone-transgenic coho salmon *Oncorhynchus kisutch* (Walbaum). *Aquacult. Res.* **30**: 479–482.

Devlin, R.H., L.F. Sundström, and R.A. Leggatt (2015). Assessing ecological and evolutionary consequences of growth-accelerated genetically engineered fishes. *BioScience* 65:685–700.

Devlin, R.H., L.F. Sundström, and W.M. Muir (2006). Interface of biotechnology and ecology for environmental risk assessments of transgenic fish. *Trends Biotechnol.* **24**(2): 89–97.

Devlin, R.H., T.Y. Yesaki, E.M. Donaldson, *et al.* (1995a). Production of germline transgenic Pacific salmonids with dramatically increased growth performance. *Can. J. Fish. Aquat. Sci.* **52**: 1376–1384.

Devlin, R.H., T.Y. Yesaki, E.M. Donaldson, *et al.* (1995b). Transmission and phenotypic effects of an antifreeze/GH gene construct in coho salmon (*Oncorhynchus kisutch*). *Aquaculture* **137**: 161–169.

DeVore, P.W., and J.G. Eaton (1983). An investigation of spinal deformity of trout (*Salmo* sp.) in the Brule River, Wisconsin. *J. Great Lakes Res.* **9**(1): 69–73.

Dill, W.A. and A.J. Cordone (1997). History and status of introduced fishes in California, 1871-1996. *Calif. Fish Game Fish. Bull*. **178**, 414 pp.

Down, N.E., E.M. Donaldson, H.M. Dye, *et al.* (1989). A potent analog of recombinant bovine somatotropin accelerates growth in juvenile Coho salmon (*Oncorhynchus kisutch*). *Can. J. Fish. Aquat. Sci.* **46**: 178–183; as cited in Kapuscinski and Hallerman, 1991.

Du, S.J., Z. Gong, G.L. Fletcher, *et al.* (1992). Growth enhancement in transgenic Atlantic salmon by the use of an "all fish" chimeric growth hormone gene construct. *Bio/Technology* **10**: 176–181.

Elliott, J.M. (1981). Some aspects of thermal stress on freshwater teleosts. In: <u>Stress and Fish</u>, A.D. Pickering (ed.), pp. 209–245. Academic Press, London.

Elliott, J.M. (1991). Tolerance and resistance to thermal stress in juvenile Atlantic salmon, *Salmo salar*. *Freshwater Biol.* **25**: 61–70.

EPA (1992). Framework for Ecological Risk Assessment. Risk Assessment Forum, U.S. Environmental Protection Agency. EPA/630/R-92/001.

FWS (2009). Species profile for Atlantic salmon (*Salmo salar*), Available at https://ecos.fws.gov/ecp0/profile/speciesProfile?spcode=E07L. Accessed 12/19/2017.

Fjelldal, P.G. and T. Hansen. (2010). Vertebral deformities in triploid Atlantic salmon (*Salmo salar* L.) underyearling smolts. *Aquaculture* **309**: 121–136.

Fjelldal, P.G., T. Hansen, O. Breck, *et al*. (2012). Vertebral deformities in farmed Atlantic salmon (*Salmo salar* L.) – etiology and pathology. *J. Appl. Ichthyol*. **28**: 433–440.

Fleming, I.A. (1996). Reproductive strategies of Atlantic salmon: ecology and evolution. *Rev. Fish Biol. Fisheries* **6**: 379–416.

Fleming, I.A. (1998). Pattern and variability in the breeding system of Atlantic salmon (*Salmo salar*), with comparisons to other salmonids. *Can. J. Fish. Aquat. Sci.* **55**(Suppl. 1): 59–76.

Fleming, I.A., K. Hindar, I.B. Mjolnered, *et al.* (2000). Lifetime success and interactions of farm salmon invading a native population. *Proc. R. Soc. Lond. B* **267**: 1517–1524.

Fleming, I.A., T. Agustsson, B. Finstad, *et al.* (2002). Effects of domestication on growth physiology and endocrinology of Atlantic salmon (*Salmo salar*). *Can. J. Fish. Aquat. Sci.* **59**: 1323–1330.

Frankham, R. (1995). Conservation genetics. *Ann. Rev. Gen*. **29**: 305–327.

Fraser, T.W.K., T. Hansen, J.E. Skjaeraasen, *et al*. (2013). The effect of triploidy on the culture performance, deformity prevalence, and heart morphology in Atlantic salmon. *Aquaculture* **416-417**: 255–264.

Galbreath, P.F. and G.H. Thorgaard (1995).Sexual maturity and fertility of diploid and triploid Atlantic salmon X brown trout hybrids. *Aquaculture* **137**: 299–311.

Garside, E.T. (1973). Ultimate upper lethal temperature of Atlantic salmon (*Salmo salar* L.). *Can. J. Zool.* **51**: 898–900; as cited in Amiro, 2006.

Gjedrem, T., H.M. Gjøen, and B. Gjerde (1991). Genetic origin of Norwegian farmed salmon. *Aquaculture* **98**: 41–50.

Gjøen, H. M., and H.B. Bentsen (1997). Past, present, and future of genetic improvement in salmon aquaculture. *ICES J. Mar. Sci.* **54**: 1009–1014.

Gray, A.K., M.A. Evans, and G.H. Thorgaard (1993). Viability and development of diploid and triploid salmonids hybrids. *Aquaculture* **112**: 125–142.

