Environmental and Indirect Human Health Risk Assessment of the AquAdvantage® Salmon

DRAFT IN REVISION

(PROTECTED B)

Office of Aquatic Biotechnology
Department of Fisheries and Oceans Canada

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1 EXECUTIVE SUMMARY

(To be completed after peer-review process)

2 TABLE OF CONTENTS

3 *LIST OF FIGURES*

4 LIST OF TABLES

5 *LIST OF TABLES IN APPENDICES*

[redacted]

6 *ACRONYMS*

AAS: AquAdvantage salmon

ABT: AquaBounty Technologies

CEPA 1999: *Canadian Environmental Protection Act, 1999*

COSEWIC: Committee on the Status of Endangered Wildlife in Canada

DU: Designatable Unit (COSEWIC)

DFO: Fisheries and Oceans Canada

DNA: Deoxyribonucleic acid

EC: Environment Canada

EPA: Environmental Protection Agency (of the United States)

FMA: Failure Modes Analysis

GE: Genetically engineered

GH: growth hormone

GxE: Gene by environment interaction

IGF-1: Insulin-like growth factor

MPA: marine protected areas

mRNA: Messenger RNA

NSNR(O): *New Substances Notification Regulations (Organisms)*

PEI: Prince Edward Island

RNA: Ribonucleic acid

SARA: *Species at Risk Act*

SOP: Standard Operating Procedures

7 *GLOSSARY*

AAS descendant: offspring of AAS that are produced in the wild environment and carry the *opAFP-GHc2* rDNA construct at the α-locus

Abiotic factors: physical, chemical and other non-living environmental factors

Abundance: the total number of individuals of a taxon or taxa in an area, community or population

AquAdvantage salmon (AAS): an Atlantic Salmon (*Salmo salar*) bearing the *opAFP-GHc2* rDNA construct at the α-locus in the EO-1α lineage

α-integrant: functional form of the opAFP-GHc2 transgene in the founder animal, EO-1.

Assessment endpoint: ecological entities that are susceptible to harm upon exposure to a stressor and should be protected to achieve established protection goals

Backcross: a mating between individuals of the parental generation (P) and the first generation of offspring (F_1)

Background genotype: the residual genotype; that part of the genome not primarily responsible for producing the phenotype

β-integrant: Non-functional form of the opAFP-GHc2 transgene in the founder animal, EO-1.

Biological containment: limiting gene flow from AAS into the receiving environment by preventing reproduction. This is typically accomplished by sterilization through induced triploidy, production of mono-sex (female only) populations, or a combination of both

Biological diversity: As defined in CEPA 1999, "biological diversity" means the variability among living organisms from all sources, including, without limiting the generality of the foregoing, terrestrial and marine and other aquatic ecosystems and the ecological complexes of which they form a part and includes the diversity within and between species and of ecosystems

CEPA Toxic: a substance or an organism that may enter the environment in a quantity or concentration or under conditions that (a) have or may have an immediate or long-term harmful effect on the environment or its biological diversity; (b) constitute or may constitute a danger to the environment on which life depends; or (c) constitute or may constitute a danger in Canada to human life or health.

Competition: the simultaneous demand by two or more organisms (competitors) or species for an essential common resource that is actually or potentially in limited supply (exploitative competition), or the detrimental interaction between two or more organisms or species seeking a common resource that is not limiting (interference competition)

Designatable Unit (DU) - COSEWIC guidelines state that "a population or group of populations may be recognized as a DU if it has attributes that make it "discrete" and evolutionarily "significant" relative to other populations". Evidence of discreteness can include "inherited traits (e.g. morphology, life history, behaviour) and/or neutral genetic markers (e.g. allozymes, DNA microsatellites…" as well as large disjunctions between populations, and occupation of different eco-geographic regions.

Diploid (2n): having two sets of homologous chromosomes, typical of most organisms derived from fertilized egg cells

Direct effect: impact resulting from interactions with AAS or AAS descendants

Dispersal: movement of an organism in its environment; movement of AAS away from its point of entry into the environment

Distribution: the geographical range of a taxon or group; the spatial pattern or arrangement of the members of a population or group

Diversity: the absolute number of species in an assemblage, community or sample; species richness; a measure of the number of species and their relative abundance in a community, assemblage or sample; the fact of being varied or different

Ecosystem: As defined in the CEPA 1999, "ecosystem" means a dynamic complex of plant, animal and micro-organism communities and their non-living environment interacting as a functional unit

Entry: loss of physical containment resulting in the release of AAS into the aquatic environment

EO-1: Mosaic, transgenic founder animal of the EO-1α line of AAS

EO-1α line: Commercial line of AAS derived from the founder animal, EO-1

EO-1α locus: Functional, stably integrated form of opAFP-GHc2 in the AAS genome

Established: growing and reproducing successfully in a given area as a self-sustaining population

Exposure: likelihood that the organism (AAS) will come into contact with susceptible species and/or environmental components in Canada

Exposure pathway: the physical route by which AAS or AAS descendants move from a source to assessment endpoints

Fate: the final outcome or expected result.

Frequency: the number of occasions that a given character, species or event occurs in a series of samples or for a given period of time

Genetic diversity: the existing genetic variation within a population; allelic composition and genomic organization of populations

Genotype x Environment (GxE) interactions: how the genotype interacts with the environment to shape the observed phenotype; the differential morphological, physiological or behavioral responses of two or more genotypes to environmental fluctuations; plasticity

Geographical containment: confinement of AAS by culturing the organism in a geographic location where it cannot survive if it enters the surrounding environment

Grow-out: in conventional fish farming, the phase during which juvenile fish are raised to market size for harvest

Habitat: Habitat is the area or type of site where an individual or wildlife species naturally occurs and depends on directly or indirectly to carry out its life processes. It includes the biological, chemical, and physical attributes of the environment that living organisms require to complete their life process and life cycle.

Habitat fragmentation: the spatial isolation of small habitat areas that compounds the effects of habitat loss on populations and biological diversity

Haploid (n): having only a single set of chromosomes; having the normal gametic chromosome number

Harmful effect: an immediate or long-term detrimental impact on the structure or function of the ecosystem including biological diversity

Hazard: potential to cause a harmful effect

Hemizygous: having one copy of a given gene or transgene in only one set of chromosomes in a diploid organism

Homozygous: having both chromosome sets in a diploid organism carry one copy of the same allele of a given gene or transgene

Horizontal gene transfer: the transfer of genes between organisms in a manner other than by conventional sexual or asexual reproduction

Hybridization: any crossing of individuals of different genetic composition, typically belonging to different strains or species

Indirect effect: impact resulting from the consequences of a direct effect

Indirect human health risk assessment: assessment of risk to human health resulting from environmental exposure to AAS

Introgression: stable integration of new genetic variation into a population by hybridization with individuals from a second population; the spread of genes from one species or population into the gene pool of another by hybridization and backcrossing

Invasiveness: property of an organism that arrived, established and spread in a new aquatic ecosystem and resulted in harmful consequences for the natural resources in the native aquatic ecosystem and/or the human use of the resource

Keystone species: a species that has a disproportionately large impact on ecosystem structure and function

Life cycle: The sequence of events from the origin as a zygote, to the death of an individual; those stages through which an organism passes between the production of gametes by one generation and the production of gametes by the next

Likelihood: the degree of belief warranted by evidence; the degree to which a proposition, model or hypothesis fits the available data

Measurement endpoint: a measurable characteristic of the selected assessment endpoint

Mesocosm: experimental water enclosure designed to provide a limited body of water with close to natural conditions, in which environmental factors can be realistically manipulated

Migration: Movement of an organism or a group from one habitat or location to another; periodic or seasonal movement, typically of a relatively long distance, from one area, stratum or climate to another

Neomale: a genotypic female that is converted to a phenotypic male by hormone treatment; masculinized genetic female

Nutrition: the life supportive constituents acquired by ingestion and/or absorption, digestion, and assimilation of food by plants and animals

Persist: survives to the reproductive stage

Physical containment: confinement of AAS by preventing its entry into the receiving environment through use of mechanical barriers, chemical treatments and through the implementation of policies and procedures to ensure that the devices and chemicals are used as prescribe

Pleiotropy: the phenomenon in which a single gene affects more than one phenotypic characteristic

Point of entry: geographical position at which and organism enters the environment or is no longer physically contained and is released into the environment

Predator: an organism that kills its victim in order to utilize resources contained in that victim

Predation pressure: the effects of predation on the dynamics of a prey population

Prey: any animal or animals actually or liable to be killed and consumed by a predator

Primary production: the assimilation of organic or inorganic matter by autotrophs (organisms that can convert inorganic carbon to organic materials and thus do not need to ingest or absorb other living things)

Productivity: the potential rate of incorporation or generation of energy or organic matter by an individual, population or trophic unit per unit time per unit area or volume; the organic fertility or capacity of a given area or habitat

Propagule: any part of an organism, produced sexually or asexually, that is capable of giving rise to a new individual

Propagule pressure: a composite measure of the number of individuals of a species that are released into a region to which they are not native and the frequency of release events; the number of viable organisms that could arrive in a geographic area over a set time period

Resilience: the capacity of a community to return to a previous state following exogenous disturbance; the ability to continue functioning after perturbation

Risk: the likelihood that a harmful effect will be realized as a result of exposure to a hazard. Risk incorporates the notion of the nature and severity of the harmful effect as well as the likelihood that the harmful effect will be realized.

Selection: non-random differential reproductive success of different genotypes in a population

Size-age structure: the number or percentage of individuals in each size class and each age class of a population; size and age distribution; size and age composition

Spatial heterogeneity: environment having a geographically non-uniform structure or composition

Spread: movement of a successfully established population beyond its distribution limit

Survival: occurs when the immediate physiological requirements of the organism are met

Triploid (3n): having three sets of homologous chromosomes; triploidy

Uncertainty: the lack of knowledge regarding the true value of a parameter resulting from either randomness, incompleteness or both

Unintentional release: accidental breach of physical containment resulting in the entry of a contained organism into the environment

Variable: the property with respect to which parameter values within a sample differ in some discernible way

Variability: the property of being variable in form or quality

The sources used for the definitions in this glossary include Burgman, 2005, Kapuscinski et al., 2007, Levin, 2009, Lincoln et al., 1998, and Mair et al., 2007, Oxford 1996.

8 INTRODUCTION

8.1 Purpose of this Document

This document comprises the environmental and indirect human health risk assessment conducted under the *Canadian Environmental Protection Act, 1999* (CEPA 1999) in respect of the *AquAdvantage salmon* (AAS), a genetically engineered Atlantic salmon notified by AquaBounty Technologies Inc. under the *New Substances Notification Regulations (Organisms)* (NSNR(O)).

This risk assessment identifies environmental and human health protection objectives and elaborates an appropriate scope and focus for the risk assessments that is based on the proposed use scenario and relevant hazards. It identifies protection goals and assessment endpoints that are aligned with legislative protection goals in CEPA 1999. The risk assessment explicitly addresses uncertainty throughout relevant areas of the document.

The environmental and indirect human health risk assessment of the *AquAdvantage Salmon* provides a focused, scientifically defensible risk assessment that can be concluded within the 120-day legislative timeframe allowed by the NSNR(O) for notifications under Schedule 5.

Further information on the CEPA 1999 and the NSNR (O), including guidance on the regulations, detailed guidance for information requirements, use of waivers, significant new activities, risk assessment outcomes and risk management can be found on the Biotechnology page of the Environment Canada website.

8.2 Legislative Context

CEPA 1999 is *an act respecting pollution prevention and the protection of the environment and human health in order to contribute to sustainable development.* The biotechnology provisions of CEPA 1999 take a preventative approach to pollution by requiring that all new living organism products of biotechnology, including genetically engineered fish, are notified and assessed prior to import or manufacture to determine whether they are "toxic" or capable of becoming "toxic".

As defined in section 64 of CEPA 1999, an organism is *"toxic" if it is entering or may enter the environment in a quantity or concentration or under conditions that*
(a) have or may have an immediate or long-term harmful effect on the environment or its biological diversity;
(b) constitute or may constitute a danger to the environment on which life depends; or,
(c) constitute or may constitute a danger in Canada to human life or health.

CEPA 1999 defines the "environment" broadly to mean *components of the Earth and includes (a) air, land and water;*
(b) all layers of the atmosphere;
(c) all organic and inorganic matter and living matter and living organisms; and,
(d) the interacting natural systems that include components referred to in paragraph (a) to (c).

CEPA 1999 defines "sustainable development" as *development that meets the needs of the present without compromising the ability of future generations to meet their own needs.*

Based on the stated purpose and effect in CEPA 1999, the timeframe associated with the environmental protection goal is for a period as long as is reasonably foreseeable.

Anyone proposing to import or manufacture a living animal product of biotechnology, including a genetically engineered fish, in Canada is required to provide Environment Canada (EC) with the information prescribed in Schedule 5 of the NSNR(O) at least 120 days prior to the commencement of import or manufacture of the organism, in Canada. This information is used to conduct an environmental risk assessment and assessment of risk to human health from environmental exposure to the living organism which will be used as the basis to determine if the organism is toxic or capable of becoming toxic. Although Schedule 5 allows for the notification of a broad range of activities, the risk assessment may be limited only to the activities proposed by the notifier.

The regulations do not apply to animals that are part of a research and development program and that are imported to, or manufactured in, a facility from which there is no release into the environment of the organism, the genetic material of the organism or material from the organism involved in toxicity.

Fisheries and Oceans Canada (DFO) has a Memorandum of Understanding with Environment Canada and Health Canada whereby DFO conducts the environmental and indirect human health risk assessments for fish products of biotechnology under the *New Substances Notification Regulations (Organisms)* of CEPA 1999 and recommends any necessary measures to manage risks. Under this arrangement, the Minister of the Environment receives advice and recommendations, but retains ultimate responsibility for regulatory decision making.

A waiver for one or more regulatory information requirements specified in Schedule 5 may be requested by the notifier. As specified under paragraph 106(8), waivers may be granted if (a) in the opinion of the Minister of the Environment and the Minister of Health, the information is not needed in order to determine whether the living organism is toxic or capable of becoming toxic; (b) a living organism is to be used for a prescribed purpose or manufactured at location where, in the opinion of the Ministers, the person requesting the waiver is able to contain the living organism so as to satisfactorily protect the environment and human health; or (c) it is not, in the opinion of the Ministers, practicable or feasible to obtain the test data necessary to generate the information. Under 106(8)(b), the organism must be contained throughout its life cycle (e.g. manufacture, transportation and handling, processing, storage, intended use, and disposal) so as to satisfactorily protect the Canadian environment and human health.

Under CEPA 1999, depending on the risk assessment outcome, options are available to manage any risks associated with the organism. These options are described in Section 4.6 on regulatory decision making.

Environment Canada is responsible for enforcement of the NSNR(O) including adherence to any imposed conditions, terms of use, or other risk management measures. Designated CEPA Analysts, including DFO staff, may also participate in an official capacity during inspections. Inspections are not undertaken outside of Canadian jurisdiction.

8.3 Risk assessment process

Regulatory decisions under CEPA 1999 are based on whether a living organism is toxic or not and are determined through scientific risk assessments. Risk is the likelihood that a harmful effect will be realized as a result of exposure to a hazard. The risk assessment will incorporate the nature and severity of the harmful effect as well as the likelihood that the harmful effect will be realized.

Both an environmental risk assessment and an indirect human health risk assessment will be conducted by DFO. The environmental risk assessment considers the potential of the organism to cause a harmful effect to the aquatic, terrestrial, and atmospheric components of the Canadian environment. The indirect human health risk assessment considers the potential of the organism to pose a risk to human health in Canada from environmental exposure to the organism. The risk assessments follow the classic paradigm in which *Risk* is proportional to the *Hazard* and the *Exposure*.

Potential food safety issues associated with human food consumption of the AAS are regulated by Health Canada under the *Food and Drugs Act* and are not considered under the NSNR(O). Risks associated with occupational health and safety are also not regulated under the CEPA 1999 and the NSNR(O) as this area falls within provincial jurisdiction.

The risk assessments of AAS conducted by DFO under the NSNR(O) will be conducted in accordance with the following principles:

- risk assessments will be science-based and do not include considerations such as socio-economics, ethics or harm/benefit ratios;
- a case-by-case approach will be taken whereby the specific use scenario notified and elaborated by AquaBounty in the regulatory submission, including any containment or mitigation measures, will set the specific parameters around the risk assessments (e.g. possible exposure pathways). If the risk assessment concludes no suspicion of 'CEPA toxic' for the proposed use scenario, but a significant new activity may alter the exposure of the organism (e.g. a change in containment procedures, location or scale of manufacture and/or production), then the Significant New Activity provisions of CEPA 1999 may be used to reassess and, if necessary, restrict the import or manufacture of the organism under the circumstances of the significant new activity;
- a comprehensive cradle-to-grave approach will be taken whereby AAS will be assessed from the time it is manufactured through production and use to disposal;
- all life stages (gametes through to reproductively mature adults), genotypes (e.g. diploids, triploids, heterozygotes, hemizygotes, homozygotes) and genders (males and females) carrying the opAFP-GHc2 rDNA construct at the α-locus that are required to generate the final egg product will be considered in the risk assessment;
- out-crossing of the AAS with thecommercial St. John River strain, as proposed by the notifier, will be considered in the risk assessment;
- in assessing the potential environmental risk, the characteristics of AAS will be compared to wild individuals and populations of unmodified Atlantic salmon;
- a predicted change to an assessment endpoint beyond the normal or historical range of variation will be used as an indicator of a potential effect;
- all available relevant information (e.g. academic, aboriginal, governmental) in addition to that submitted by AquaBounty under the NSNR(O) will be used

The scope of the current risk assessments and regulatory decision will be limited only to the production and grow-out scenario proposed by AquaBounty, namely, egg production and broodstock maintenance at the PEI production facility, egg transportation from the

Canadian to the Panamanian facility and commercial grow-out at the Panamanian facility under the containment conditions specified for each facility and during transportation. Where a production range is notified, the highest proposed values in the range will be used for the risk assessments. Activities and exposure events in Panama are only relevant where they may result in an exposure to the Canadian environment (i.e. the potential for AAS to be released and swim back to Canadian waters).

The determination of the potential risks of the AAS to the Canadian environment and human health in Canada will be done through a combination of an extensive assessment of exposure detailing the potential for entry, survival and reproduction (with consideration of the sterility containment strategy) of the AAS in the environment, and an assessment of the hazards and their potential direct, indirect, short-term and long-term effects and severity (see Figure 8.1).

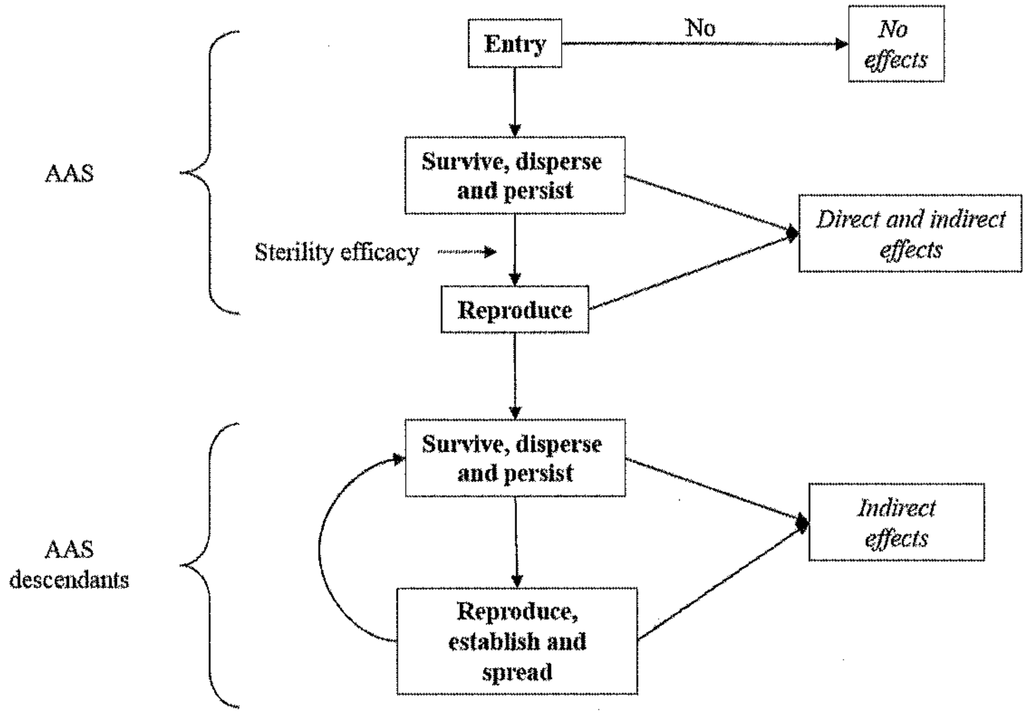

Figure 8.1 Logic model for the environmental risk assessment process. ***Adapted from Devlin et al., 2006.***

8.3.1 Protection goals

The protection goals, in accordance with CEPA 1999, are to protect the Canadian environment and its biological diversity from immediate or long-term harmful effects, to protect the environment on which life depends, and to protect human life and health in Canada.

In the current environmental assessment, key to protecting the Canadian environment is to maintain in a sustainable form all components of the ecosystem that may interact directly or indirectly with AAS.

DFO recommendations to Environment Canada regarding regulatory decision making and risk management in respect of AAS will be aligned with these CEPA 1999 environmental and human health protection goals. In particular, DFO will recommend a regulatory decision based on the likelihood, severity and reversibility of harmful effects, if any, to the structure and function of the Canadian ecosystem or to indirect human health that are expected to be realized as a consequence of environmental exposure to AAS.

8.3.2 Exposure

The exposure assessment is focused on the Canadian environment. AquaBounty will submit their regulatory package under Schedule 5 of the NSNR(O) and may submit a waiver request for regulatory information item 5(a) in respect of the ecological effects of the AAS (i.e. data from a test conducted to determine its invasiveness) on the basis that the organism is contained [see CEPA 1999, paragraph 106(8)(b)] (ABT 2011). If such a waiver request is received, the robustness of containment measures at both the Canadian and Panamanian facilities and during transportation will be assessed to determine if the legislative test is met such that all life stages of the AAS are contained so as to satisfactorily protect the Canadian environment and human health in Canada. If this is demonstrated then the recommendation will be made to the Minister of the Environment to grant the waiver request, however, the Minister's power to grant the waiver is discretionary.

The assessment of exposure of the AAS to the Canadian environment will include both its potential to enter the environment and its fate once in the environment. In considering the physical, geographical, and biological containment strategies used for all life stages of the AAS, the exposure assessment will focus on:

1. The potential for unintentional release(s) of AAS into the receiving environment (i.e. entry) at both the Canadian and Panamanian facilities and during transport between the two locations;
2. The potential of AAS to survive, disperse and persist in the Canadian and Panamanian receiving environments (i.e. fate). If applicable, the magnitude and frequency of dispersal (i.e. propagule pressure) will also be assessed;

3. The potential of AAS to reproduce, establish and spread in the Canadian and Panamanian environments (i.e. fate). If applicable, the magnitude and frequency of reproduction, establishment and spread will also be assessed; and
4. The potential for the disposal of AAS carcasses in Canada to act as an exposure pathway.

Although containment at both the Canadian and Panamanian facilities will be examined, the assessment will only consider the exposure of AAS to the Canadian environment. Consequently, assessment of potential exposure from activities in Panama will focus primarily on the potential of AAS to return to Canadian waters, including the Atlantic and Pacific Oceans. Table 8-1 Categorization for exposure of AAS in the Canadian environmentTable 8-1 describes the ranking for exposure of AAS in the Canadian environment based on entry and fate elements that will be considered in the assessment.

A final ranking for exposure will require consideration of multiple elements related to the biological, geographical and physical containment of AAS, including a variety of pathways, that determine the entry and fate of AAS in the Canadian environment. In many cases, the significance of one element will be limited by, or dependent on, another. For example, survival or reproduction in the Canadian environment will be dependent on entry into the Canadian environment. Similarly, entry into the Canadian environment will be dependent on the likelihood of physical containment failure. When considering physical containment alone, the likelihood of AAS bypassing a downstream barrier will be dependent on the failure of all upstream barriers that are along the same pathway to entry. This latter example emphasises the likelihood of AAS to bypass a particular barrier and should not be confused with the likelihood of failure at two or more different barriers, which are independent events and far less likely to occur simultaneously.

Table 8-1 Categorization for exposure of AAS in the Canadian environment

Rank	Description
Negligible	AAS will not be present in the Canadian environment (i.e. no entry or no survival at the point of entry)
Low	AAS may enter in very low numbers and survive in the Canadian environment but will not reproduce (low-level, single generation presence)
Moderate	AAS may enter in significant numbers and survive in the Canadian environment but will not reproduce (significant, single generation presence)
High	AAS may reproduce, establish or spread within the Canadian environment (established presence, including through hybridization with wild populations)

When elements are dependent, the final ranking for exposure is the ranking associated with the determining element. For example, if AAS will not enter the Canadian environment but can reproduce, then the final exposure ranking would be negligible since reproduction in the Canadian environment is precluded by the lack of entry into the Canadian environment.

In other cases, the significance of one element will be independent of other elements or pathways. For example, entry into the Canadian environment from Panama will not influence entry into the Canadian environment from the facility in PEI. Likewise, the likelihood of physical containment failure along one pathway of entry, or drainage route, will not influence the likelihood of failure along a discrete pathway.

When events are independent from one another, it is value of the highest ranking element that ultimately determines the exposure outcome and final ranking. For example, if AAS will not enter into the Canadian environment from Panama but may enter the Canadian environment from the PEI facility in moderate numbers and survive then the final exposure ranking would be moderate.

8.3.3 Hazard

The hazard identification process will consider the potential toxicity, allergenicity, capacity to act as a vector for pathogens, and invasiveness of AAS. In addition, and as part of the invasiveness assessment, other potential ecological effects will be identified through consideration of the AAS phenotypes that may result in a harmful effect.

Harmful effect refers to an immediate or long-term detrimental impact on the structure or function of the ecosystem or on human health from environmental exposure. The structure of the ecosystem refers to the spatial and temporal distribution of the biotic and abiotic elements including dominant species, rare species, and keystone species. The function of the ecosystem refers to interactions between species (e.g. competition, predation, disease) and with abiotic elements that contribute to the provision of ecosystem services (e.g. nutrient dispersal and cycling, primary production, decomposition). Changes to the structure or function of the ecosystem will be assessed based on changes to the assessment endpoints (see section 11.2). Table 8-2 categorizes the severity of the biological consequences of each potential hazard into four categories based on the severity and reversibility of effects to the structure and function of the ecosystem. Hazard would be ranked as negligible when no effects are expected, for example if a specific hormone is not expected to be bioactive between species. Table 8-3 categorizes the severity of the (indirect) human health hazards based on severity of effects to individuals and the community as well as on the availability of prophylactic treatments.

Table 8-2 Categorization of biological consequences of environmental hazards

Rank	Description
Negligible	No effects[1]
Low	No harmful[2] effects
Moderate	Reversible harmful effects
High	Irreversible harmful effects

[1]No effects: when no biological responses are expected, for example if hormones are not expected to be bioactive. [2]Harmful: an immediate or long-term detrimental impact on the structure or function of the ecosystem including biological diversity beyond natural background variability.

Table 8-3 Categorization of human health hazards

Rank	Description
Negligible	No effects on human health
Low	Effects on human health are expected to be mild, asymptomatic, or benign in healthy individuals. Effective prophylactic treatments are available. Case reports of human disease are rare and without potential for community-level effects.
Moderate	Effects on human health are expected to be moderate but rapidly self-resolving in healthy individuals and/or effective prophylactic treatments are available. Some potential for community-level effects.
High	Effects on human health are expected to be severe, of long duration and/or sequelae in healthy individuals or may be lethal. Prophylactic treatments are not available or are of limited benefit. High potential for community-level effects.

As is the case for exposure, the final ranking for both environmental and human health hazard will require consideration of multiple elements. The final ranking for hazard is that associated with the highest ranked assessment endpoint for either the environmental or human health hazard.

8.3.4 Uncertainty

The risk assessment includes explicit consideration of the uncertainty associated with all elements of the exposure and hazard assessments. Uncertainty has important implications related to regulatory decision making, and is closely tied to the application of precaution. In accordance with Canada's policy on the application of precaution in regulatory decision making elaborated in the *Government of Canada Framework for the Application of Precaution in Science-based Decision Making about Risk* (Government of Canada, 2003), in cases where uncertainty about risk is high, precautionary measures that are applied should be proportional to the potential severity of the risk being addressed and to society's chosen level of protection.

Factors influencing uncertainty include the availability of detailed information about AAS, its history of use and the proposed use scenario, as well as the extent, relevance (e.g. specific to the notified organism rather than surrogate) and quality of peer-reviewed information and/or empirical data. Prevalence of knowledge gaps, inherent variability of biological systems and experimental data, and the strength of logical deduction and inferences from knowledge of the species will also influence uncertainty.

While some forms of uncertainty can be reduced by filling knowledge gaps or through larger data sets, others cannot due to factors such as the inherent complexity and variability of biological systems or the occurrence of chance events.

In conducting environmental and indirect human health risk assessments, the following measures will be employed to ensure an accurate understanding of uncertainty and to reduce uncertainty to the greatest extent possible:

- A comprehensive scientific peer-review process will be undertaken on the risk assessment and any recommended risk management measures to ensure that expert advice is available in all key areas and that knowledge gaps and differences in scientific opinion are identified and adequately resolved wherever possible; and,
- Uncertainty will be explicitly estimated and stated separately for each element of the exposure and hazard assessments so that the overall risk will not be over- or under-represented by the inclusion of cautionary or dismissive assumptions.

8.3.5 Uncertainty in the exposure assessment

The exposure assessment will require two distinct approaches to assessing uncertainty; one for the physical containment (i.e. entry) and a second for the biological and geographical containment (i.e. fate).

Since exposure related to physical containment relies on both the design and operational management of facilities, the evaluation of uncertainty relies upon the availability of accurate and detailed information that adequately demonstrates the efficacy and redundancy of mechanical barriers, and the efficacy of standard operating procedures. This may include diagrams of mechanical barriers and containment systems, incident reports, and training and compliance documentation. It may also include information on the occurrence of chance events such as fires, floods, hurricanes and earthquakes that could lead to a failure of containment (Table 8-4).

Table 8-4 Categorization of exposure uncertainty based on the assessment of the physical containment (i.e. entry) of the AAS in the Canadian and Panamanian facilities

Rank	Description
Highly certain	Detailed information on facility design, containment structures, water treatment equipment, SOPs, internal compliance documentation, facility incident reports, and inspection reports is available. Long-term, reliable historical data on relevant chance events at or near the location of each facility are available.
Reasonably certain	Detailed information on facility design, containment structures, water treatment equipment, SOPs is available. Historical data on relevant chance events in the region of each facility is available.
Reasonably uncertain	Information on facility design, containment structures, and water treatment equipment is available however, no SOPs or historical data on chance events available.
Highly uncertain	Limited information of facility design, containment structures and water treatment equipment.

In contrast, the evaluation of uncertainty associated with exposure that may result from the failure of biological and geographical containment will depend the availability and robustness of scientific information related to biological and ecological parameters of AAS, valid surrogates and the receiving environment. The lack of empirical data around the survival, fitness and ability of AAS to reproduce in the natural environment (i.e. knowledge gaps) will also contribute uncertainty to the exposure assessment (Table 8-5).

Table 8-5 Categorization of exposure uncertainty based on the assessment of effectiveness of biological and geographical containment (i.e. fate) of the AAS

Rank	Description
Highly certain	High quality data on AAS (e.g. sterility, temperature tolerance, fitness). Data on environmental parameters of the receiving environment and at the point of entry. Demonstration of absence of GxE effects or complete understanding of GxE effects across relevant environmental conditions. Evidence of low variability.
Reasonably certain	High quality data on AAS-relatives or valid surrogate. Data on environmental parameters of the receiving environment. Understanding of potential GxE effects across relevant environmental conditions. Some variability.
Reasonably uncertain	Limited data on AAS, AAS-relatives or valid surrogate. Limited data on environmental parameters in the receiving environment. Knowledge gaps. Reliance on expert opinion.
Highly uncertain	Significant knowledge gaps. Significant reliance on expert opinion.

Quality of data refers to, for each parameter being examined as well as the integration of this information, the number of replications, breadth of experimental conditions examined, sample size and appropriateness of controls, statistical analysis, experimental design and interpretations of the results. Variability refers to both the range of phenotypic differences between individuals or strains within the same environment as well as the range of physical, chemical and biological conditions that may be experienced by AAS in the receiving environment.

8.3.6 Uncertainty in the hazard assessment

Uncertainty around the hazard assessment may be significant due to clear knowledge gaps and lack of empirical data around the behavior and effects of genetically engineered (GE) fish, and AAS in particular, in the natural environment. In addition, the knowledge gap associated with disease susceptibility and ability to act as a reservoir and spread infectious disease agents to other fish populations in the natural environment, as well as the complex interaction of the pathogen and host (behavioral and immune function) with environmental parameters in disease expression will also contribute to uncertainty in the hazard assessment.

Fisheries and Oceans Canada's (DFO) Centre of Expertise for Aquatic Biotechnology Regulatory Research has conducted a significant amount of laboratory research on the fitness and behavior of GE fishes to aid in estimating the fitness of GE fishes in the natural environment through use and comparison of results of studies conducted in tanks, semi-natural streams and mesocosms. Although this research was not conducted on AAS per se, it has highlighted several broad principles that may also be applicable to AAS and that represent potential sources of uncertainty about the extent to which laboratory data can be depended upon as a reliable indicator of how GE fishes would behave in the natural environment. These findings are described below:

- The environment in which fish are reared can significantly affect the phenotypic expression of the transgene (Devlin et al. 2004; Sundström et al. 2007). The influence of rearing environment limits our ability to extrapolate laboratory data as a reliable indicator of how a GE fish may behave (e.g. compete, survive) in the natural environment unless it can be demonstrated that wild-type controls reared in the laboratory environment behave the same way as wild-type fish in the natural environment. In the absence of such control data, there is uncertainty around the extent to which we can rely upon laboratory data as an accurate indicator of behavior in the natural environment;
- The phenotypic effects of the transgene can vary significantly with the genetic background of the parent (e.g. wild-type vs. domesticated, species). For example, the performance of a wild-type fish with an inserted growth hormone gene construct may be very different from the performance of a domesticated fish of the same species into which the same construct has been inserted (Devlin et al. 2001). Consequently, regulators must scrutinize the background genetics of experimental controls when evaluating the scientific validity of experimental data to assess whether the phenotype is durable across multiple genotypes as would be encountered in nature. Experimental data on transgene expression in one species or strain should be interpreted with caution as it may or may not be representative of the expression of the same transgene in a different species or strain;

- A single transgene may result in several phenotypic expressions, termed pleiotropic effects. For example, some empirical data demonstrates that increased growth in some fish species may also affect metabolism and swimming ability (Farrell et al. 1997), disease resistance (Jhingan et al., 2003), ability to compete for food (Devlin et al. 2001) and hormonal regulation (Devlin et al. 2000). Thus, unless the investigator has specifically directed attention towards an unintended effect, it may go undetected; and
- DFO research has demonstrated that insufficient sample sizes may also be a source of error when determining triploid efficacy induction rates (Devlin et al. 2010).

Given the lack of empirical data around the behavior and fitness of AAS in the natural environment, significant attention to uncertainty considerations in the hazard assessment will be required. Table 8-6 and Table 8-7 respectively describe the ranking for uncertainty around the potential hazards of the AAS in the environment and to human health.

Table 8-6 Categorization of uncertainty related to environmental hazard

Rank	Description
Highly certain	High quality data on AAS. Demonstration of absence of GxE effects or complete understanding of GxE effects across relevant environmental conditions. Evidence of low variability.
Reasonably certain	High quality data on AAS-relatives or valid surrogate. Understanding of GxE effects across relevant environmental conditions. Some variability.
Reasonably uncertain	Limited data on AAS, AAS-relatives or valid surrogate. Limited understanding of GxE effects across relevant environmental conditions. Knowledge gaps. Reliance on expert opinion.
Highly uncertain	Significant knowledge gaps. Significant reliance on expert opinion.

Table 8-7 Categorization of uncertainty related indirect human health hazard

Rank	Description
Highly certain	There are many reports of human health effects related to the hazard, and the nature and severity of the reported effects are consistent (i.e. low variability); OR The potential for human health effects in individuals exposed to the organism has been monitored and there are no reports of effects.
Reasonably certain	There are some reports of human health effects related to the hazard, and the nature and severity of the effects are fairly consistent; OR There are no reports of human health effects and there are no effects related to the hazard reported for other mammals.
Reasonably uncertain	There are some reports of human health effects that may be related to the hazard, but the nature and severity of the effects are inconsistent; OR There are reports of effects related to the hazard in other mammals but not in humans.
Highly uncertain	Significant knowledge gaps (e.g. there have been a few reports of effects in individuals exposed to the organism but the effects have not been attributed to the organism).

The overall uncertainty ranking associated with exposure or hazard is that associated with the element that determines the final exposure or hazard ranking. For example, if a final exposure ranking of negligible is determined by entry into the environment at the PEI facility, and the uncertainty associated with that ranking is reasonably certain, then the overall uncertainty ranking for exposure would be reasonably certain. Whereas, if there is high certainty that only very numbers may enter the Canadian environment but it is reasonably uncertain whether they will survive and reproduce (i.e. fate) then the final ranking for exposure would be low and reasonably uncertain.

8.3.7 Risk estimation

DFO's recommendation to Environment Canada for a regulatory decision will be based on both the overall risk of AAS in the context of AquaBounty's proposed use scenario and the associated level of uncertainty.

Figure 8.2 illustrates how the final exposure and hazard rankings will be integrated to determine an overall estimate of risk. Each of the four rankings for both exposure and hazard are assigned a numerical value that increases (from 1 to 4) with increasing likelihood of exposure or severity of hazard (from negligible to high) respectively. In accordance with the classic risk assessment paradigm, where Risk = Exposure X Hazard, the values along the X and Y axis are multiplied, creating a two-dimensional risk matrix where the numerical value within each cell indicates an increasing level of risk. Each cell is then assigned to one of four risk categories according to the severity of its numerical value as indicated in the legend to the right of the risk matrix.

Uncertainty associated with the risk assessment will also be explicitly communicated in the recommendation to Environment Canada.

DFO will recommend to Environment Canada a regulatory decision of "CEPA toxic" if the risk is moderate or high. In general, a recommendation of not "CEPA toxic" will be made if the risk is negligible or low risk with reasonable certainty. If the rankings for uncertainty in the hazard and exposure assessments differ, the higher uncertainty ranking will generally be assigned to the risk. An exception to this would be when either hazard or exposure fall within the negligible category, in which case a greater level of uncertainty may be tolerated on the opposing axis. Accordingly, as exposure or hazard becomes more extreme along one axis, there must be a higher level of certainty associated with the opposing axis before a recommendation of not "CEPA toxic" can be made. For example; if the hazard assessment is high, then exposure must be negligible to result in a risk estimation of low, but would have to be negligible with high certainty in order for a recommendation of not "CEPA toxic" to be made.

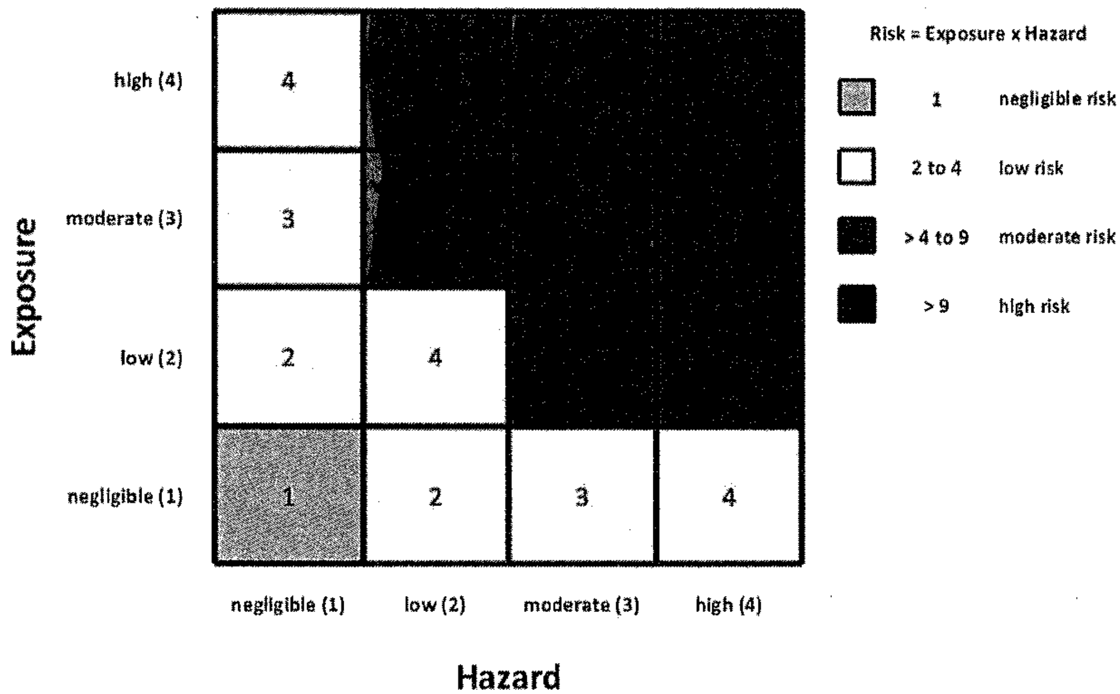

Figure 8.2 Overall estimate of risk

The colored risk matrix indicates the level of risk based on the integration of exposure and hazard by multiplying the assigned exposure and hazard values as indicated in the legend.

8.3.8 Regulatory decision-making

Under CEPA 1999, options for managing risks associated with the organism are available depending on the risk assessment outcome.

As depicted in Figure 8.3, risk assessments under CEPA 1999 result in one of the following outcomes:

1. a determination that the organism is not suspected of being "CEPA toxic" or capable of becoming "CEPA toxic"; or,

2. a determination that the organism is not suspected of being "CEPA toxic" under the proposed use scenario, but that a significant new activity in relation to the organism may result in the organism becoming "CEPA toxic". In this case, a significant new activity notice will be issued to reassess and, if necessary, restrict the import or manufacture of the organism under any other use scenario.
3. a suspicion that the organism is "CEPA toxic" or capable of becoming "CEPA toxic", which may require:
    a. the establishment of conditions on the manufacture, import, use or disposal of the organism;
    b. prohibition of the manufacture or import of the organism; or,
    c. prohibition pending submission and assessment of additional information determined to be required.

If the risk assessment concludes no suspicion of 'CEPA toxic' for the proposed use scenario, but a significant new activity may alter the exposure of the organism (e.g. a change in containment procedures, location or scale of manufacture and/or production), then the Significant New Activity provisions of CEPA 1999 may be used to reassess and, if necessary, restrict the import or manufacture of the organism under the circumstances of the significant new activity.

If the risk assessment concludes a suspicion of 'CEPA toxic' for the current proposed activity, then import or manufacture may be prohibited or conditions may be placed on the import, manufacture and use of the organism, such as minimal standards for containment of the organism.

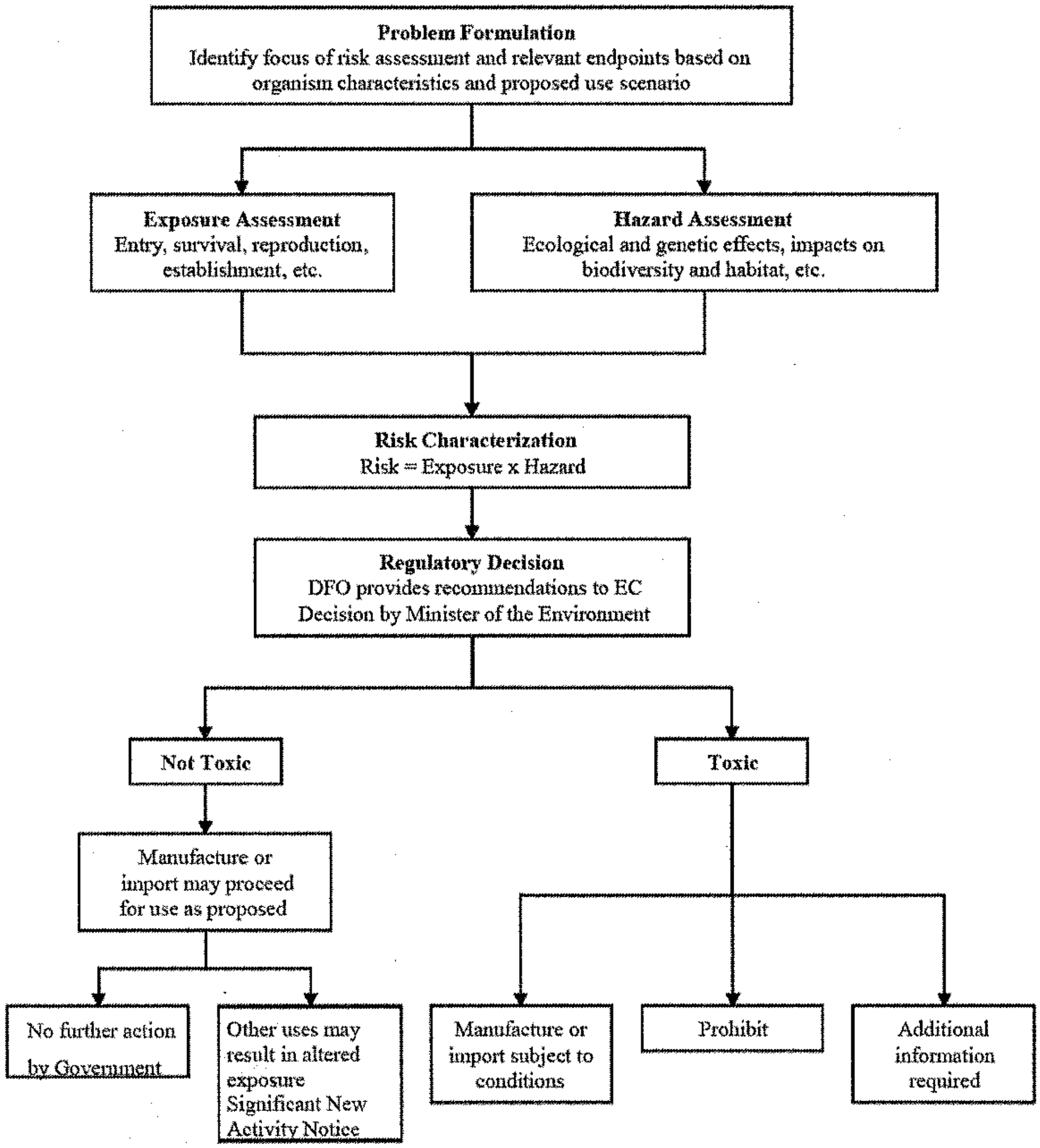

Figure 8.3 Regulatory Decision-Making Framework for environmental and indirect human health risk assessments of fish products of biotechnology conducted at Fisheries and Oceans Canada. ***Adapted from Shahsavarani et al., 2008.***

9 BACKGROUND

9.1 AquaBounty and the AquAdvantage Salmon

9.1.1 Company Structure

AquaBounty Technologies Inc. is an American biotechnology company with a land-based, contained research and development facility in Prince Edward Island (PEI). AquaBounty has genetically engineered an Atlantic salmon (*Salmo salar*) referred to as *AquAdvantage* salmon (AAS hereinafter) intended for human food consumption that is claimed to grow faster than its non-genetically engineered counterpart.

As described in the Notification the corporate headquarters (principal place of business in Canada) of Aqua Bounty Canada, Inc. is 0718 Bay Fortune, RR No.4 , Souris, Prince Edward Island, postal code C0A 2B0.

AquaBounty Canada, Inc. is a wholly owned subsidiary of AquaBounty Technologies with corporate headquarters at Suite 395, Two Clock Tower Place, Maynard, Massachusetts, United States of America, 01754. According to the Company web site (www.aquabounty.com, accessed on 19 JUN 2013), AquaBounty was originally incorporated in1991, under the name A/F Protein, to pursue the commercial development of antifreeze protein-based technology under license from the University of California at Berkeley.

In 1996, A/F Protein acquired a license to the AquAdvantage® technology from the University of Toronto and Memorial University of Newfoundland, and was subsequently reorganized in 2000, into two separate entities: A/F Protein, which retained the antifreeze protein technology; and, AquaBounty Farms, which obtained the AquAdvantage® technology. The company changed its name in 2004 to AquaBounty Technologies.

In 2006, AquaBounty Technologies was listed in the London Stock Exchange's Alternative Investment Market (AIM) raising $28 million in an initial public offering of stock. The company is incorporated in the State of Delaware, United States of America under the Delaware General Corporation Law.

In the instance of receiving approvals from the Canadian and United States of America regulatory bodies, and for the express purpose of product launch, AquaBounty Technologies will manufacture triploid eyed-eggs at a single facility in Atlantic Canada for the commercial production (i.e., grow-out) of sterile, all-female *AquAdvantage* Salmon (*AAS*) at a single facility in Panama, at the following locations:

Manufacturing Site
AquaBounty Canada, Inc.
0718 Bay Fortune, RR No. 4
Souris, PE C0A 2B0 Canada

Production Site
AquaBounty Panama, SA
[redacted]
District of Boquete, Chiriqui
Panama

AquaBounty Canada will be the manufacturer-seller of *AquAdvantage* Salmon eyed-eggs; and, AquaBounty Panama will be the buyer of those eyed-eggs for commercial production. Since both entities are wholly-owned and operated by AquaBounty Technologies, the latter will exercise singular and direct control over these critical aspects of manufacture and production involving live animals (AquaBounty Technologies, 2013. Notification package).

9.1.2 Proposed (Notified) Activities

9.1.2.1 Manufacture of AAS (Commercial Egg Production) in PEI

Male, female and neomale diploid brood stock are maintained at the PEI facility where AAS eggs and milt are produced and where eggs are fertilized to generate both diploid brood stock and, using hydrostatic pressure shocking technology, triploid production fish.

The company (AquaBounty Canada) has indicated its intent to commercially produce sterile female AAS eggs at its land-based aquaculture facility in PEI for export to a land-based, grow-out facility in the highlands of western Panama. No more than 100,000 eggs will be exported to Panama in any given year. In Panama, AAS will be grown to a commercial weight of 1 to 3 kg, then harvested, euthanized and transported to a processing plant in close proximity to the Panamanian grow-out facility where they will be processed and shipped to the United States for human food consumption.

AquaBounty has also committed to ensuring that live eggs exported from the facility in PEI to the facility in Panama, will be reared only at the production site described in the notification and that no live fish of any life stage will be sold or given by AquaBounty Panama to a third party for grow-out. This is also the basis of the application made to the US FDA and a condition of sale as outlined on the formal label that can be found on p. 579 of the notification (ABT 2013).

Although the proposed AAS product for export to Panama is all-female triploid eyed-eggs from the EO-1α line bearing a single copy of the opAFC-GHc2 transgene, all life-stages (gametes through to sexually mature adults), all genotypes (i.e. diploids, triploids, hemizygotes, homozygotes) and all genders (regular males and females, and neomales) are and will continue to be reared at the PEI facility as broodstock for egg production and for research and development purposes.

9.1.2.2 Production and Processing of AAS in Panama

AAS will be grown at the Panama facility to a commercial weight of 1 to 3 kg, then harvested, euthanized and transported to a processing plant in close proximity to the Panamanian grow-out facility where they will be processed and exported to the United States for human food consumption.

9.1.2.3 Transportation from Manufacturing Site to Production Site

Based on the Notification (AquaBounty Technologies, 2013, Notification package) the packaged triploid, transgenic AAS eyed-eggs will be transported by ABC staff for air transport from either Charlottetown, PE (YYG) or Halifax, NS (YHZ) to the grow-out facility. All procedures as required by SOP/ABPEI/4260 will be completed. [redacted] Transport from Charlottetown and Halifax will be affected by a freight-forwarder to maintain chain-of-custody through and including arrival [redacted] where ABP staff will receive the shipment directly for transport to the production facility [redacted]. Coordination of the effort via freight-forwarding will assure compliance with permitting and other customs requirements. [redacted]

9.1.2.4 2.1.2.4. Disposal of Waste

In their submission, AquaBounty Canada have included a standard operating procedure for the disposal of transgenic and / or bio-hazardous waste, which includes dead eggs, alevins, fry, parr, smolt, and adult fish [redacted] as well as an operational protocol (OP) for the disposal of dead or discarded fish at the Aqua Bounty Panama facility. [redacted]

9.2 Characterization of AAS

9.2.1 Taxonomic Identification

The taxonomic identification of the notified organism is adequate. The AAS line is a genetically engineered Atlantic salmon (Salmo salar) containing a single copy of the opAFP-GHc2 transgene at the EO-1α locus. The opAFP-GHc2 transgene contains a growth-hormone gene from the Chinook salmon (Oncorhynchus tshawytscha) under the control of the ocean pout (Zoarces americanus) anti-freeze promoter.

[redacted] The assembly of the opAFP-GHc2 transgene involved genomic DNA isolated from ocean pout (*Zoarces americanus*) testes and mRNA isolated from chinook salmon (*Oncorhynchus tshawytscha*) pituitary gland[1] (ABT 2013). The details of the creation of the construct are reviewed in the Genetic modification section (section 9.2.3).

The AAS line can be distinguished from other Atlantic salmon lines using one of two variations on the same method: [redacted] The notifier provided all the information to perform the two above procedures.

9.2.2 Strain History and Genealogy

The AAS includes the genetic background of several strains of Atlantic salmon. Early generations were from and crossed with individuals from the Exploits, Colinet and Northeast Rivers in Newfoundland. Later generations bred at the AquaBounty Canada facility in Prince Edward Island were mainly crossed with the domesticated St. John River strain from New Brunswick. Manufacture of the eyed-eggs will continue to involve crossing individuals from the brood stock with non-transgenic salmon from the St. John River strain.

[redacted]

In 1989, Atlantic salmon eggs originating from the Exploits and Colinet Rivers were microinjected with the transgenic constructs at the Ocean Science Centre (OSC) in Memorial University in Newfoundland, Canada. In 1990, a F_0 transgenic fry, referred to as the EO-1 female, with a greater body weight than the non-transgenic individuals was selected for further development. In 1992, once the EO-1 female reached sexual maturity, her eggs were fertilized with milt of a wild Atlantic salmon (Figure 9.1). Two rapidly-growing F_1 progeny, [redacted], were selected for further development. [redacted] In 2013, the AAS was reported to be at its [redacted] generation (Stotish, 2013). [redacted]

[redacted]

[redacted]

At the time of writing the risk assessment, in 2013, there is no AAS line of Atlantic salmon manufactured in Canada for commercial applications. [redacted] (ABT 2013, p. [redacted]).

9.2.3 Genetic Modifications

The notifier appropriately described the elements of the opAFP-GHc2 construct and sequencing results confirmed the absence of sequences coding for toxic protein. Although insertion of the transgene through microinjection is not of concern, uncertainty remains about the potential integration of small fragments of the plasmid due to its co-injection with the transgene. Absence of a complete ampicillin resistance gene and sequences for toxic proteins alleviate these concerns. Uncertainties also remain about potential for the transgene integrant to disrupt surrounding endogenous genes which are alleviated through characterization of the phenotype. We conclude with reasonable certainty that the nature of the transgene integrant at the EO-1α locus is not of concern for the risk assessment.

Molecular characterization including construction, insertion, integration, expression, inheritance and stability of the inserted gene informs risk assessments about potential hazards. Careful examination of the transgene design, the transgene insertion method and locus, the transgene expression level and the transgene transmission rates through generations can contribute to identify unintended genotypic and phenotypic effects (Gong et al. 2007).

9.2.3.1 Characterization of the Transgene Construct

The notifier appropriately described the transgene construct and demonstrated the ability of the promoter to drive geneexpression in salmonids. The nature of the transgene was confirmed through complete sequencing, which also confirmed the absence of sequences coding for toxic protein. The transgene construct is not a concern in the context of the risk assessment.

In the context of a risk assessment, it should be determined if all the critical elements to ensure gene expression, i.e. a promoter, a protein coding region and a transcriptional terminator sequence were included in the transgene and are functional (Gong et al. 2007). Concerns should be raised if the construct includes sequences for toxic proteins or production of antibiotic molecules.

Developed in the late 1980s in Canada, the construction of the transgene involved standard molecular biology and cloning techniques of which the main steps included the isolation of the target sequences and their sequential ligation into plasmid vectors into a functional transcriptional unit (Du et al. 1992, ABT 2013). The opAFP-GHc2 construct contains a transgene comprised of a 5'-flanking region (5'-FLANK) from the Ocean pout (*Zoarces americanus*) antifreeze protein (AFP) gene, the promoter (5'OP) from the Ocean pout AFP gene, a synthesized 5'untranslated region (5'UTR) derived from the 5'-UTR of an Ocean pout AFP gene, the coding region of the Chinook Salmon

(*Oncorhynchus tshawytscha*) growth hormone (GH) gene and a 3' regulatory sequence (3'OP) from the Ocean pout AFP gene (Figure 9.2).

A promoter was included in the op-AFP-GHc2 construct. The op-AFP flanking region and the regulatory sequences, including the promoter, originated from a genomic clone of the Type III AFP gene isolated from a Charon 30 library which had been prepared from Ocean pout testes genomic DNA. Sub-clones of the genomic clones were sequenced to identify regions coding for the 5'-flanking sequence (in pUC18), the regulatory-control elements and the anti-freeze protein gene (in pUC9). Sequencing of the plasmid construct confirmed the promoter to contain the appropriate regulatory sequences including a CAAT and a TATA boxes and a transcriptional start site [redacted]. Salmonids do not possess antifreeze proteins, hence expression of genes driven by this promoter is not expected to be affected by the host genome not having homologous endogenous gene (Du et al. 1992). Evidence of the functionality of the promoter to drive expression of the transgene in Rainbow Trout and chinook salmon cell lines is provided through *in-vitro* assays with the opAFP promoter and the bacterial chloramphenicol acetyltransferase (CAT) reporter gene (Du et al. 1992). The opAFP promoter also drove reporter gene expression *in-vivo* in medaka embryos (Du et al. 1992) and in Atlantic salmon (Hobbs and Fletcher, 2008). The tissue distribution of genes driven by opAFP promoter suggested that the promoter lacks tissue specific elements being expressed in most tissues (Hobbs and Fletcher, 2008) as seen for AFP genes in the Ocean pout (Gong et al. 1992; Hobbs and Fletcher, 2008). The notifier adequately demonstrated the functionality of the promoter to drive gene expression in salmonids, including in Atlantic salmon. The nature of the selected promoter does not represent a concern for the risk assessment.

The protein coding region for growth hormone included in the op-AFP-GHc2 construct was derived from a cDNA clone of the Chinook Salmon growth hormone gene isolated from a pUC13 library which had been prepared from pituitary gland mRNA. Sequencing of the plasmid construct confirmed the transgene to contain the expected translational start codon (ATG) and a translational stop codon (TAG) [redacted]

[redacted]. Differences between the expected and observed sequence include two additional nucleotides detected in the 3'UTR GH-coding and 3 changes on the third nucleotide on a codon. Differences between expected and observed sequences did not affect the coding sequence of the GH (ABT [redacted]. The GH clone was demonstrated to contain a full-length sequence encoding for a mature hormone homologous to the endogenous GH-1 Chinook Salmon gene. The deduced amino acid sequence of the GH from Chinook Salmon differs by 10 amino acids from the Atlantic salmon (95% homology) (ABT 2013). The nature of the protein coding region of the transgene does not represent a concern for the risk assessment.

A transcriptional terminator sequence from the AFP was included in the op-AFP-GHc2 construct. The terminator sequence (3'OP) originated from the same genomic clone, but different sub-clone (in pUC9) than the promoter. The fragment incorporated the expected polyadenylation site involved in transcription termination [redacted].

The step-wise assembly of the AFP regulatory sequences and the GH coding gene into the final plasmid opAFP-GHc2 construct was done through the use of standard molecular biology tools such as plasmids (including pUCs with reporter genes), bacteriophage, restriction enzymes, linearization and ligation (ABT 2013, [redacted]). The reported differences between the expected and observed sequences of the plasmid construct are not of concern[2]. The final opAFP-GHc2 construct is a recombinant plasmid (6721 bp) composed of inserted transgene DNA (4061 bp) and vector DNA (2660 bp) mainly from pUC18 but also from pUC9 (Figure 9.2).

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[2] Three nucleotides differed from the expected sequence of the plasmid. Of the three, two were in the GH-coding sequence and one in the 3'UTR. In all cases, the differences were on the third nucleotide of the codon and did not cause a change in amino acid or reading frame.

Finally, complete sequencing of the construct did not reveal coding sequences for foreign toxic proteins nor did a BLAST search of the sequence (accession number AY687640.1) on the NCBI website[3].

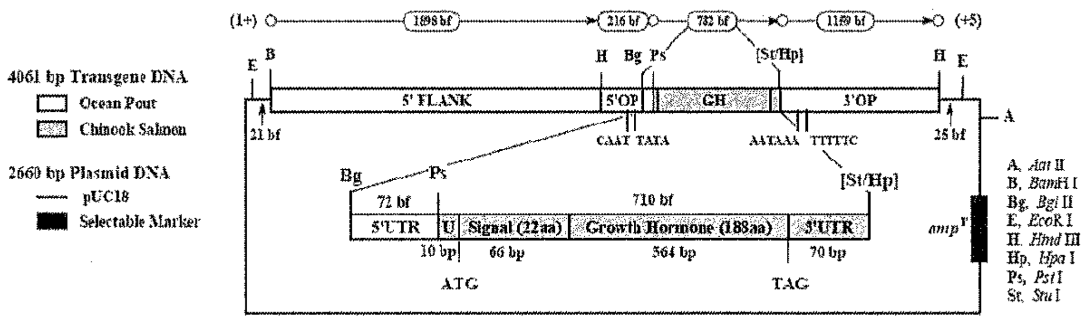

Figure 9.2 Physical characterization of the opAFP-GHc2. Modified from ABT 2013.

9.2.3.2 Insertion Methodology

The transgene was inserted into the host genome through microinjection. As this method results in random integration of the transgene in the host genome, appropriate tests are required to ensure that no endogenous host genes were interrupted. The notifier provided extensive characterisation of the integrated transgene (see section 9.2.5). No mobile genetic elements were used. Overall, microinjection is not considered to be a transgene insertion method of concern for the risk assessment.

The AAS was created through microinjection of the final construct plasmid after digestion with restriction enzyme without further purification (ABT 2013, [redacted] which raises two concerns. First, microinjection is a common transgene delivery method in fish (Nam et al. 2007) but is often reported to cause multiple copies of the transgene to be integrated in the host genome (MacDonald and Ekker, 2012). This concern is alleviated with an appropriate characterization of the transgene, including evidence of the number of copies and integration sites in the host genome (see section 9.2.5). Second, the

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[3] Further details are provided on the BLAST search conducted on the integrant (see section 11.1.1.1)

microinjection of the digested but unpurified final construct raises concern for potential plasmid-vector sequence integration, including antibiotic resistance gene, in the host genome (Gong et al. 2007). This concern can be alleviated with appropriate evidence of the absence of integration of the vector, or parts of, in the host genome (see section 9.2.5.4).

Mobile genetic elements, such as viral vectors and transposons, are used to improve transgene integration but are also reported to increase risks of mobilization and hence considered as a concern in the context of an environmental risk assessment (Gong et al. 2007). No mobile genetic elements were reported in the development of the opAFP-GHc2 construct and no nucleotides from the bacteriophage DNA were inserted in the final construct.

9.2.4 Other Modifications

The notifier adequately described the method of production of an all-female, sterile triploid product for purposes of biological containment through gynogenesis, sex-reversal, and triploidization. However, triploidization is identified as less that 100% efficient, with up to 0.5% diploid present per batch. While biological containment through the detailed methods is highly efficient, 100% containment cannot be assumed in all batches given current evidence. ABT provided supporting evidence, although not derived from studies, of successful generation of an all-female population through gynogenesis. Evidence was sufficient to demonstrate maintenance of the gender under the rearing conditions at the PEI facility but no information was available about for the Panamanian facility.

The manufacture of the notified sponsor product involves three additional methodologies to manipulate the reproductive biology of the Atlantic salmon (see Figure 9.3). Gynogenesis and sex-reversal are used to develop an all-female population of AAS, and triploidization is used to develop a sterile generation. The efficiency of these other modifications and their potential for concern in the context of the current risk assessment are discussed below.

[redacted]

[redacted]

The notifier described the method for gynogenesis induction in sufficient detail, including a [redacted]

Gynogenesis was used during early commercial brood stock development and is no longer used for maintenance of the commercial brood stock (ABT 2013, [redacted]

Salmonid genetic sex determination is by female homogamety, where XX results in genetic female, and XY results in genetic male. The purpose of gynogenesis is to create a genetically all-female population that can then be sex-reversed to XX-phenotypic males. [redacted]

[redacted] Production of gynogenetic Atlantic salmon using irradiated Atlantic salmon, rainbow trout or brook trout sperm in other studies has resulted in 100% female gynogenes (Johnstone and Stet 1995, Quillet and Gaignon 1990, Pepper et al. 2004). However, there have been several reports of phenotypic males being produced through gynogenesis in other salmonid models (see Pandian and Koteeswaran 1998). While the cause of these is not always determined, they include paternal genetic contamination (see Pandian and Koteeswaran 1998), which in the current case would be identified by Atlantic salmon x Arctic charr phenotype and removed in addition to be sterile (reviewed by Chevassus 1975). Gynogenesis in rainbow trout has occasionally resulted in production of phenotypic males due to a genetic mutation overriding the sex gene in some individuals (Quillet et al. 2002). These phenotypic males can pass the male phenotype to their offspring, resulting in >0% male offspring. Whether this mutation is present in other salmonid species has not been identified. However, should such a mutation be present in the AAS line, the result could potentially be XX AAS product with genetic predisposition to develop as male. This would result in bisexual population that could theoretically reproduce among itself. Current literature on Atlantic salmon gynogenetic production has not identified such a gene. However, Eisbrenner et al. (2013) identified phenotypic males in Tasmanian Atlantic salmon populations that were genetically predicted to be female, suggesting sex determination in Atlantic salmon may not be solely genetic. Preliminary evidence suggests that sex differentiation in Atlantic salmon may be thermolabile (King et al.).While current gynogenetic techniques are expected to produce all-female populations, this should be confirmed under all conditions used.

[redacted]

[4] Genetic males (XY)

[redacted] We conclude that the successful generation of an all-female population through gynogenesis has been performed, however there has been limited sampling to confirm the on-going maintenance of the female sex under the rearing conditions at the PEI facility. No information is available for the animals reared under Panamanian facility. The peer review committee suggested that the recommendation be made to AquaBounty to adopt a standard operating procedure to verify the genetic sex of neomale broodstock on an on-going basis.

9.2.4.2 Sex-reversal

[redacted] The process of sex-reversal is not of concern in the context of the following risk assessment.

In order to produce an all-female product, gynogenetic fish homozygous for the EO-1α transgene were treated with 17α-methyltestosterone to produce phenotypic male fish that are genetically female. This is done either by 1 or 2 immersion treatments of yolk-sac fry in 17α-methyl testosterone, or by feeding fry with feed sprayed with 17α-methyl testosterone [redacted] There are no expected complications in production of sponsor product from the described sex-reversal process, provided all fish are genetically predispositioned to be female.

9.2.4.3 Triploidization

The notifier adequately explains the process of triploidization of the final product, and estimation of percent triploidy success on a per batch basis [redacted]

Induction of triploidy in fish is a common method for sterilization. Hydrostatic, heat or other shocks applied to eggs shortly after fertilization results in retention of the egg's second polar body. The resulting triploid organism has three sets of chromosomes, two of maternal and one of paternal origin. While male triploid salmonids can sexually mature and produce non-viable offspring, female triploid salmonids do not generally mature (e.g. Benfey et al. 1989). Consequently triploid fish, particularly all-female triploid fish, are a useful method of biological containment. However, triploid methods are not 100%, although reported failure rates are usually less than 1%.

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

9.2.5 Characterization of the Transgene Integrant

The company thoroughly characterized the inserted transgene and provided sufficient evidence to conclude that that AAS only contains one copy of the opAFP-GHc2 integrant at a single locus (EO-1α). Characterisation of the inserted transgene revealed a rearrangement of the integrant relative to the construct and short non-coding pUC sequences which are not of concern. Two integration sites were initially identified in the founder animal but there is enough evidence to ensure that only the α-integrant remains in fish included in the AAS broodstock. Although microinjection is not of concern, uncertainty remains about the potential integration of small fragments of the plasmid due to its co-injection with the transgene. Absence of a complete ampicillin resistance gene and sequences for toxic proteins alleviate these concerns. The integrant does not appear to have been inserted in the coding region of an endogenous gene; however uncertainties remain about the potential for the transgene integrant to disrupt surrounding endogenous genes. The latest are alleviated through characterization of the phenotype. We conclude with reasonable certainty that the nature of the transgene integrant at the EO-1α locus is not of concern for the risk assessment.

Relevant genotypic changes are both related to the integration of the transgene and the triploidization of the eyed-eggs. Aspects of the integration of the transgene of considerations include the sequence of the integrant, number of integration sites, number of copies integrated, positions of the integrants, and determination of presence or absence of plasmid-vector sequence in the host genome.

9.2.5.1 Sequence of the Integrant

The opAFP-GHc2 construct was rearranged upon insertion into the host genome positioning a portion of the ocean pout AFP promoter downstream from the construct. In addition, short non-coding pUC sequences were included in the integrant. Excluding the above differences, sequencing demonstrated complete identity of the integrant in the host genome and the construct.

The initial structure of the integrant was initially analysed through PCR and linker-mediated PCR (MLPCR) through which a rearrangement of the integrant was suspected.

Complete sequencing of the EO-1α integrant provided solid evidence of the rearrangement of the opAFP-GHc2 construct upon insertion to the host genome (ABT 2013, [redacted]). The 4205 bp EO-1α integrant consists of the last 613 bp of the ocean pout AFP 5'regulatory sequence (which was originally made of 2186 bp including the 5'-flanking region, the promoter and the 5'UTR region in the construct prior to injection), followed by the intact Chinook Salmon GH cDNA, the complete Ocean pout antifreeze 3' regulatory sequence, 25 bp of pUC9, 20 bp of pUC18 and the first 1678 bp of the Ocean pout antifreeze 5'region.

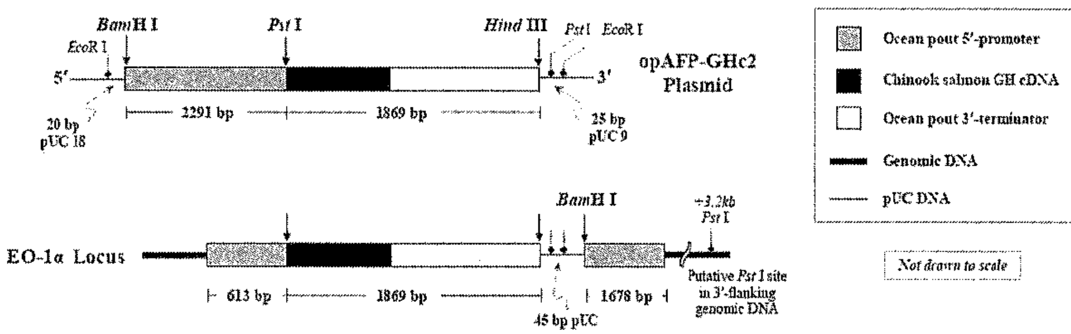

Figure 9.4 Comparison of the physical characterizations of the microinjected plasmid construct (opAFO-GHc2 Plasmid) and the integrated transgene (EO-1α Locus) in the Atlantic salmon (*Salmo salar*) genome.

***Source*: ABT 2013, p.177**

[redacted]

[redacted]

9.2.5.2 Number of Integrants

Two integration sites, referred to as the α- and β-integrants, were identified in the founder animal. As only the α-integrant confer the enhanced growth phenotype the company eliminated the non-functional β-integrant from the AAS EO-1α line. Sufficient evidence was provided to demonstrate the absence of the β-integrant in all fish included in the AAS broodstock maintained at the PEI facility. The company provided sufficient evidence to conclude that that AAS only contains one copy of the opAFP-GHc2 integrant at a single locus (EO-1α).

The assessment of the number of integrants includes considerations to both the number of integration sites, i.e., locus, and the number of copies at each loci. Southern blot analysis was provided as evidence to support the number of integration sites in the AAS. Results suggested two integration sites, referred to as the α- and β-integrants, in the founder animal (EO-1♀) and early generations (F_1 and F_2) (ABT 2013, [redacted]). Further analysis determined that the integrants were independently segregating and that only the α-integrant confer the enhanced growth phenotype. The notifier selectively bread later generations to only retain the α-integrant in transgenic individuals and eliminated the non-functional β-integrant from the AAS EO-1α line (ABT 2013, [redacted]).

The probes used in the Southern blot were designed to anneal the ocean pout antifreeze protein, which are not present in the Atlantic salmon hence making them specific to the transgene. Genomic DNA was digested with different restriction enzymes (*Pst*I, *Hind*III or *Bgl*II) prior to Southern blot hybridization in which the α-integrant was represented by

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[redacted]

a single band, indicating a single integration site (ABT 2013, AAS-MFG-004, Supplement 2 to AAS-MFG-004). The notifier provided sufficient evidence across five generations that the α-integrant is present at a single site in the AAS line. Genomic Southern blot hybridization has been routinely used in plants (OECD, 2010) and in fish (Du et al. 1992; MacDonald and Fletcher, 2012) to determine the number of integration sites. In the assessment of the evidence provided to support the number of integrant, it is important to consider recent information about the molecular characterisation of transgenic cattle in which detection of multiple copies of incomplete transgene and vector backbone was reported in a host genome through next-generation sequencing while other techniques had failed to detect them (Zhang et al. 2012). This new information raises uncertainties around the nature of the integrated transgenic material in the host genome of the AAS.

Of relevance to the current risk assessment is the number of integrants in the fish used in the production of the AAS line, hence the evidence provided in support of the elimination of the non-functional β-integrant from the AAS line needs to be considered. Based on the summary genealogy provided by the notifier (ABT 2013, p. 46), [redacted] Genomic Southern blot hybridization was conducted on DNA isolated from blood samples from individuals from the F_2, F_4 and F_6 generations descendant from [redacted] and [redacted] that are representative of the AAS population [redacted] Southern blot hybridization conducted on the *Pst*I digested genomic DNA using a probe in the ocean pout AFP region of the transgene confirmed the presence of a single band representing the α-integrant and providing evidence of the absence of the β-integrant in F_4 and F_6 descendants from the three individuals selected from [redacted], in the F_2 AS200 and in F_4 and F_6 descendants from the three individuals selected from [redacted] (ABT 2013, [redacted]. [redacted] A multiplex PCR was used to confirm the absence of the β-integrant. The [redacted]

Concatemerization of a transgene at a single locus has been reported in transgenic salmonids (Uh et al. 2006). The complete sequencing of the α-integrant provide sufficient evidence of a single copy of the transgene in the EO-1α AAS line.

9.2.5.3 Position of Integrants

The exact location of the integrant in the host genome is not known. Sequencing of the flanking regions of the integrant provides sufficient evidence to conclude that the integrant was not inserted in the coding region of an endogenous gene. However, uncertainty remains about the potential for the integrant to disturb surrounding genes.