Hallerman, E.M., E. McLean, and I.A. Fleming (2007). Effects of growth hormone transgenes on the behavior and welfare of aquacultured fishes: A review identifying research needs. Appl. Anim. Behav. Sci. 104(3-4): 265–294.

Hansen, L.P. (2006). Migration and survival of farmed Atlantic salmon (*Salmo salar* L.) released from two Norwegian fish farms. *ICES J. Marine Science* **63**: 1211–1217.

Hansen, T.J., R.E. Olsen, L. Stien, F. Oppedal, T. Torgerson, O. Breck, M. Remen, T. Vågseth, and P.G. Fjelldal (2015).Effect of water oxygen level on performance of diploid and triploid Atlantic salmon post-smolts reared at high temperature. *Aquaculture* **435**: 354–360.

Hindar, K. (1993). Genetically engineered fish and their possible environmental impact, Norsk Institutt for Naturforskning (NINA), *Oppdragsmelding* **215**: 1–48.

Hindar, K. (2001). Chapter 3. Interactions of cultured and wild species. In: <u>Marine Aquaculture in the Environment: A meeting for stakeholders in the Northeast,</u> M.F. Tlusty, D.A. Bengston, H.O. Halverson, *et al*. (eds.); Cape Cod Press, Falmouth, MA, 325 pp.

Hislop, J.R.G. and J.H. Webb (1992). Escaped farmed Atlantic salmon, *Salmo salar* L., feeding in Scottish coastal waters. *Aquacult. Fish. Manage*. **23**: 721–723.

Hurrell, R.H. and D.J. Price (1991). Natural hybrids between Atlantic salmon, *Salmo salar*, and brown trout, *Salmo trutta*, in juvenile salmonids populations in south-west England. *J. Fish Biol*., **39** (Suppl.A): 355–341.

Hutchings, J.A., and M.E.B. Jones (1998). Life history variation and growth rate thresholds for maturity in Atlantic salmon, *Salmo salar. Can. J. Fish. Aquat. Sci*. **55**(Suppl. 1): 22–47.

ICES [International Council for Exploration of the Sea] (2009). Report of the Working Group on North Atlantic Salmon (WGNAS). 30 March–8 April, Copenhagen, Denmark. ICES CM 2009/ACOM:06. 282 pp. Available at: http://www.ices.dk/sites/pub/Publication Reports/ICES Annual Report/ICES_Annual_Report_09.pdf . Accessed 12/20/2017.

Indiana Department of Environmental Management (IDEM). (2016). Total Maximum Daily Load Report for the Upper Mississinewa River Watershed (Draft). Available online at: http://www.in.gov/idem/nps/3918.htm. Accessed 11/09/2017.

Indiana Department of Natural Resources (IDNR) (2014). Mississinewa River trout stocking under review. Available at: http://www.in.gov/activecalendar_dnr/EventList.aspx?view=EventDetails&eventidn=6954&information_id=14080&type=&syndicate=syndicate. Accessed 11/09/2017.

Indiana Department of Natural Resources (IDNR) (2017). Indiana Floodplain Information Portal. Available at: http://dnrmaps.dnr.in.gov/appsphp/fdms/. Accessed 11/09/2017.

Inglis, V., G.N. Frerichs, S.D. Millar, *et al.* (1991). Antibiotic resistance of *Aeromonas salmonicida* isolated from Atlantic salmon, *Salmo salar* L., in Scotland. *J. Fish Dis.* **14**(3): 353–358.

Jansson, H., I. Holmgren, K. Wedin, and T. Andersson (1991). High frequency of natural hybrids between Atlantic salmon, *Salmo salar*, and brown trout, *Salmo trutta*, in a Swedish river. *J. Fish Biol*., **39**(Suppl.A): 343–348.

Johnsen, B., and A.J. Jensen (1991). The *Gyrodactylus* story in Norway. *Aquaculture* **98**: 289–302.

Johnsen, B.O., and A.J. Jensen (1994). The spread of furunculosis in salmonids in Norwegian rivers. *J. Fish Biol*. **45**(1): 47–55.

Johnsson, J.I., and B.Th. Björnsson (2001). Growth-enhanced fish can be competitive in the wild. *Funct. Ecol.* **15**: 654–659.

Jonsson, B., N. Jonsson, and L.P. Hansen (1991). Differences in life history and migratory behaviour between wild and hatchery-reared Atlantic salmon in nature. *Aquaculture* **98**: 69–78.

Jonsson, N., B. Jonsson, and L.P. Hansen (1997). Changes in proximate composition and estimates of energetic costs during upstream migration and spawning in Atlantic salmon *Salmo salar* L. *Anim. Ecol.* **66**: 425–436, as cited in Klemetsen *et al*., 2003.

Jonsson, N., B. Jonsson, and I.A. Fleming (1996). Does early growth cause a phenotypically plastic response in egg production of Atlantic salmon? *Funct. Ecol.* **10**: 89–96, as cited in Klemetsen *et al*., 2003.

Julien, H.P., and N.E. Bergeron (2006). Effect of fine sediment infiltration during the incubation period on Atlantic salmon (*Salmo salar*) embryo survival. *Hydrobiologia* **563**: 61–71.