Knowledge of the position of the integrant can have two main uses in the context of a risk assessment. First, it can contribute to the determination of the stability of the transgene through generations and second, it can be useful to determine the genes surrounding the integrated transgene and hence provide the possibility to generate evidence to determine if the insertion disrupts the expression of gene surrounding the transgenic integration site (Ohigashi et al. 2010). Determination of the position of transgene integration in fish is achievable through fluorescence in-situ hybridization (Phillips and Devlin, 2010).

The position of integration of the transgene, i.e. specific location of the EO-1α locus, in AAS is unknown and the NSNR(O) do not require the notifier to determine the position of the integrant in the host genome. Evidence from other methodologies to demonstrate the stability of the inserted transgene (reviewed in Inheritance and stability section) will be required. Evidence provided to demonstrate that the inserted transgene did not disrupt endogenous genes relies on sequencing of the genomic flanking regions of the EO-1α locus which revealed 35 bp repeat sequences for at least 1136 bp upstream and 730 bp downstream of the α-integrant (ABT 2013 – [redacted] and Yaskowiak et al. 2006[6]). Additional evidence for the lack of disturbance of surrounding genes can be substantiated with considerations of unintended effects (see section 9.2.7).

9.2.5.4 Vector Sequence in the Host Genome

Considering that the transgene was co-injected with the vector. The company provided sufficient evidence to conclude that no fragment larger than 161 pb from the pUC vector, which was co-injected with the transgene, was incorporated into the genome of AAS.

Evidence of absence of plasmid backbone integration is important to ensure no expression of additional proteins or altered endogenous gene expression (OECD, 2010).

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6 [redacted]

This is of concern in this risk assessment as the plasmid was co-injected with the transgene[7] (ABT 2013). Provided evidence includes absence of bands in Southern blot analyses using genomic [redacted] Although the nature of the probe is not of concern, its size (2686 pb) raises the concern for detection of small integrants of the vector in the host genome especially in light of detection of multiple copies of incomplete transgene and vector backbone through next-generation sequencing (Zhang et al. 2012). This new information raises uncertainties around the nature of the integrated transgenic material in the host genome of the AAS. However, the company also conducted multiplex PCR analyses to verify if AAS had incorporated or inherited the ampicillin resistance gene. Appropriate primers and internal controls were included. Blood samples from individuals from multiple families from the F_2- through F_6- generation were included. Results support the conclusion [redacted] pUC vector, including the ampicillin resistance gene, was incorporated into the genome of AAS.

9.2.6 Expression of the Transgene

Despite the truncated nature of the promoter in the integrant, there is enough in-vitro and in-vivo evidence to conclude that the remaining portion can drive expression of the transgene. [redacted] Available information about

[7] The opAFP-GHc2 was liberated from the plasmid construct by restriction enzyme digestion with *Eco*RI for microinjections but was not further purified resulting in microinjection of both the transgene and the vector DNA (ABT 2011, [redacted]

[redacted]

Relevant to this assessment is the activity of the trunked promoter, expression of the growth hormone transgene, expression of any allergenic or toxic proteins in transgenic individuals.

9.2.6.1 Activity of the Truncated Promoter

Despite the truncated nature of the promoter in the integrant, there is enough in-vitro and in-vivo evidence to conclude that the remaining portion can drive expression of the transgene.

Indirect evidence of the ability of the truncated promoter to drive expression of the GH transgene is available through *in-vitro* analysis. A series of opAFP promoter of varying sizes were fused to the bacterial chloramphenicol acetyltransferase (CAT) gene and microinjected into medaka embryos (*Oryzias latipes*) and transfected in rainbow trout hepatoma cells, chinook salmon embryonic cells and chum salmon (*Oncorhynchus keta*) embryonic cells. Results demonstrate the basal activity of the promoter to be retained despite the 5'end, including the CAAT sequence, being truncated (reported in ABT 2013, [redacted]). Additional indirect *in-vitro* evidence includes the transfusion of eleven AFP constructs of various sizes fused to a luciferase reporter gene and transfected in salmon and human cell lines (Butler and Fletcher 2009). The authors concluded that the expression of the EO-1α transgene in the AAS line to be driven by nucleotide elements within the promoter truncated upstream of the TATA box and to a small degree, by the relocated downstream sequence. Since the relocated 1579 bp downstream of the transgenic growth-hormone did not fully restore the promoter regulatory activity, no major enhancers appear to be part of the promoter. The relocated sequence is not expected to affect gene expression downstream from the EO-1α locus which gene sequencing demonstrated to be 35-bp repeat region. The authors provided enough evidence of presence of positive and negative regulatory regions in the promoter. Any remaining uncertainty around the ability of the trunked promoter to regulate the expression of the EO-1α transgene is alleviated by the demonstration of the expression of the transgenic growth hormone in the AAS (see section 9.2.6.2). Finally, the promoter appears to lack tissue specificity (Hobbs and Fletcher 2008).

9.2.6.2 Transgenic Growth Hormone Expression

Available data demonstrate active transcription of the GH transgene in several tissues at varying levels but does not provide a complete temporal profile of the transgenic GH expression through the life cycle of the AAS. Low levels of transgenic GH mRNA expression were detected using RT-PCR in several tissues in a small sample of AAS juvenile fish; however the use of [redacted] Available data does not provide a complete temporal and tissue expression profile of the transgenic GH protein levels through the life cycle of the AAS. No data on plasma GH levels are available for AAS. [redacted]

A complete characterisation of a transgene expression should include a description of the temporal and spatial distribution of the expression of the gene (transcript and proteins) and evidence for the phenotypic expression. Relevant evidence of the transgenic growth hormone expression includes analysis of the expression of the transcript, protein levels and phenotypes in representatives of the AAS line. Evidence from AAS-relatives is also included but weighted differently.

Transcriptional evidence of the transgenic GH in AAS juveniles of approximately 400g[8] is provided in 12 different tissues[9] but not in blood cells using reverse transcriptase PCR (Hobbs and Fletcher 2008). Primers used in the study targeted the 5'-junction between

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[8] Age and size are not specified in Hobbs and Fletcher (2008) publication but the size is reported to be around 400g in the notification.

[9] Tested tissues were heart, mouth skin, intestine, spleen, liver, kidney, stomach, ovary, gills, muscles, skin, brain, blood and pituitary.

the promoter and the transgenic GH coding sequence amplifying a 331 bp fragment in transgenic individuals and failure to do so in non-transgenic controls. Northern blot analysis in the same study using the same samples with a probe designed to anneal to the same region than the PCR primers, only detected a signal in the spleen. The above study provides very little information about the transcription of the transgenic GH in AAS considering the small sample size (n=2) and the unique life stage. [redacted] Overall, the available data does not provide a complete temporal expression profile of the transgenic GH through the life cycle of the AAS. Available data is limited to transgene mRNA expression at varying levels in several tissues in two juvenile fish of unknown position in the AAS genealogy and in muscle and skin samples of eight AAS progenies at market size using methodologies with different sensitivity.

Analyses of plasma levels of GH analyses were conducted in F_4 AAS progenies. With over 95% amino acid sequence homology between the Chinook and the Atlantic salmon (ABT 2013), contrary to the mRNA levels, it is not possible to distinguish the GH protein derived from the transgene from the GH protein derived from the endogenous gene. Investigation of the expression of the transgene thereby relies on the comparative total

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10 [redacted].

GH levels in transgenic and non-transgenic individuals. [redacted] transgenic (n=30), non-transgenic controls (n=33) and farmed comparators that are not from ABT (n=10) [redacted]

Information about plasma GH levels is available in AAS-relatives fry (n = 5 to 7) in which there was no statistical difference between the plasma GH levels in the transgenic (39.9 ± 14.8 ng/ml), five biggest aged-matched non-transgenic siblings (28.2 ± 8.8 ng/ml) and other non-transgenic siblings (20.5 ± 7.8 ng/ml) (Du et al. 1992). It should be noted that fry differed greatly in average weight (37.0 ± 10.2 g for transgenic and 5.94 ± 0.14 g for non-transgenic).

Overall, the available data does not provide a complete temporal and tissue expression profile of the transgenic GH protein levels through the life cycle of the AAS. Available data is limited to GH levels below detection limit in AAS muscle-skin of commercial size AAS fish and detectable levels in plasma of AAS-related fry. Knowing that levels of plasma GH vary with life stages and environmental factors (Björnsson 1997, Ebbesson et al 2008), we conclude that the available information about the GH levels in AAS might not be representative of potential highest levels.

[redacted]

[redacted]

9.2.7 Biological and Ecological Properties

This section focuses on the biological and ecological properties of the AAS. Information about AAS-relatives, i.e. Atlantic salmon injected with the same construct as AAS but resulting from different insertion events, is considered as surrogate information as opposed to information about the notified organism as transgene expression and physiological effects are strain specific and depend on the integration site(s)[13] (Devlin et al. 2004). AAS-relatives are therefore included in this section but less weight is attributed to this information. Biological and ecological properties of other transgenic growth-enhanced salmonids presented in numerous studies will be incorporated in the hazard assessment with related uncertainty level.

9.2.7.1 Size and Growth

The magnitude of the effect on growth rates reported in studies provided by the company, combined with evidence published in the scientific literature provides evidence of an enhanced growth phenotype in AAS under hatchery conditions. [redacted] or that AAS would not reach a larger size than their non-transgenic comparators in the natural environment. Uncertainty remains around the growth enhanced phenotype of AAS across different environments including those in low food availability.

Increased growth rate associated with the presence of the EO-1α transgene has been reported in several studies conducted by the notifier, and published in the scientific literature. The notifier conducted a study to compare body mensurements of AAS (2N and 3N) and control siblings (2N and 3N) at 2700 degrees-days, corresponding approximately to 7 to 8 months old (189 to 223 days at reported temperatures) ([redacted]). [redacted]

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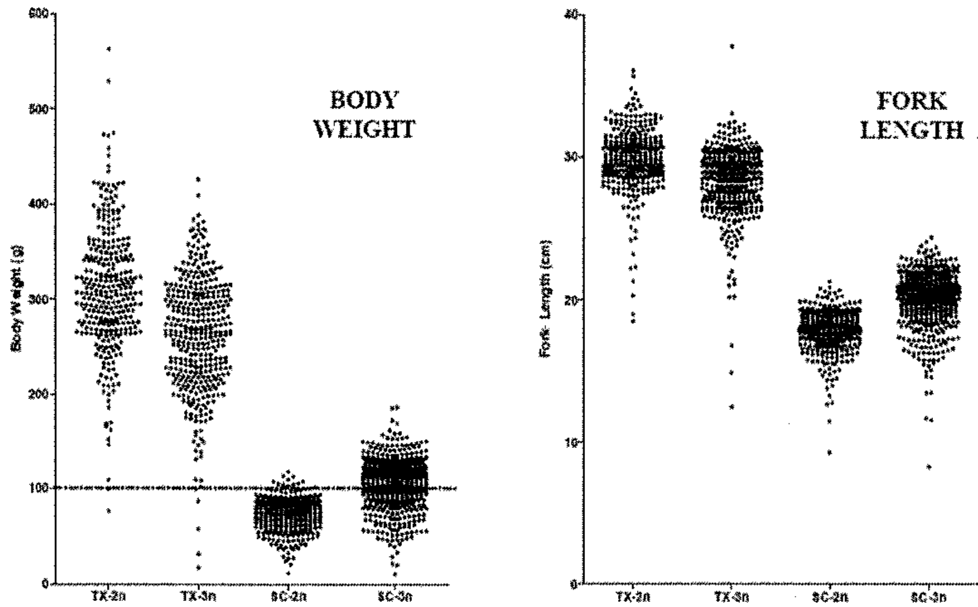
[13] Individuals strains of transgenic growth-enhanced salmon injected with the OnMTGH1 construct have different survival rates and different fry and juvenile growth rates suggesting that the insertion site and transgene structure affects transgene expression (Devlin et al. 2004). Hence, one cannot assume the reported phenotypes for AAS-relatives to be the same in AAS.

[redacted]

BW (g)	TX-2n	TX-3n	SC-2n	SC-3n
Sample (n)	309	369	306	464
Mean	309.90	261.00	72.63	104.21
SD	65.63	60.93	17.80	27.82
Max	563.3	426.3	118.0	186.5
Min	76.8	16.9	11.0	10.2
n > 100g	307	364	15	276
% > 100g	99.35%	98.64%	4.90%	59.48%

FL (cm)	TX-2n	TX-3n	SC-2n	SC-3n
Sample (n)	309	368	306	464
Mean	29.85	28.22	17.81	20.03
SD	2.30	2.53	1.35	1.99
Max	36.1	37.8	21.3	24.4
Min	18.5	12.5	9.3	8.3



* *BW*, body weight; *FL*, fork length; *Max*, maximum value; *Min*, minimum value; *SC-2n*, diploid, non-transgenic control salmon; *SC-3n*, triploid, non-transgenic control salmon; *SD*, standard deviation; *TX-2n*, diploid *AquAdvantage* Salmon; *TX-3n*, triploid *AquAdvantage* Salmon.

Figure 9.5 Body mensuration data for AAS (2N and 3N) and control siblings (2N and 3N) at 2700 degrees-days

[redacted] **Taken from ABT 2013:** [redacted]

[redacted]

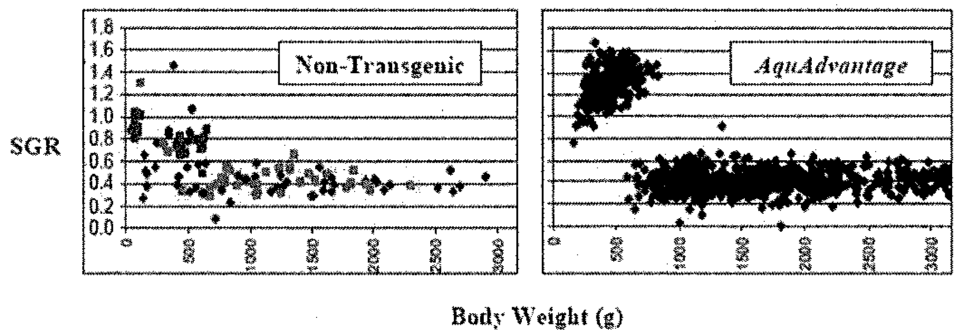


Figure 9.6 Change in specific growth rate during growth of AAS and non-transgenic comparators. Taken from ABT 2013: Narrative Response, p. 139.

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted] compared to non-transgenic counterparts (0.72 ± 0.10 % per day). [redacted]

[redacted]

The notifier indicated that there have been no controlled studies comparing the growth rate of AAS with wild-type Atlantic salmon or any [redacted]

[redacted]

Information in support of an increase growth rate of the AAS is also available in the scientific literature. Levesque et al.(2008) report significantly higher mass and length for AAS (14.33 ± 3.32 g and 11.75 6.57 ± 0.81 cm) compared to their non-transgenic siblings (2.83 ± 0.75 g and 6.57 ± 0.49 cm) at 4 months of age. AAS was also reported to outgrow its non-transgenic sibling resulting in significantly higher mass during the first and second year of life under low and high feeding conditions (Moreau and Fleming, 2012). Finally, Deitch et al. (2006) reported a 3.6 times faster growth rate in F_5 AAS compared to non-transgenic comparators from the St. John River strain (not siblings).

[redacted]

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[14] [redacted]

[redacted]

We concluded that there is sufficient evidence for the enhanced growth phenotype of the AAS under hatchery conditions. However, we consider that there is no evidence to support the claim that AAS does not grow larger than their non-transgenic counterparts (ABT 2013, [redacted]). Although anecdotal information was provided to demonstrate that the accelerated growth-rate of the AAS is not sustained over later stages of development, appropriate data would include size and growth rates of AAS and non-transgenic siblings after 4 years of age.

Of relevance to this risk assessment is the growth enhanced phenotype of AAS under food limited conditions. In a study in which AAS and non-transgenic Atlantic salmon were split between high and low feed levels (4 to 8% and 1 to 2% tank biomass per day, respectively), AAS outgrew non-transgenic fish in both feed levels (Moreau and Fleming, 2012) (Figure 9.9). The size of transgenic fish varied with feed levels suggesting that transgenic growth is limited by feed availability. On the other hand, Moreau et al. (2011b) also reported similar growth performance of GH transgenic and non-transgenic Atlantic salmon during first feeding in food-limited stream microcosms. Results from this study should be carefully interpreted due to the reported weight loss for all fish, including the non-transgenic controls, over the 37-day assessment period and to the different genetic background (i.e. AAS being crossed with wild adults from the Exploit and Colinet rivers as opposed to domesticated individuals from the St. John River strain). Using the same fish, Oke et al. (2013) confirmed the growth rates of transgenic and the non-transgenic in a naturalized stream to be lower than in hatchery conditions. AAS has higher growth rates in hatchery than the non-transgenic controls (approximately 1.8% and 1.4% g per day, respectively) as opposed to in naturalized streams in which AAS has lower growth rates than the non-transgenic controls (approximately 0.65% and 1% g per day, respectively), representing an approximate 64% and 40% reduction for the AAS and the non-transgenic fish, respectively.

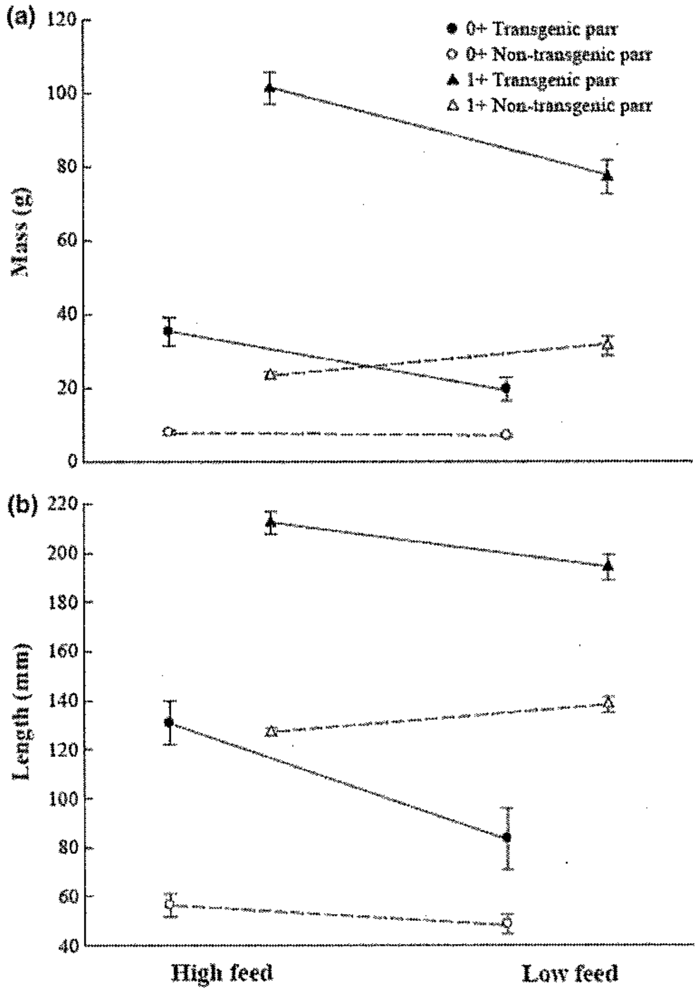

Figure 9.9 Mass and fork length of transgenic (AAS) and non-transgenic precocious male Atlantic salmon during the first (0+) and second (1+) years of life.

High and low feed levels were applied only during the first year of life. Data represent means ± 95% confidence intervals. Taken from Moreau and Fleming, 2012

AAS-relatives were also reported to have greater growth rates than their non-transgenic comparators. Study of AAS-relatives provides estimates of growth rates 2- to 6-fold greater than non-transgenic comparators during the first year of life (Du et al. 1992), 2.62- to 2.85-fold greater in transgenic pre-smolts compared to control fish when fed to satiation (Cook et al. 2000a) and to be significantly higher in GH transgenic compared to non-transgenic juvenile Atlantic salmon (Stevens and Sutterlin, 1999, Stevens et al. 1999). Abrahams and Sutterlin (1999) also demonstrated the growth rate of AAS-relatives (1.53% per day) to be significantly greater than in their non-transgenic counterpart (fish from the same strain but not siblings) (1.05% per day) over the weight interval of 1 to 10g.

Despite the lack of statistical analysis in some of the reports summited by the company, the magnitude of the effect on growth rates, combined with evidence published in the scientific literature, is considered to be adequate evidence of an enhanced growth phenotype in AAS. Nevertheless, there is [redacted] (ABT 2013, [redacted]). In addition, despite reduced thermal growth coefficient (ABT 2013: [redacted]) and reduced specific growth rates (Levesque et al. 2008) with time, there is no evidence that the AAS would not reach a higher mass and grow to a larger size than their non-transgenic comparators. Appropriate supportive data would require growth curves, ideally under naturalized conditions, for diploid and triploid AAS and non-transgenic siblings over longer periods of time. The maximum size of the AAS therefore remains unknown.

9.2.7.2 Morphology

Based on several studies and observations, the notifier has asserted that there were no significant differences between the AAS and non-transgenic comparators. They also concluded that the morphological irregularities in some AAS specimens were of low magnitude, limited distribution, and of non-debilitating nature.

The notifier provided gross morphology data for diploid and triploid AAS and non-transgenic comparators (n=12 fish per group)[15] ([redacted]). Transgenic and non-transgenic fish included in this study share common parents without being full siblings

[15] The experimental design includes 6 males and 6 females for each group, corresponding to 12 fish per group, 24 fish per genotype and a total of 48 fish ([redacted]).

but the triploid and diploid fish, within a same genotype, are full sibling[16]. A total of eight external (jaw, operculum, gills, fin structure, vertebral column, eyes-cornea, skin, and color-markings), ten internal (gonad, GI tract, liver-gall bladder, spleen, swim bladder, kidney, heart, body wall, cranium-ant spine, and gills) features and weights of five organs (GI tract, heart, liver, gall bladder and spleen) were assessed. No statistical analysis was reported on the gross morphology results. Abnormality in external features were mostly mild and of similar rates in transgenic and non-transgenic fish with most of the cases were observed in the gills and appeared to be related to the triploid state rather than to the genotype. Abnormalities in the internal features appeared to be similar in transgenic and non-transgenic fish ([redacted]). The report authors concluded that gross and microscopic findings for *AAS* in the aggregate were of low magnitude, limited distribution, and a non-debilitating nature are were unlikely to compromise the overall health of these fish during commercial production (ABT 2013: [redacted]).

Several morphological features were also reported for F_5 AAS (8 months old) and size-matched non-transgenic comparators (20 months old) from the St. John River strain, i.e. not siblings of the AAS (Deitch et al., 2006). No differences between AAS and the non-transgenic comparators were reported for general morphology features including fork length, body depth, opercular length, caudal peduncle depth and tail area or for gill morphology features, including number and length of filaments, lamellar density and area and total gill area. Although no differences were reported for optical surface area of erythrocytes, their perimeters and compactness in the transgenic fish were significantly smaller than in the controls. Atrium and bulbus heart mass were not different but ventricle mass and relative ventricular mass were higher in transgenic than in non-transgenic comparators. The authors also reported *in situ hearts* of the AAS to exhibit a marked 18% increases in maximum cardiac output compared to the controls.

[16] [redacted]

Morphological differences were also reported in the gills and gastrointestinal tract of AAS-relatives compared to their non-transgenic comparators[17]. GH transgenic Atlantic salmon have longer intestinal folds leading to a 1.5 times larger digestive surface area in the anterior intestine and 1.2 times in the pyloric caeca (Stevens et al. 1999). In addition, contrarily to adult AAS (Deitch et al. 2009), pre-smolts GH transgenic AAS-relatives have significantly longer gill filaments than their non-transgenic comparators leading to a 1.24 times larger gill surface area (Stevens and Suttelin, 1999). Deitch et al. 2009 attributed the reported differences to the higher mass-specific oxygen requirements of the freshwater pre-smolts.

9.2.7.3 Life-history

Based on the information available in the literature, the effects of the EO-1α on early life stages appear to be small with less than a day of advance on the hatching time and less than 2% in weight and 1% in size for the AAS compared to the non-transgenic counterparts. There is concurring evidence of early smoltification in both the AAS and AAS-relatives compared to their non-transgenic Atlantic salmon. There is no information about the timing of emergence of the AAS alevins from the gravel and on the maturation rate of female AAS. However, AAS reaches smolt and adult maturity stages faster than wild conspecifics suggesting a shortened life-cycle. Overall, limited available information suggests that the transgene has a larger effect on later freshwater stages than earlier ones.

The notifier anecdotally reported to have never observed any acceleration or delay in time to hatch for diploid, triploid, hemizygous or homozygous growth hormone transgenic Atlantic salmon relative to their non-transgenic counterparts at the R&D facility on PEI (ABT 2013: [redacted]92). In addition, the notifier reported [redacted]

[17] The control fish were reported to be from a non-transgenic cross from the same stock and spawned on the same day as the transgenic fish. The authors do not report them to be full siblings.

Hemizygous AAS males were crossed with wild non-transgenic Atlantic salmon females from the Exploit River (Moreau 2011). The cross resulted in approximately half of the offspring inheriting the transgene as expected for Mendelian inheritance patterns. Such an experimental design enables the comparison of full siblings differing for the presence of the transgene hence controlling for maternal effects and genetic background (Moreau 2011). Over 60% of the fish in each family of transgenic and non-transgenic Atlantic salmon hatched over a period of three to four days (Figure 9.10) (Moreau, 2011). The transgenic fish hatched on average less than one day earlier than their non-transgenic full siblings (493 ± 8.2 and 497.2 ± 8.1 degree days, respectively) and was dependent on family. Figure 9.10 also demonstrates the interfamily variation in the onset and rate of hatching over several days which is within the range that has been typically reported elsewhere (Darek Moreau, personal communication). In the same study, the amount of yolk remaining near emergence time in the transgenic (13.38 ± 0.27) was slightly greater than in the non-transgenic (12.99 ± 0.26) fish. Finally, the transgenic fish weigh less than their non-transgenic counterparts (0.148 ± 0.001 g vs. 0.151 ± 0.001 g, respectively) and were smaller (25.08 ± 0.09 mm vs. 25.26 ± 0.12 mm) at time of emergence.

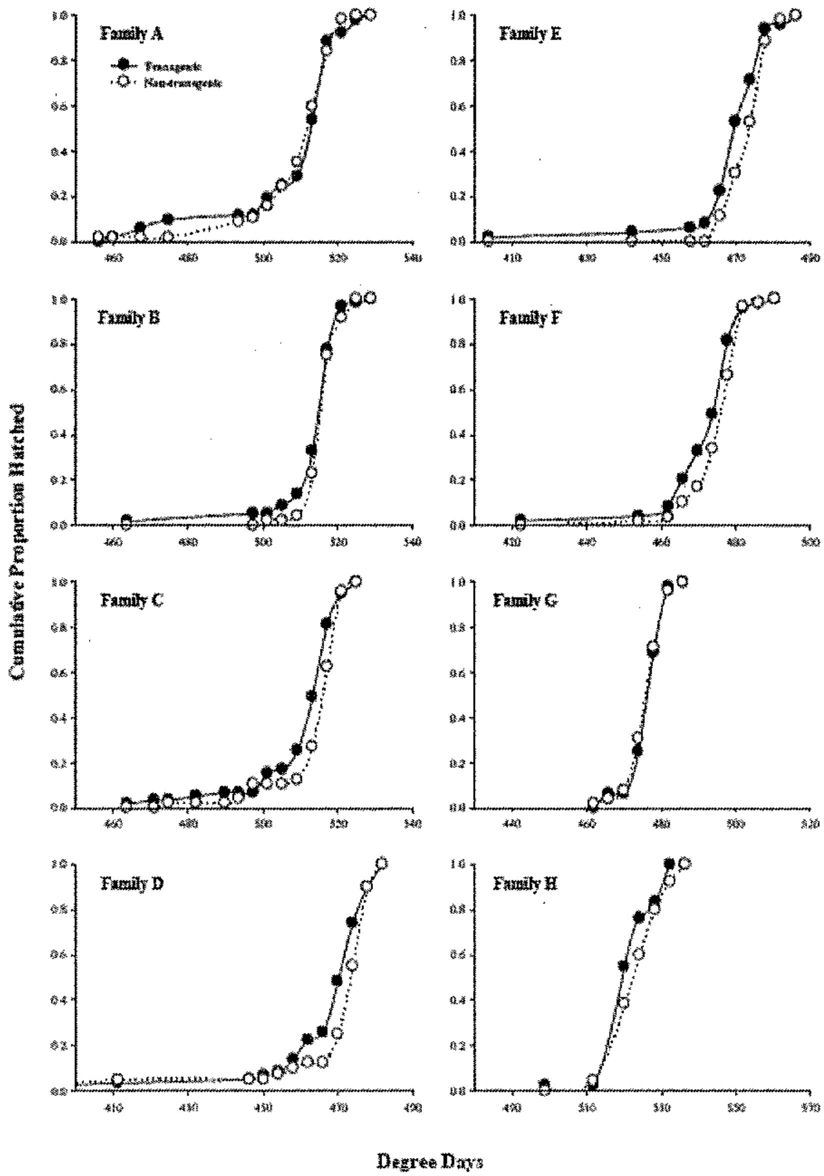

Figure 9.10 Time of hatch (degree days) of full sibling GH-enhanced transgenic and non-transgenic Atlantic salmon (*Salmo salar*) from eight families (n = 100 eyed-eggs for each family).

Taken from Moreau, 2011, Ph.D. Thesis

The number of maturing AAS transgenic parr was the same as their non-transgenic siblings from the Exploit River over the first year of life but was only half during the second year of life from which the authors concluded in reduced precocial male maturation in AAS (Figure 9.11) (Moreau and Fleming, 2011). In addition, although not quantified, observations of secondary smolt characteristics in the immature transgenic parrs (of silver colouration and loss for parr marks), as opposed to the non-transgenic fish, suggest the transgene preferentially include physiological pathways towards smoltification.

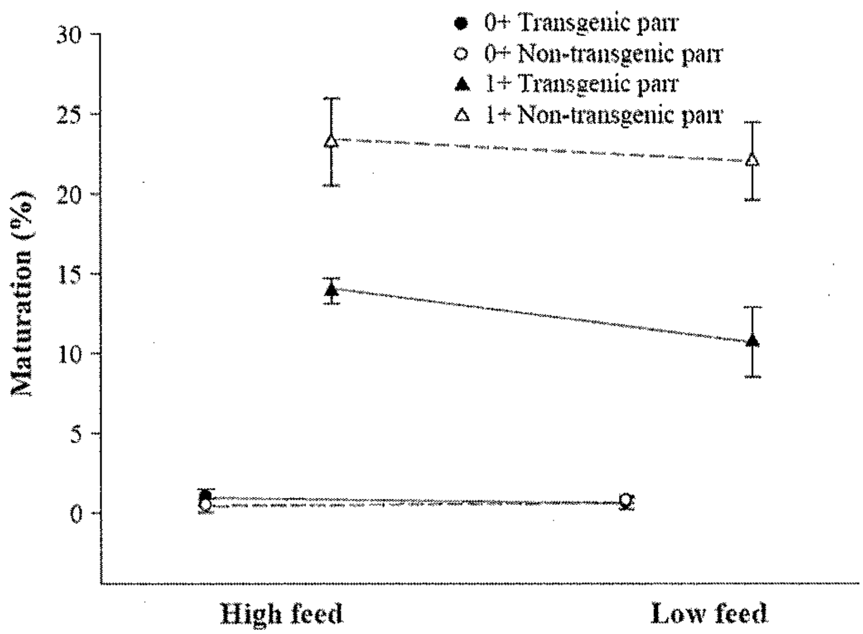

Figure 9.11 Incidence of mature male transgenic and non-transgenic Atlantic salmon parr during the first (0+) and second (1+) years of life.

High and low feed levels were applied in the first year of life only. Transgenic fish are AAS in the Exploit River genetic background. Taken from Moreau and Fleming, 2011

Changes in life history have also been reported in AAS-relatives. AAS-relatives reach smolt size and completed smoltification at 6 months of age (Saunders et al. 1998) and transgenic AAS-relatives appeared to undergo precocious smoltification based on silver coloration and lost of dark vertical parr marks at a smaller size than their non-transgenic counterparts (Cook et al. 2000b).

9.2.7.4 Metabolism and Physiology

Metabolic and physiological differences between AAS and non-transgenic counterparts include higher feed consumption rates, lower feed conversion ratios, increased oxygen consumption rates and reduced metabolic scope and swimming performance. Available information does not provide a complete profile over the entire life cycle of the AAS.

Serum analysis revealed significant lower serum glucose and cholesterol in AAS compared to their non-transgenic comparators from common parentage (Figure 9.12) (ABT 2013: [redacted])[18]. Several other parameters were demonstrated to be different, while remaining within the normal range observed in Atlantic salmon, between AAS and non-transgenic comparators including chloride, aspartate aminotransferase, biliburin, total protein, albumin, globulin, calcium, and phosphorous while no difference were reported for sodium potassium, alanine aminotransferase and creatine phosphokinase. Together, these results suggest a difference in the metabolic rates of sexually immature AAS compared to non-transgenic controls. However, due to the small sample size of the above study (n=12), and the single time point, the results are considered preliminary and not necessarily representative of the whole population.

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[18] Transgenic and non-transgenic fish were 1213.0 ± 125.9 g (n=48) and 45.9 ± 1.7 cm and shared a common parentage with the commercial broodstock without being full siblings. Transgenic fish were derived from the F_5 generation of AAS. Triploids were derived from hydrostatic pressure shock of approximately half of the fertilized eggs from the respective crosses ([redacted]).

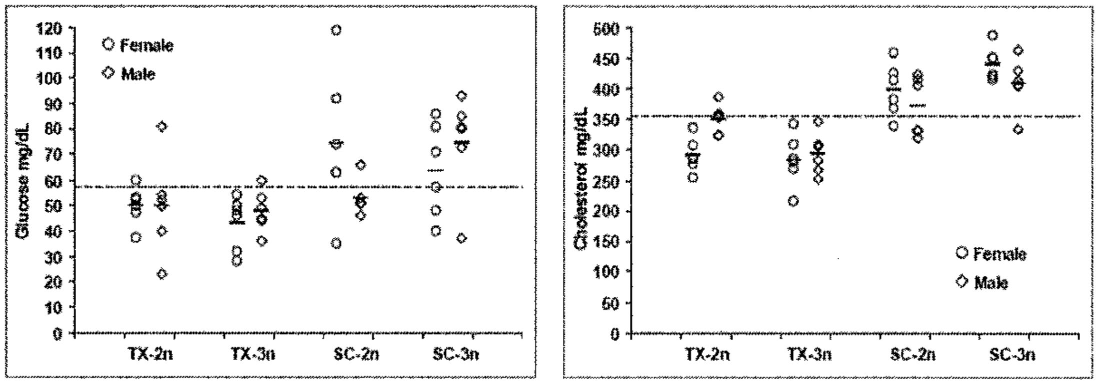

Figure 9.12 Scatter plots of serum glucose and cholesterol values by genotype (TX: transgenic, SC: non-transgenic) and ploidy (2N: diploid and 3N: triploid).

N = 12 per group including equal numbers of sexually immature males and females weighing 1000 to 1500g. Data taken from [redacted].

[redacted]

The respiratory metabolism of AAS during early ontogeny has been examined in several families resulting from crosses between the AAS and wild fish from the Exploit River, Newfoundland (Moreau 2011). The oxygen consumption in AAS eyed-eggs and alevins was not significantly different from their full sibling comparators (Figure 9.13) (Moreau 2011). In addition, the mean oxygen consumption in AAS (0.170 ± 0.004 mg O_2 g^{-1} hr^{-1}) and non-transgenic siblings (0.164 ± 0.007 mg O_2 g^{-1} hr^{-1}) first-feeding fish was also not

[19] Sample size reported to be 6 tanks, without the number of fish per tanks. No details on the statistical analysis were provided.

significantly affected by the transgene. The results from this study suggest that the metabolic differences between AAS and non-transgenic Atlantic salmon to be minimal at critical early life stages.

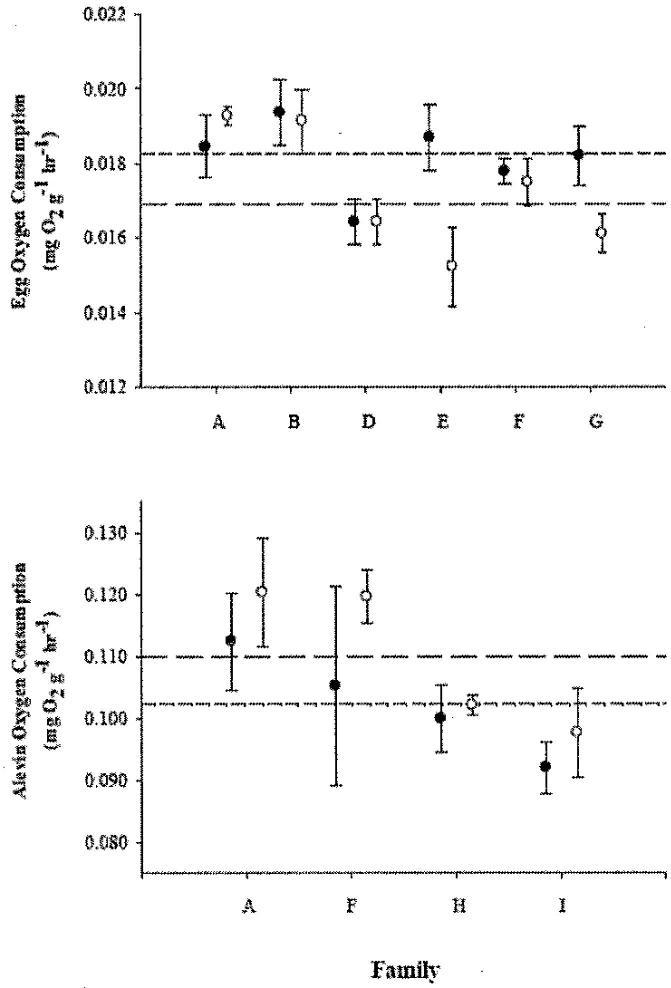

Figure 9.13 Oxygen consumption in transgenic (black circles) and non-transgenic (white circles) full siblings Atlantic salmon eggs (top panel) and alevins (bottom panel).

Data represent mean values within different families. The overall transgenic and non-transgenic means are represented by the short and long dashed lines, respectively. Transgenic fish are AAS with an Exploit River genetic background. Taken from Moreau 2011 Ph.D. Thesis

Metabolic differences attributed to the transgene appear to be more important at the post-smolt adult life stages than at early life stages. Deitch and colleagues (2006) reported a 21 to 25% higher oxygen consumption rates in F_5 AAS (828 ± 40g) compared to their size-matched non-transgenic comparators (884 ± 86g) from the St. John River strain (not siblings) (Deitch et al. 2006). The same study also reported on several other metabolic and physiological differences between AAS and non-transgenic comparators.. AAS has a 18% lower metabolic scope and a 9% lower critical swimming speed compared to the non-transgenic counterparts despite having higher oxygen consumption rates, 29% larger hearts, 18% greater mass-specific in-situ maximum cardiac output, 14% higher post-stress blood hemoglobin concentrations and 5 to 10% higher aerobic enzyme activities (Deitch et al., 2006). The authors concluded that the gill surface area, which did not increase in the AAS, may limit the ability of the adult AAS to elevate its maximum metabolic rate and swimming performance[20]. Overall, the above study

Metabolic and physiological differences are also reported in AAS-relatives. Abrahams and Sutterlin (1999) demonstrated the rates of consumption of AAS-relatives to be approximately five times that of their non-transgenic counterpart (fish from the same strain but not siblings) over the weight interval of 1 to 10g. Daily feed consumption over a pre-smolt body eight interval of 8 to 55 g is 2.14- to 2.62-fold greater for AAS-relatives than their non-transgenic counterparts, suggesting an increased appetite, and exhibit a 10% improvement in gross feed conversion efficiency (Cook et al. 2000a). Transgenic fish have less body fat than their comparators, which is reported to be a function of their elevated metabolic rates (Cook et al. 2000a). Routine oxygen consumption rates are 1.54 to 1.70-fold higher in the AAS-relative than in their non-transgenic counterparts over the same weight interval (Cook et al. 2000b). Oxygen consumption remained 1.58 to 2.30-fold higher in transgenic fish than in non-transgenic comparator after 24 hours starvation (Cook et al. 2000b). As starvation progressed over 8 weeks, transgenic fish exhibit a more rapid decline in oxygen consumption as well as in body protein, lipid and energy (Cook et al. 2000c). Oxygen uptake in transgenic fish is 1.7 times higher than in control

[20] Sample size varied between 7 to 8 fish

fish resulting in a critical oxygen uptake level of 6 mg/L for transgenic fish compared to 4 mg/L for controls (Stevens et al. 1998). Oxygen uptake of transgenic fish was 1.6 times higher than in control fish during forced swimming activity but critical swimming speed was not different between groups (Stevens et al. 1998).

9.2.7.5 Endocrinology

Available data for the GH concentrations in the AAS is scarce. Plasma GH concentrations across the life cycle of the AAS have not been reported. Muscle-skin of commercial size AAS and non-transgenic counterparts have GH levels below detection limit and suggested no difference between the different genotypes for IGF-1, estradiol, testosterone and thyroid hormones. Juvenile AAS appears to have a different hormonal response to stress than its non-transgenic counterparts.

Growth hormone expression profile in the AAS is addressed under the *Expression of the transgene* section (9.2.6). This section specifically addresses the profiles of other hormones in the AAS.

The notifier conducted a blinded comparative muscle-skin hormonal composition study for Atlantic salmon and AAS (ABT 2013: [redacted]). In this study, muscle-skin samples were collected from F_4 AAS individuals[21] (n=30), non-transgenic controls[22] (n=33) and farmed comparators from other sources (n=10). The AAS and non-transgenic salmon were of commercial size (2 to 7.5 kg) [redacted] Samples were analysed for growth hormone (GH), insulin-like growth factor-1 (IGF), triiodothyronine (T3), tetraiodothyronine (T4), estradiol (EST), testosterone (TT), and 11-keto-testosterone (11kT) content by a private

[21] Specific position in the genealogy of sampled fish is not specified in the study report (AAS-HFS-001) nevertheless, summary genealogy in the notification (p. 46) suggests that the fish are from individuals transferred to the PEI facility from which the AAS line descent.

[22] Sponsor Control animal subjects were the progeny of wild-type male and female Atlantic salmon maintained at the facility (ABT 2013: AAS-HFS-001, p. 17).

laboratory in accordance with GLP regulations under approved SOPs using radioimmunoassays (RIAs) and an enzyme immunoassay (EIA) developed and validated using commercial biomaterials, reagents, and supplies. Complete procedures and validation reports for all methodologies were provided by the company (ABT 2013: [redacted]). Summary of the hormone results are presented in Table 9-3. It is should be noted that the farmed comparators were not included in the statistical analysis and that a sub-sample of the non-transgenic fish (the Sponsor Control, i.e. SC) was selected for the analysis. Analysis concluded in no significant differences in the hormone levels in the muscle-skin sample between the AAS and the non-transgenic control fish (ABT 2013: AAS-HFS-001).

[redacted]

In addition to the data provided by the notifier, Deitch and colleagues (2006) reported eight month old F_5 AAS to have similar resting cortisol levels than their size-matched twenty-month-old non-transgenic comparators[23] (Table 9-4). Epinephrine,

[23] Controls are from the St. John River strain but not reported to be AAS siblings

norephinephrine and total cathecholamines plasma levels were significantly higher in transgenic fish compared to the control, with the exception of epinephrine in the rested fish. Results suggest a potential impaired cortisol response in the AAS compared to non-transgenic fish suggesting a potential impact on the hypothalamic-pituitary-adrenal axis. In addition, results suggest a different catecholamine response in the AAS and their non-transgenic counterparts.

Table 9-4 Plasma cortisol and catecholamine levels in rested and stressed GH transgenic and control Atlantic salmon. Taken from Deitch et al. (2007).

		Control	Transgenic	Trans/Con ratio	P value
Cortisol (ng ml^{-1})	Rest	12.1±1.7	11.6±2.3	0.95	0.86
	Stress	24.7±2.3†	17.8±1.3†	0.72	0.02*
Epinephrine (nmol l^{-1})	Rest	3.3±0.6	5.8±1.7	1.76	0.17
	Stress	12.3±2.1†	20.6±2.8†	1.67	0.03*
Norepinephrine (nmol l^{-1})	Rest	1.7±0.3	4.3±0.8	2.53	0.02*
	Stress	5.0±0.8†	8.9±0.7†	1.78	0.004*
Total catecholamines (nmol l^{-1})	Rest	4.9±0.9	10.2±2.1	2.08	0.04*
	Stress	17.4±2.9†	29.6±3.4†	1.70	0.02*

Resting measurements were taken 48 h after cannulation and black box confinement. Post-stress catecholamine levels were measured immediately after a 45 s net stress, whereas post-stress cortisol levels were assessed 30 min later.
Values are means ± 1 standard error (N=8).
*Significant difference (P<0.05) between transgenic and control salmon; †significant difference (P<0.05) between resting and stressed fish.

Information is also available in a small sample (n = 3 to 5) of AAS-relatives fry in which plasma T_3 levels in the five biggest aged-matched non-transgenic siblings (2.8 ± 0.5 ng/ml) was significantly higher than in the transgenic (1.1 ± 0.5 ng/ml), and other non-transgenic siblings (1.9 ± 0.1 ng/ml) (Du et al. 1992).

9.2.7.6 Behaviour

Little information about the behaviour of the AAS is available. The notifier reported normal avoidance, feeding and postural behaviour of juveniles AAS in a hatchery environment. Competition for territory is related to prior dominance rather than transgenesis and there is no information available about the predatory behaviour of the AAS in the environment. Information is limited to foraging behaviour in AAS-relatives which appear to be more willing to be exposed to predators than the non-transgenic comparators.

Under hatchery conditions, the AAS and the non-transgenic domesticated counterparts show no abnormal behaviour in avoidance, feeding and posture[24] during feeding ([redacted]).

Studies conducted in experimental stream mesocosms suggest that territorial dominance is advantaged by prior residency at the fry stage rather than genotype as the presence of the GH transgene[25] was reported not to influence survival or territorial dominance (Moreau et al. 2011a). There is no study available specifically for the AAS on their prey selection or food preference (ABT 2013, [redacted]), schooling tendency, predator avoidance, territorial defence and migration.

Behavioural information is also reported for AAS-relatives which were demonstrated to spend significantly more time feeding in presence of a predator than the non-transgenic controls and have a significantly higher average speed of movement (328 cm/min) than to the control fish (96 cm/min) (Abrahams and Sutterlin, 1999). Together, these observations suggest that AAS-relatives are more willing to be exposed to predators while foraging than non-transgenic comparators.

9.2.7.7 Reproduction

Potential reproduction of AAS of the fertile broodstock and sterile triploid females is considered separately. Despite reduced reproductive performance in male, fertile male AAS can participate in natural spawning events and offspring can survive past first feeding stage under food limited conditions. AAS has reduced occurrence of sexually mature male parr. Significant knowledge gap relies in the absence of information about

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[redacted]

[25] Fish used in this study were AAS crossed with wild fish from the Exploit River in Newfoundland.

the fecundity and reproductive behaviour of fertile female AAS. Triploid AAS females are expected to be functionally sterile but up to 2%, but likely less than 0.5%, diploid AAS potentially heterozygous for the transgene can be expected among triploid eyed-eggs for exportation.

AAS includes all the genotypes, life stages and ploidy states required in its manufacture. The reproductive capacity of the diploid broodstock and the triploid AAS are therefore considered separately.

The fecundity and fertility relative to wild conspecifics has not been examined in AAS. However, studies conducted under naturalized stream mesocosm reported wild anadromous males to outperformed captively reared AAS anadromous males in terms of nest fidelity, quivering frequency and spawn participation (Moreau et al. 2011a). In addition, despite displaying less aggression, captively reared non-transgenic mature parr were superior competitors relative to AAS parr in terms of nest fidelity and spawn participation (Moreau et al. 2011). Studies examining alternative male breeding phenotypes of AAS reported a reduced occurrence of sexually mature parr in tanks under low and high food abundance conditions (Moreau and Fleming 2012). Together, the above studies provide evidence of (1) the ability of male AAS to participate in natural spawning events, (2) an overall reduced breeding performance of male AAS relative to wild conspecifics and (3) reduced occurrence of sexually mature male parr relative to wild conspecifics. The reproductive breeding behaviour of female AAS has not been examined. The knowledge gap about fecundity and breeding behaviour of female AAS significantly limits prediction of the overall reproductive fitness of AAS in the natural environment as Atlantic salmon females spend more energy in offspring production than males (Fleming 1996). Finally the overall reproductive performance also depends on the survival rates of the offspring. Under simulated natural rearing conditions, the GH transgene did not influence the survival or growth at the onset of exogenous feeding (Moreau et al. 2011a). However, it is important to note that all fish in the studies, including controls, loss weight hence the results requires cautious interpretation.

Nevertheless, the study provides evidence that AAS, after hatching under naturalized conditions, can survive past first-feeding stage under food limited conditions.

Reproduction of triploid AAS is also relevant as AquaBounty proposes to export triploid female eyed-eggs (ABT 2013). As reviewed under the triploidization section (see section 9.2.4.3), triploid AAS, as other triploid fish, are not expected to mature sexually hence to be functionally sterile. [redacted]

9.2.7.8 Health Status

We conclude with reasonable certainty that AAS is more susceptible to A. salmonicida than domesticated comparators, however, the relative disease susceptibility to A. salmonicida of AAS compared to wild Atlantic salmon is not known. It is highly certain that AAS is highly susceptible to ISAV. In addition, AAS and domesticated non-transgenic comparators likely have comparable susceptibility to ISAV, but this is reasonably uncertain. The relative susceptibility of AAS to ISAV compared to wild Atlantic salmon is not known. [redacted]. Based on this Fish Health Certificate data, we conclude that disease risk at the AquaBounty facility in PEI is well managed. Based on studies and observations of gross morphological and clinical pathologies, we conclude with reasonable uncertainty, that morphological irregularities derived from the transgene do not represent serious fish health issues.

Disease susceptibility:

[redacted]

[redacted]

[redacted] Note that in a similar disease challenge experiment using GH transgenic coho salmon, Kim et al. 2013 concluded that the transgenics were more susceptible to *A. salmonicida* challenge than non-transgenics (wild) controls based, in part, on a difference in mortality profiles of only day. For the purposes of the current risk assessment, we are interested in the relative disease susceptibility of AAS compared to wild Atlantic salmon and the ability of AAS to act as a vector for pathogens. Although this difference in *A. salmonicida* infection and mortality between AAS and domesticated controls can be attributed to the presence of the transgene, it is not clear how the susceptibility of AAS to *A. salmonicida* would compare to that of wild Atlantic salmon. From the foregoing, we conclude with reasonable certainty that AAS is [redacted], however, no data is available to assess the relative disease susceptibility to *A. salmonicida* of AAS versus wild Atlantic salmon.

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

Preliminary results from functional genomic and QPCR analysis conducted by Hori et al. 2013 showed no significant difference within a given AAS family in immune-relevant transcript expression (Mx1 gene is a key gene in anti-viral defence) between diploid and triploid AAS injected with a viral mimic (pIC). However, transcript expression did vary significantly between families suggesting that genetic background may be important in immune response, a finding that is consistent with the preliminary data on ISA susceptibility data presented directly above.

[redacted]

[redacted] Based on this Fish Health Certificate data, we conclude that disease risk at the AquaBounty facility in PEI is well managed.

Also of relevance to any differential susceptibility of AAS to fish pathogens are the observations that necropsy of moribund and dead fish at the PEI facility [redacted]

Morphological irregularities:

AquaBounty provided a Study Report (2013 [redacted] in which gross and microscopic morphology, clinical and histological pathologies of diploid and triploid AAS, non-transgenic size-matched controls are presented. In addition to these primary Study groups, one additional group of three male and three female "satellite" comparators of matching ploidy (but not size) was enrolled contemporaneously with each Treated cohort: since Treated and Sponsor Control fish achieved target body weight and were enrolled at different times of the year, the diploid (***SAT-2n***) and triploid (***SAT-3n***) satellite controls were used to facilitate the distinction of phenotypic differences associated with seasonal variation in culture conditions. The study was intended to identify any acute, and clinically-relevant phenotypic changes associated with transgenesis or triploidization in AAS as an indication of general health and welfare of AAS.

Pre-qualified pools of candidate animal subjects were established in Phase I of the study whereby prospective-candidate animal subjects were selected from pre-Study inventory and subjected to remote observation of behavior for at least two weeks prior to Phase II screening-enrollment. [redacted]

[redacted]

Screening-enrollment process: At screening-enrollment for each Study group, candidate animal subjects in the pre-qualified pool [redacted]

[redacted]

Findings with regard to gross morphology: Findings with regard to gross morphology are summarized Table 9-10and ranked as indicated in the table description. Of the 384 observations for external morphology, 8 features x 48 fish (24 Treated and 24 Sponsor Control), made during external examination, abnormal findings were reported 18 times (9%) for the former and 25 times (13%) for the latter. The number of findings was considerably larger for triploid fish of both treatments, which findings were related predominately to abnormalities in gill structure; a similar pattern of abnormal findings was observed for triploid satellite controls. The overall rank scores for change in external appearance of the animal subjects enrolled were either a 1 (none) or 2 (slight), with the exception of one triploid Sponsor Control with a rank score of 3 (moderate). Of the 216 observations for internal morphology (9 organs-structures x 24 fish) made during the internal examinations for *each* of the Treated and Sponsor Control groups, abnormal findings were reported 12 times (6%) for the former and 10 times (5%) for the latter. The number of abnormal findings was similar among diploid and triploid fish of both treatments; and, a similar pattern of abnormal findings was observed for the satellite controls. No obvious or remarkable difference in relative organ weights (gastrointestinal tract, heart, liver and gall bladder, and spleen) between the Treated and Sponsor Control animal subjects, or between the diploid and triploid fish of either treatment, was noted.

A few types of gross findings involving gill arches and fins were substantially more prevalent among triploid than diploid salmon. [redacted] that were subject to erosion, shortening, twisting, bifurcation, and/or the presence of nodules. With the exception of a nodule associated with an epidermal cyst (SC-2n fish) and a shortened, twisted dorsal fin associated with a skeletal deformity (SC-3n fish), these structural abnormalities were not generally correlated with specific microscopic changes. A marginally higher prevalence of cardiac shape abnormalities (e.g., loss of pyramidal profile) was observed in triploid than diploid salmon for which no microscopic correlates were observed; and, a low occurrence of jaw erosion was observed exclusively in male and female, diploid AAS. Diploid AquAdvantage fish had an increased prevalence of focal inflammatory lesions. The lesions, which were generally minimal-to-mild, were observed in a variety of tissue types and tended to be more common in diploid than triploid animals, and more common among diploid transgenic (and to a lesser extent, triploid transgenic) salmon than either the Sponsor or satellite controls. Inflammation was most frequently characterized as granulomatous, consisting chiefly of macrophages in spherical nodular aggregates, with or without multinucleated giant cells or central areas of necrosis. Other categories of inflammatory lesions (e.g., acute, chronic active, necrogranulomatous, pyogranulomatous) were less regularly represented. The most commonly affected sites for inflammation were the abdominal mesentery, cranium, and trunk kidney. Etiologic agents were not evident in any of the lesions. A higher prevalence of inflammatory and hyperplastic changes was associated with those structural abnormalities in triploid than diploid fish. Absent filaments were observed most often in a single region of the gill arch (usually at the apex) and the filaments flanking each bare region were frequently misaligned; little (if any) inflammation was associated with filament absence. [redacted]. A substantially higher prevalence of minimal-to-moderate *mineralization* was observed in a variety of soft tissues for the triploid *AquAdvantage* and satellite control groups compared to diploid and triploid Sponsor Controls. This was generally the case for male *vs.* female fish, although females had a higher prevalence of mineralization than males among transgenics and satellite controls. Commonly affected sites were the eye, heart, liver, and trunk kidney. Microscopically, most instances of mineralization were graded as minimal, although mild-to-moderate mineralization was seen on occasion and a few mineralized lesions of the urinary tract were identified macroscopically at necropsy. Minimal-to-moderate *hepatocellular vacuolization* tended to be higher in triploid than diploid salmon (with the exception of diploid, female Sponsor Controls), and was characterized by single or multiple, variably-sized, discrete, round, sharply-defined spaces within the cytoplasm of hepatocytes; larger vacuoles displaced the nucleus toward the cell periphery. [redacted]

In sum, the pathology findings associated with AAS were 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. The majority of other findings, which included gill and fin abnormalities,

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

The data in the above tables and the study report has been reviewed by a DVM of Fisheries and Oceans Canada who provided the following comments:

- Collectively the results, descriptive summaries and discussion/interpretations were reasonable and satisfactory in clarity and sophistication;
- Some areas in the report ([redacted]) needed further explanation and / or would have benefitted from an expanded study design scope.
- AquaBounty's statement that there was 'no indication of serious health issues deriving specifically from *AquAdvantage* transgenesis that would be cause to prevent the deployment of the *AquAdvantage* Salmon line in commercial production' [redacted] was a reasonable conclusion based on the findings that were presented but this conclusion is less certain given the short comings of the study design and lack of additional diagnostic work-up done for the pre-study and enrolled fish at the time of post mortem or Necropsy, respectively;
- Specific pathological changes that were associated with AquAdvantage (transgenic) fish, included 'increased presence of focal inflammation, especially among diploid fish, and a low occurrence of jaw erosions among both male and female diploids' [redacted] These changes are somewhat unusual (especially the inflammation) but ultimately were not considered further by the

authors. Presumably, after the authors took into consideration clinical, growth, gross and remaining histopathological findings and the inflammatory lesions in the transgenic fish were deemed incidental. I have no objection to this conclusion based on the results and diagnostic materials considered in the Study. However, the study was restricted to such a small number of animals at one point in time. The issue of determining whether there are health or welfare concerns with transgenic fish that are to be cultured in a commercial setting would have benefitted from a more wide ranging study involving fish selected from different ages and sizes throughout a grow-out cycle, under actual commercial conditions. A greater scope for this study would have improved the strength of the conclusions (more fish over a greater time period; repeated.

- AquaBounty states: ' In the aggregate, these findings were generally of low magnitude, limited distribution, and non-debilitating nature that would be unlikely to compromise the overall health of *AquAdvantage* Salmon in commercial production. '. This presumably alludes to at least a part of the study objective of the client that involved a determination as to whether the AquAdvantage triploid fish are healthy enough to withstand the rigors of commercial production. To this question the study design was too restrictive in scope to provide a satisfactory answer.

Based on studies and observations of gross morphological and clinical pathologies, we conclude with reasonable uncertainty, that morphological irregularities derived from the transgene do not represent serious fish health issues.

9.2.7.9 Tolerance to Physical Factors

There is no empirical data on the range of temperature, salinity and pH tolerance of the AAS compared to non-transgenic Atlantic salmon. Physiological data suggest that the AAS would have a reduced capacity to survive in lower dissolved oxygen water. Evidence of preferential physiological pathways towards smoltification may render the AAS able to sustain changes in salinity at an earlier stage but there is no comparative data on the tolerable salinity range.

There are no studies specifically reporting on the comparative tolerance of the AAS and non-transgenic comparators to a range of physical factors such as temperature, salinity, oxygen, pH, etc. Nevertheless, the increased requirement of AAS for oxygen at rest and the lack of increase in gill surface area (Deitch et al. 2006) provides indirect evidence of a reduced capacity for survival of the AAS under lower dissolved oxygen concentration.

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

AAS-relatives are able to compete smolting at the low age of 6 months and hence tolerate direct transfer from freshwater to 35‰ salinity and survive over 96 hours contrarily to their aged-matched non-transgenic counterparts that all died within 24 hours[26] (Saunders et al. 1998)[27]. Although this study provides evidence that AAS-relatives can undergo smoltification earlier than their non-transgenic counterparts, it does not provide comparative tolerance to a range of salinity between transgenic and non-transgenic smolts.

9.2.7.10 Body Composition

There is supporting evidence that AAS feed commercial diet has a body composition within the range of commercial Atlantic salmon strains. Nevertheless, there is no information about the body composition of AAS at other life stages than commercial size and while feeding on natural preys.

The notifier provided information about the body composition of AAS at a market-size (Erisman, 2004). This information is mainly relevant to the safety and fitness for human consumption, nevertheless extreme deviations from the body composition of wild Atlantic salmon could potentially affect predators of Atlantic salmon.

ABT reported body composition for market-sized (2.0 to 7.5 kg) Atlantic salmon including AAS (referred to as TX by ABT), non-transgenic controls (SC) and farmed

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[26] Fish were transferred to seawater when they had reached (for transgenic) or were approaching (non-transgenic) smolt size (14 to 16 cm).
[27] The genotype of fish in this study is determine by the growth rate, fish in the upper modal groups and in the lower modal groups were designated to be transgenic and non-transgenic, respectively without confirmation of the genotype.

comparators[28] (Erisman, 2004). Fish were automatically feed Moore-Clarke commercial salmon diet (Erisman, 2004) of three different protein (ranging from 37 to 46%) and fat (25 to 36%) content (USFAD, 2010) depending on stages. Nevertheless, AAS and non-transgenic controls were fed similar diets during the three months prior to sample collection (USFAD, 2010). Main conclusions from the study report 71% higher total fat, a 13% lower pantothenic acid, a 21% lower vitamin B1, and lower 30% vitamin C content in the AAS compared to the non-transgenic control salmon (Table 9-15). Other reported small differences [redacted] in the AAS compared to the non-transgenic controls. Despite differences, all reported values were similar to farmed salmon body composition (Erisman, 2004).

[28] [redacted]

[redacted]

[redacted]

We did not conduct a full analysis of the compositional and nutritional raw data provided by ABT considering the remote potential hazard of body composition of AAS on predators. Based on the data provided by ABT, USDA and the literature, we conclude that market size AAS, fed the identified commercial diets, has a similar body composition to other commercial Atlantic salmon strains (Table 9-16). We also conclude that in the context of the environmental risk assessment, the body composition of the AAS at other life stages, including highly predated upon juvenile stages, and the body composition of the AAS based on a diet representative of what would be found in nature also remains unknown.

Table 9-16. USDA and ABT nutrition profile of raw Atlantic salmon. Taken from Erisman, 2004.

		- USDA No. 15076 -			SPONSOR DATA									- USDA No. 15236 -			
Value/100g tissue		Wild Atlantic			Treated Salmon			Sponsor Control			Farmed Control			Farmed Atlantic			
Proximate	(g)	Mean	SE	n	Mean	SE	n	Mean	SE	n	Mean	...	n	Mean	SE	n	
Moisture	...	68.50	1.146	21	65.20	0.593	30	69.36	0.377	30	64.43	...	10	68.90	...	2	
Protein	...	19.84	0.662	9	19.13	0.245	30	20.15	0.185	30	18.85	...	10	19.90	...	2	
Total Fat	...	6.34	1.772	7	14.59	0.685	30	8.99	0.267	30	15.17	...	10	10.85	...	2	
Ash	...	2.54	0.894	6	1.14	0.040	30	1.17	0.029	30	1.13	...	10	1.05	...	2	
Carb	...	0.00	...	0	...	...	..	...	...	..	...	...	..	0.00	...	0	
Mineral	(mg)																
Ca	...	12	4.117	3	27.6	1.192	30	29.4	1.118	30	31.5	...	10	12	...	0	
Cu	...	0.250	...	1	0.067	0.002	30	0.070	0.003	30	0.064	...	10	0.049	...	2	
Fe	...	0.80	...	1	0.490	0.019	30	0.482	0.015	30	0.518	...	10	0.36	...	2	
Mg	...	29	...	1	24.7	0.414	30	26.9	0.260	30	25.6	...	10	28	...	2	
Mn	...	0.016	...	1	0.026	0.001	30	0.025	0.001	30	0.028	...	10	0.015	...	2	
P	...	200	...	1	256.4	3.129	30	267.2	2.478	30	260.7	...	10	233	...	2	
K	...	490	30.234	3	368.6	4.527	30	393.6	4.148	30	375.5	...	10	362	...	2	
Na	...	44	...	2	32.5	1.154	30	35.7	0.810	30	32.5	...	10	59	...	2	
Zn	...	0.64	...	1	0.509	0.014	30	0.517	0.014	30	0.568	...	10	0.40	...	2	
Se	(µg)	36.5	...	0	16.94	0.160	30	17.98	0.260	30	20.17	...	10	36.5	...	0	ppm*10^2
Vitamin	(mg)																
FOL	(µg)	25	...	1	21.78	1.33	30	25.21	1.72	30	28.89	...	10	26	...	1	µg/g*10^2
NIA	...	7.860	0.618	13	9.746	0.167	30	8.849	0.157	30	8.889	...	10	7.505	...	2	µg/g*10^3
PAN	...	1.664	0.375	5	1.100	0.040	30	1.293	0.044	30	1.340	...	10	1.380	...	2	µg/g*10^3
A	(IU)	40	...	1	<50	...	30	<50	...	30	<50	...	10	50	...	2	
B_1	...	0.226	0.038	11	0.068	0.002	30	0.078	0.002	30	0.064	...	10	0.340	...	2	
B_2	...	0.380	0.147	6	0.108	0.002	30	0.114	0.003	30	0.101	...	10	0.120	...	2	µg/g*10^3
B_6	...	0.818	0.035	4	0.932	0.018	30	0.868	0.016	30	0.800	...	10	0.637	...	2	µg/g*10^3
B_{12}	(µg)	3.18	0.312	10	3.03	0.150	30	2.75	0.140	30	3.29	...	10	2.80	...	2	µg/g*10^2
C	...	0.0	...	0	2.98	0.142	30	3.84	0.230	30	2.77	...	10	3.9	...	2	

9.2.8 Inheritance and Stability of the Transgene

There is sufficient evidence over 3 generations to conclude that the opAFP-GHc2 inserted at the EO-1α locus is transmitted through Mendelian inheritance. There is also sufficient evidence over 5 generations to conclude in the molecular stability of the transgene at the EO-1α locus. However, the accelerated growth phenotype of AAS appears to be very plastic, and is strongly influenced by environmental conditions.

9.2.8.1 Inheritance of the Transgene

There is sufficient evidence, based on the transgenic ratios in different generations, to conclude in a Mendelian inheritance of the opAFP-GHc2 transgene integrated at the EO-1α locus in the AAS.

The determination of the inheritance mechanism is based on ratios of non-transgenics to transgenic individuals as determined by PCR[29]. The notifier provided inheritance ratios for 80 different crosses over 5 generations of AAS in both the ASC3482 and ASC3513 families of AAS. Progenies of crosses of the ASC3482 and ASC3513 males from the F_1 generation with non-transgenic females resulted in in 73% and 67% of transgenic fry suggesting a Mendelian inheritance of two independently segregated integrants (named α and β) at chromosomally distinct loci (ABT 2013, Shears and Yaskowiak 2004 [redacted]). Selective breeding was applied to increase growth performance and lead to the establishment of an AAS brood stock that only bears the α-integrant. Progenies of crosses of [redacted], [redacted] and [redacted] males from the F_2 generation, derived from 3482αβ, with wild-type females all resulted in 50% inheritance of the GH transgene in the offspring, which represents the expected ratio for Mendelian inheritance for crosses between transgenic hemizygous with wild-type individuals (Fletcher et al. 2004, [redacted]). The notifier also provided evidence of transgene inheritance percentage of 0% and 100% for crosses between wild-type fish and involving transgenic homozygous fish, respectively (Shears and Yaskowiak 2004, [redacted]). The evidence is considered adequate to conclude in a Mendelian inheritance of the opAFP-GHc2 transgene across generations in the AAS.

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[29] Primers used to confirm the Mendelian inheritance are different from the ones officially reported to detect a transgenic fish bearing the opAFP-GHc2 construct at the EO-1α locus [redacted]

9.2.8.2 Genotypic Stability

There is sufficient evidence, based on multi-generational sequencing and multiplex PCR, to conclude in the molecular stability of the opAFP-GHc2 transgene at the EO-1α locus.

Brood stock is maintained over several generations, hence the importance of demonstrating molecular stability of the transgene. The stability of the opAFP-GHc2 at the EO-1α locus is demonstrated over three generations through consensus nucleotide sequencing results of the EO-1α integrant and genomic flanking regions in F_2 and F_4 individuals (Yaskowiak et al. 2006 and ABT 2013 – AAS-MFG-004). [redacted] Additional demonstration of the stability is also provided for a broad sampling of AAS individuals from F_2, F_4 and F_6 generations through a diagnostic PCR assay that detects the 5' and 3' junctions of the EO-1α integrant (ABT 2013, Supplement 1 to Study Report AAS-MFG-004). For every sample, [redacted] and considered indicative of molecular-genetic stability of the EO-1α integrant. The combination of the multi-generational sequencing

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[redacted] This is acceptable considering the complexity of optimizing a PCR reaction for several primer sets, and considering that the bands that discriminate between the transgenic and non-transgenic and the ones that demonstrate the stable integration into the genome at the 5' and 3' junction are clear.

and multiplex PCR are considered sufficient evidence to conclude in the molecular stability of the transgene at the EO-1α locus and thereby in low potential for mobilization of or recombination of the EO-1α. However, it should be noted that the insertion of the transgene in a simple sequence repeat region of the genome has the potential to alter locus structure but only over evolutionary timeframes (Greckho 2011).

[redacted]

9.2.8.3 Phenotypic Stability

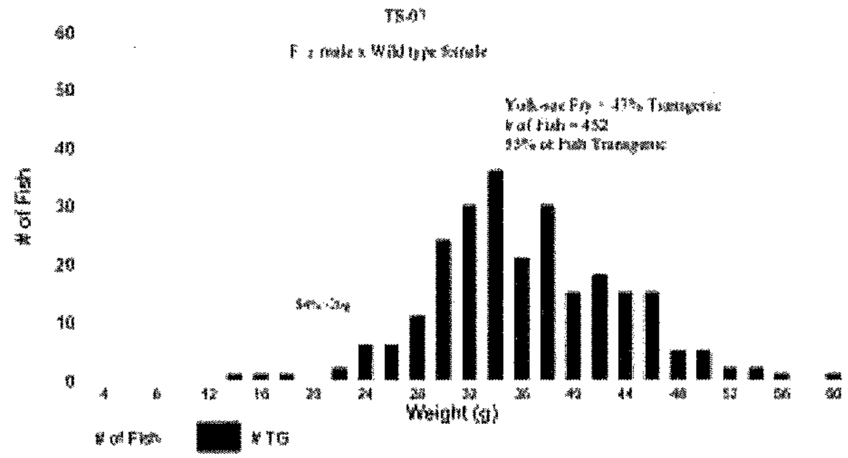
The primary phenotypic change of AAS is increased growth and size at equivalent age relative to non-transgenic siblings. This phenotype is consistently observed in standard hatchery practices by ABT and in numerous published papers. Variation in growth of AAS between and within generations has not been well examined, but appears to be slightly greater than variation of non-transgenics. Accelerated growth of AAS appears to be moderately influenced by different standard culture conditions, and strongly influenced by natural versus culture conditions. As such, the accelerated growth phenotype of AAS appears to be very plastic, and is strongly influenced by environmental conditions.

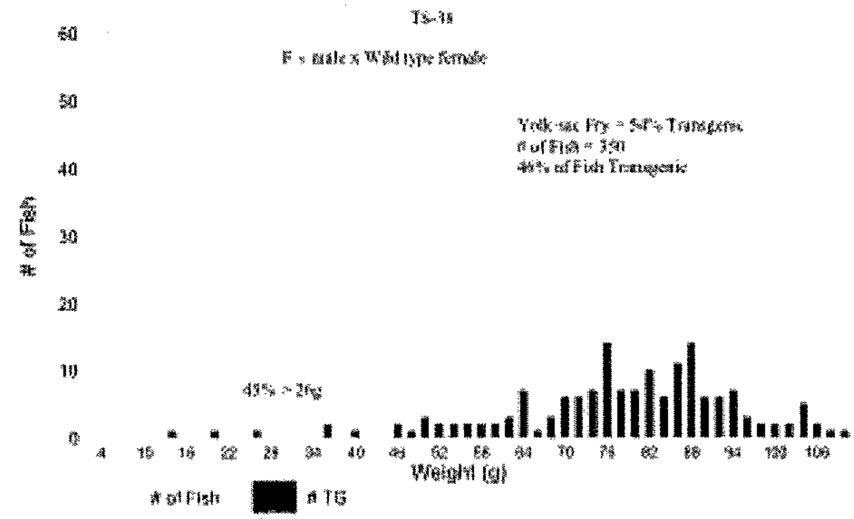
The primary phenotypic change of AAS is increased growth rate and increased size at equivalent age relative to non-transgenic siblings. This phenotype is consistently observed in both diploid and triploid AAS in standard hatchery practices by ABT and in numerous published papers on AAS and relatives (see Section 9.2.7.1). However, there is limited data on the stability of accelerated growth over generations, as well as in different environments. While more information is needed, accelerated growth of AAS fish can vary to a moderate degree between different generations and standard culture conditions, and to a large degree between different environmental conditions (e.g. hatchery versus artificial stream).

Direct comparisons of growth rates of AAS between generations have not been specifically examined, but some information regarding generation-effect on growth rate can be ascertained from data provided by ABT. While inconsistencies between age at reported size make comparisons of data between generations difficult, there appears to be noteworthy variation in growth rate of AAS fish between generations. [redacted]

[redacted] Taken together, size and growth rate appears to vary to a greater degree between and within generations in AAS fish than in non-transgenic fish, although further work is required to confirm this.

The effects of different standard culture conditions on accelerated growth of AAS fish have not been directly assessed. AAS fish maintained high growth rate and body size at the PEI facility (see Section 2.2.7.1), at the Ocean Science Center (see Figures 9.17 and 9.18), and at the grow-out site in Panama (see Figure 9.19). However, direct comparison of these studies is difficult as there are no data between studies that are consistent in format (i.e. degree versus calendar days), in time at measurement, or in year/generation of measurement. Increased growth relative to non-transgenic controls does appear to vary between studies conducted at different facilities. For example, AAS fish are approximately 1.5 times greater in size than non-transgenic fish at 20 months when grown at the Panama site (see Figure 9.19), and 4 or more times greater in size at 15-18 months when grown at the PEI facility (in 2010, see Figure 9.7). However, whether this is due to differing environmental conditions at the two sites, generational effects, or a combination of these and other factors, is not known, and further work is required to determine the phenotypic stability of high growth in AAS fish across standard culture conditions.





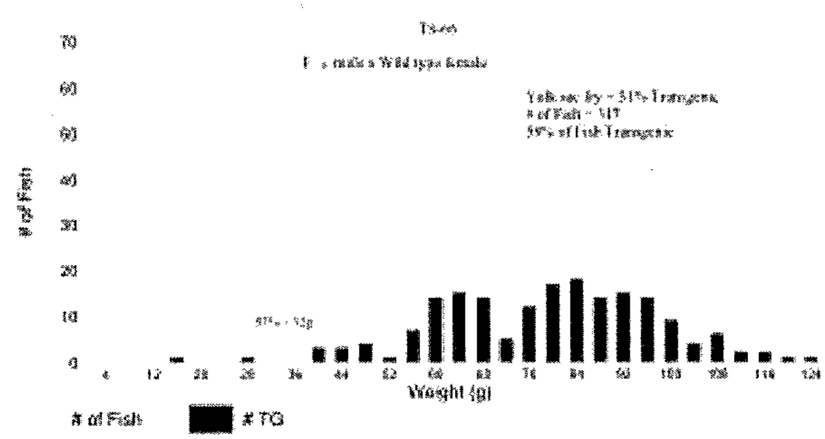


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Figure 9.17 (previous page) Size-frequency distribution (by weight) of representative F_3, F_4 and F_5 generations of AAS at the Ocean Science Center, measured at 11-12 months post first-feed.