Kapuscinski, A.R. (2005). Current scientific understanding of the environmental biosafety of transgenic fish and shellfish. *Rev. Sci. Tech. Off. Int. Epiz.* **24**(1): 309–322.

Kapuscinski, A.R., and D.J. Brister (2001). Genetic impacts of aquaculture. In: Environmental Impacts of Aquaculture, K.D. Black, (ed.), Sheffield, UK, Sheffield Academic Press, pp. 385-–415.

Kapuscinski, A.R., and E.M. Hallerman (1990). Transgenic fish and public policy: anticipating environmental impacts of transgenic fish. *Fisheries (Bethesda)* **15**(1): 2–11.

Kapuscinski, A.R., and E.M. Hallerman (1991). Implications of introduction of transgenic fish into natural ecosystems. *Can. J. Fish. Aquat. Sci.* **48**(Suppl.1): 99–107.

Kapuscinski, A.R., J.J. Hard, K.M. Paulson, *et al.* (2007). Approaches to assessing gene flow, In: Environmental Risk Assessment of Genetically Modified Organisms, Volume 3: Methods for Transgenic Fish, CAB International, Wallingford, Oxfordshire, UK.

King, T.L., S.T. Kalinowski, W.B. Schill, *et al.* (2001). Population structure of Atlantic salmon (*Salmo salar* L.): a range-wide perspective from microsatellite DNA variation. *Mol. Ecol.* **10**(4): 807–21.

Klemetsen, A., P.A. Amundsen, J.B. Dempson, *et al.* (2003). Atlantic salmon *Salmo salar* L., brown trout *Salmo trutta* L., and Arctic charr *Salvelinus alpinus* (L.): a review of aspects of their life histories. *Ecol. Freshw. Fish* **12**: 1–59.

Knapp, G., C.A. Roheim, and J.L. Anderson (2007). The great salmon run: Competition between wild and farmed salmon. TRAFFIC North America, World Wildlife Fund, Washington DC. Available at:
http://www.iser.uaa.alaska.edu/people/knapp/personal/pubs/TRAFFIC/The_Great_Salmon_Run.pdf. Accessed 12/19/2017.

Kvellestad, A., S. Høie, K. Thorud, *et al.* (2000). Platyspondyly and shortness of vertebral column in farmed Atlantic salmon *Salmo salar* in Norway - description and interpretation of pathologic changes. *Dis. Aquat. Org.* **39**(2): 97–108.

Lacroix, G.L., and I.A. Fleming (1998). Ecological and behavioural interactions between farmed and wild Atlantic salmon: consequences for wild salmon. Canadian Stock Assessment Secretariat, Department of Fisheries and Oceans, Res. Doc. 98/162, 25 pp.

Leclercq, E., J.F. Taylor, D. Fison, *et al.* (2011). Comparative seawater performance and deformity prevalence in out-of-season diploid and triploid Atlantic salmon (*Salmo salar*) post-smolts. *Comp. Biochem. Physiol.*, Part A **158**: 116–125.

Leggatt, R.A., C.A. Biagi, D. Sakhrani, R. Dominelli, E.J. Eliason, A.P. Farrell, and R.H. Devlin (2017a). Fitness component assessments of wild-type and growth hormone transgenic coho salmon reared in seawater mesocosms. *Aquaculture* **473**: 31–42.

Leggatt, R.A., L.F. Sundström, K. Woodward, and R.H. Devlin (2017b). Growth-enhanced transgenic coho salmon (Oncorhynchus kisutch) strains have varied success in simulated streams: implications for risk assessment. PLoS ONE **12(1)**: e0169991.

Lõhmus, M., L.F. Sundström, M. Björklund, and R.H. Devlin (2010). Genotype-temperature interaction in the regulation of development, growth, and morphometrics in wild-type, and growth-hormone transgenic coho salmon. PLoS ONE **5(4)**: e9980. doi:10.1371/journal.pone.0009980.

Lura, H., and H. Sægrov (1991). Documentation of successful spawning of escaped farmed female Atlantic salmon, *Salmo salar*, in Norwegian rivers. *Aquaculture* **98**(1-3): 151–159.

Madsen, L., and I. Dalsgaard (1999). Vertebral column deformities in farmed rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* **17**(1-2): 41–48.

Mair, G.C., Y.K. Nam, and I.I. Solar (2007). Risk management: reducing risk through confinement of transgenic fish. In: Environmental Risk Assessment of Genetically Modified Organisms, Volume 3: Methods for Transgenic Fish, A. R. Kapuscinski *et al.* (eds.), CAB International, Wallingford, Oxfordshire, UK.

Mbuthia, P.G. (1994). Scoliosis of farmed rainbow trout (*Salmon gairdneri*, Richardson) in Kiambu District, Kenya. *Bull. Anim. Health Prod. Africa* **42**: 111–115.

McConnell, S.K., P. O'Reilly, L. Hamilton, *et al.* (1995). Polymorphic microsatellite loci from Atlantic salmon (*Salmo salar*): genetic differentiation of North American and European populations. *Can. J. Fish. Aquat. Sci.* **52**: 1863–1872.

McCormick, S.D., R.L. Saunders, E.B. Henderson *et al.* (1987). Photoperiod control of parr-smolt transformation in Atlantic salmon (*Salmo salar*): changes in salinity tolerance, gill Na$^+$,K$^+$-ATPase activity, and plasma thyroid hormones. *Can. J. Fish. Aquat. Sci.* **44**: 1462–1468.