Shaded areas indicate transgenic offspring, white areas indicate non-transgenic offspring. Taken from Shears and Yaskowiak (2004).

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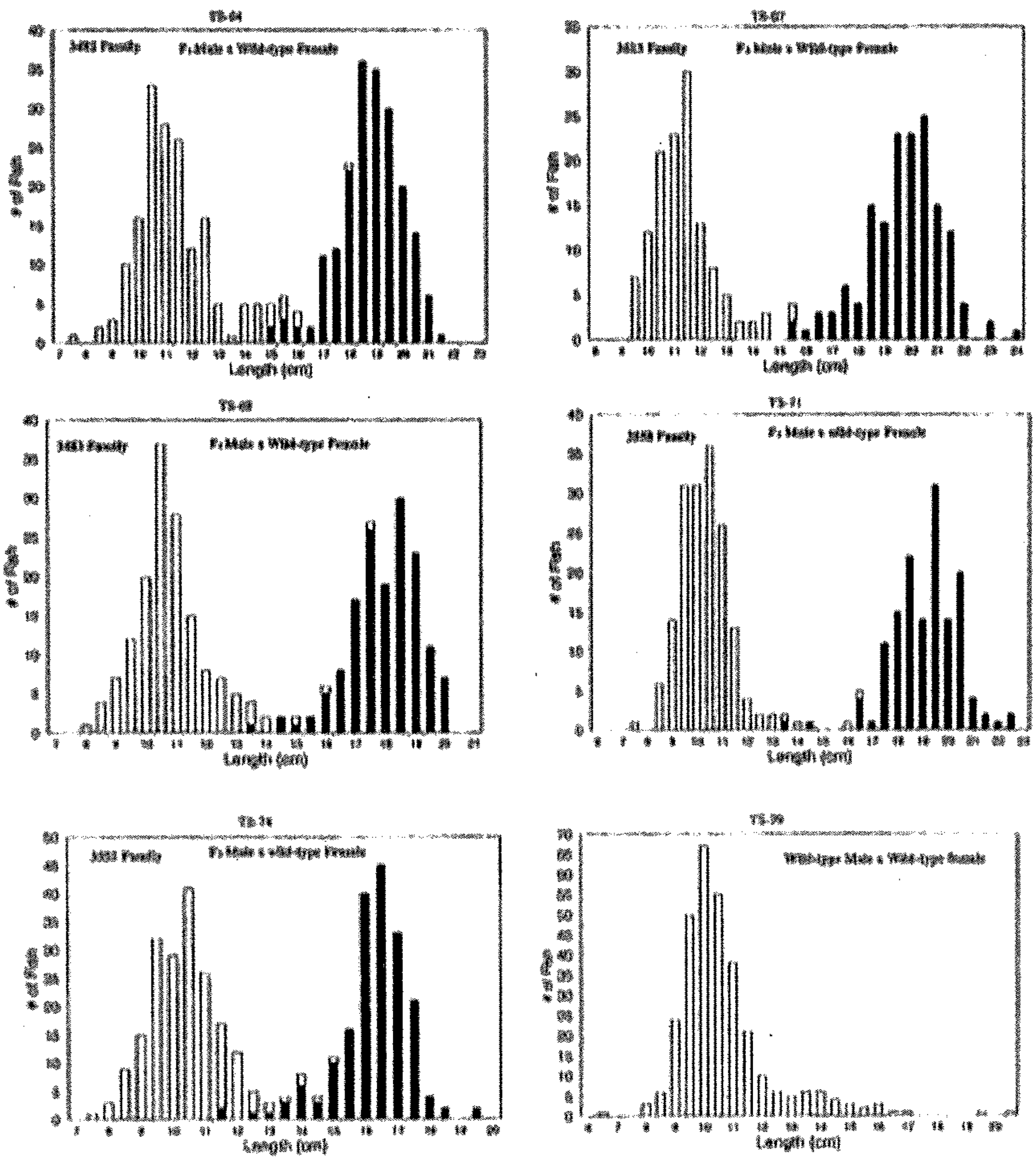
Figure 9.18 (next page) Size-frequency distribution (by length) of representative F_4 and F_5 generations of AAS at the Ocean Science Center, measured at 7 months post first-feed

Shaded areas indicate transgenic offspring, white areas indicate non-transgenic offspring. Taken from Shears and Yaskowiak (2004) page 46.



☐ # of Fish ■ # of Transgenics

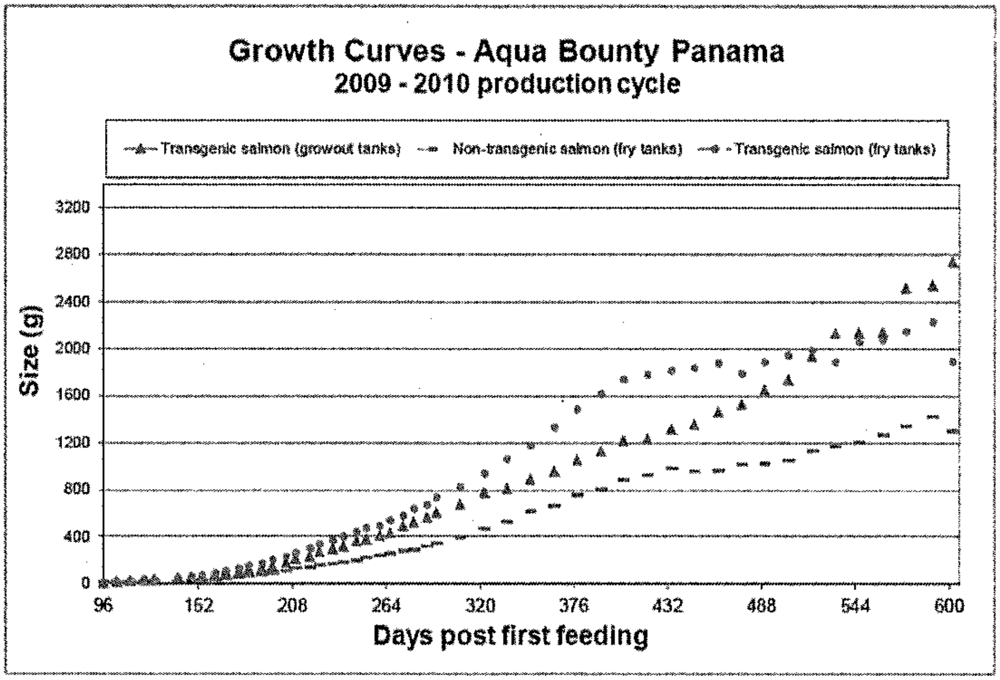


Figure 9.19 Growth performance of AAS at the AquaBounty Canada research and development grow-out facility in Panama. Taken from ABT (2013, p. 147).

Of particular interest is whether AAS fish would maintain high growth rates if released to natural environments. Oke et al. (2013) found AAS fry grown in a hatchery had growth rate 1.29 times greater than non-transgenic fry, but only 0.65 times that of non-transgenic fish when grown in a semi-natural stream environment with limited live feed. In this environment, AAS fish lost their phenotypic high growth rate, to the point of having lower growth than that of non-transgenic fish. Moreau (2011) also found AAS fish did not have increased growth or size above non-transgenic fish for 2 weeks post-emergence in an artificial stream with limited live food and low or high density. This later experiment should be interpreted with caution as all fish had negative growth rate over the course of the experiment. The effect of feeding levels has not been directly assessed in AAS. However, Moreau and Fleming (2012) found AAS x wild salmon mature male parr maintained larger size than non-transgenic mature male parr at both low and high feeding levels under culture conditions. Taken together, the above studies indicate the ability to predict whether AAS fish may maintain high growth phenotype in natural environments is highly problematic, although current studies suggest accelerated growth may be limited in many circumstances.

9.3 Biology of Wild Atlantic salmon

Atlantic salmon (*Salmo salar*), with its spectacular life history, resolve in ascending rivers, and flavourful and tender flesh, has captivated the interest and imagination of inhabitants of Europe and eastern North America for centuries, has been the subject of some of the earliest British laws, and is one of the most studied fish species in the world. By some estimates there are currently tens of thousands of scholarly papers and monographs on the ecology, distribution, behaviour, physiology, genetics, taxonomy and all other aspects of Atlantic salmon life, as well as numerous policies, position papers, memorandums, popular science and media articles, related to its utilization, management, cultivation, and preservation.

9.3.1 Taxonomic Status of Atlantic salmon

Atlantic salmon has been classified as a distinct species over 250 years ago. It is accepted that it is a monotypic species with a high degree of phenotypic plasticity (King et al., 2007). Its closest relative is the brown trout (*Salmo trutta*), of European origin.

Linnaeus classified the Atlantic salmon as the species *Salmo salar* in 1758. It is one of the approximately 20 species in the sub-family *Salmoninae*, of the *Salmonidae* family. It is accepted that the entire Salmonidae family appears to have evolved from a common ancestor following genome duplication. This facilitated species radiation driven by the adaptive benefits of the tetraploidisation event. At the current stage of the family's evolution, the initially tetraploid genome has evolved in each species as to it again behaves as a diploid (King et al., 2007).

The genus *Salmo* comprises two species – the Atlantic salmon and brown trout (*Salmo trutta*). In the past the species has been viewed as composed of a number of distinct evolutionary lineages (polytypic origin); however, the species has been considered monotypic by most contemporary researchers (Webb et al., 2007). The genetic diversity and species structure is further discussed in Section Background Genetics.

9.3.2 Distribution

Atlantic salmon is native to the temperate and subarctic regions of the North Atlantic Ocean and its marginal seas. Although the migratory ranges of many populations overlap during the marine stage of their life cycle, the freshwater spawning and rearing habitat is highly fragmented.

Atlantic salmon are distributed throughout the North Atlantic Ocean and the associated freshwater drainage basins (Thorstad et al. 2011; Webb et al. 2007; MacCrimmon and Gots 1979, Scott and Crossman, 1973).

In North America, anadromous populations of Atlantic salmon can be found in the coastal rivers and streams of the New England States, the Maritime Provinces, Newfoundland, Quebec and in Labrador as far north as Siugak Brook in Okak Bay (57 35 N, 62 06 W). There have been unconfirmed reports of populations further north (Ian Bradbury, personal communication). Isolated populations can also be found in the Nastapoka River on the eastern side of Hudson Bay, Ungava Bay and one population on the western side of Greenland. Resident freshwater populations are found throughout north-eastern Quebec, Labrador, Newfoundland, southern New Brunswick and New England.

In Europe, Atlantic salmon are distributed from northern Portugal to the Kara and Barents Seas in north-western Russia and can be found in the Baltic Sea, the United Kingdom, Ireland and Iceland.

During its marine phase, Atlantic salmon feed throughout the North Atlantic Ocean. Most salmon originating from rivers in North America will spend the winter feeding in waters surrounding the Grand Bank, north eastern Newfoundland and southern Labrador, before migrating back to their natal streams as one sea-winter (1-SW) adults, commonly known as grilse (reviewed by Reddin 2006). Multiple sea-winter (M-SW) salmon tend to migrate further north into the Labrador Sea and east of Greenland, where they inter-mingle with multiple sea-winter salmon that originate from Europe. On rare occasions, multiple sea-winter fish originating from North America will continue to the Eastern Atlantic, where they will ascend European rivers to spawn (Reddin et al. 1984 cited in Reddin 2006. However, other authors have demonstrated that there are no contemporary, or recent intercontinental gene exchanges and that the presence of European allelles in some populations in Europe and of North American alleles in northern Europe and Russia are considered to be remnants of post-glaciation colonization events (G. Chaput, personal communication, King et al., 2007). An exception to the migratory pattern in North America consists of several populations of Atlantic salmon that originate from rivers in the inner Bay of Fundy, who tend to remain in the bay and in the surrounding areas throughout their entire life-cycle (Ritter 1989 cited in Reddin 2006, Webb et al., 2007). Atlantic salmon populations originating from rivers draining into Ungava Bay, Quebec exhibit a similar migratory behaviour (Power et al. 1987 cited in Reddin 2006).

Over the past century, declining numbers of Atlantic salmon has resulted in a contracted distribution that has become increasingly fragmented, especially at the southern fringe of the species range (G. Chaput, personal communication, Thorstad et al. 2011; Webb et al. 2007; Parrish et al. 1998). In those southern areas of its historical range, populations have either been extirpated, or are in danger of disappearing without the support of supplemental stocking programs, including river- and stock—specific products and captive bred and in river gene banks. However, in many cases, historical practices used by such programs have introduced fish not native to local waters into areas where endemic populations are endangered or extinct.

Industrial and agricultural practices that have resulted in habitat destruction and changes to both the freshwater and marine environments are broadly implicated in the decline of wild Atlantic salmon populations (Chaput 2012; Parrish et al. 1998).

9.3.3 Physical and Biological Requirements

Atlantic salmon populations have complex and flexible life histories that begin in freshwater and may involve extensive migrations through freshwater and marine environments before returning to fresh water to spawn. The transition between the life history stages is accompanied by profound hormonal, physiological, and morphological changes. Availability and quality of habitat as well as water's physicochemical parameters are considered limiting factors during the freshwater stages of life cycle.

9.3.3.1 Physical Habitat

Rivers used by Atlantic salmon for spawning and rearing are generally clear, cool and well oxygenated, with low to moderate gradient, and possessing bottom substrates of gravel, cobble and boulder. Freshwater habitat is considered a limiting resource to freshwater production and is used to set conservation requirements for Canadian rivers. There have been substantial declines in habitat quantity and quality in the southern portion of the species' Canadian range. This loss of freshwater habitat may be an important risk factor for declining abundance in several southern DUs. Trends in the quality and quantity of marine habitat are not well understood, but large-scale changes in ocean ecosystems may be adversely affecting Atlantic salmon across their range (COSEWIC, 2010).

The migratory behaviour exhibited by Atlantic salmon makes them particularly vulnerable to the negative effects of obstructions. Both natural and man-made barriers to fish passage severely reduce the production of salmon by restricting mature salmon from reaching spawning habitat and preventing juveniles from reaching feeding and refuge habitats. In general, most obstructions in excess of 3.4 m in height will block the upstream passage of adult salmon (Powers and Orsborn 1985). Ideally, a passable falls will have a vertical drop into a plunge pool with a depth 1.25 times the height. Depending on the shape of the falls and plunge pool, the maximum height can be considerably less. Furthermore, since jumping and swimming capacity is a function of body length (Reiser and Peacock 1985), the ability of juveniles to surmount barriers is greatly reduced relative to adults (COSEWIC, 2010).

9.3.3.2 Dissolved Oxygen Content

Salmonids are known to have a greater requirement for dissolved oxygen (DO) than warm-water fish (Scott and Crossman, 1973, Grant and Lee, 2004, O'Connell et al., 2006). Oxygen requirements and tolerance to low dissolved oxygen vary depending on the life stage, but it is generally accepted that concentrations above 9 mg/L are optimal, and concentrations above 7 mg/L are tolerable in the 50th percentile (Hendry and Cragg-Hine, 2003).

9.3.3.3 Water Temperature

Atlantic salmon, are ectothermic and so are dependent upon the surrounding water temperature to cue migratory patterns, to drive metabolic processes, and to determine the rate of progression from one life stage to the next. Some authors view Atlantic salmon as having the most narrowly defined thermal requirements, in terms of survival, feeding, and growth, of all salmonids species (Elliott, 1991, cited in Webb et al., 2007). However, the range of water temperatures experienced by different populations is highly variable, and some might experience the full range of tolerable temperatures for the species within a year.

Relative change in water temperature affects both local movement and seaward migration of salmon; at the extremes of the tolerable range, feeding and survival are affected. The optimal range is estimated to be ~4-10°C, with the lower- and upper-limits for survival being ~0°C and ~28°C, respectively. The upper-limit of tolerance for juveniles (< 100 g) in fresh water has been estimated at ~24°C;for parr and 28^{0}C for fry,. Since thermal sensitivity is size –specific, adults are more temperature intolerant than juveniles and the incipient lethal temperature has been estimated to be near 25^{0}C (DFO 2012)

Lethal seawater temperatures for both wild and farmed salmon smolts adapting to seawater were reported to occur at both low and high temperatures. At the lower end of the temperature range, mortalities of postsmolts occurred at sea temperatures of 6-7°C while at the higher end, mortalities occurred at temperatures over 14°C. This suggests that there may also be environmental windows for successful smolt transition into the sea. (COSEWIC, 2010). On the other hand, smolts enter saltwater at temperatures above 140C in the southern Gulf of St. Lawrence (G. Chaput, personal communication).

Adult and juvenile salmon may live for short periods above the incipient lethal temperature. A sudden increase in incipient temperature in excess of 10°C may bring about the death of resident salmon at temperatures considerably below the upper lethal temperature. (COSEWIC, 2010; Center for Veterinary Medicine, 2012a).

Post smolt and adult salmon are found at sea in water with temperatures of 1-12.5°C, with peak abundance at 6-8°C. In the Labrador Sea, 80% of the salmon were found in waters with surface temperature between 4-10°C. Similarly, tagged Atlantic salmon kelts were found in temperatures ranging from a low near 0°C to over 25°C, although most of the time kelts stayed in seawater of 5-15°C. Lethal temperatures for adult salmon occur below 0°C. This may explain the tendency of salmon to avoid ice-covered water (COSEWIC, 2010).

Little else is known about the upper-limit of tolerance for adult Atlantic salmon in the marine environment, but feeding and general activity continue to occur at temperatures up to ~20°C and according to some authorsmortality does occur at ~22°C (CVM, 2012a). However, salmon stage in estuaries and coastal waters of the Gulf of St. Lawrence through summer in water temperatures exceeding 22°C frequently without observed negative effects, and salmon returning from the ocean were frequently sampled in estuary trapnets when temperatures exceed 22°C (G. Chaput, personal communication.

9.3.3.4 Hydrogen Ion Activity (pH) and Acidification

Acidification is an important freshwater stressor for Atlantic salmon in some regions. Increased H+ ion concentrations, coupled with the low concentrations of Ca++ are responsible for increased mortality of salmon in acidified rivers of Nova Scotia. In fresh water, the osmotic gradient results in the passive diffusion of water into the blood and of ions out of the blood. Passive losses of ions are countered by active uptake of Na+ and Cl- from the water to maintain a balanced state. When pH is ≤ 5.0, active uptake of Na+ and Cl- is reduced and passive efflux is increased resulting in a net loss of both ions. The loss of ions results in a shift of water from the extracellular fluids (e.g., plasma) to the intracellular fluids, causing a reduction in blood volume. In addition, red blood cells swell and additional cells are released from the spleen. The reduced blood volume and increased number and size of the red blood cells may cause a doubling of blood viscosity and arterial pressure. Death is a result of failure of the circulatory system.

Other determinants of the negative effects of acidification are the concentrations of calcium, dissolved organic carbon (DOC), and aluminum. High levels of DOC (5-30 mg/L) chelate the free (i.e., cationic) form of aluminum that is toxic to fish; however, in the absence of free aluminum, low pH and calcium alone can cause salmon mortality. Generally mortality due to exposure to low pH in fresh water varies with the life stage of salmon. All freshwater stages are unaffected when pH is above 5.4 but mortality of fry (19- 71%) and smolts (1-5%) occurs when pH is below about 5.0. Mortality of parr and smolts is relatively high (72-100%) when pH declines to the 4.6-4.7 range. Eggs and alevins begin to experience lethal effects at pH's below 4.8. Levels of pH ≤5.0 also interfere with the smoltification process and seawater adaptation. Due to the natural buffering capacity of the ocean, acidification issues for Atlantic salmon are restricted to freshwater environments (COSEWIC 2010).

9.3.4 Life-history

Atlantic salmon display considerable phenotypic plasticity and variability in life-history characters ranging from fully freshwater resident forms, where females can mature at approximately 10 cm in length, to anadromous populations characterized by 3–5 sea-winter (5SW) salmon.

Atlantic salmon have a complex and highly variable life-history (Hutchings and Jones 1998) undergoing a series of anatomical, physiological and behavioural changes that enable individuals and populations to survive and adapt to constant variation in both the freshwater and marine environment. Even within simple 1SW populations, 20 or more spawning life-history types can be identified (Klemetsen et al., 2003).

Atlantic salmon are, for the most part, anadromous, spending their embryonic (egg and alevin) and juvenile (fry and parr) life-stages in fresh water streams before migrating (as smolts) to the Atlantic Ocean where they become adults (reviewed by Thorstad et al. 2011). After a period of growth at sea, sexually mature adults migrate back, with variable fidelity, to their natal streams where they spawn and deposit fertilized eggs into the river's gravely substrate. Resident freshwater populations, spending their entire lives in fresh-water without a marine migration, are commonly observed throughout the native range of the species. These include landlocked (whose migrations are prevented by impassable barriers), and resident freshwater populations as well as juvenile males that become sexually mature as parr (reviewed by Fleming and Einum 2011). Following spawning some mature parr may undergo smoltification; migrate to sea, and return to spawn again later as full-size adults (Garcia de Leaniz, cited in Webb et al., 2007).

A generalised life cycle of Atlantic salmon is presented in Figure 9.20.

Figure 9.20 Generalized life history of Atlantic salmon (source COSEVIC, 2010, from O'Connell et al., 2006)

9.3.4.1 Eggs and Alevins

The embryonic stage for Atlantic salmon lasts from fertilization until just after absorption of the egg-sac. During this period of development, cells differentiate, organs take form and the organism grows until it eventually breaks free from the egg. After hatching, the alevin, or sac-fry, is still dependant on the nutrients originally supplied by the mother with the egg, which are now contained within the egg-sac. It is not until this sac has been completely absorbed and the digestive tract is fully functional, that the fry is ready to swim up to the surface, fill its air-bladder and commence feeding on exogenous nutrients. Throughout this stage of development, the embryo is restricted to an environment in which physical and chemical factors such as temperature, dissolved oxygen, pH, salinity and mechanical stress must be maintained within acceptable limits for normal development.

9.3.4.2 Fry

The Atlantic salmon fry stage is relatively short lived, occurring when the fish emerges from the river's gravel bed and start to feed on exogenous nutrients. Soon after emergence, fry start to disperse from the area surrounding the redd (gravel nest), typically moving downstream and avoiding pools. The fry stage ends when the fish settle and establish small territories, which they defend against conspecifics of the same year-class. After this point, they are referred to as parr.

9.3.4.3 Parr

The Atlantic salmon parr stage starts once the newly emerged fry have dispersed from the redd and establish small territories, which they defend against conspecifics of the same year-class. It is the predominant freshwater stage and, in the wild, may last from one to eight years depending on the growth conditions of the nursery stream. In some cases, parr may leave the territory it initially established as a fry and move upstream in search of more favourable habitat (McCormick et al. 1998; Hutchings 1986) or downstream to occupy an estuarine environment if there is improved food availability (Cunjak 1992). The parr stage ends when the fish becomes a smolt and undergoes a physiological transformation that enables it to survive and grow in the marine environment.

9.3.4.4 Sexual Maturation as Parr

The life cycle of most anadromous *Salmoninae* encompasses migration to sea. However, a proportion of the fish, usually male, become mature at the parr stage, without leaving fresh water. These fish have the capability to reproduce with anadromous and resident partners. Mature female parr have been detected in some anadromous populations, but are considered relatively rare (Fleming 1996 cited in Webb et al., 2007). Precocial maturation of parr is widespread, and likely the dominant male phenotype, in Newfoundland rivers where sex ratios of anadromous individuals are significantly skewed towards females (~70%) (I. Bradbury, personal communication, Daley et al., 1983).

Maturity among resident parr has been regarded as a 'conditional strategy', whereby the expression of the phenotype is not predetermined genetically, but is most probably linked to the individual growth rate and size and condition at age. It is also a manifestation of the phenotypic plasticity of the Atlantic salmon. Laboratory studies have demonstrated the relationship between the predisposition of individuals to mature and body size and lipid content (Rowe et al., 1991, Berglund, 1995 cited in Webb 2007). It has been noted that in wild populations with larger individuals there is a higher proportion of mature male parr. At the individual level, the initial fastest-growing individuals tend to mature in higher proportions, than slower-growing individuals, though the latter may overcome the former by the fall. Consequently, it has been suggested that size divergence of fish destined to mature begins soon after the emergence from the spawning nests, and that pre-maturing male parr exhibit a size advantage a year before maturation (Webb et al., 2007). Results of studies on the reproductive success of mature male parr at the group and individual level have shown a high variability; however, it appears that mature parr as a group contribute to the fertilization of eggs in egg nests and redds, and on the basis of relative body mass, reproductive success can be substantial (Jordan et al., 2007). Dionne et al. (2012) estimate that the contribution of mature parr is approximately 40 % in Newfoundland streams. As well, they may be playing a critical role in maintaining diversity in Newfoundland rivers (Johnstone et al., 2013, I. Breadbury, personal communication).

9.3.4.5 Smolts

The Atlantic salmon smolt stage is a period of transition in which freshwater parr undergo morphological, physiological and behavioural changes that prepare it for life in the marine environment (Thorstad et al. 2011, McCormick et al. 1998). This transformation typically involves the acquisition of a slimmer body form, colour changes that help to conceal it in the pelagic environment, increased salinity tolerance and the behavioural drive to leave its territory and migrate downstream toward the sea.

9.3.4.6 Smolt Migration

Throughout the natural distribution of Atlantic salmon, there is considerable inter-population and inter-regional variation in both the timing and the destination of seaward migrations (reviewed by Thorstad et al. 2011). While the age at which a parr becomes a smolt may vary depending on growth rate or productivity of the stream, the timing of seaward migration within a particular river is coordinated and is believed to be highly dependent on variables such as the river's water temperatures and the diurnal cycle.

9.3.4.7 Post-smolts and adults

Once wild Atlantic salmon smolts have left freshwater and have completed the transition to the marine environment, they are referred to as post-smolts and will spend the next one to four years at sea, growing into sexually mature adults that will ascend suitable rivers in an attempt to reproduce (Thorstad et al. 2011).

As previously noted, most salmon originating from rivers in North America will spend the winter feeding in waters surrounding the Grand Bank, northeastern Newfoundland and southern Labrador, before migrating back to their natal streams as one sea-winter (1-SW) adults, commonly known as grilse (reviewed by Reddin 2006). Multiple sea-winter (M-SW) salmon tend to migrate further north into the Labrador Sea and east of Greenland, where they inter-mingle with multiple sea-winter salmon that originate from Europe.

Several populations of Atlantic salmon that originate from rivers in the inner Bay of Fundy, tend to remain in the bay and the immediate surrounding areas throughout their entire life-cycle (Ritter 1989 cited in Reddin 20068, Webb et al., 2007). Atlantic salmon originating from rivers draining into Ungava Bay, Quebec display an even more spatially restricted migratory pattern, limiting their marine phase to estuaries in some years (Power et al. 1987 cited in Reddin 2006, Webb wet al., 2007)).

Unlike the Pacific salmon, which die after spawning, the Atlantic salmon is iteroparous and is capable of spawning more than once in their life-time. After spawning, spent Atlantic salmon will spend the winter in fresh water as kelts before migrating back to sea in the spring, to repeat the salt-water phase of its life-cycle and the related migratory patterns (Thorstad et al. 2011). In some instances, including Bay of Fundy populations, kelts treturn to sea in November and December (Lacroix 2013)

9.3.4.8 Changes in Growth Hormone Levels during the Life Cycle of Wild Atlantic salmon

Growth hormone (GH) is a hormone produced by the pituitary gland in bony fish and other vertebrates. In fish GH participates in almost all major physiological processes in the body including the regulation of ionic and osmotic balance, lipid, protein, and carbohydrate metabolism, skeletal and soft tissue growth, reproduction and immune function. Recent studies have indicated that GH affects several aspects of behaviour, including appetite, foraging behaviour, aggression, and predator avoidance, which in turn have ecological consequences (for reviews, see Björnsson, 1997, Björnsson et al., 2004; Peter and Marchant, 1995, Reinecke et al., 2005).

Literature data suggests that plasma growth hormone levels in hatchery reared juveniles varied depending on the light regime and other clues, but were generally between 0 and 4 ng / ml, with the higher values recorded in April, May and June (Augustsson et al., 2001) and generally lower values under winter photoperiod (Ebbesson et al., 2008). Smoltification brought higher concentrations of growth hormone, with increases between 3 to 9 ng/ml (Ebbesson et al., 2008) , 4 to 18 ng/ml (Boeuf et al., 1989) and 10 to 30 ng/ml (Prunet et al., 1989).

In maturing males and females, plasma growth hormone levels were around 1 ng / ml through February to September, rising to about 2 ng / ml in October (Bjornsson et al., 1994).

9.3.5 Background Genetics

Atlantic salmon populations form a single species, *Salmo salar*, though divergence between groups in the Eastern and Western Atlantic has resulted from limited genetic exchange for over 500,000 years. The species frequents a wide range of diverse and fragmented freshwater habitats which, coupled with the homing behaviour, has resulted in a large number of reproductively distinct and adaptively differentiated genetic populations.

Atlantic salmon live in the North Atlantic Ocean, its marginal seas, and the Barents and Kara Seas. Although the migratory ranges of many populations overlap during the marine stage of their life cycle, the freshwater spawning and rearing habitat is highly fragmented and the species is subdivided into a high number of spatially disconnected groups of breeders (King et al., 2007). Because of relatively high homing fidelity these groups demonstrate limited mixing among different rivers, even within a river system. As a consequence, Atlantic salmon has demonstrated a considerable level of evolutionary diversity and population structuring. However, the species has shown very narrow scope of morphological differentiation between populations (King et al., 2007).

Based on genetic data (King et al., 2007, COSEWIC, 2010) Atlantic salmon in Western and Eastern Atlantic Ocean belong to two distinct, deeply divergent phylogeographic groups, that have experienced limited gene exchange for approximately 500, 000 years. Nevertheless there is evidence that there have been gene exchanges between the two groups, most likely due to secondary contact early in the current post-glacial period. Within the continental populations individual groups have been isolated for less than 15,000 years and their structure reflects the repeated expansion and contraction of the Atlantic salmon range due to the Pleistocene glaciations, post-glacial colonisation, and environmental conditions. Generally, the following groups have been identified (King et al., 2007):

Eastern Atlantic

- Iceland / Greenland
- Northern Russia / Norway
- Southern Norway / Sweden
- Baltic Sea
- Northern British Isles
- Southern British Isles / Northern France
- Southern France / Spain

Western Atlantic

- Labrador (and Ungava Bay)
- Newfoundland
- Gulf of St. Lawrence
- Nova Scotia
- Inner Bay of Fundy
- Outer Bay of Fundy
- Maine

It should be noted that there is evidence of weaker geographic structuring in the Western Atlantic populations than in the Eastern Atlantic.

Non-genetic data, including variations in life history support much of the broad-scale population structure inferred from the genetic data, including smolt age, small and large salmon proportions in returns, sea-age at maturity, proportion of small and large females, and fork length of small and large fish (Chaput et al., 2006, cited in COSEWIC, 2010). Based on several criteria, both genetic and non-genetic, DFO proposed 28 Atlantic salmon Conservation Units (DFO and MRNF 2008).

In a similar manner, COSEWIC proposed 16 designatable units (DUs, Figure 9.21, COSEWIC 2010) as follows:

- DU 1- Nunavik population,

DU 2- Labrador population,
DU 3- Northeast Newfoundland population,
DU 4- South Newfoundland population,
DU 5- Southwest Newfoundland population,
DU 6- Northwest Newfoundland population,
DU 7- Quebec Eastern North Shore population,
DU 8- Quebec Western North Shore population,
DU 9- Anticosti Island population,
DU 10- Inner St. Lawrence population,
DU 11- Lake Ontario population,
DU 12- Gaspé-Southern Gulf of St. Lawrence population,
DU 13- Eastern Cape Breton population,
DU 14- Nova Scotia Southern Upland population,
DU 15- Inner Bay of Fundy population, and
DU 16- Outer Bay of Fundy population

These units recognise population or group of populations that have attributes that make them discrete and evolutionarily significant relative to other populations, including both inherited traits (e.g. morphology, life history, behaviour) and/or neutral genetic markers (e.g. allozymes, DNA microsatellites, as well as large disjunctions between populations, and occupation of different eco-geographic regions.

The microinjected eggs that initiated the AAS lineage were from the Exploits and Colinet rivers , which belong to DU 3. Further crosses were made with Atlantic salmon captured in in Exploits and Northeast rivers, also in DU 3. The development of the intended AAS commercial line involved predominantly domesticate salmon from the St. John River lineage. As well, the manufacture of eyed-eggs will involve crossing individuals from the brood stock with the domesticated St. John River strain (please refer to Section 9.2.2 for further details. The wild St. John River population of Atlantic salmon is in DU 16.

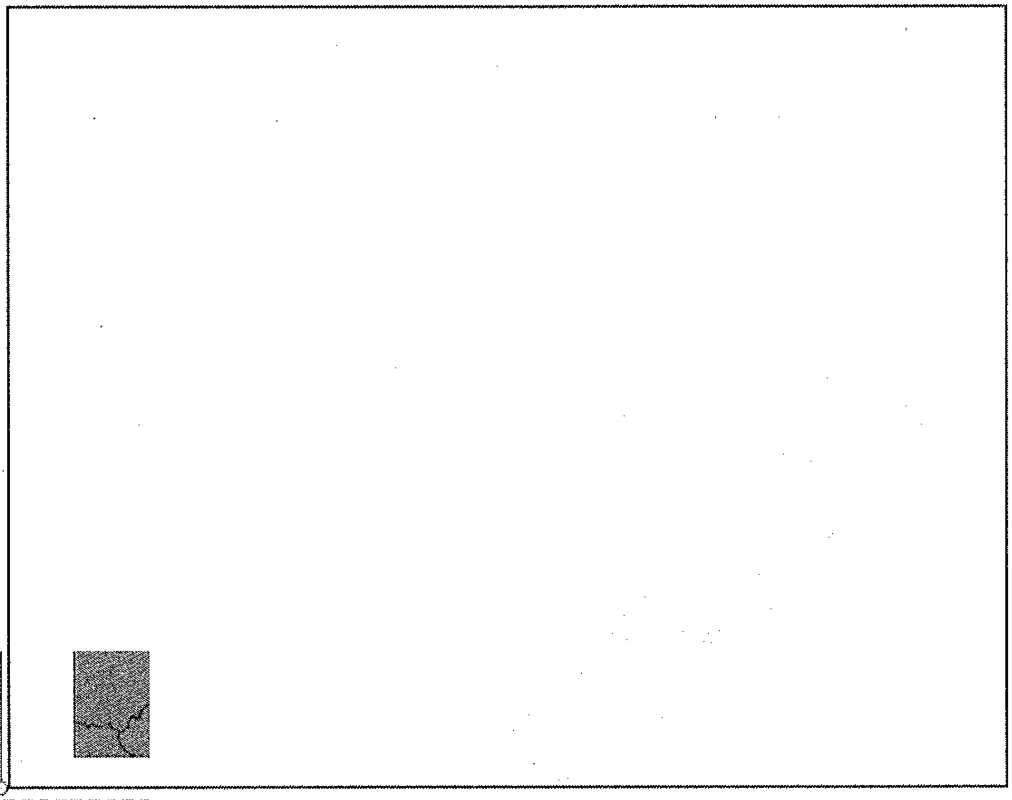


Figure 9.21 Proposed designatable units (DU) for Atlantic salmon in eastern Canada (COSEWIC 2010)

9.3.6 History of Invasiveness

Unlike its close relative, the brown trout, Atlantic salmon are not predisposed to invasiveness to territories outside their North Atlantic native range. With few struggling exemptions, attempts to establish Atlantic salmon populations outside the Atlantic Ocean have failed.

When compared with some other salmonid species, such as brown trout (*Salmo trutta*), rainbow trout (*Oncorhynchus mykiss*), or brook trout (*Salvelinus fontinalis*), Atlantic salmon is considered a poor coloniser outside of its native range (Thorstad et al. 2011). Numerous attempts to establish self-sustaining populations of Atlantic salmon outside of their native or historic range in Canada have occurred in the western provinces of British Columbia and Alberta; however, no permanent populations were ever established (MacCrimmon and Gots 1979). Internationally, repeated attempts to establish anadromous populations of Atlantic salmon in various countries have failed (Thorstad et al. 2011; FAO Database on Introductions of Aquatic Species), though self-sustaining freshwater populations have been established in Argentina, New Zealand and the Kerguelen Islands (MacCrimmon and Gots 1979; Valiente et al 2010; Lecomte et al. 2013).

9.4 Biology of Domesticated Atlantic salmon

The environment and selective pressures in hatcheries and fish farms differ drastically from these in the natural habitat of Atlantic salmon. As a result cultivated fish are subject to morphological, physiological, ecological, and behavioural changes. When cultured Atlantic salmon are released into nature their competitive and survival abilities differ from their wild conspecifics, often putting them in a disadvantageous position. Nevertheless, due to sheer numbers and genetic effects, escaped farm salmon can have detrimental effect on wild salmon populations.

Domestication of Atlantic salmon has three main forms, whereby the domesticated fish spends different time under human husbandry: hatchery rearing and stocking, ocean ranching, and fish farming and aquaculture. Each of these schemes has its own purpose and goals, brings different levels of domestication, and has different impacts on the environment and the receiving ecosystems.

In fish stocking fish are raised in a hatchery and released into the wild at an early age to supplement existing populations, or to create a population where none exists. Stocking may be done for the benefit of commercial, recreational, or Aboriginal fishery, or to restore or increase a population of threatened or endangered fish (Cross et al., 2007, Egglishaw et al., 1984). Under most stocking schemes, indigenous broodstock, or non-indigenous broodstock with desirable traits are caught and stripped of eggs and milt. The fertilized eggs are then either released in the wild or cultured in a hatchery where they hatch and after the reabsorption of the yolk sack, the alevins are reared to a stage when they can be released in wild habitats.