McGinnity, P., C. Stone, J.B. Taggart, *et al.* (1997). Genetic impact of escaped farmed Atlantic salmon (*Salmo salar* L.) on native populations: use of DNA profiling to assess freshwater performance of wild, farmed, and hybrid progeny in a natural river environment. *ICES J. Mar. Sci*. **54**: 998–1008.

McGinnity, P., P. Prodöhl, A. Ferguson, *et al.* (2003). Fitness reduction and potential extinction of wild populations of Atlantic salmon, *Salmo salar*, as a result of interactions with escaped farm salmon. *Proc. R. Soc. Lond. B* **270**: 2443–2450.

McGowan, C. and W.S. Davidson (1992). Unidirectional natural hybridization between brown trout (*Salmo trutta*) and Atlantic salmon (*S. salar*) in Newfoundland. *Canadian Journal of Fisheries and Aquatic Sciences* **49(9)**: 1953–1958.

McVicar, A.H. (1997). Disease and parasite implications of the coexistence of wild and cultured Atlantic salmon populations*. ICES J. Mar. Sci*. **54**(6): 1093–1103.

McVicar, A.H, G. Olivier, G.S. Traxler, *et al.* (2006). Cultured and wild fish disease interactions in the Canadian marine environment. In: A Scientific Review of the Potential Environmental Effects of Aquaculture in Aquatic Ecosystems - Volume 4. Fisheries and Oceans Canada. Can. Tech. Rep. Fish. Aquat. Sci., 2450: x + 139 p. Available at: http://www.dfo-mpo.gc.ca/aquaculture/publications/sok-edc/volume4/mcvicar-eng.html. Accessed 12/20/2017.

Metcalfe, N.B., F.A. Huntingford, and J.E. Thorpe (1988). Feeding intensity, growth rates, and the establishment of life-history patterns in juvenile Atlantic salmon *Salmo salar*. *J. Anim. Ecol.* **57**: 463–474; as cited in Klemetsen *et al*., 2003.

Metcalfe, N.B., S.K. Valdimarsson, and I.J. Morgan (2003). The relative roles of domestication, rearing environment, prior residence and body size in deciding territorial contests between hatchery and wild juvenile salmon. *J. App. Ecol*. **40**: 535–544, as cited in Klemetsen *et al.,* 2003.

Moreau, D.T.R. (2011). Potential for ecological effects and gene flow resulting from growth hormone transgenic Atlantic salmon (*Salmo salar*) interactions with wild specific. Ph.D. Thesis. Memorial University of Newfoundland. St. John's, Newfoundland & Labrador, Canada.

Moreau, D.T.R. (2014) Ecological Risk Analysis and Genetically Modified Salmon: Management in the Face of Uncertainty. *Annu. Rev. Anim. Biosci.* **2**: 515–533.

Moreau, D.T.R. and I.A. Fleming (2011). Enhanced growth reduces precocial male maturation in Atlantic salmon. *Functional Ecology* **26**: 399–405.

Moreau, D.T.R., C. Conway, and I.A. Fleming (2011a). Reproductive performance of alternative male phenotypes of growth hormone transgenic Atlantic salmon (*Salmo salar*). *Evolutionary Applications* **4:** 736-748.

Moreau, D.T.R., I.A. Fleming, G.L. Fletcher, and J.A. Brown (2011b). Growth hormone transgenesis does not influence territorial dominance or growth and survival or first-feeding Atlantic salmon *Salmo salar* in food-limited stream microcosms. *J. Fish Biol*., **78**: 726–740.

Moreau, D.T.R., A.K. Gamperl, G.L. Fletcher, and I.A. Fleming (2014). Delayed phenotypic expression of growth hormone transgenesis during early ontogeny in Atlantic salmon (*Salmo salar*)? PLoS ONE **9(4)**: e95853.

Mori, T. and R. Devlin. (1999).Transgene and host growth hormone gene expression in pituitary and nonpituitary tissues of normal and growth hormone transgenic salmon. *Molecular and Cellular Endocrinology* **149**: 129–139.

Muir, W.M. (2004). The threats and benefits of GM fish. *EMBO Rep.* **5**(7): 654–659.

MUNLV (2001). [Migratory Fish Program Nordrhein-Westfalen - Status report through the first program phase]. NRW, Dusseldorf, 112 pp; as cited in Teufel *et al*., 2002.

NASCO (2007). Report of the 24th meeting of the council, 362pp. Available at: http://www.nasco.int/pdf/reports_annual/2007%20Council%20Report.pdf . Accessed 12/20/2107.

Nielsen, E.E., M.M. Hansen, and V. Loeschcke (1997). Analysis of microsatellite DNA from old scale samples of Atlantic salmon *Salmo salar*: a comparison of genetic composition over 60 years. *Molec. Ecol.* **6**: 487–492.

National Oceanic and Atmospheric Administration (NOAA). (2017). National weather Service. Delaware County Tornadoes, 1950-2016. Available at: https://www.weather.gov/ind/delaware_torn. Accessed 11/09/2017.

National Oceanic and Atmospheric Administration (NOAA) (2018). National Centers for Environmental Information: Climate at a Glance. Available at: https://www.ncdc.noaa.gov/cag/time-series/us/12/6/tavg/all/1/1987-2017?base_prd=true&firstbaseyear=1901&lastbaseyear=2000. Accessed 4/18/2018.

NRC (2002). Animal Biotechnology: Science-based Concerns. Board on Agriculture and Natural Resources, Board on Life Sciences, The National Academies Press, Washington, DC, 200 pp.

NRC (2003). Atlantic salmon in Maine. Board on Environmental Studies and Toxicology, The National Academies Press, Washington, DC, 276 pp.

NRC (2004). Biological Confinement of Genetically Engineered Organisms. Board on Agriculture and Natural Resources, Board on Life Sciences, The National Academies Press, Washington, DC, 255 pp.

Oke, K.B., P.A. Westley, D.T.R. Moreau, and I.A. Fleming (2013). Hybridization between genetically modified Atlantic salmon and wild brown trout reveals novel ecological interactions. *Proc. R. Soc. B* **280**: 20131047. http://dx.doi.org/10.1098/rspb.2013.1047.

Oliver, G. (2002). Disease interactions between wild and cultured fish – Perspectives from the American Northeast (Atlantic Provinces). Bull. Eur. Ass. Fish Pathol., **22**(2): 103–109.

Olsen, R.E. and O.T. Skilbrei (2010). Feeding preference of recaptured Atlantic salmon *Salmo salar* following simulated escape from fish pens during autumn. *Aquacult. Environ. Interact.* **1**: 167–174.

Organisation for Economic Cooperation and Development (OECD) (2017). Consensus Document on the Biology of Atlantic Salmon (Salmo salar). Sereis on Harmonisation of Regulatory Oversight in Biotechnology No. 64. ENV/JM/MONO(2017)64.

Ørnsrud, R., L. Gil, and R. Waagbø (2004). Teratogenicity of elevated egg incubation temperature and egg vitamin A status in Atlantic salmon, *Salmo salar* L. *J. Fish. Dis.* **27**(4): 213–223.

PCHB (Pollution Control Hearing Board of Washington) (1998). Final Findings of Fact, Conclusions of Law and Order. PCHB No. 96-257 et seq. NPDES Permit Appeals, November 30, 1998.

Piferrer, F., A. Beaumont, J.-C. Falguière, *et al.* (2009). Polyploid fish and shellfish: production, biology and applications to aquaculture for performance improvement and genetic containment. *Aquaculture* **293**: 125–156.

Pimm, S.L. (1984). The complexity and stability of ecosystems. *Nature* **307**: 321–326; as cited in NRC, 2002.

Polymeropoulous, E.T., D. Plouffe, S. LeBlanc, N.G. Elliott, S. Currie, and P.B. Frappell (2014). Growth hormone transgenesis and polyploidy increase metabolic rate, alter the cardiorespiratory response to acute hypoxia in Atlantic salmon (*Salmo salar*) yolk-sac alevins. *Journal of Experimental Biology* **217**: 2268–2276.

Power, M.E. (1990). Effects of fish in river food webs. *Science* **250**: 811–814.

Powers, D. (1989). Fish as model systems. *Science* **246**: 352–357; as cited in Kapuscinski & Hallerman, 1991.

Poynton, S.L. (1987). Vertebral column abnormalities in brown trout, *Salmo trutta* L. *J. Fish. Dis.* **10**(1): 53–57.

Raven, P.A., R.H. Devlin, and D.A. Diggs (2006). Influence of dietary digestible energy content on growth, protein and energy utilization and body composition of growth hormone transgenic and non-transgenic coho salmon (*Oncorhynchus kisutch*). *Aquaculture* **254**: 730–747.

Reddin, D.G. (2006). Perspectives on the marine ecology of Atlantic salmon (*Salmo salar*) in the Northwest Atlantic. Canadian Science Advisory Secretariat, Department of Fisheries and Oceans, Res. Doc. 2006/018, 44 pp.

Refstie, T. and T. Gjedrem (1975). Hybrids between Salmonidae species, hatchability and growth rate in the freshwater period. *Aquaculture* **6**: 333–342.

Roberts, R.J., R.W. Hardy, and S.H. Sugiura (2001). Screamer disease in Atlantic salmon, *Salmo salar* L., in Chile. *J. Fish. Dis.* **24**(9): 543–549.

Ryman, N., and F. Utter (eds., 1987). Population Genetics and Fishery Management. University of Washington Press, Seattle.

Ryman, N., F. Utter, and L. Laikre (1995). Protection of intraspecific biodiversity of exploited fishes. *Rev. Fish Biol. Fisheries* **5**(4): 417–446.

Sægrov, H., K. Hindar, S. Kålås, *et al.* (1997). Escaped farmed Atlantic salmon replace the original salmon stock in the River Vosso, western Norway. *ICES J. Mar. Sci.* **54**(6): 1166–1172.

Sambraus, F., R.E. Olsen, M. Remen, T.J. Hansen, T. Torgerson, and P.G. Fjelldal (2017). Water temperature and oxygen: The effect of triploidy on performance and metabolism in farmed Atlantic salmon (*Salmo salar* L.) post-smolts. Aquaculture **473**: 1–12.

Saunders, R.L. (1991). Potential interaction between cultured and wild Atlantic salmon. *Aquaculture* **98**: 51–60.