Similar to stocking, in salmon ranching local salmon broodstock are caught and stripped of eggs and milt. The fertilized eggs are then cultured in a hatchery where they spend the first stages of their life cycle. Mimicking the natural life cycle of a wild salmon, the smolts are then transferred from freshwater hatcheries to saltwater fish farms. While in net pens, salmon are fed feed pellets to gain size and strength. Also, during that time the salmon are "imprinted" to the area where they are temporarily farmed. Imprinting ensures that these cultured salmon return to the same place where they were "born" – similar to natural, wild salmon. Once large enough to survive, these cultured salmon are released into the ocean to forage for food (hence the reference to "ranching"). Salmon ranching does not always involve rearing smolts in sea pens before releasing. In many instances smolts are released in the river and imprint to the river and therefore return after rearing at sea (G. Chaput, personal communication).

Upon return, a mixture of wild and ranched salmon are caught in the commercial and sports fisheries. In many instances (e.g. Iceland), there are no wild populations in the "natal" rivers (G. Chaput, personal communication). Selected salmon are also retained by the source hatchery to be used again for eggs and milt and the process is repeated.

In salmon farming (or salmon aquaculture), the entire life cycle of the fish, from fertilization to harvesting or gamete production is under controlled conditions. Generally salmon are farmed in two stages. First, broodstock with desirable traits, usually non-indigenous and cultivated in artificial habitats for generations, are stripped of eggs and milt. The fertilized eggs are incubated and the juveniles reared in fresh water to the smolt stage, then transferred to net cages in the sea for grow-out to market size and harvested. Because under the Notification AAS is intended for salmon farming in closed land facilities, and not for salmon stocking or ranching, mostly the biology of farmed Atlantic salmon is being reviewed. However, for the sake of more completed review, the life history of Atlantic salmon produced by other forms of domestication, as well as other domesticated salmonids was considered, when warranted.

Unlike carp (*Ciprinus carpio*), goldfish, and other ciprinids, which have been domesticated and reared for food more than thousand years, the aquaculture of Atlantic salmon (*Salmo salar*) began in the early 1970s, when Norwegian entrepreneurs successfully harvested fish that had been stocked as smolts and reared in pens at sea for two years. Since then the industry has expanded exponentially and in 2011 approximately 1.7 million tonnes were produced worldwide, with a value of over $ 9.7 billion (FAO, 2013).

In most instances Atlantic salmon farming mimics broadly the life cycle of wild salmon. First, the salmon are hatched from eggs and raised on land in freshwater tanks. When they are 12 to 18 months old, the smolt are transferred to floating sea cages or net pens anchored in sheltered bays or fjords along a coast. There they are fed pelleted feed for another 12 to 24 months, when they are harvested.

Rearing in artificial environments exposes domesticated fish to new selective forces (space restrictions, high density, selection for desirable traits, sensory deprivation, manipulation and handling) while other pressures are alleviated (abundant food, lack of predators, medication, artificial reproduction) . Throughout the generations these forces have led to significant morphological, physiological, behavioural, and life cycle changes. Unless indicated otherwise, the following sections were summarized from the reviews provided by Cross et al. (2007), Ferguson et al. (2007), Jonsson and Jonsson (2006), and Gross (1998).

9.4.1 Morphology and Anatomy

Phenotypic divergences can be shaped by environmental conditions early in life. In artificial conditions the protected environment permits fish to allocate more energy to protein growth and lipid deposition, and several morphological changes occur in association with this. For example, cultured Atlantic salmon parr have smaller heads and rayed fins and narrower caudal peduncles than wild parr. Similar changes have been observed in other salmonids reared under hatchery conditions.

Morphological divergences were observed between wild and cultured Atlantic salmon smolts from the Irish Burrishoole and Corrib stocks. In Burrishoole stocks, wild smolts were thinner and smaller. Atlantic salmon parr grown from the eyed-egg stage with a non-sibling group in a hatchery environment came to resemble the body shape of the cultured non-sibling fish more closely than that of full siblings grown in their natal habitat. Moreover, the shape of wild smolts differed from that of cultured offspring. Although this difference was less pronounced, it was still significant when the fish were captured after free-swimming at sea for 1 year.

At maturity, farmed Atlantic salmon may display a morphology that differs greatly from that of a wild fish. Farmed adults have longer heads, smaller rayed fins, larger adipose fins, and shorter horizontal trusses in the trunk region, and more distorted jaws (Fleming et al., 1994). Farmed males display more damage to their kypes and jaw distortion than wild males, which are almost free of such deformities.

Other changes that were noted in cultured salmonids include the following:

1- It was found (Lema et al., 2005 cited in Jonsson and Jonsson, 2006) that cultured rainbow trout and coho salmon have smaller brains than wild conspecifics of similar size and it is not known whether the reduced cell proliferation of the telencephalon of juvenile fish is associated with swimming activity, sensory input, or social structure in the hatchery tanks. However this may influence cultured salmonids' subsequent behavioural performance in nature.

2- Poppe et al. (2003, cited in Jonsson and Jonsson, 2006) found that the hearts of farmed Atlantic salmon and rainbow trout were rounder than those of their wild counterparts and that the angle between the ventricular axis and the axis of the *bulbus arteriosus* was less acute in farmed fish than in their wild counterparts. The normal shape of the salmonids ventricle is a triangular pyramid with the apex pointing caudo-ventrally.

3- Poole et al. (2003 cited in Jonsson and Jonsson, 2006) found that cultured smolts have significantly higher concentrations of mucous cells in both skin and secondary gill lamellae, which may influence subsequent marine survival.

9.4.2 Physiology and Biochemistry

In order to optimise production, the characteristics of the produced fish, and the timing of harvest, most fish farm operators select broodstock with desirable traits and manipulate many of the environmental variables that guide salmon's life cycle. These deviations from the "natural" environment and selection pressure bring forth not only changes in the morphology and anatomy of cultured fish, but also changes in their physiological functions and biochemical characteristics.

In Burrishoole stocks, cultivated smolts have lower basal cortisol levels in April and May and exhibit a strong cortisol responses to capture stress, which is lacking in wild smolts. Similar differences appear in serum glucose levels. These physiological changes, together with lower gill Na /K ATPase activity, lower growth hormone and plasma chloride levels found in cultured smolts (as compared with wild smolts), and differences in survival on transfer to full-strength seawater at different temperatures (Handeland et al., 2003 cited in Jonsson and Jonsson, 2006), indicate that wild Atlantic salmon smolts may tolerate the transfer better than cultured smolts.

Fleming et al. (2002) found higher levels of growth hormone in domesticated than in wild Atlantic salmon, which is not surprising, considering that individuals with a fast growing phenotype are targeted during the domestication process.

9.4.3 Behaviour and Life History

Farm salmon demonstrate not only physiological and morphological differences, but also dissimilarities in behaviour and life history, both in the artificial environment that has brought forth these changes, but also in the wild, following accidental or intentional releases (Ferguson et al., 2007). The difference in sensory stimulation between cultured and wild salmon may also influence different behavioural aspects, such as territoriality and dominance, feeding, predator avoidance, migration, and reproductive behaviour in nature. The following behavioural traits were observed:

9.4.3.1 Aggression and Dominance

The results of experimental tests of feeding competition between wild and cultured salmon differ. Einum and Fleming (1997) observed that parr of farmed Atlantic salmon dominated wild fish in one-on-one challenges, with hybrids exhibiting intermediate success. The authors related this to greater aggression in farmed fish, as compared with wild fish. Similar dominance of cultured fish was reported by Rhodes and Quinn (1998, cited in Jonsson and Jonsson, 2006) for coho salmon. Furthermore, it was found (Berejikian et al., 1999 cited in Jonsson and Jonsson, 2006) that juvenile coho salmon with cultured mothers won dominance challenges in a laboratory flume more frequently than parental half-siblings with wild mothers, suggesting that dominance may be a maternal effect. Other authors (Riley et al., 2005) found no evidence that rearing environments caused more aggression in cultured steelhead fry than in wild steelhead fry. The greater aggression observed in some cultured fish populations, and the outcome of aggressive and dominant behaviour were shown to be modified by the environment, with wild salmon being better adapted to more complex environments, as well as by previous residence of the conflicting parties.

9.4.3.2 Predator Avoidance

In predator-response experiments domesticated parr had a shorter time until reappearance from cover following a simulated predator attack, and had a lower heart rate and less pronounced flight and heart responses to model predator attack (Jonsson and Jonsson, 2006). Shorter reappearance time of farmed Atlantic salmon was also observed by Houde et al (2009). The authors, who used St. John River farmed stock, concluded that farmed fry exhibited significantly reduced antipredator responses relative to fry from both wild populations. The anti-predator responses of wild-farmed hybrid fry were intermediate to those of the parental populations (pure farmed or wild). The magnitude by which wild X farmed hybrids differed in anti-predator responses from pure wild fish also depended on the wild population.

9.4.3.3 Feeding

Following experiments with masu salmon it was postulated that the tendency of farmed salmon to feed on the surface was at least partially learned. Over time wild fish were feeding closer to the surface, although not as high in the water column as cultured ones Reiriz et al., 1998 cited in Jonsson and Jonsson, 2006).

In the North Atlantic, cultured post-smolts have considerably more food items in their stomachs, especially amphipods and krill, than do wild post-smolts. Amphipods were the most abundant item in the stomachs of cultured postsmolts, whereas krill was the most abundant food item of wild post-smolts. Fish, mostly sandlances (*Ammodytidae*), the largest prey item consumed, were almost twice as abundant in the diet of cultured post-smolts as in that of their wild counterparts. In the northeastern Atlantic, mesopelagic fish such as lanternfish (*Myctophidae*), pearlsides (*Sternoptychidae*), and barracudinas (*Paralepididae*) were more important than amphipods, which were more important than krill (Jacobsen and Hansen, 2001 cited in Jonsson and Jonsson, 2006). In the open ocean, the diet of wild and cultured salmon is similar, indicating that at least some cultured fish are well adapted to ocean life.

9.4.3.4 Smolt Emigration

When released into rivers, cultured Atlantic salmon smolts move quickly to the sea, even when released in daylight. Wild smolts usually move to the sea over a longer period, starting in cool temperature and moving downstream by night, and gradually becoming day-active as temperatures rise above ca. 13^{0}C (Thorpe et al., 1994 cited in Jonsson and Jonsson, 2006). Wild smolts may also be entrained during the day in schools consisting chiefly of hatchery fish (Hansen and Jonsson, 1985).

Juvenile Atlantic salmon migrate actively through fjords into the ocean (Finstad et al., 2005). Sexually maturing cultured post-smolts, on the other hand, seem more inclined to stay in coastal areas and to enter rivers as they migrate (Hansen et al., 1987 Jonsson et al., 1993).

9.4.3.5 Reproduction

When sexually mature, both wild and cultured salmon enter rivers to spawn, and may home to the area of their origin (Jonsson et al., 1990). However, cultured salmon may not originate in a specific river and may return to areas adjacent to the hatchery outflow (Clifford et al., 1998). Their homing precision appears to be less accurate than that of wild fish, even when the two leave the river together as smolts (Jonsson et al., 2003). Mean rates of straying for sea-ranched and wild Atlantic salmon of the River Imsa stock were estimated at 15% and 6%, respectively, when both types of fish left the river as smolts in May. Moreover, the straying rate was higher for Atlantic salmon attaining sexual maturity and returning to fresh water after two years at sea rather than one year. The longer the time fish stayed away from their home river, the greater the chance of straying. Both cultured and wild salmon strayed to many of the same rivers, ca. 80% of which drain into the fjord of the River Imsa within 60 km of the outlet. Therefore, the chance of entering the ''wrong'' river increases with time or distance moved at sea. Farmed post-smolt salmon that escape to sea in winter do not return to any specific area when sexually mature (Hansen and Jonsson, 1991), and fish released when maturing in their second summer also appear to have lost their ability to navigate back to their home river or place of release (Hansen et al., 1987). There is probably a finite period when Atlantic salmon are able to choose navigational cues that they use during the return migration. Sub-adults and adults have lost this ability (Hansen et al., 1993; Hansen and Jonsson, 1994). Therefore, many cultured fish may reproduce in rivers other than the one that they left as smolts.

It was observed that cultured Atlantic salmon entered the rivers to spawn later in the season, moved about more, and stayed in the river for a shorter time than wild fish (Jonsson et al., 1990; Økland et al., 1995).

Thorstad et al. (1998) found that unlike wild salmon, cultured fish were not homing to any particular spawning area, and moved as far upstream as possible, instead of utilizing the spawning grounds of wild fish lower downstream. Once at the spawning grounds sea-ranched male Atlantic salmon, probably resulting from their experience of feeding competition in hatchery tanks, took part in more prolonged aggressive encounters, incurred greater wounding, and sustained greater mortality than wild males originating in the same population, even though both cohorts showed similar levels of aggression. Furthermore, farmed males did not establish dominance hierarchies as effectively as wild males, courted less, spawned with females in larger numbers and participate in fewer spawning events, and frequently failed to release sperm when the females released their eggs. Consequently, experimental evidence suggests that they achieve only a low percentage of the reproductive success of wild males (Fleming et al., 1996; Weir et al., 2004, 2005). Similarly, in other salmonids such as coho, reproductive success is greater for wild than for cultured males (Fleming and Gross, 1992, 1993; Berejikian et al., 1997 cited in Jonsson and Jonsson, 2006).

Studies in an experimental stream indicated that cultured brown trout males appear to have less reproductive success than wild males, but a similar effect was not found for females (Dannewitz et al., 2004 cited in Jonsson and Jonsson, 2006). In Atlantic salmon, the inferiority of cultured fish is often sex-biased, being more pronounced in males than in females and resulting in cross-breeding between cultured females and wild males. As a consequence more hybrids are produced in the wild than pure farm offspring.
Nevertheless, experimental evidence suggests that female cultured salmon have also reduced fitness caused by morphological maladaptation (Fleming et al., 1994; Gross, 1998), being less active, displaying less breeding behaviours, constructing fewer nests, retaining a greater mass of unreleased eggs, incurring more nest destruction, being less efficient at nest covering, and suffering greater egg mortality than wild females.
As a consequence of domestication, farmed salmon, their offspring, and hybrids show substantially reduced lifetime success with poorer survival in the early juvenile stages and, later in the life cycle at sea and during spawning. This results in loss of overall fitness in individual salmon population. Because farm escapes occur on a regular basis small and vulnerable populations could be severely affected and may eventually become extinct.

9.4.4 History of Invasiveness

In contrast to wild Atlantic salmon, the invasiveness and potential detrimental impact of domesticated Atlantic salmon has received considerable attention (Morris et al. 2008; Hindar et al. 2006; Thorstad et al. 2007; Naylor et al. 2005; McGinnity et al. 2003; Youngson and Verspoor 1998; McGinnity et al. 1997). Accidental releases of cultured Atlantic salmon into the environment that result from activities in the aquaculture industry have been implicated in the spread of disease and parasites (Amundrud and Murray 2009; Naylor et al. 2005), increased competition for resources among native fish species (Volpe et al. 2001; Fiske 2006) and temporal changes in the genetic integrity of wild Atlantic salmon populations (Bourret et al. 2011; Skaala et al. 2006). However, frequent accidental releases over a period of many years have not resulted in known established population of Atlantic salmon outside of its natural range. Follow-up studies following the successful spawning of adults and rearing of juveniles in Tsitika River, British Columbia, reported by Volpe et al. (2000) did not document presence of either adult or juvenile Atlantic salmon in the river (Piccolo and Orlikowska, 2012).

10 EXPOSURE CHARACTERIZATION

10.1 Characterization of Exposure

The characterization of exposure will consider the potential for AAS to enter, survive, reproduce and establish in both the Canadian and Panamanian environments, however, the final assessment will only consider exposure to the Canadian environment.

The assessment of exposure of the AAS to the Canadian environment will include both its potential to enter the environment and its fate once in the environment. In considering the physical, geographical, and biological containment strategies used for all life stages of the AAS, the exposure assessment will focus on:

1. The potential for unintentional release(s) of AAS into the receiving environment (i.e. entry) at both the Canadian and Panamanian facilities and during transport between the two locations;

2. The potential of AAS to survive, disperse and persist in the Canadian and Panamanian receiving environments (i.e. fate). If applicable, the magnitude and frequency of dispersal (i.e. propagule pressure) will also be assessed;

3. The potential of AAS to reproduce, establish and spread in the Canadian and Panamanian environments (i.e. fate). If applicable, the magnitude and frequency of reproduction, establishment and spread will also be assessed; and

4. The potential for the disposal of AAS carcasses in Canada to act as an exposure pathway.

Although containment at both the Canadian and Panamanian facilities will be examined, the assessment will only consider the exposure of AAS to the Canadian environment. Consequently, assessment of potential exposure from activities in Panama will focus primarily on the potential of AAS to return to Canadian waters, including the Atlantic and Pacific Oceans. Measurement endpoints will include relevant information about physical, geographical and biological containment strategies used for all life stages of the AAS. The likelihood of natural events (e.g. hurricanes, earthquakes) and security violations that may lead to a failure of physical containment will also be considered and weighed against the adequacy of the reasonable measures (e.g. facility siting, design, security) employed by AquaBounty to prevent an accidental release under an extreme circumstance.

10.1.1 Scenarios under which AAS May Enter the Receiving Environment

Both acute failures in physical containment, caused by natural events or security violation, and chronic failures in physical containment will be considered in the assessment of exposure.

Though land-based hatcheries and grow-out facilities offer the potential for high-level confinement of aquatic organisms, the prevention of accidental releases from such facilities requires considerable forethought regarding its design, security, staff, operational procedures and oversight, as well as the facility's geographic location and siting. There are three principle scenarios by which AAS may come to breach physical containment and enter the receiving environment.

Natural events, such as earthquakes, tsunamis, hurricanes, tidal surges, mud slides and flooding, may cause significant damage to a facility and possibly result in a large scale or acute release of organisms. This type of event would be expected to occur at a low frequency, but has the potential to release a large number of organisms. It is a common experience when farming salmon in open net-pens (Morris et al. 2008) and has also occurred at land-based fish hatcheries. In Eastern Canada, floods have occurred at land-based hatcheries that are close to streams and there have been breaches associated with the flooding of ponds (Gerard Chaput, DFO, personal communication). Similar incidents have been reported in the United States (http://www.boston.com/news/local/massachusetts/2013/01/20/fish-hatchery-damaged-irene-almost-fixed/aNvyNIj5Ny2dTPBdxMOn4O/story.html. http://recovery.doi.gov/press/us-fish-and-wildlife-service/dc-booth-historic-national-fish-hatchery/). Although it is difficult to predict an event of this nature, it is important to consider its potential when assessing the geographic location of a facility, its siting, construction and emergency procedures in place to prevent the possibility of containment failure under this scenario.

Security violations committed by unauthorized individuals who gain access to the site may result in the escape of AAS into the environment through either the deliberate release of organisms or the failure of mechanical barriers that may result from vandalism or theft (Morris et al. 2008). As with natural events, this type of scenario is difficult to predict and is not expected to occur with any predictable frequency, however it is important to consider especially given the contentious stance that several private interest groups have taken towards this product. Consequently, it is important to review measures AquaBounty has put in place to prevent security violations.

Finally, chronic failure of physical containment is commonly recognised as a predominant circumstance by which domesticated salmonids may enter the environment (Carr and Whoriskey 2006; Morris et al 2008; Arismendi et al. 2009). Even if the number of individuals released during discreet events is small, persistent and repeated entry may be sufficient to result in significant impacts or further exposure through reproduction and establishment. Assessment of the potential for chronic failure of physical containment will consider the suitability and redundancy of mechanical barriers as well as the operation procedures and oversight in place to ensure that physical barriers are properly used and maintained so to prevent the accidental release of AAS.

The prospect of recapturing an organism such as Atlantic salmon once it has entered a suitable aquatic environment may be limited by a variety of factors (Chittenden et al. 2011; Skilbrei and Jørgensen 2010 ; Skilbrei et al. 2009) and is therefore not considered as an acceptable mitigation measure for the accidental release of AAS.

10.1.2 Standards and Methodologies for the Assessment of Physical Containment

A minimum number of three mechanical barriers will be accepted as adequate physical containment of AAS. A Failure Mode Analysis will provide further guidance on the efficacy of physical containment.

Standards for the physical containment of genetically modified fish are currently not available. The U.S. Department of Agriculture's 'Performance Standards for Safely Conducting Research with Genetically Modified Fish and Shellfish' (ABRAC 1995) emphasizes the importance of mechanical barriers, security and the operational procedures that are in place to maintain physical containment and mitigate catastrophic events. It has suggested that 3 to 5 independent barriers along a single pathway are sufficiently redundant to effectively contain an organism. However, it acknowledges that an adequate level of redundancy may depend on the specific location of the facility or the nature of the proposed research. Some guidance on containment standards for salmonids is also provided by the New Brunswick Rainbow Trout Aquaculture Policy (http://www.gnb.ca/0168/Trout.pdf) which also advocates at least three barriers. In addition, to facilitate the assessment of the physical containment of AAS in both the Canadian and Panamanian facilities a Failure Modes Analyses (FMA) will be conducted following guidance from Stamatis 2003 and McDermott et al., 2009.

Failure Modes Analyses (also known as Failure Modes and Effects Analysis) was first adopted by the automotive industry to be used as a systematic method for identifying and preventing product problems before they occur (McDermott et al. 2009). It has since been extended to a variety of industries that are concerned with quality and safety during design and improvement when a product is in use (Stamatis 2003). The ISO/TS 16949:2002 standard (part of the ISO 9000 family of certifications) requires that suppliers to the automotive industry conduct product design and process FMAs in an effort to prevent failures before they happen (McDermott et al. 2009). FMA has also been extended to the interface of mechanical and biological systems by Hayes (2002), who used it to identify the potential spread of marine organisms via human vectors.

In this assessment, FMA is extended to the mechanical and operational processes of physical containment of AAS at both AB PEI and AB Panama and during transport between the two facilities with the objective of identifying potential weaknesses along all pathways of entry. The FMA also provides a systematic method for the examination and assessment of each and every element of physical containment. Both the mechanical barriers and the operational procedures in place to maintain and ensure the proper employment of each barrier to entry will be considered along with the potential consequences of a failure at each barrier.

Briefly, each element of physical containment is ranked according to the severity of a failure (based on the redundancy of downstream containment), its likelihood of occurrence (based on incident records provided by AquaBounty) of and the mitigation in place to prevent a potential failure (based on SOPs and oversight documentation provided within the notification). Severity (S), occurrence (O) and mitigation (M) are ranked according to Table 10-1, Table 10-2 and Table 10-3. The product of the three rankings generates a risk priority number (RPN) that is used to identify where potentially severe failure modes are most likely to occur, assess the consistency of containment across all individual pathways and indicate where a recommendation of additional mitigation may be required (able 10-4).

Table 10-1 Rankings for the Severity (S) of potential failures in physical containment based on the redundancy of downstream containment

Rank	**Severity (S)**
1	Low; No entry possible; ≥ 2 downstream barriers still present
2	Medium; No entry possible; 1suitable downstream barrier still present
3	High; entry possible; no suitable downstream barrier present

Table 10-2 Ranking for Occurrence (O) of potential failure in physical containment based records of incidents provided by AquaBounty

Rank	**Occurrence (O)**
1	Low; $O < 1$ recorded incidents per year
2	Medium; $1 \leq O < 5$ recorded incidents per year
3	High; $O \geq 5$ recorded incidents per year or no records available

Table 10-3 Ranking for Mitigation (M) to prevent potential failure in physical containment based on SOPs and oversight documentation provided within the notification

Rank	Mitigation (M)
1	High; written SOPs include daily inspection and compliance documentation
2	Medium; written SOPs do not include daily inspection and compliance documentation
3	Low; no written SOPs, daily inspections or compliance documentation

able 10-4 Rankings for concern based on Risk Priority Numbers (RPNs)

RPN	Concern
1 to 3	Low
4 to 9	Medium
10 to 27	High

The FMA is intended to provide a qualitative estimate for the likelihood of an unintentional release, through the examination of every element of physical containment at each life-stage of AAS along all pathways to entry. Though accurate estimations of RPNs relies heavily upon documented occurrences of failure, in the absence of data, the FMA still provides a systematic means by which potential problems with containment can be identified or where additional oversight may be required. In the absence of data, uncertainty regarding the assessment of a particular pathway is likely to increase. Consequently, the assessment of physical containment will take into consideration not only the redundancy of mechanical barriers for a particular pathway to entry, but will also address the potential for failure of each barrier and the operational mitigation in place to prevent failures from occurring. Under specific circumstances, this type of analysis may lead to conclusions about elements that underestimate the overall risk. For example, the system would yield an RPN of Low (3) if a breach occurred once every 2 years (Occurrence <1), even if there is no downstream barrier (Severity = 3), but excellent procedures are in place (Mitigation = 1). Consequently, the FMA is meant more as guide to identify where there may be weaknesses in containment, not as an absolute test of efficacy.

Failure Mode Analysis tables for the containment of AAS along pathways at AB PEI, AB Panama and during transport between the two locations are presented in Appendix B, C and D respectively.

10.2 Potential for Entry of AAS into the Receiving Environment at both the Canadian and Panamanian Facilities and during Transport between the Two Locations

The likelihood and magnitude of exposure of the AAS to the Canadian aquatic environment resulting from any failed containment at the facilities and during transportation will be assessed. This will include unintentional releases that may result from equipment failure and human error as well as potential catastrophic events. Measures proposed by AquaBounty to prevent and mitigate unintentional releases from the failure of physical containment and security violations will be evaluated. In addition, the likelihood of natural event (e.g. hurricanes, tidal surges) that could lead to a containment failure will be considered and weighed against the adequacy of reasonable measures, such as facility siting, design and emergency provisions, to prevent unintentional release under such circumstances.

10.2.1 Potential for Entry of AAS into the Receiving Environment at the Canadian Facility

All life-history stages of both sterile triploid (3n) and fertile diploid (2n) AAS will be housed at the facility in PEI, which is land-based, includes extensive mechanical and operational containment provisions and has been subject to regulatory oversight since 1996.

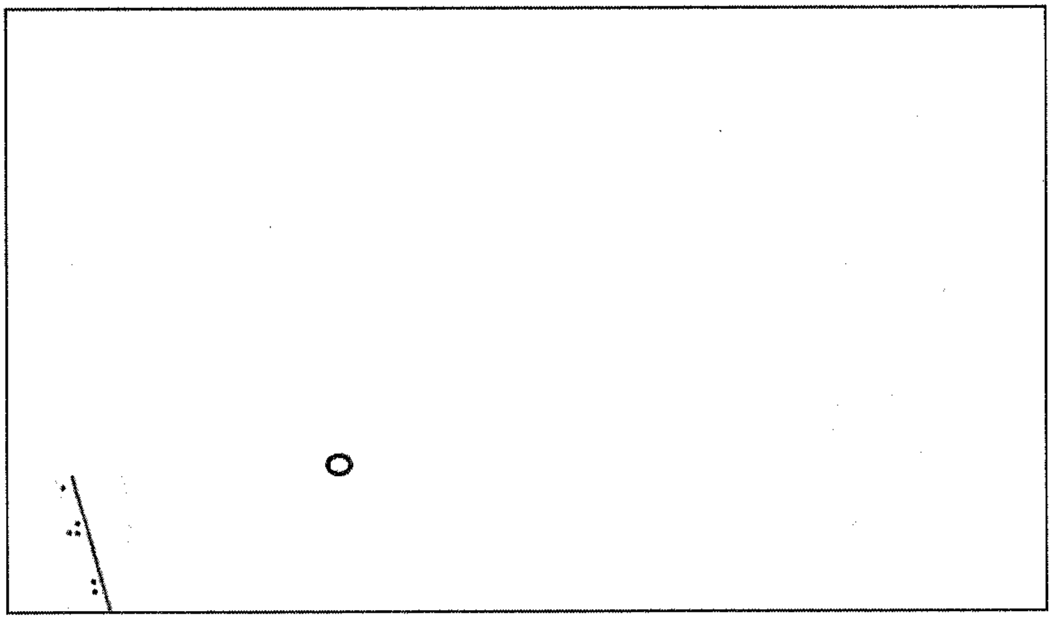


Figure 10.1 Map indicating approximate location of AquaBounty facility, next to Bay Fortune on PEI.

The manufacturing site, where triploid eggs are produced and broodstock are maintained, is located southwest of Souris, Prince Edward Island, Canada, on a parcel of land that is adjacent to the south bank of the Fortune River, approximately 50 meters from the Bay Fortune estuary and approximately 1000 meters from a spit of land that extends into Rollo Bay and the Northumberland Straight (Figure 10.1).

The facility is entirely land-based. All organisms are maintained within the confines of a two-story main building [redacted]

All life-stages of AAS, both diploid (2n) and triploid (3n), are housed in various locations [redacted]

[redacted] In addition, a variety of mechanical and chemical barriers designed to prevent the accidental release of AAS into the environment are in place, along with standardized operational procedures (SOPs) and internal compliance documentation, to ensure that all containment provisions are properly employed and maintained. Since 1996, the facility has been subject to oversight by the Department of Fisheries and Oceans (DFO) and Environment Canada (EC) pursuant to its use for R&D involving transgenic organisms (ABT 2013, p. 486).

AquaBounty has indicated its intent to produce annually, no more than 100,000 female triploid AAS eyed-eggs for commercial export. In order to meet this demand, the facility in PEI will need to maintain a diploid commercial broodstock (see section 9.1.2.1 and Figure 9.3) and will require additional female triploid eggs as insurance against natural and unexpected mortality or possible changes to shipping schedules.

[redacted]

[redacted]

[redacted]

[redacted]

10.2.1.1 The Potential for Natural Events in PEI to Result in the Accidental Release of AAS

Given the information of the facility's siting, structural integrity, construction and design, the SOPs and emergency procedures currently in place and current knowledge of extreme natural events that may occur in the region and may challenge the containment of AAS, the likelihood of an accidental release resulting from a natural event such as a hurricane or a tidal surge is considered to be <u>negligible</u>, with <u>high certainty</u>.

An acute release of AAS resulting from a natural disaster, such as earthquakes, tsunamis, tornados, hurricanes, tidal surges, flooding or fires, is highly unlikely. The facility is not located in an area of significant seismic activity and tsunamis in the region are extremely rare. Those that have occurred in the past did not affect the inner Gulf of St. Lawrence (http://www.earthquakescanada.nrcan.gc.ca/zones/eastcan-eng.php). Though several tornados have been reported in New Brunswick, Nova Scotia and Quebec over the past

100 years, none have been reported on PEI (http://cdd.publicsafety.gc.ca/srchpg-eng.aspx).

The most likely natural disaster to challenge the facilities infrastructure and physical containment of AAS would be a hurricane or the flooding that may result from the tidal surge that often accompanies intense depressions in barometric pressure. Indeed, Canada and its Atlantic waters are threatened by an average of six tropical storms per year (http://www.ec.gc.ca/meteo-weather/default.asp?lang=En&n=9F6732DB-1). Prince Edward Island's official hurricane season runs from June 1st to November 30th and peaks between mid-August and the end of September. According to Environment Canada, there have been six land falling hurricanes on PEI since 1891, three of which have been category 2 (winds between 154 and 177 km/hr.).

The building itself is structurally sound, built to local building codes by professional contractors with a [redacted] that has withstood several severe storms including the 120 km/hr. winds of Hurricane Juan in September of 2003. This challenge was shortly followed by the "White Juan" blizzard of February 2004 which dropped approximately 1meter of snow on the region without damaging the facility. In addition to a sturdy above-ground construction, [redacted] During the winter, snow accumulation on the roof is monitored and is professionally removed when it becomes too deep. Consequently, it is reasonable to conclude that the facility's structure will continue to withstand the extreme winds and snowfall that it may be subjected to in this region of the country.

Though the province of PEI has a history of flooding, the effect of tidal surges tends to be at its worst around Charlottetown and diminishes towards the northeastern part of the island (Vasseur and Catto 2008)). For example, on January 21st 2000, an intense low pressure system brought a storm surge of approximately 1.36 meters to the Maritimes. When combined with the normal tide height and waves, water levels around Charlottetown (Approximately 78 km southwest of the facility) reached a total height of

4.23 meters above chart datum, the highest levels recorded on the island in 100 years (http://cdd.publicsafety.gc.ca/srchpg-eng.aspx?cultureCode=en-Ca&provinces=10&eventTypes=%27SS%27&normalizedCostYear=1). At the same time, in Souris (approximately 12 km to the northeast of the facility) the combined surge, tide and waves reached a land elevation of only 1.75 meters (Climate Change Vulnerability Assessment – Souris and Souris West, PEI, 2012 http://atlanticadaptation.ca/sites/discoveryspace.upei.ca.acasa/files/CC%20Vulnerability%20Assessment%20-%20Souris%20FINAL%20Combined_1.pdf).

The PEI facility is located at latitude N49:19:53.3 (46.331472) and longitude W062:21:50.7 (-62.364083), adjacent to the south bank of the Fortune Estuary and on a rise of land that prevents damage from heavy rain (p.503). Although a hand held GPS unit places the facility at approximately 12 meters (39 feet) above the high water line of the Fortune River (p.503), according the Canadian Topographic Series, the given coordinates correspond to a height of approximately 25 feet above chart datum, or just less than 7.6 meters above the mean lower low tide. A more conservative and acceptable estimate would be that the floor of the facility's foundation lies somewhere between 6 and 7 meters above chart datum. Consequently, given the history of flooding caused by storm surges on the island, and the siting of the facility above the Fortune Estuary, it is highly unlikely that a storm or tidal surge would ever reach the facility or cause damage to the facility's infrastructure.

In addition to the above physical limitations to natural events which may lead to a catastrophic release, facility staff are trained on emergency procedures and SOPs designed to limit the effects of catastrophic events or a loss of operational capacity.

Therefore, given the information of the facility's siting, structural integrity, construction and design, the SOPs and emergency procedures currently in place and knowledge of extreme natural events that may occur in the region and may challenge the containment of AAS, the likelihood of an accidental release resulting from a natural event such as a hurricane or a tidal surge is negligible, with high certainty.

10.2.1.2 The Potential for Security Violations in PEI to Result in the Accidental Release of AAS

Given its remote and peaceful location, extensive measures in place to prevent illegal access and history of no security violations, the likelihood of an accidental release of AAS resulting from a security violation is considered to be <u>negligible</u> with <u>high certainty</u>.

Like natural events, security violations are difficult to predict, but carry the potential to result in large scale releases of AAS. Regardless, AquaBounty has, in prudence, put in place several security measures to protect both its property and personnel. These measures include:

- An 8 foot high, galvanized chain-linked perimeter fence, with locked gates that encloses the facility's main [redacted]

- [redacted]

- There is exterior lighting throughout the premises at night.

- There are steel exterior doors, key control and entry logs.

- Intercoms and a remote unlock are used to confirm the identity of and enable access to approved visitors.

- All ground floor windows have steel bars.

- [redacted]

- [redacted]

- AquaBounty has also explored [redacted]

These provisions have been put in place despite the very limited number of threats to the facility (several small and peaceful protests) that have occurred without incident. There has never been a security violation at the facility since AquaBounty took possession. Turnover of staff is low and most employees have been with the company for over five years.

Consequently, given its remote and peaceful location, extensive measures in place to prevent illegal access and history of no security violations, the likelihood of an accidental release of AAS resulting from a security violation is considered to be <u>negligible</u> with <u>high certainty.</u>

10.2.1.3 The Potential for Chronic Failure of Physical Containment in PEI to Result in the Release of AAS

Physical containment strategies for all life-history stages (gametes through to sexually mature adults) of AAS and all potential pathways to entry will be individually assessed.

[redacted]

[redacted]

Housing and husbandry for all life-stages of Atlantic salmon requires a broad variety of tank sizes, incubators, water flow rates, operational procedures and mechanical barriers to prevent accidental releases. [redacted] The following assessment will consider all mechanical or chemical barriers for each pathway and for all life-history stages of AAS.

The notification provides detailed information regarding the facility's floor plan, drainage system, operational procedures and redundant barriers designed to contain AAS. Over the past 17 years there have been a number of modifications made to the facility in order to improve the physical containment of AAS as well as operational oversight (ABT 2013, p.491). [redacted]

10.2.1.3.1 Physical Containment of AAS Gametes

The potential for exposure resulting from the accidental release of AAS gametes is low, though limited information and the absence of oversight documentation make this assessment reasonably uncertain. Regardless of any likelihood that AAS gametes may be released, the viability of Atlantic salmon gametes exposed to an aqueous environment is extremely limited and likely to negate any potential for exposure.

Spawning of AAS takes place from late October to late December. Eggs and milt are collected from individual adult broodstock [redacted] which is not sufficient to prevent the loss of gametes into the exterior environment (Heinimaa and Heinimaa 2004; Reid and Chaput 2012).

[redacted]

[redacted]

Failure Modes Analysis (FMA) for this stage of development identifies only [redacted] components of physical containment and 6 potential failure modes (Appendix Table B-1). The majority of these failure modes are likely to be the result of human errors such as the accidental spilling of gametes on the floor during collection or a failure to properly secure floor drain covers.

[redacted]

Despite these mitigation measures, RPNs (risk priority numbers) associated with the various failure modes are high (9 to 12); a result of limited redundancy in physical containment during this activity and uncertainty with regards to the frequency and occurrence of failure modes [redacted] SOPs and internal compliance oversight will likely limit the incidence of release to a very low frequency; however there is a chance that AAS gametes will accidentally enter the environment. Consequently, the potential for exposure resulting from the accidental release of AAS gametes is low, though limited information and the absence of oversight documentation make this assessment <u>reasonably uncertain</u>. Regardless of any likelihood that AAS gametes may be released, the viability of Atlantic salmon gametes exposed to an aqueous environment is extremely limited and likely to negate any potential for exposure (see section 9.3.4.1).