Saunders, R.L., G.L. Fletcher, and C.L. Hew (1998). Smolt development in growth hormone transgenic salmon. *Aquaculture* **168**: 177–193.

Scott, W.B., and E.J. Crossman (1973). Freshwater fishes of Canada. Fisheries Research Board of Canada; as cited in Teufel *et al.*, 2002.

Shepherd, J., and N. Bromage (1995). Intensive Fish Farming. Oxford, UK. 404 pp; as cited in Teufel *et al.*, 2002.

Silverstone, A.M. and L. Hammell (2002). Spinal deformities in Atlantic salmon. *Can. Vet. J.* **43**(10): 782–784.

Snow, A.A., D.A. Andow, P. Gepts, *et al.* (2005). Genetically engineered organisms and the environment: current status and recommendations. *Ecol. Appl.* **15**(2): 377–404.

Soto, D., F. Jara, and C. Moreno (2001). Escaped salmon in the inner seas, southern Chile: facing ecological and social conflicts. *Ecological Applications* **11**: 1750–1762

Ståhl, G. (1987). Genetic population structure of Atlantic salmon, In: Population Genetics and Fishery Management, N. Ryman and F. Utter (eds.), pp. 121–140. University of Washington Press, Seattle, WA.

Stead, S.M., and L. Laird (2002). Handbook of Salmon Farming. Springer-Praxis, Ltd.

Stevens, E.D., and A. Sutterlin (1999). Gill morphology in growth hormone transgenic salmon. *Environ. Biol. Fish.* **54**: 411–415; as cited in NRC, 2002.

Stevens, E.D., A. Sutterlin, and T.J. Cook (1998). Respiratory metabolism and swimming performance in growth hormone transgenic Atlantic salmon. *Can. J. Fish. Aquat. Sci.* **55**: 2028–2035.

Sundström, L.F., M. Lõhmus, R.H. Devlin, *et al.* (2004). Feeding on profitable and unprofitable prey: comparing behaviour of growth-enhanced transgenic and normal coho salmon (*Oncorhynchus kisutch*). *Ethology* **110**: 381–396.

Sundström, L.F., M. Lõhmus, R.H. Devlin (2016). Gene-environment interactions influence feeding and anti-predator behavior in wild and transgenic coho salmon. Ecological Applications **26(1):** 67–76.

Sundström, L.F., M. Lõhmus, W.E. Tymchuk *et al.* (2007). Gene–environment interactions influence ecological consequences of transgenic animals. *Proc. Nat. Acad. Sci. USA* **104**(10): 3889–3894.

Sutterlin, A.M., J. Holder, and T.J. Benfey (1987). Early survival rates and subsequent morphological abnormalities in landlocked, anadromous and hybrid (landlocked x anadromous) diploid and triploid Atlantic salmon. *Aquaculture* **64**: 157–164.

Taggart, J.B., E. Verspoor, P.T. Galvin, *et al.* (1995). A minisatellite DNA marker for discriminating between European and North American Atlantic salmon (*Salmo salar*). *Can. J. Fish. Aquat. Sci.* **52**: 2305–2311.

Taylor, J.F., A.C. Preston, D. Guy, and H. Migaud (2011). Ploidy effects on hatchery survival, deformities, and performance in Atlantic salmon (*Salmo salar*). *Aquaculture* **315**: 61–68.

Templeton, A.R. (1986). Coadaptation and outbreeding depression. In: <u>Conservation Biology: The Science of Scarcity and Diversity</u>, M. E. Soule (ed.), p. 105–116. Sinauer Assoc., Sunderland, MA.

Tessier, N., and L. Bernatchez (1999). Stability of population structure and genetic diversity across generations assessed by microsatellites among sympatric populations of landlocked Atlantic salmon (*Salmo salar*). *Molec. Ecol.* **8**(2): 169–179.

Teufel, J., F. Pätzold, and C. Potthof (2002). Specific research on transgenic fish considering especially the biology of trout and salmon. Öko-Institut e.V., Institut für Angewandte Ökologie and Pätzolg Gewässerökologie. Research Report 360 05 023, 177 pp.

Thorgaard, G.H. (1983). Chromosome set manipulation and sex control in fish. In: <u>Fish Physiology</u>, Vol. 9B, W.S. Hoar, D.J. Randall, and E.M. Donaldson (eds.), pp. 405–434. Academic Press, New York.

Thorpe, J.E., M.S. Miles, and D.S. Keay (1984). Developmental rate, fecundity and egg size in Atlantic salmon, *Salmo salar* L. *Aquaculture* **43**: 289–305, as cited in Klemetsen *et al,.* 2003.

Tibbets, S.M., C.L. Wall, V. Barbosa-Solomieu, M.D. Bryenton, D.A. Plouffe, J.T. Buchanan, and S.P. Lall (2013). Effects of combined 'all-fish' growth hormone transgenics and triploidy on growth and nutrient utilization of Atlantic salmon (*Salmo salar* L.) fed a practical grower diet of known composition. *Aquaculture* **406-407**: 141–152.

Vågsholm, I., and H.O. Djupvik (1998). Risk factors for spinal deformities in Atlantic salmon, *Salmo salar* L. *J. Fish. Dis.* **21**(1): 47–53.

Verspoor, E. (1988). Widespread hybridization between native Atlantic salon, *Salmo salar*, and introduced brown trout, *S. trutta*, in eastern Newfoundland. *J. Fish Biology* **32**: 327–334.

Waknitz, F., R.N. Iwamoto, and M.S. Strom (2003). Interactions of Atlantic salmon in the Pacific Northwest. IV. Impacts on the local ecosystems. *Fisheries Research* **62**: 307–328.

Webb, J.H., D.W. Hay, P.D. Cunningham, *et al.* (1991). The spawning behaviour of escaped farmed and wild adult Atlantic salmon (*Salmo salar* L.) in a northern Scottish river. *Aquaculture* **98**(1-3): 97–110.

Webb, J.H., A.F. Youngson, C.E. Thompson, *et al.* (1993). Spawning of escaped farmed Atlantic salmon, *Salmo salar* L, in western and northern Scottish rivers: egg deposition by females. *Aquacult. Fish. Manage*. **24**: 663–670.

Webb, J., E. Verspoor, N. Aubin-Horth, A. Romakkaniemi, and P. Amiro (2007). The Atlantic Salmon. In: The Atlantic Salmon: Genetics, Conservation, and Management, pp. 17–56. E. Verspoor, L. Strademeyer, and J.L. Nielsen (eds.). Blackwell Publishing Ltd.

White, H.C. (1940). "Sea lice" (*Lepeophtheirus*) and death of salmon. *J. Fish. Res. Bd. Can.* **5**: 172–175.

Whoriskey, F.G., P. Brooking, G. Doucette, S. Tinker, and J.W. Carr. (2006). Movements and survival of sonically tagged farmed Atlantic salmon released in Cobscook Bay, Maine, USA. *ICES J. Marine Science* **63**: 1218–1223.

Wilkins, N.P., D. Cotter, and N. O'Maoiléidigh (2001). Ocean migration and recaptures of tagged, triploid, mixed sex and all-female Atlantic salmon (*Salmo salar* L.) released from rivers in Ireland. *Genetica* **111**: 107–212, as cited in Pifferer *et al.*, 2009.

Willoughby, S. (1999). Manual of Salmonid Farming. Fishing News Books, Blackwell Science.

Wong, A.C., and A.L. Van Eenennaam (2008). Transgenic approaches for the reproductive containment of genetically engineered fish. *Aquaculture* **275**: 1–12.

Yeager, B.L., and R.J. Neves (1986). Reproductive cycle and fish hosts of the rabbit's foot mussel, *Quadrula cylindrical strigillata* (Mollusca: Unionidae) in the Upper Tennessee River drainage. *The American Midland Naturalist:* 329–340.

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

The eggs are buried in gravel at a depth of about 12-25 cm (Bigelow, 1963; Scott and Crossman, 1973). The female rests after spawning and then repeats the operation, creating a new redd, depositing more eggs, and resting again until spawning is complete. The male continues to guard the female, and to drive away competitors aggressively until she has completed making redds and depositing her eggs. This may take as long as a week, and require the building of up to seven redds to deposit her nearly 7,500 eggs. Thereafter, the post-spawn adult fish, or kelt, may return to the ocean without delay, move to a pool down-river for a period of rest, or over-winter in the nursery river and return to sea in the spring. Many kelt do not survive the first mating; some survive to mate twice, but very few mature 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, which are referred to as **parr marks**, aid in camouflaging the young fish, which are preyed upon by other fish, as well as mammals and birds that live along rivers and streams. At this stage, the juveniles are referred to as "**parr.**" They remain in their **natal** (birth) **streams**, feeding on the larvae of insects, worms, and shellfish, and sometimes each other or related species (such as trout).

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

Parr may spend between one and eight years (usually two to three years) in their natal streams; at some point, if they are not in land-locked lakes, they begin their downstream migration and prepare for life in the sea. They are usually about 10-22 cm long at this point in their development (OECD, 2017).

The seaward migration involves a change in physiology which allows the young salmon to adapt to salt water conditions. This transformation in physiology is referred to as "smoltification" and the young fish that migrate to the sea are called "**smolts.**" In general, smolts tend to live for a while in brackish (part salt) water, such as bays and estuaries while they complete their adaptation to salt water. It is thought that the "imprinting" of the natal river occurs during smoltification[25]. 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 sometime in April or May, and can be found in the waters off Labrador and Newfoundland by mid-summer. They then migrate to take advantage of available food supplies and generally spend their first winter at sea off the coast of Greenland. 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)

---

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

fish. In general, the longer a salmon spends at sea feeding, the larger it becomes, although Atlantic salmon rarely get bigger than about 25 pounds.

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

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

A few salmon never make the transition to 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[26] 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

---

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

expenditure due to spawning: 1-SW 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 and 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 approximately 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 Services' (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 (Knapp *et al*., 2007).

The Northeast Fishery Management Council developed a Fishery Management Plan for Atlantic Salmon in 1988. This authority extends over all Atlantic salmon of United States origin, and prohibits "possession" of Atlantic salmon, either as the intended catch of commercial fishing, or as the indirect (by-catch) result of fishing for other fish. Commercial fishing of wild Atlantic salmon is now prohibited in U.S. federal waters, although recreational fishing is allowed. (Commercial fishing of wild Atlantic salmon still occurs off the coast of Greenland, where adult Atlantic salmon feed).