10.2.1.3.2 Physical Containment of AAS Diploid and Triploid Embryos (Eggs and Alevins)

[redacted] The likelihood of AAS embryos entering the environment is considered to be negligible. For all pathways, this assessment is made with high certainty.

AAS embryos (both diploid and triploid) will be physically contained in several different locations within the PEI facility and for variable periods of time depending on the location, type of incubator, temperature of incubation or the organism's end use (broodstock, R&D or commercial production). During fertilization procedures, which include pressure shocking to induce triploid sterilization, [redacted] Care is taken to move them, though if an accident did occur, eggs would simply fall on the grass (or snow) and whatever can't be picked up immediately would die. Depending on the type of incubation unit or the end use of the organism, embryos may be removed from an incubator prior to hatching, or maintained within a unit until the egg has hatched and the egg-sac is partially absorbed. Consequently, the assessment of physical containment during this stage of AAS development must consider several independent pathways to entry over a period of several months.

[redacted]

[redacted]

Atlantic salmon egg diameters may range between 4.5 and 7 mm (Heinimaa and Heinimaa 2004; Reid and Chaput 2012) and developing alevins are capable of fitting through spaces greater than 5 mm in diameter (New Brunswick Rainbow Trout Aquaculture Policy). The small size of embryos makes it possible for them to pass through containment screens with a mesh pore size greater than 6 mm in diameter. Consequently, the assessment of various entry pathways of AAS embryos does not include the facility containment sump as an element of physical containment since the basket filters at this point have a minimum pore size of 6.2 mm. However, it should be noted that in ten years of collecting compliance documentation, no AAS embryos have been detected in the ERA containment sump.

There are a total of 6 distinct pathways to entry for AAS embryos that are under physical containment at the PEI facility. This includes containment during pressure shocking procedures to induce triploidy. Each pathway will be considered, in turn, below.

10.2.1.3.2.1 Containment of AAS Diploid and Triploid Embryos during Fertilization and Pressure Shocking Activities in the Loading Dock Area of the GOA

Given the limited time frame for this activity, the redundant mechanical and chemical containment and the operational oversight, the likelihood of entry into the environment of viable AAS embryos by means of this pathway is <u>negligible</u>. Detailed information available on facility design, containment features, water treatment, SOPs and internal compliance documentation result in a <u>highly certain</u> assessment.

[redacted]

[redacted] A small number of triploid AAS will also be reared at the PEI facility as part of AquaBounty's R&D program. During these activities, which may be limited to a few hours per day over a course of several months, there is a potential for both diploid and triploid AAS to spill on the floor [redacted] and enter the environment via [redacted] Should fertilized eggs enter [redacted] drainage system, the only downstream containment measure is a screen located at the exterior containment sump with a minimum pore size of 6.2 mm, which is not sufficient to prevent entry of fertile eggs into the exterior environment. In response to this possibility, AquaBounty has put in place several physical, chemical and operational containment provisions to mitigate the potential for entry.

For a fertilized AAS egg to enter [redacted] To further mitigate the highly unlikely event of failure [redacted] (also subject to oversight and compliance documentation, [redacted]). This chemical barrier would effectively kill any AAS embryos that may enter the drain, provided they are exposed to the chemical for a sufficient period of time. [redacted]

The FMA for these activities identified [redacted] components to physical (and chemical) containment and 10 potential failure modes (Table B2). The majority of these failure modes are likely to be the result of human errors such as the accidental spilling of eggs on the floor during transfers between egg containers and pressure shocking cylinders (SOP/ABPEI/4350), or a failure to inspect and confirm the correct placement of all containment elements. The notification appropriately recognizes the inevitability of spilled eggs and addresses this issue in [redacted]. Multiple containment features, which include chemical treatment of the drainage pathway, have been put in place to prevent the accidental release of AAS eggs during fertilization and pressure shocking activities and there is written compliance documentation [redacted]) to ensure all elements of containment are in place at the appropriate time. The RPNs associated with potential failure modes during these activities are ranked as low to medium (3 to 9). This is primarily the result of limited information regarding the frequency of occurrence of failures and values would likely drop to within the low range if occurrence of failure modes is provided.

Given the limited time frame for this activity, the redundant mechanical and chemical containment and the operational oversight, the likelihood of entry into the environment of viable AAS embryos by means of this pathway is negligible. Detailed information available on facility design, containment features, water treatment, SOPs and internal compliance documentation result in a highly certain assessment of negligible exposure to the environment from AAS that may result from containment failures along this pathway.

Once fertilization and pressure shocking activities are complete, 2n and 3n eggs are relocated [redacted] where they are transferred to one of several different incubation units.

10.2.1.3.2.2 Containment of AAS Diploid and Triploid Embryos in Upwelling Incubation Units [redacted]

Given the limited time frame for this activity, the redundant mechanical and chemical containment and the operational oversight, the likelihood of entry into the environment of viable AAS embryos by means of this pathway is negligible. Detailed information available on facility design, containment features, water treatment, SOPs and internal compliance documentation result in a highly certain assessment of negligible exposure to the environment from AAS that may result from containment failures along this pathway.

[redacted]

[redacted]

Given the redundant mechanical containment and the operational oversight, the likelihood of entry into the environment of viable AAS embryos by means of this pathway is negligible.

Detailed information available on facility design, containment features, water treatment, SOPs, internal compliance documentation and information on the frequency of containment failure result in an assessment that is highly certain for this pathway.

10.2.1.3.2.3 Containment of AAS diploid and triploid embryos in Heath stacks located [redacted]

Given the redundant mechanical containment and the operational oversight, the likelihood of entry into the environment of viable AAS embryos by means of this pathway is negligible. Detailed information available on facility design, containment features, and water treatment, SOPs, internal compliance documentation and information related to the frequency of past containment failures result in an assessment that is highly certain.

Heath stack incubators [redacted] Water drains from the system, [redacted] Embryos may be removed from an incubation unit prior to hatching, or as alevins, just prior to egg-sac absorption.

The Heath stack units [redacted]

[redacted]

[redacted]

For a fertilized AAS egg to enter [redacted] system from the Heath stacks [redacted], there must be a simultaneous failure of [redacted] mechanical barriers, [redacted] are subject to daily oversight (inspections) and internal compliance documentation ([redacted]) and [redacted]

The FMA for this pathway identified [redacted] and 24 potential failure modes that may result from material failures, electrical failures, human error, or a combination of these (Appendix Table B-4). In addition to multiple and redundant containment features, written SOPs in the form of daily inspection documentation promote compliance and enable the immediate detection and correction for the majority of failure modes. The RPNs associated with potential failure modes during these activities are ranked as low to medium (2 to 6), though the majority of failure modes are ranked as low. Moderate rankings are primarily the result of an inability to check [redacted]. However, the severity of a failure [redacted] is ranked as low since there are [redacted] additional barriers downstream to maintain containment.

Given the redundant mechanical containment and the operational oversight, the likelihood of entry into the environment of viable AAS embryos by means of this pathway is negligible. Detailed information available on facility design, containment features, and water treatment, SOPs, internal compliance documentation and information related to the frequency of past containment failures result in an assessment that is highly certain.

10.2.1.3.2.4 Containment of AAS diploid and triploid embryos in individual egg trays in the A, B or C tanks in [redacted]

Given the redundant mechanical containment and the operational oversight, the likelihood of entry into the environment of viable AAS embryos by means of this pathway is <u>negligible</u>. Detailed information available on facility design, containment features, and water treatment, SOPs, internal compliance documentation and information related to the frequency of past containment failures result in an assessment that is <u>highly certain.</u>

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

The FMA for this pathway identified [redacted] components to physical containment and 20 potential failure modes that may result from material failures, electrical failures, human error, or a combination of these (Appendix Table B-5). In addition to multiple and redundant containment features, written SOPs in the form of daily inspection documentation promote compliance and enable the immediate detection and correction for the majority of failure modes ([redacted]). The RPNs associated with potential failure modes during these activities are ranked as low to medium (2 to 6), though the majority of failure modes are ranked as low. Moderate rankings are primarily the result of an inability to check the bottom screen on a daily basis when a tray is in use. However, the severity of a failure at the bottom screen is ranked as low since there are [redacted] additional barriers downstream to maintain containment.

Given the redundant mechanical containment and the operational oversight, the likelihood of entry into the environment of viable AAS embryos by means of this pathway is negligible. Detailed information available on facility design, containment features, and water treatment, SOPs, internal compliance documentation and information related to the frequency of past containment failures result in an assessment that is highly certain.

10.2.1.3.2.5 Containment of AAS diploid and triploid embryos in individual egg trays [redacted]

Given the redundant mechanical containment and the operational oversight, the likelihood of entry into the environment of viable AAS embryos by means of this pathway is negligible. Detailed information available on facility design, containment features, and water treatment, SOPs, internal compliance documentation and information related to the frequency of past containment failures result in an assessment that is highly certain.

Individual egg trays [redacted] these units may be used to incubate AAS diploid or triploid embryos, [redacted] Depending on water temperatures, [redacted]

[redacted] Embryos may be removed from the unit prior to hatching, or as alevins, prior to egg-sac absorption.

For a fertilized AAS egg to ente[redacted] drainage system from the individual egg trays [redacted] there must be a simultaneous failure of at least [redacted] mechanical barriers, all of which are subject to daily oversight (inspections) and internal compliance documentation ([redacted]).

The FMA for this pathway identified [redacted] components to physical containment and 13 potential failure modes that may result from material failures, electrical failures, human error, or a combination of these (Appendix Table B-6). In addition to multiple and redundant containment features, written SOPs in the form of daily inspection documentation promote compliance and enable the immediate detection and correction for the majority of failure modes ([redacted]). The RPNs associated with potential failure modes during these activities are ranked as low to medium (2 to 6), though the majority of failure modes are ranked as low. Moderate rankings are primarily the result of an [redacted]. However, the severity of a failure at the bottom screen is ranked as low since [redacted] additional barriers downstream to maintain containment.

Given the redundant mechanical containment and the operational oversight, the likelihood of entry into the environment of viable AAS embryos by means of this pathway is <u>negligible</u>. Detailed information available on facility design, containment features, and water treatment, SOPs, internal compliance documentation and information related to the frequency of past containment failures result in an assessment that is <u>highly certain.</u>

10.2.1.3.2.6 Containment of AAS diploid and triploid embryos in Heath stacks [redacted]

Given the redundant mechanical containment and the operational oversight, the likelihood of entry into the environment of viable AAS embryos by means of this pathway is <u>negligible</u>. Detailed information available on facility design, containment features, and water treatment, SOPs, internal compliance documentation and information related to the frequency of past containment failures result in an assessment that is <u>highly certain.</u>

Depending on the circumstances, Heath stack incubators [redacted]

[redacted]

For a fertilized AAS egg or alevin to enter [redacted] from a Heath stack tray [redacted], there must be a simultaneous failure of at least [redacted] mechanical barriers, the majority of which are subject to daily oversight (inspections) and internal compliance documentation [redacted].

The FMA for this pathway identified [redacted] and 16 potential failure modes that may result from material failures, electrical failures, human error, or a combination of these (Appendix Table B-7). In addition to multiple and redundant containment features, written SOPs in the form of daily inspection documentation promote compliance and enable the immediate detection and correction for the majority of failure modes [redacted]. The RPNs associated with potential failure modes during these activities are ranked as low to medium (2 to 6), though the majority of failure modes are ranked as low. Moderate rankings are primarily the result of an inability to check the Heath tray screens on a daily basis when they are in use. However, the severity of a failure at a Heath tray screen is ranked as low [redacted] additional barriers downstream to maintain containment.

Given the redundant mechanical containment and the operational oversight, the likelihood of entry into the environment of viable AAS embryos by means of this pathway is <u>negligible</u>. Detailed information available on facility design, containment features, and water treatment, SOPs, internal compliance documentation and information related to the frequency of past containment failures result in an assessment that is <u>highly certain.</u>

10.2.1.3.3 Physical containment of AAS diploid and triploid fry

There are a total of [redacted] pathways to entry for AAS fry that are under physical containment at the PEI facility. The likelihood of AAS fry entering the environment is considered to be <u>negligible</u>. For all pathways, this assessment is made with <u>high certainty.</u>

For the practical purposes of this exposure assessment, AAS at the PEI facility are considered to be fry just after egg sac absorption, when fish are first 'ponded' into fry tanks, until they have grown to a size at which they will be retained [redacted] According to provincial standards for the containment of rainbow trout in New Brunswick (see New Brunswick Rainbow Trout Aquaculture Policy, http://www.gnb.ca/0168/Trout.pdf), when fish are at a weight of 1.5 grams, the standard for round screen openings is 5 mm or less. Therefore, Atlantic salmon at 3 grams will, in all likelihood, be retained by a screen with an opening of 6.2 mm. Therefore, the fry stage is expected to last for a period of approximately 3 month post first feeding, [redacted]

AAS fry will be physically contained in several different locations within the PEI facility. Once egg-sacs are close to being fully absorbed, fish are transferred from incubation units to fry tanks [redacted] The small size of fry makes it possible for them to pass through containment screens with a mesh pore size greater than 6 mm in diameter. Consequently, the FMA for the various entry pathways of AAS fry [redacted]

There are a total of [redacted] distinct pathways to entry for AAS fry that are under physical containment at the PEI facility. Each pathway will be considered, in turn, below.

10.2.1.3.3.1 Containment of AAS diploid and triploid fry in [redacted]

Given the redundant mechanical containment and the operational oversight, the likelihood of entry into the environment of AAS fry by means of this pathway is negligible. Detailed information available on facility design, containment features, and water treatment, SOPs, internal compliance documentation and information related to the frequency of past containment failures result in an assessment that is highly certain.

[redacted]

[redacted]

For an AAS fry to enter [redacted], there must be a simultaneous failure of at least [redacted] mechanical barriers, all of which are subject to daily oversight (inspections) and internal compliance documentation ([redacted]).

The FMA for this pathway identifies [redacted] components to [redacted] containment and examined 25 potential failure modes that may result from material failures, electrical failures, human error, or a combination of these (Appendix Table B-8). In addition to multiple and redundant containment features, written SOPs in the form of daily inspection documentation promote compliance and enable the immediate detection and correction for the majority of failure modes ([redacted]). The RPNs associated with potential failure modes along this pathway are ranked as low (2 to 3).

[redacted]

Given the redundant mechanical containment and the operational oversight, the likelihood of entry into the environment of AAS fry by means of this pathway is negligible. Detailed information available on facility design, containment features, and

water treatment, SOPs, internal compliance documentation and information related to the frequency of past containment failures result in an assessment that is highly certain.

10.2.1.3.3.2 Containment of AAS diploid and triploid fry in [redacted]

Given the redundant mechanical containment and the operational oversight, the likelihood of entry into the environment of AAS fry by means of this pathway is negligible. Detailed information available on facility design, containment features, and water treatment, SOPs, internal compliance documentation and information related to the frequency of past containment failures result in an assessment that is highly certain.

[redacted]

[redacted]

[redacted]

For an AAS fry to enter [redacted], there must be a simultaneous failure of at least [redacted] barriers, all of which are subject to daily oversight (inspections) and internal compliance documentation [redacted]).

The FMA for this pathway identifies [redacted] containment and examined 24 potential failure modes that may result from material failures, electrical failures, human error, or a combination of these (Appendix Table B-9). In addition to multiple and redundant containment features, written SOPs in the form of daily inspection documentation promote compliance and enable the immediate detection and correction for the majority of failure modes [redacted]). The RPNs associated with potential failure modes along this pathway are ranked as low (2 to 3).

[redacted]

[redacted]

Given the redundant mechanical containment and the operational oversight, the likelihood of entry into the environment of AAS fry by means of this pathway is negligible. Detailed information available on facility design, containment features, and water treatment, SOPs, internal compliance documentation and information related to the frequency of past containment failures result in an assessment that is highly certain.

10.2.1.3.3.3 Containment of AAS diploid and triploid fry [redacted]

Given the redundant mechanical containment and the operational oversight, the likelihood of entry into the environment of AAS fry by means of this pathway is negligible. Detailed information available on facility design, containment features, and water treatment, SOPs, internal compliance documentation and information related to the frequency of past containment failures result in an assessment that is highly certain.

[redacted]

[redacted]

[redacted] there must be a simultaneous failure of at least [redacted], all of which are subject to daily oversight (inspections) and internal compliance documentation ([redacted]).

The FMA for this pathway identifies [redacted] and examined 18 potential failure modes that may result from material failures, electrical failure, human error, or a combination of these (Appendix Table B-10). In addition to multiple and redundant containment features, written SOPs in the form of daily inspection documentation promote compliance and enable the immediate detection and correction for the majority of failure modes ([redacted]). The RPNs associated with potential failure modes along this pathway are ranked as low (2 to 3).

[redacted]

Given the redundant mechanical containment and the operational oversight, the likelihood of entry into the environment of AAS fry by means of this pathway is negligible. Detailed information available on facility design, containment features, and

water treatment, SOPs, internal compliance documentation and information related to the frequency of past containment failures result in an assessment that is highly certain.

10.2.1.3.4 Physical containment of AAS diploid and triploid parr

There are a total of [redacted] pathways to entry for AAS parr that are under physical containment at the PEI facility. The likelihood of AAS parr entering the environment is considered to be negligible. For all pathways, this assessment is made with high certainty.

For the practical purposes of this exposure assessment, AAS at the PEI facility are considered to be parr once their weight is greater than 3 grams (see section 9.2.1.3.5) until they have reached a fork length of approximately 16 cm and are capable of transfer to full strength sea water as smolts (Saunders et al. 1998). This is expected to cover a period of approximately 3 months.

[redacted]

There are a total of [redacted] pathways to entry for AAS parr that are under physical containment at the PEI facility. Each pathway will be considered, in turn, below.

10.2.1.3.4.1 Containment of AAS diploid and triploid parr [redacted]

Given the redundant mechanical containment and the operational oversight, the likelihood of entry into the environment of AAS fry by means of this pathway is negligible. Detailed information available on facility design, containment features, and water treatment, SOPs, internal compliance documentation and information related to the frequency of past containment failures result in an assessment that is highly certain.

[redacted]

The [redacted] and associated water systems have been described earlier in section 9.2.1.3.3.1. As parr grow into smolts, they can continue to be held in these tanks or transferred into larger tanks to maintain appropriate biomass concentrations.

For an AAS parr to enter environment outside the facility [redacted], there must be a simultaneous failure of at least [redacted] mechanical barriers, all of which are subject to daily oversight (inspections) and internal compliance documentation ([redacted]).

The FMA for this pathway identifies [redacted] components to physical containment and examined 31 potential failure modes that may result from material failures, electrical failures, human error, or a combination of these (Appendix Table B-11). In addition to multiple and redundant containment features, written SOPs in the form of daily inspection documentation, promote compliance and enable the immediate detection and correction for the majority of failure modes ([redacted]). The RPNs associated with potential failure modes along this pathway are all ranked as low (1 to 3), a result of redundant containment which includes both the ERA and the facility containment sumps.

[redacted]

Given the redundant mechanical containment and the operational oversight, the likelihood of entry into the environment of AAS fry by means of this pathway is <u>negligible</u>. Detailed information available on facility design, containment features, and water treatment, SOPs, internal compliance documentation and information related to the frequency of past containment failures result in an assessment that is <u>highly certain.</u>

10.2.1.3.4.2 Containment of AAS diploid and triploid parr [redacted]

Given the redundant mechanical containment and the operational oversight, the likelihood of entry into the environment of AAS fry by means of this pathway is negligible. Detailed information available on facility design, containment features, and water treatment, SOPs, internal compliance documentation and information related to the frequency of past containment failures result in an assessment that is highly certain.

[redacted]

For an AAS parr to enter the environment outside the facility [redacted], there must be a simultaneous failure of at least [redacted] barriers, all of which are subject to daily oversight (inspections) and internal compliance documentation ([redacted]).

The FMA for this pathway identifies [redacted] containment and examined 35 potential failure modes that may result from material failures, electrical failures, human error, or a combination of these (Appendix Table B-12). In addition to multiple and redundant containment features, written SOPs in the form of daily inspection documentation promote compliance and enable the immediate detection and correction for the majority of failure modes ([redacted]). The RPNs associated with potential failure modes along this pathway are all ranked as low (1 to 3), a result of redundant containment which includes both the ERA and the facility containment sumps.

[redacted]

Given the redundant mechanical containment and the operational oversight, the likelihood of entry into the environment of AAS fry by means of this pathway is <u>negligible</u>. Detailed information available on facility design, containment features, and water treatment, SOPs, internal compliance documentation and information related to the frequency of past containment failures result in an assessment that is <u>highly certain.</u>

10.2.1.3.4.3 Containment of AAS diploid and triploid parr [redacted]

Given the redundant mechanical containment and the operational oversight, the likelihood of entry into the environment of AAS fry by means of this pathway is negligible. Detailed information available on facility design, containment features, and water treatment, SOPs, internal compliance documentation and information related to the frequency of past containment failures result in an assessment that is highly certain.

[redacted]

For an AAS parr to enter environment outside the facility from the [redacted], there must be a simultaneous failure of at least [redacted] barriers, all of which are subject to daily oversight (inspections) and internal compliance documentation [redacted]).

The FMA for this pathway identifies [redacted] containment and examined 24 potential failure modes that may result from material failures, electrical failures, human error, or a combination of these (Appendix Table B-13). In addition to multiple and redundant containment features, written SOPs in the form of daily inspection documentation promote compliance and enable the immediate detection and correction for the majority of failure modes ([redacted]). The RPNs associated with potential failure modes along this pathway are all ranked as low (1 to 3), a result of redundant containment which includes both the ERA and the facility containment sumps.

[redacted]

Given the redundant mechanical containment and the operational oversight, the likelihood of entry into the environment of AAS fry by means of this pathway is <u>negligible</u>. Detailed information available on facility design, containment features, and water treatment, SOPs, internal compliance documentation and information related to the frequency of past containment failures result in an assessment that is <u>highly certain.</u>

10.2.1.3.4.4 Containment of AAS diploid and triploid parr [redacted]

Given the redundant mechanical containment and the operational oversight, the likelihood of entry into the environment of AAS fry by means of this pathway is <u>negligible</u>. Detailed information available on facility design, containment features, and water treatment, SOPs, internal compliance documentation and information related to the frequency of past containment failures result in an assessment that is <u>highly certain.</u>

[redacted]

[redacted]

[redacted]

For an AAS parr to enter environment outside the facility from [redacted], there must be a simultaneous failure of at least [redacted] barriers, all of which are subject to daily oversight (inspections) and internal compliance documentation ([redacted]

The FMA for this pathway identifies [redacted] containment and examined 24 potential failure modes that may result from material failures, electrical failures, human error, or a combination of these (Appendix Table B-14). In addition to multiple and redundant containment features, written SOPs in the form of daily inspection documentation promote compliance and enable the immediate detection and correction for the majority of failure modes [redacted]. The RPNs associated with potential failure modes along this pathway are all ranked as low (2 to 3), a result of redundant containment which includes both the ERA and the facility containment sumps.

[redacted]

[redacted]

Given the redundant mechanical containment and the operational oversight, the likelihood of entry into the environment of AAS fry by means of this pathway is <u>negligible</u>. Detailed information available on facility design, containment features, and water treatment, SOPs, internal compliance documentation and information related to the frequency of past containment failures result in an assessment that is <u>highly certain.</u>

10.2.1.3.4.5 Containment of AAS diploid and triploid parr [redacted]

Given the redundant mechanical containment and the operational oversight, the likelihood of entry into the environment of AAS fry by means of this pathway is <u>negligible</u>. Detailed information available on facility design, containment features, and water treatment, SOPs, internal compliance documentation and information related to the frequency of past containment failures result in an assessment that is <u>highly certain.</u>

[redacted]

[redacted]

For an AAS pair to enter environment outside the facility from [redacted], there must be a simultaneous failure of at least [redacted] barriers, most of which are subject to daily oversight (inspections) and internal compliance documentation ([redacted]).

The FMA for this pathway identifies [redacted] containment and examined 20 potential failure modes that may result from material failures, human error, or a combination of these (Appendix Table B-15). In addition to multiple and redundant containment features, written SOPs in the form of daily inspection documentation promote compliance and enable the immediate detection and correction for the majority of failure modes ([redacted]. The RPNs associated with potential failure modes along this pathway are ranked as low to medium (2 to 6), though the majority of failure modes are ranked as low.

[redacted]

[redacted]

[redacted]

Given the redundant mechanical containment and the operational oversight, the likelihood of entry into the environment of AAS fry by means of this pathway is negligible. Detailed information available on facility design, containment features, and water treatment, SOPs, internal compliance documentation and information related to the frequency of past containment failures result in an assessment that is highly certain.

10.2.1.3.4.6 Containment of AAS diploid and triploid par[redacted]

Given the redundant mechanical containment and the operational oversight, the likelihood of entry into the environment of AAS fry by means of this pathway is <u>negligible</u>. Detailed information available on facility design, containment features, and water treatment, SOPs, internal compliance documentation and information related to the frequency of past containment failures result in an assessment that is <u>highly certain.</u>

[redacted]

[redacted]

For an AAS parr to enter environment outside the facility from [redacted], there must be a simultaneous failure of at least [redacted] barriers, all of which are subject to daily oversight (inspections) and internal compliance documentation ([redacted].

The FMA for this pathway identified [redacted] containment and examined 16 potential failure modes that may result from material failures, human error, or a combination of these (Appendix Table B-16). In addition to multiple and redundant containment features, written SOPs in the form of daily inspection documentation promote compliance and enable the immediate detection and correction for the majority of failure modes ([redacted]). The RPNs associated with potential failure modes along this pathway are all ranked as low (1 to 3), a result of the limited number of documented failures and redundant containment [redacted]

[redacted]

Given the redundant mechanical containment and the operational oversight, the likelihood of entry into the environment of AAS fry by means of this pathway is <u>negligible</u>. Detailed information available on facility design, containment features, and

water treatment, SOPs, internal compliance documentation and information related to the frequency of past containment failures result in an assessment that is highly certain.

10.2.1.3.4.7Physical containment of AAS diploid and triploid smolts, post-smolt juveniles and adults

Given the redundant mechanical containment and the operational oversight, the likelihood of entry into the environment of AAS fry by means of this pathway is negligible. Detailed information available on facility design, containment features and water treatment, SOPs, internal compliance documentation and information related to the frequency of past containment failures result in an assessment that is highly certain.

The Atlantic salmon smolt stage is a period of transition in which freshwater parr undergo morphological, physiological and behavioural changes that prepare it for life in the marine environment (Thorstad et al. 2011, McCormick et al. 1998). This transformation typically involves the acquisition of a slimmer body form, colour changes that help to conceal it in the pelagic environment, increased salinity tolerance and the behavioural drive to leave its territory and migrate downstream toward the sea.

After completing the smolt stage (in their first year of life), AAS will continue to be reared in freshwater throughout the post-smolt juvenile stage and into their development as sexually mature adults. [redacted] adult AAS broodstock (both hemizygous and homozygous) at the PEI facility are expected to become sexually mature and will be used in the propagation of triploid all-female AAS for commercial production or the propagation of diploid AAS for future broodstock and for research and development needs. All AAS will continue to be maintained in freshwater throughout this stage of their life-cycle [redacted]

[redacted]

[redacted] Since AAS smolts at the PEI facility are kept in the same locations and same tanks as AAS parr, the analysis of physical containment and conclusions regarding the likelihood of entry into the environment are essentially the same (negligible with high certainty).

All diploid and triploid post-smolt and adult AAS will be physically contained [redacted] Since AAS post-smolt and adults are kept in the same locations and same tanks as AAS smolt, the analysis of physical containment and conclusions regarding the likelihood of entry into the environment are essentially the same (negligible with high certainty).

Details of physical containment for the tanks used to house smolts, post-smolt and adult AAS at the PEI facility are provided for parr in section 9.2.1.3.4. The FMA for the various pathways to entry can be found in appendix A.

Given the redundant mechanical containment and the operational oversight, the likelihood of entry into the environment of AAS fry by means of this pathway is negligible. Detailed information available on facility design, containment features and water treatment, SOPs, internal compliance documentation and information related to the frequency of past containment failures result in an assessment that is highly certain.

10.2.2 Potential for entry of AAS into the receiving environment at the Panamanian facility

Only sterile triploid (3n) all female stocks, from the eyed-egg stage to market weight (~3kg), will be housed at the facility in Panama, which is land-based, includes extensive mechanical and operational containment provisions and has been subject to regulatory oversight since 2009

[redacted]

The grow-out site, where triploid all-female AAS eggs will be received, hatched, grown and harvested for US retail markets, is located in a secluded region of the high altitude, tropical rainforest of Chiriquí, the western most province of Panama, near the town of Boquete and [redacted]

[redacted] The facility is entirely land-based, sited on a five acre parcel of land that is adjacent to the Caldera River (part of the Chiriquí River watershed) and approximately 130 km inland from where the Chiriquí River empties into the Bahía de Muertos and the Pacific Ocean.

[redacted]

[redacted]

To prevent the accidental release of AAS into the environment a variety of mechanical barriers are in place along all potential pathways to entry. Standardized operational procedures (SOPs) and internal compliance documentation are also in place to ensure that all containment provisions are properly employed and maintained. The facility is subject to oversight by a number of Panamanian authorities including the Autoridad Nacional del Ambient (National Environmental Authority).

In addition to physical containment measures, AquaBounty has indicated a number of biological and geographical containment provisions aimed at mitigating environmental hazards by preventing the potential establishment and dispersal of any AAS that may be accidentally released from the facility in Panama. The populations of AAS to be reared at the facility will be limited to female-only, sterile triploids. Also, regional environmental conditions are considered hostile to the long term survival of AAS and are expected to prevent the dispersal of any escaped salmon from the facility's immediate location. Both these factors will be considered in the assessment with respect to the likelihood of AAS entering Canadian waters via the Pacific Ocean.

10.2.2.1 The potential for natural events in Panama to result in the accidental release of AAS

The likelihood of an accidental release resulting from a natural event such as an earthquake, flooding or landslides is considered low, with reasonable certainty.

A catastrophic release of fish resulting from a natural disaster, such as earthquakes, tsunamis, tornados, hurricanes, tidal surges, flooding or fires, is unlikely. The western province of Chiriquí does experience the greatest frequency of seismic activity in Panama; a result of its proximity to borders between the Cocos, Nazca and Caribbean tectonic plates (Benz et al. 2011). However, the majority of significant earthquake are centered in the Gulf of Chiriquí or further west in Costa Rica. Tremors that are felt in Boquete tend to be mild and there have been no reports of significant damage to infrastructure resulting from earthquakes in the area. It should also be notes that the facility is sited on the eastern flank of an active volcano, Volcán Barú, which may have erupted as recently as 1550 AD (Sherrod et al. 2007). Several notable 'earthquake swarms' have been reported around the volcano over the past 50 years and as recently as 2006, but they are not known to have resulted in significant property damage. An eruption would likely be explosive, but would be preceded by days or months of intensifying seismic activity, giving residents the opportunity to prepare for the event (Sherrod et al. 2007). Tsunamis and tidal surges are highly unlikely to affect the facility given its elevation of approximately 1600 meters above sea level and though tornados are known to occur in Panama, there have been no reports of tornados forming in the province of Chiriquí.

The most likely natural disaster to challenge the facility's infrastructure and physical containment of AAS would be flooding or landslides that may result from excessive amounts of rain. Although hurricanes and tropical storms rarely make landfall in Panama (Hurricane Martha in 1969 is the only event on record), many have formed or tracked within the Central American region (Williams et al. 1989). Low pressure weather systems that form in the Pacific Ocean or track through the Gulf of Mexico can often force moist air into the area resulting in above average rainfalls. This can be of particular significance late in the rainy season (October - November) when drainage systems are saturated and there is an increased susceptibility to flash floods and mud slides. There is an extensive history of flooding and mud slides in Central American countries including Costa Rica and Panama. In November 2008, excessive rainfall in Chiriquí province caused significant property damage in the town of Boquete, but did not have a significant affect the aquaculture facility (still under construction at the time) that is sited approximately 20 km north of Boquete and is much closer to the headwaters of the Caldera River. [redacted]

Since 2008, there have been additional reports of flooding and mud slides in the province of Chiriquí with no reported impact to the facility. Consequently, it is likely that the siting of the facility at a high elevation and closer to the headwaters of regional watersheds will continue to protect it from weather related events.

Therefore, given the information of the facility's siting, knowledge of extreme natural events that may occur in the region and may challenge the containment of AAS, the likelihood of an accidental release resulting from a natural event such as an earthquake, flooding or landslides is considered low, with reasonable certainty.

10.2.2.2 The potential for security violations in Panama to result in the accidental release of AAS

Given its remote location and the information regarding measures in place to prevent trespassing, security violations are expected to be rare. Consequently, the likelihood of an accidental release of AAS resulting from a security violation is considered to be negligible, with reasonable certainty.

Regardless of its remote and peaceable location, AquaBounty has, in prudence, put in place several security measures to protect both its property and personnel. [redacted]

Given its remote location and the information regarding measures in place to prevent trespassing, security violations are expected to be rare. Consequently, the likelihood of an accidental release of AAS resulting from a security violation is considered to be negligible, with reasonable certainty.

10.2.2.3 The potential for chronic failure of physical containment in Panama to result in the release of AAS

Physical containment strategies for all life-history stages (eyed-eggs through to market weight) of AAS and all potential pathways to entry will be individually assessed.

[redacted]

Although the facility in Panama is of a much simpler design than the facility in PEI, there are still several pathways by which AAS may enter the environment and multiple containment points that may be subject to failure for a variety of reasons. Therefore, in addition to providing a critical review of the suitability and redundancy of physical containment, the assessment will include a failure modes analysis (FMA) for all life-stages present and for all potential pathways to entry. Tables for each FMA can be found in appendix C. They provide a detailed look at all containment provisions and all measures in place to mitigate or prevent potential failures.

10.2.2.3.1 Physical containment of all-female triploid AAS embryos

Given the redundant mechanical containment, but the absence of operational oversight documentation, the likelihood of entry into the environment of viable AAS embryos by means of this pathway is considered to be low. Detailed information available on facility design, containment features, and water treatment, SOPs, internal compliance documentation and information related to the frequency of past containment failures result in an assessment that is reasonably certain.

[redacted]

In Panama, there is only one notified location to be used for the embryonic development of AAS.

Once the triploid (3n) all-female AAS eggs are received at the ABP site, they will be removed from an insulated 'egg-crate' under the supervision of an official from the National Animal Health Authority (DINASA, a division of the Ministry of Agriculture, MIDA) and [redacted].

[redacted]

[redacted] Consequently the FMA for this pathway does not consider physical containment features beyond the Fry tank drainage system (see section 9.2.1.3.2).

When eggs or alevins are being held in the Heath stack incubators, [redacted] physical barriers in place to prevent entry into the Caldera River. Consequently, a simultaneous failure at all [redacted] is required for an accidental release to occur.

The FMA for this pathway identified [redacted] containment and 16 potential failure modes that may result from material failures, human error, or a combination of both (Appendix Table C-1). In addition to multiple and redundant containment features, there are written SOPs directing staff to inspect most containment features on a daily basis, however, there is no internal compliance documentation such as a daily check-list that must be signed by the attending staff member.

The RPNs associated with potential failure modes during these activities are ranked as low to medium (1 to 6). Moderate rankings are primarily the result of an inability to check the Heath tray screens on a daily basis when a tray is in use and limited information regarding the failure frequency of heath tray screens. [redacted]

[redacted]

Consequently, given the redundant mechanical containment, but the absence of operational oversight documentation, the likelihood of entry into the environment of viable AAS embryos by means of this pathway is considered to be <u>low</u>. Detailed information available on facility design, containment features, and water treatment, SOPs, internal compliance documentation and information related to the frequency of past containment failures result in an assessment that is <u>reasonably certain.</u>

10.2.2.3.2 Physical containment of all-female triploid AAS fry

Given the redundant mechanical containment, but the absence of operational oversight documentation, the likelihood of entry into the environment of viable AAS fry by means of this pathway is considered to be low. Detailed information available on facility design, containment features, and water treatment, SOPs, internal compliance documentation and information related to the frequency of past containment failures result in an assessment that is reasonably certain.

[redacted]

[redacted]

For the practical purposes of this exposure assessment, AAS at the Panama facility are considered to be fry from just after egg sac absorption when fish are first 'ponded' into fry tanks, until they have grown to a size 2 grams and can be effectively retained by mechanical barriers with a mesh size smaller than or equal to 6 mm. (a period of approximately 30 days).

[redacted] Consequently the FMA for this pathway does not consider physical containment features beyond the Fry tank drainage system.

When fry are being held in the fry-tank inserts, there are [redacted] barriers in place to prevent entry into the Caldera River. Consequently, a simultaneous failure at all [redacted]

The FMA for this pathway identifies [redacted] containment and examined 12 potential failure modes that may result from material failures, human error, or a combination of both (Appendix Table C-2). In addition to multiple and redundant containment features, there are written SOPs directing staff to inspect most containment features on a daily basis, however [redacted] The RPNs associated with potential failure modes along this pathway are all ranked as low to moderate (2 to 6). Moderate rankings primarily result from the absence of internal compliance documentation, such as a daily check-list to ensure that all relevant mechanical barriers are in place and functioning properly. Consequently, given the redundant mechanical containment, but the absence of operational oversight documentation, the likelihood of entry into the environment of viable AAS fry by means of this pathway is considered to be low. Detailed information available on facility design, containment features, and water treatment, SOPs, internal compliance documentation and information related to the frequency of past containment failures result in an assessment that is reasonably certain.

10.2.2.3.3 Physical containment of all-female triploid AAS parr

Given the redundant mechanical containment, but the absence of operational oversight documentation, the likelihood of entry into the environment of viable AAS parr by means of this pathway is considered to be low. Detailed information available on facility design, containment features, and water treatment, SOPs, internal compliance documentation and information related to the frequency of past containment failures result in an assessment that is reasonably certain.