There is now a Recovery Plan for the Gulf of Maine Population Segment of Atlantic salmon, which identifies steps that need to be taken to stop the decline of the population.[27] 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

---

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

in pools. It is difficult to characterize the extent of competitive interactions in marine waters due to the vast scale of the habitat that is used.

Predators of smolt and juvenile salmon in fresh water include birds, reptiles, mammals, and other fish (including salmon and trout); predators in estuaries, coastal waters, and the sea include birds, fish, and mammals.

In fresh water, juvenile salmon are opportunistic predators of invertebrates, especially those drifting at the surface (including mayflies, stoneflies, caddisflies, midges, and beetles). Larger parr eat fish (including smaller trout and salmon) and their eggs. In marine waters, post-smolts feed primarily on small fish and crustaceans such as euphausiids (krill), amphipods (scud), copepods, and crab larvae. Large juveniles prey mostly upon fish.

A.6     Domesticated and Wild Salmon

General practices used in salmon aquaculture are presented in this section; specific production and grow-out practices for AquAdvantage Salmon are described in Section 5 of this EA. This section of the appendix discusses information about the interaction of domestic salmon with their wild counterparts to provide context for predicting how AquAdvantage Salmon might fare in the unlikely event that they would be released into the wild.

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 local line), achieved a growth rate improvement of about 10% per generation over the first seven generations of development (Gjøen and Bentsen, 1997).

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

Under ambient light and temperature conditions, the freshwater phase typically takes 14-16 months, but is often shortened to eight months by increasing the early-rearing temperature

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

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 are:

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

- Genetic disturbance caused by transmission of fitness-reducing alleles (Ryman and Utter, 1987; Frankham, 1995), disruption of locally-evolved allelic combinations (Templeton, 1986; Ryman *et al*., 1995; McGinnity *et al*., 2003), or "swamping" of the native gene pool (Sægrov *et al*., 1997);

- Direct competition for environmental resources, such as habitat, food, or mating opportunities (McGinnity *et al*., 1997; Fleming *et al*., 2000); and

- Ecological disturbance through interference competition or disruption of local equilibria in complex systems, such as food webs, predator-prey relationships, or migration patterns (Lacroix and Fleming, 1998).

To provide additional context for potential application to 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 and Jensen, 1994; Inglis *et al*., 1991). There is little direct evidence of bacterial disease transmission from commercial to wild salmon. None of the reviews that have evaluated the available scientific literature on the potential for disease interchange between wild and farmed salmon has found irrevocable evidence that fish farming has contributed to detectable adverse changes in wild fish populations (McVicar *et al*., 2006).

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

- o The occurrence and persistence of the infection in the source population;

- o The availability of susceptible potential new hosts;

- o The viability and concentration of the infectious organism in the environment; and

- o 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 and Harris, 1998). Salmon lice, *Lepeophtheirus salmonis*, are endemic throughout the native range of Atlantic salmon, making a direct link to salmon aquaculture difficult to establish. White (1940) associated the occurrence of "white spot" and salmon mortalities with sea lice infections in wild Atlantic salmon populations in eastern Canada as early as 1940, well before the advent of commercial salmon farming. Natural populations of parasites may be amplified in areas associated with salmon farming (Bakke and Harris, 1998), but sea lice abundance may be associated with rising marine temperatures as much as with the availability of hosts.

*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 and brown trout) salmonid predators. Despite these challenges, evidence of genetically-differentiated population structuring is still evident for salmon at local, regional, and continental scale based on allozyme, mitochondrial, and nuclear DNA analyses (Ståhl, 1987; Bourke *et al*., 1997; Bermingham *et al*., 1991; McConnell *et al*., 1995; Taggart *et al*., 1995; King *et al*., 2001). The temporal stability of this structure has been traced over decades

through the analysis of genetic material contained in archived scales (Nielsen *et al*., 1997; Tessier and Bernatchez, 1999).

Farmed salmonid strains are typically genetically distinct from local wild populations because of breeding and selection practices that have been designed primarily to optimize growth rates and other commercially desirable traits. As a result, many farmed strains used in Ireland and Scotland are of Norwegian origin. Escaped farmed salmon can interbreed with local populations, intermixing their genomes with the locally adapted populations (Teufel *et al*., 2002). The persistence of genetic population structuring, even in the extreme circumstance of low population abundance and significant management intervention, indicates a degree of genetic resilience in locally-adapted wild populations (NRC, 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 and Sægrov, 1991; Webb *et al*., 1991), only a small proportion of the number that escape from farms actually breed, (Webb *et al*., 1993; Clifford *et al*., 1998) and then at a fraction of the spawning rate of wild Atlantic salmon (Fleming, 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 and Fleming, 1998).

Although farmed salmon have been known to enter marine systems in large numbers by escape from containment nets, they can only become established by reproducing in adjacent freshwater ecosystems. Consequently, the fitness and behavior of feral[28] Atlantic salmon is of continuing interest as a matter of risk management in Atlantic salmon aquaculture, specifically with respect to the extent to which any homing migration imprinting may have occurred, the extent to which feral Atlantic salmon succeed in spawning, and the relative survival of their offspring. Escaped farmed salmon feed poorly in fresh and salt water and may not begin feeding on wild prey for a considerable period after escape owing to their acclimation to pelleted feed. For example, only 5-15% of escaped Atlantic salmon recovered from British Columbian and Alaskan waters had fed after their release (Alverson and Ruggerone, 1997).

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

---

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