[redacted]

[redacted]

For the practical purposes of this exposure assessment, AAS at the Panama facility are considered to be parr once they have reached a weight of 2 grams (are no longer fry) until they have grown to a size of approximately 25 grams and can be effectively retained by mechanical barriers with a mesh size smaller than or equal to 12 mm (a period of approximately 60 days).

[redacted]

[redacted] Fish below this minimum size are euthanized and disposed of according to SOPs for ABP.

[redacted] Consequently, all containment features with a mesh size greater than 6 mm are not considered in the FMA. There is only one pathway to entry into the Caldera River for AAS parr that are under physical containment at the facility in Panama.

When parr are being held in the fry-tank building, there are [redacted] independent physical barriers in place to prevent entry into the Caldera River. [redacted] Consequently, failure at all [redacted] barriers, with simultaneous failures at the first [redacted] barriers, is required for an accidental release to occur. [redacted]

The FMA for this pathway identifies [redacted] components to physical containment and examined 35 potential failure modes that may result from material failures, human error, or a combination of both (Appendix Table C-3). The majority of potential failure modes are mitigated by written SOPs in the form of daily inspection; however, [redacted] The RPNs associated with potential failure modes along this pathway are all ranked as low to moderate (2 to 6). Moderate rankings primarily result from the absence of internal compliance documentation, such as a daily check-list to ensure that all relevant mechanical barriers are in place and functioning properly.

Consequently, given the redundant mechanical containment, but the absence of operational oversight documentation, the likelihood of entry into the environment of viable AAS parr by means of this pathway is considered to be low. Detailed information available on facility design, containment features, and water treatment, SOPs, internal compliance documentation and information related to the frequency of past containment failures result in an assessment that is reasonably certain.

10.2.2.3.4 Physical containment of all-female triploid AAS juveniles and adults in grow-out

Given the redundant mechanical containment, but the absence of operational oversight documentation, the likelihood of entry into the environment of viable AAS juveniles and adults by means of this pathway is considered to be low. Detailed information available on facility design, containment features, and water treatment, SOPs, internal compliance documentation and information related to the frequency of past containment failures result in an assessment that is reasonably certain.

[redacted]

[redacted]

[redacted]

[redacted]

When juvenile or adult AAS are being held in the grow-out tanks, there are [redacted] independent physical barriers in place to prevent entry into the Caldera River. [redacted] Consequently, failure at all [redacted] barriers, with a simultaneous failure at the [redacted] barriers, is required for an accidental release to occur. [redacted]

The FMA for this pathway identifies [redacted] components to physical containment and examined 45 potential failure modes that may result from material failures, human error, or a combination of both (Appendix Table C-4). The majority of potential failure modes are mitigated by written SOPs in the form of daily inspection; however, [redacted]

The RPNs associated with potential failure modes along this pathway are all ranked as low to moderate (2 to 6), though the majority of ranking are low. [redacted] [redacted].

Consequently, given the redundant mechanical containment, but the [redacted], the likelihood of entry into the environment of viable AAS juveniles and adults by means of this pathway is considered to be <u>low</u>. Detailed information available on facility design, containment features, and water treatment, SOPs, internal compliance documentation and information related to the frequency of past containment failures result in an assessment that is <u>reasonably certain.</u>

10.2.3 Potential for entry of AAS into a receiving environment during transport

Given the redundant mechanical containment and operational oversight, the likelihood of entry into the environment of viable AAS embryos by means of this pathway is negligible. Detailed information available on containment features, SOPs, internal and international compliance documentation and information related to the frequency of past containment failures result in an assessment that is highly certain.

[redacted]

The shipping and handling of eyed triploid all-female AAS eggs from the site in PEI to the site in Panama represents an additional pathway by which AAS may enter the environment.

[redacted] The entire activity is subject to standard operational procedures [redacted], internal compliance documentation and international compliance documentation and oversight. Preparing eggs in PEI for shipping is an activity during which many physical containment barriers [redacted]

[redacted]

During ground transport from the facility to either the Halifax or Charlottetown airport, the eggs will be in the possession of AquaBounty Canada staff. Air transport will be facilitated by a commercial freight-forward company to maintain a chain-of-custody through to its arrival [redacted]. The AAS eggs will be received and transported to the ABP facility under the supervision of an official from the Ministry of Agriculture's (MIDA) Quarantine Department and will be unpacked and inspected at the facility under the supervision of an official from the National Animal health Authority (DINASA, also a division of MIDA).

Eyed triploid all-female AAS eggs received at ABP will be acclimated and disinfected according to SOPs, [redacted]

The FMA for this pathway identifies [redacted] components to physical containment and examined 17 potential failure modes that may result from material failures, human error, or a combination of both (Appendix Table D-1). The RPNs associated with potential failure modes along this pathway are all ranked as low (1 to 3), though rankings are difficult to make given the unpredictable nature of incidents involving significant impact or their severity, which may depend heavily on the location of an event. During transport, when triploid all-female AAS eggs are in the possession of ABC or ABP personnel, containment is mainly determined by the company's SOPs and is equally stringent as when conducting other activities such as the collection of gametes, pressure shocking or tank transfers. When the eggs are in the possession of shipping agents, airlines or government authorities, they are subject to SOPs designed to ensure the proper handling of valued merchandise and public protection.

Consequently, given the redundant mechanical containment and operational oversight, the likelihood of entry into the environment of viable AAS embryos by means of this pathway is <u>negligible</u>. Detailed information available on containment features, SOPs, internal and international compliance documentation and information related to the frequency of past containment failures result in an assessment that is <u>highly certain.</u>

10.3 The potential of AAS to survive, disperse and persist in the Canadian and Panamanian receiving environments

The ability to survive in the receiving environment is limited to parr, smolt, post-smolt and adults that may enter the environment at the facility in PEI and all life-stages that may enter the environment in Panama. The capacity of AAS to disperse from the point of entry and enter the Canadian environment is limited to parr, smolt, post-smolt and adults that may enter the environment at the facility in PEI.

The assessment of the potential of the AAS to survive, disperse and persist in the environment will rely on available fitness data. Relevant measurement endpoints will include the physical tolerance of the different life stages of the AAS, or valid comparators, to environmental parameters such as temperature, salinity and pH at potential sites of entry. Tolerance to seasonal or sudden changes in physical and physiological parameters will also be assessed along with availability of habitat and resources within dispersal range of the facilities. Since the physical requirements and physiological tolerances of Atlantic salmon and AAS are known to change according to its life-history stage, the potential for AAS to survive at, disperse from and persist in the environments at both the ABC and ABP facilities will be considered for all relevant life-stages. The potential effects of triploidy, gynogenesis, sex-reversal and domestication and transgenesis on the ability of AAS to survive, disperse and persist are also taken into consideration.

10.3.1 Potential effects of triploidy, gynogenesis and sex-reversal on the capacity of AAS to survive and disperse and persist in the receiving environment

Triploidy, gynogenesis and sex-reversal are expected to decrease or have no effect on survival, persistence and dispersal of the organism in most circumstances. Triploidy can decrease survival during non-optimal conditions, although combined with all-female technology triploidy can increase survival during spawning season. Triploidy greatly decreases spawning migrations of salmon, particularly in all-female fish. Otherwise, these technologies are expected to have little effect on survival, persistence and dispersal of the organism.

AquaBounty has demonstrated that triploid AAS fish had more minor external abnormalities and some minor alterations in organ function, but equal internal abnormalities relative to diploid AAS fish (Erisman et al. 2009). However, whether triploidy, gynogenesis, or sex-reversal would influence the ability of AAS or AAS broodstock to survive, persist, or disperse in a receiving environment has not been addressed. Studies of other triploid salmonids in stocking programs, aquaculture, or laboratory conditions indicate triploid fish may have equal, lesser or greater survival than diploid fish, depending on the conditions. In particular, triploid fish may perform poorly in systems of low productivity (Kozfkay et al. 2006), during exposure to disease (Parsons et al. 1986, Yamamoto and Iida 1994, Ojolick et al. 1995, Cotter et al. 2002, Jhingan et al. 2003, Ozerov et al. 2010), when oxygen is limiting (Yamamoto and Iida 1994, Ojolick et al. 1995), during smolting or early seawater rearing (Benfey 2001, O'Flynn et al. 1997, Johnson et al. 1986, Galbreath and Thorgaard 1995, Withler et al. 1995, McCarthy et al. 1996, Taylor et al. 2007), and high temperature (Atkins and Benfey 2008). All-female triploid fish may survive longer in some circumstances as they do not have spawning-related mortality observed in diploid fish or triploid males (Teuscher et al. 2003, Chatterji et al. 2008, Koenig et al. 2011). As well, Berrill et al. (2013) found triploid rainbow trout had overall greater survival than diploid fish in aquaculture within the UK. Overall, triploidy is expected to decrease persistence of AAS fish in poor conditions, but combined with all-female technology may increase persistence post-spawning.

The effects of gynogenesis and sex-reversal on potential survival of escaped organisms have not been examined to our knowledge. Gynogenesis can result in low early survival in founder fish, but offspring of gynogenetic fish generally have normal or near-normal survival (see Pandian and Koteeswaran 1998, Kome and Thorgaard 2007). The effect of sex-reversal on survival of fish has been poorly studied, but environmental chemical sex-reversal appears to have little effect on survival (see McNair et al. 2012). However, when all-female technology is combined with triploidy, this can result in increased survival during spawning season (see above).

Triploidy combined with all-female technology is expected to decrease dispersal of fish, as triploid salmon have greatly decreased spawning migrations relative to diploids, particularly female fish (Warrillow et al. 1997; Cotter et al. 2000; Wilkins et al. 2001; Chatterji et al. 2008). The effect of gynogenesis or sex-reversal on dispersal has not been examined, but is not expected to increase potential dispersal of notified organism.

10.3.2 Potential effects of domestication on the capacity of AAS to survive and disperse and persist in the receiving environment

Domestication is expected to diminish the fitness of AAS, but will not prevent it from surviving and dispersing from the point of entry, nor is expected to prevent AAS from reaching the adult life-stage.

The physical requirements and tolerances of AAS are not expected to be significantly affected by the process of domestication. In general, the temperatures, salinities and pH at which domesticated salmon are able to survive are expected to fall within the ranges observed for the wild populations from which they have been derived.

Most studies investigating the survival of domesticated Atlantic salmon in the wild have focused on how the changes to morphology, physiology and behavior brought about by selection (or adaptation) in the hatchery environment, or the conditions imposed by intensive aquaculture, can affect their fitness, relative to wild Atlantic salmon, in the natural environment (Fisheries and Oceans Canada 2006; Jonsson and Jonsson 2006; Moreau and Fleming 2012). For example, faster growth rates and aggressive behavior observed in juvenile domesticated Atlantic salmon may provide an advantage over competitors in the wild; however a greater motivation to risk predation in order to feed can also lead to higher rates of mortality (Einum and Fleming 1997; McGinnity et al. 1997; Biro et al 2004). Diminished swimming performance (Enders et al. 2004) and stress response (Johnsson et al. 2001) may also lead to inferior fitness and reduced survival of domesticated Atlantic salmon in the wild. Additional factors such as the timing and location of release may also play a role (Fisheries and Oceans Canada 2006).

Direct measurements of the survival of domesticated Atlantic salmon in the wild are limited. Survival of farmed lines during the pre-smolt freshwater phase has been observed as equivalent to (Einum and Fleming 1997) or inferior (McGinnity et al. 1997) to wild salmon when both are reared in the wild. Studies investigating marine mortality have demonstrated a lower rate of survival for farmed Atlantic salmon compared to wild salmon when the hatchery smolts are released at the same time as wild smolts are migrating (Jonsson et al. 1991; Jonsson et al. 2003; Saloniemi et al. 2004). The conclusion drawn from all of these studies is that, provided all physiological requirements are met, domesticated Atlantic salmon can survive in the wild environment long enough to reach maturity and have a genetic impact on wild populations.

Much of what is known about the migratory patterns of domesticated Atlantic salmon comes from a small number of studies designed to understand the potential impact of accidental releases from the aquaculture industry. Taken together, these studies suggest that the survival and migratory behaviour of domesticated Atlantic salmon that are released into the wild is dependent on the location and time release as well as the age or life-stage at the time of release. Skilbrei (2010) observed that smolts released in the spring demonstrated much stronger migratory behaviour, dispersing quickly from fiords when released in the spring and little or no migratory or dispersal behaviour when released in the fall. When released as adults, domesticated Atlantic salmon tend to follow prevailing currents, demonstrate little homing ability and enter non-natal streams to spawn. Hanson and Youngson (2010) found that domesticated Atlantic salmon released at a sight in Norway during the spring, remained within 150 km of the release sight, where they eventually entered local fresh water systems. However, in the same study, salmon from a similar release in Scotland drifted east with prevailing ocean currents and were recaptured in Norwegian rivers. Hanson (2006) demonstrated that adult domesticated Atlantic salmon released in Norway at various times of the year, tend to follow prevailing currents northward before entering non-natal streams to spawn (in one case over 2000 km from the release site). Whoriskey et al. (2006) used telemetry to establish the fate of adult domesticated Atlantic salmon released from cage sites in Maine, USA. They concluded that fish released in winter and spring dispersed from coastal areas and followed currents into the Bay of Fundy, but were never reported entering fresh water. To date there is no evidence of escaped domesticated smolts or adult Atlantic salmon migrating from eastern North America to winter feeding regions in the western North Atlantic.

10.3.3 Potential effects of growth hormone transgenesis on AAS survival, dispersal and persistence in the receiving environment

Though the effects of growth hormone transgenesis may result in diminished fitness of AAS, but will not prevent it from surviving and dispersing from the point of entry, nor is expected to prevent AAS from reaching the adult life-stage.

Changes to the physical requirements and tolerances of growth enhanced transgenic salmonids have received little attention. Most often, transgenic salmonids are raised, in captivity, under physical conditions that best represent either a natural environment or a standard hatchery and grow-out facility. Deitch et al. (2006) have demonstrated that AAS have a smaller metabolic scope than non-transgenic Atlantic salmon, which may diminish its ability to thrive at higher than optimal water temperatures or lower than optimal oxygen concentrations.

Many of the physiological and behavioural changes that may result from the domestication have also been observed in salmonids that have undergone transgenesis with growth enhancement genes. Growth enhanced transgenic coho salmon (*Oncorhynchus kisutch*) may also demonstrate increased feeding motivation (Sundström et al. 2003) and an increased ability to compete for food (Devlin et al. 1999), but suffer greater mortality due to diminished predator avoidance behaviour (Sundström et al. 2004). These similarities in the physiology and behaviour of domesticated and transgenic coho salmon are complimented by similarities in gene expression (Overturf et al. 2009). Abrahams and Sutterlin (1999) demonstrated that AAS relatives are also willing to accept a greater predation risk in order to satisfy its enhanced motivation for food. Diminished swimming performance and reduced metabolic scope (Deitch et al. 2006) may also lead to reduced fitness and lower survival of AAS when compared to wild salmon in the natural environment. However, in the absence of predators and during early life-stages, AAS and wild Atlantic salmon have similar rates of survival when fish are reared in naturalized environments (Moreau et al. 2011).

Few studies have investigated the effects of growth enhanced transgenesis on dispersal or the migratory behaviour of salmonids. Sundström et al. (2007) observed that growth enhanced transgenic coho salmon were less likely to disperse upstream, but equally likely to disperse downstream as their non-transgenic, wild counterpart. They also found that transgenic fish had a greater tendency to move about and explore; a behaviour similar to that observed in brown trout (*Salmo trutta*) treated with bovine growth hormone (Sundt-

Hansen 2009) and attributed an increased foraging activity. No difference in the survival or the migratory timing of growth enhanced and wild coho salmon was observed when both are raised from the first feeding stage under naturalized conditions (Sundström et al. 2010).

10.3.4 Potential for diploid and triploid AAS to survive and disperse and persist under the physical conditions at the point of entry in PEI

The saline condition of the marine environment at the point of entry in PEI is the principal factor that may limit the survival and dispersal of AAS in Canadian waters. Though conditions will likely prevent the survival of AAS at early life-stages (embryos to fry), they are not expected to prevent the survival and dispersal of AAS at later life-stages (parr to adult).

[redacted]

The estuary receives fresh water from the Fortune River watershed; a system that has not supported any populations of Atlantic salmon since the early 1900s, despite several attempts at stocking the river in the 1920s and 1930s (Cairns et al. 2010). Like many of the island's rivers, degradation, resulting from agricultural practices and the activities of beavers, has resulted in a habitat that is no longer suitable to support viable Atlantic salmon populations (Guignion 2009).

The notification states that local waters [the Bay Fortune Estuary] are quite saline (~20 to 30 ppt), which makes the local environs inhospitable year-round to early salmonid life-stages (p. 858). Indeed, data collected by Environment Canada (as part of the Canadian Shellfish Sanitation Program and provided to AquaBounty) indicate that salinity 'upstream' of the facility varies between 23 and 30 ppt from May to October. Surface salinity in the Northumberland straight tends to range between 26 ppt during the summer months and 30 ppt during the winter (http://www2.mar.dfo-mpo.gc.ca/science/ocean/gsl/gslmap.html).

The notification also states that during the winter months, the Bay Fortune estuary is covered with ice and that water temperatures range between -2 and 2^{0}C, making local conditions in the area of the facility inhospitable to salmonids at all life-stages during the coldest months of winter (p. 857). Average sea surface temperatures in this part of the Northumberland Straight fall below 0^{0}C during February and March and below 1^{0}C during January and April (http://www2.mar.dfo-mpo.gc.ca/science/ocean/gsl/gslmap.html).

Since the physical requirements and physiological tolerances of Atlantic salmon and AAS are known to change according to its life-history stage, the potential for AAS to survive at, disperse from and persist in the environment at the ABC facility will be considered for all relevant life-stages.

10.3.4.1 Survival, dispersal and persistence of AAS gametes

Exposure resulting from the survival, dispersal and persistence of AAS fertile gametes is expected to be negligible. The availability of peer reviewed data supporting rapid activation and subsequent loss of fertility in salmonid gametes and detailed information about the physical parameters of the receiving environment, result in an assessment that is highly certain.

Gametes are typically present at the facility in PEI [redacted] during egg collection and fertilization activities. By the time any unfertilized gametes enter the environment outside of the PEI facility, it is highly unlikely that they will be viable or capable of being fertilized. Upon entry into the aqueous environment, Atlantic salmon gametes are 'activated' in advance of fertilization, but lose fertility rapidly in the absence of zygosis. In the wild, eggs and milt must be in close proximity of one another at the time of release in order for fertilization to be successful. When milt is activated by water, the gametes become motile, using flagella to propel the germ cell towards an egg.

Within five minutes of activation, the energy reserves within the sperm cell are depleted, the milt is no longer viable and the gametes expire (Vladic and Jarvi 1997). The exact length of time that Atlantic salmon eggs will remain viable after activating in fresh water is unknown. Vladic and Jarvi (1997) found that Atlantic salmon eggs activated in water temperatures between 2 and 16^{0}C, remained viable for 8.5 minutes, but they did not assess egg viability beyond this time period. Studies in brown trout (*Salmo trutta*) have demonstrated that eggs are completely infertile after 10 minutes in fresh water (Lahnsteiner 2002). During this time, the outer membrane (the chorion) of the egg slowly hardens; covering the micropyle and preventing penetration of sperm cells. It is reasonable to expect a similar time frame for this process to occur in Atlantic salmon, given that brown trout is its closest living relative. Since eggs and milt are collected at different times [redacted], the simultaneous entry of eggs and milt into the environment, followed by successful fertilization, is highly unlikely.

Consequently, exposure resulting from the survival, dispersal and persistence of AAS fertile gametes is expected to be <u>negligible</u>. The availability of peer reviewed data supporting rapid activation and subsequent loss of fertility in salmonid gametes and detailed information about the physical parameters of the receiving environment, result in an assessment that is <u>highly certain</u>.

10.3.4.2 Survival, dispersal and persistence of AAS diploid and triploid embryos (eggs and alevins)

Exposure resulting from the survival, dispersal and persistence of AAS embryos (eggs and alevins) is expected to be <u>negligible</u>. The availability of peer reviewed data describing the physical requirements and tolerances of Atlantic salmon at this stage of development and detailed information about the physical parameters of the receiving environment, result in an assessment that is <u>highly certain</u>.

Depending on water temperatures, [redacted] Throughout the embryonic stage of development, the AAS is restricted to an environment in which physical and chemical factors such as temperature, dissolved oxygen, pH, salinity and mechanical stress must be maintained within acceptable limits for normal development.

Atlantic salmon embryo development is restricted to freshwater. Salinities greater than 2 ppt have been observed to result in osmotic abnormities in the egg which lead to irregular or arrested development of the embryo (Li et al 1989). According to Parry (1960) one week old alevins will last up to 8 hrs at 30 ppt or 45 hours at 7.5 ppt. Six week old alevins will only last 0.5 hours at 30 ppt or 96 hours at 7.5 ppt. Dispersal of AAS alevins to areas of lower salinity (well upstream of the entry point) is unlikely given the limited swimming abilities and poor mobility of Atlantic salmon at this life-stage. Thus, with salinities of greater than 20 ppt at the point of entry, any AAS embryos that are accidentally released from the PEI facility are not expected to survive at the point of entry. In addition, during the later stages of embryonic development [redacted] water temperatures at the point of entry are expected to range below 2^{0}C and would severely limit the survival of both AAS eggs and alevins which have lower temperature limits of 0 to 2^{0}C (Elliott and Elliott 2010).

Consequently, exposure resulting from the survival, dispersal and persistence of AAS embryos is expected to be <u>negligible</u>. The availability of peer reviewed data describing the physical requirements and tolerances of Atlantic salmon and detailed information about the physical parameters of the receiving environment, result in an assessment that is <u>highly certain</u>.

10.3.4.3 Survival, dispersal and persistence of AAS diploid and triploid fry

Exposure resulting from the survival, dispersal and persistence of AAS fry is expected to be <u>negligible</u>. The availability of peer reviewed data describing the physical requirements and tolerances of Atlantic salmon at this stage of development and detailed information about the physical parameters of the receiving environment, result in an assessment that is highly certain.

In the wild, the Atlantic salmon fry stage is a relatively short lived, transitional period, lasting several days from emergence and dispersal, until the establishment of small territories. In a hatchery, this period is more difficult to define, but typically starts just after egg sac absorption when fish are 'ponded' into early rearing fry tanks and slowly encouraged to start feeding. Within several weeks of first feeding, early rates of mortality in the tank will have diminished substantially and fish will have grown strong enough to swim and maintain a position in the stronger currents that are above the bottom of the tank, where they can actively pursue food offerings. The fry stage at the facility in PEI is expected to last for a period of approximately 3 month post first feeding, [redacted]

Though less fragile than alevins, fry are still sensitive to physical conditions that exceed those for normal freshwater development. According to Parry (1960), Atlantic salmon fry up to three month post-hatch and less than 2 cm in length will survive at a salinity of approximately 15 ppt for only 7 hours and will survive less than 4 hours if salinities are greater than 22.5 ppt. Saunders and Henderson (1969b) observed that Atlantic salmon fry greater than 5 cm in length may survive indefinitely at salinities of 12 ppt, but die when exposed to salinities of 15 ppt. Dispersal of AAS fry to areas of lower salinity (upstream) is unlikely given their small size, limited swimming abilities and the distance they would have to travel upstream to reach suitable habitat. Thus, with salinities of greater than 20 ppt at the point of entry, any AAS embryos that are accidentally released from the PEI facility are not expected to survive at the point of entry.
In addition, during the early stages of fry development [redacted] water temperatures at the point of entry are expected to range below 2^{0}C and would severely limit the survival of AAS fry which have a lower temperature limit of 0 to 2^{0}C (Elliott and Elliott 2010).

Consequently, exposure resulting from the survival, dispersal and persistence of AAS fry is expected to be negligible. The availability of peer reviewed data describing the physical requirements and tolerances of Atlantic salmon and detailed information about the physical parameters of the receiving environment, result in an assessment that is highly certain.

10.3.4.4 Survival, dispersal and persistence of AAS diploid and triploid parr

The potential exposure resulting from the survival, dispersal and persistence of AAS parr that may be accidentally released into the environment may be <u>high</u>. The availability of peer reviewed data describing the physical requirements and tolerances of Atlantic salmon parr and detailed information about the physical parameters of the receiving environment, result in an assessment that is <u>highly certain</u>.

In the wild, the Atlantic salmon parr stage may last from one to eight years depending on the growth conditions of the nursery stream. Atlantic salmon in the Gulf of St. Lawrence can spend anywhere from 1 to 7 years in freshwater before undergoing a seaward migration (O'Connell et al. 2006). It ends when fish become physiologically able to survive in the marine environment and undertakes a seaward migration as smolt. For AAS, the period of time spent as a parr is likely to be much shorter, given that it can reach a size of 30 cm in only 2700 degree-days post hatch. Saunders et al (1998) observed that AAS relatives were capable of direct transfer to full-strength sea water (35 ppt) at a size range of 15 to 25 cm and approximately six months post first feeding.

There is a high likelihood that any AAS parr entering the Bay Fortune Estuary would be able to survive and persist for an extended period of time. Saunders and Henderson (1969a) observed that Atlantic salmon parr greater than 10 cm in length will survive and grow indefinitely in salinities of up to 22 ppt while fish greater than 5 cm in length will persist at salinities of 12 ppt (Saunders and Henderson 1969b). Cunjak (1992) has reported that Atlantic salmon parr in Newfoundland may occupy the estuarine environment during juvenile stages and prior to the smolt stage and suggested an improvement in food availability as a possible reason for this alternate life-history tactic. In addition, the ability for parr to leave the territory it initially established as a fry, and move upstream in search of more favourable habitat (McCormick et al. 1998; Hutchings 1986), opens the possibility that parr escaping into the Bay Fortune Estuary could move upstream into the freshwater section of the Fortune River. Any AAS parr surviving entry into this environment would likely develop into smolt and acquire the physiological ability to survive and grow in full strength seawater (>30 ppt; Saunders et al. 1998).

Given the timing of fertilization and embryo development at the PEI facility, [redacted] From May to July, mean surface water temperatures in the Northumberland Straight range from 5.68 to 17.08^{0}C (http://www2.mar.dfo-mpo.gc.ca/science/ocean/gsl/gslmap.html). This water temperature range is not expected to affect the survival of AAS parr in any way (Elliott and Elliott 2010).

Consequently, the potential exposure resulting from the survival, dispersal and persistence of AAS parr that are accidentally released into the environment may be high. The availability of peer reviewed data describing the physical requirements and tolerances of Atlantic salmon parr and detailed information about the physical parameters of the receiving environment, result in an assessment that is highly certain.

10.3.4.5 Survival, dispersal and persistence of AAS diploid and triploid smolt, post smolts and adults

The potential exposure resulting from the survival, dispersal and persistence of AAS smolts, post-smolts and adults that may be accidentally released into the environment may be high. The availability of peer reviewed data describing the physical requirements and tolerances of AAS and Atlantic salmon smolts, post-smolts and adults and detailed information about the physical parameters of the receiving environment, result in an assessment that is highly certain with regards to survival. Though several peer reviewed studies suggest that escaped domesticated Atlantic salmon have the potential to disperse over long distances and ascend rivers to spawn, variability between studies and knowledge gaps with regards to behaviour in the marine environment, result in an assessment that is reasonably uncertain with regards to dispersal.

Throughout the natural distribution of Atlantic salmon, there is considerable inter-population and inter-regional variation in both the timing and the destination of seaward migrations (Thorstad et al. 2011, McCormick et al. 1998). While the age at which a parr becomes a smolt may vary depending on growth rate or productivity of the stream, the timing of seaward migration within a particular river is coordinated and is believed to be highly dependent on variables such as water temperatures and diurnal cycle and typically occurs once the fish has reached a minimum fork length of approximately 10 cm (Thorstad et al. 2011). Atlantic salmon in the Gulf of St. Lawrence tend to become smolts when they are between 1 and 7 years of age or at a size of 12 to 14 cm in length (O'Connell et al. 2006).

The notification states that AAS reach an average fork length of greater than 25 cm within 2700 degree days of hatching (p. 56). [redacted] Similar results for time to smolt status have been reported for AAS relatives (Saunders et al 1998).

From May to July, mean surface water temperatures in the Northumberland Straight range from 5.68 to 17.08^{0}C (http://www2.mar.dfo-mpo.gc.ca/science/ocean/gsl/gslmap.html). This water temperature range is not expected to affect the survival of AAS parr in any way (Elliott and Elliott 2010). Similarly, the salinity of the Bay Fortune Estuary is not expected to prohibit the survival of AAS smolts should they enter the environment (p. 415 of Notification). Saunders et al (1998) observed that AAS relatives were capable of direct transfer to full-strength sea water (35 ppt) at a size range of 15 to 25 cm, six months after hatching.

Environmental conditions at the point of entry are not expected to limit the survival, dispersal and persistence of post-smolt and adult AAS that may be accidentally released from the facility in PEI. From January to April, mean surface water temperatures in the Northumberland Straight range below 1^{0}C (http://www2.mar.dfo-mpo.gc.ca/science/ocean/gsl/gslmap.html) and could have a limiting effect on the survival of Atlantic salmon (Elliott and Elliott 2010). However, from May to December, mean surface temperatures range between 5.06 and 18.17^{0}C and would have no effect on the survival, dispersal and persistence of post-smolt and adult Atlantic salmon or AAS.

The salinity of the Bay Fortune estuary is also expected to have limited effects on survival of post-smolt and adult AAS. When Atlantic salmon smolts are prevented from entering seawater, a partial re-adaptation to freshwater, termed 'desmoltification' may occur through the abandonment of mechanisms permitting survival in sea water and a re-establishment of mechanisms to enable survival in the freshwater environment (Stefansson et al. 1998, Hoar 1988). It is generally believed that that direct transfer of salmonids from fresh water to salt water during or after desmoltification may result in higher mortality or poor growth (Arnesen et al. 2003). However, the results of Arnesen et al. (2003) and Mortensen and Damsgard (1998) suggest that the period of diminished salt water tolerance is short lived and that Atlantic salmon smolts and post-smolts held in freshwater are capable of direct transfer to sea water.

It is difficult, if not impossible, to predict the specific dispersal pattern that a domesticated Atlantic salmon smolt, post-smolt or adult would take if it were to enter the marine environment from a release site in the southern Gulf of St. Lawrence. There is no scientific data describing the migratory behaviour of domesticated Atlantic salmon in the southern Gulf of St. Lawrence. The Atlantic salmon aquaculture industry in this region is limited one or two facilities at the eastern end of Prince Edward Island. Consequently, research on the potential impact of escaped domesticated salmon in this area has never been a priority. The majority of Atlantic salmon culture in the Gulf region has involved the stocking of streams and rivers for the purpose of maintaining or rebuilding natural populations. These activities occur throughout the region, but only involve the husbandry of early life-stages, using the ova and milt obtained from wild Atlantic salmon as they return to spawn in fresh water systems. Consequently, these fish are not fully domesticated and would not serve as suitable comparators for the dispersal or migration patterns of domesticated Atlantic salmon, should they be released in the area. Instead, it is probably more suitable to model predictions of dispersal from research on escaped domesticated Atlantic salmon that has been conducted in Norway, Scotland and the Gulf of Maine and has been described in section 9.3.2.

What is known about escaped domesticated Atlantic salmon is that they are able to disperse long distances (Hanson and Youngson 2010, Hanson 2006, Whoriskey et al. 2006) and are capable of ascending natural river systems to successfully spawn with wild or naturalized con-specifics (Bourret et al. 2011, Thorstad et al. 2008, Morris et al. 2008, Ferguson et al. 2007, Skaala et al. 2006, Saegrov et al. 1997). Consequently, it is reasonable to assume that all Atlantic salmon populations supported by rivers that drain into the southern Gulf of St. Lawrence (approximately 85) could be exposed to domesticated GE Atlantic salmon that are released from the AquaBounty facility at Fortune Bay, PEI.

The direction and distance of dispersal would likely depend on a variety of factors such as the time of the release, the life-stage of the escapee and prevailing ocean currents at the point of release. Spatial and temporal patterns of dispersal would not be expected to follow the coordinated strategy observed in wild populations. Dispersal is more likely to be random in nature, with individuals remaining close to the site of release (within 150 km), or drifting away from the area on prevailing ocean currents. In addition, prevailing ocean currents in this region move eastward past PEI, then push north along the western shore of Cape Breton Island before exiting the region through the Cabot Straight. The prevailing current then travels south-west along the shores of Nova Scotia before entering the Bay of Fundy and Gulf of Maine (Drinkwater and Gilbert 2004). Therefore, it is also reasonable to assume that all Atlantic salmon populations along the eastern seaboard, from Cape Breton, Nova Scotia southward to Maine (approximately 140) would also be at risk of exposure.

Consequently, the potential exposure resulting from the survival, dispersal and persistence of AAS parr that are accidentally released into the environment may be <u>high</u>. The availability of peer reviewed data describing the physical requirements and tolerances of AAS and Atlantic salmon smolts, post-smolts and adults and detailed information about the physical parameters of the receiving environment, result in an assessment that is highly certain with regards to survival.

Though several peer reviewed studies suggest that escaped domesticated Atlantic salmon have the potential to disperse over long distances and ascend rivers to spawn, variability between studies and knowledge gaps with regards to behaviour in the marine environment, result in an assessment that is reasonably uncertain with regards to dispersal.

10.3.5 Potential for triploid AAS females to survive and disperse and persist under the physical conditions at the point of entry in Panama

High water temperatures in the region of Panama are the principal factor that may limit the survival and dispersal of AAS at the point of entry. Though conditions at the point of entry will likely allow the survival of AAS that may be released, regional freshwater and marine temperatures will likely prevent AAS from dispersing to a lower elevation, or surviving long enough to reach the territorial waters of Canada.

The point of entry for any triploid AAS females that may be accidentally released from the facility in Panama is the upper reaches of the Caldera River, a tributary within the Chiriquí River watershed which drains into the Pacific Ocean at the Gulf of Chiriquí. The notification states that, "the upper basin of the Caldera River has conditions that favour the establishment of salmonids" (ABT 2013, p. 805). This is evident from the naturally reproducing and self-sustaining populations of rainbow trout (*Oncorhynchus mykiss*) that are reported to have been introduced to this area for the purpose of sport fishing (Welcomme 1988). Indeed, values for water temperature, dissolved oxygen, and turbidity in the upper-basin (provided on p.806) are all within the known tolerances of Atlantic salmon (Danie et al. 1984, Amiro 2006). Consequently, if triploid AAS females at any life-stage were unintentionally released into the Caldera River from the Panamanian facility, it is reasonable to assume that they would be able to survive and grow for an extended period of time. However, dispersal of AAS downstream from the facility would in all likelihood be limited.

Average water temperatures in the lower basin of the Chiriquí watershed are reported to range between 23.6 and 25.8^{0}C (p. 806) and, similar to monthly average air temperatures for this region, are expected to remain consistent throughout the year, (p.805). Downstream from where the Caldera River enters the Chiriquí River, water temperatures of 26.0^{0}C or higher have been recorded (p. 802). The notification suggests that these water temperatures exceed the lethal limit of ~23^{0}C for Atlantic salmon at all life stages (p. 805). Though the temperature tolerance range for AAS is not known, its reduced metabolic scope relative to Atlantic salmon (Deitch et al. 2006) suggests that it will not have an upper temperature tolerance that is greater than Atlantic salmon.

Water temperature is indeed a key abiotic factor that effects both the survival and production of most freshwater fish populations, and is a pervasive determinant of habitat suitability (Elliott and Elliott 2010, Amiro 2006, Joblin 1981, Magnuson et al. 1979). Still, it is difficult to predict whether or not Atlantic salmon can tolerate an environment where temperatures may range between 23 and 27^{0}C throughout the year. During the summer months, streams that support populations of Atlantic salmon in New Brunswick, Canada, are known to reach temperatures exceeding 23^{0}C for prolonged periods of time and have been recorded at temperatures above 29^{0}C (Caissie 2000). However, extreme conditions of this nature occur for limited periods of time in Canada and the Atlantic salmon in these streams likely have opportunities to move into cooler areas within the system, such as deep ponds or lakes.

The temperature requirements of Atlantic salmon have been reviewed by Elliott and Elliott (2010). Estimates of incipient and lethal temperature limits tend to vary depending on the strain of salmon, the life-stage, and the methodology used to obtain critical values. Fertilized Atlantic salmon eggs will not survive above 16^{0}C and alevins, or sac fry, will not survive above 25^{0}C (Elliott and Elliott 2010). Garside (1973) estimated 27.5^{0}C to be the upper temperature limit at which Atlantic salmon parr can survive. Studies by Elliott (1991) recorded survival of parr for short periods of time (100 minutes) at 31.1^{0}C, but determined that prolonged survival (over 7 days) was limited by an upper temperature of 27.8^{0}C and that feeding only occurred at temperatures below 22.5^{0}C. According to Elliott and Elliott (2010), estimates of upper incipient and lethal water temperatures for parr range between 22 and 33^{0}C, but feeding will not occur above 28^{0}C. Similar estimates have been proposed for smolts (Elliott and Elliott 2010), however Alabaster (1967) found that in fresh water, smolts are more sensitive to water temperatures than parr and will not survive prolonged exposure (>100 minutes) to temperatures above 25^{0}C. Smolts are most sensitive to temperature when making the transition from fresh to salt water, but improve slightly once acclimated (Alabaster 1967). Experimental results that identify the upper-incipient and lethal water temperature for adult Atlantic salmon cannot be found. According to Danie et al. (1984), Atlantic salmon adults are rarely found in water temperatures above 20^{0}C and mortality is expected at temperatures above 28^{0}C. Temperatures of 20^{0}C to 27^{0}C reduce resistance to disease and are therefore considered to be indirectly lethal (Danie et al. 1984). Amiro (2006) has proposed 27.8^{0}C as the maximum incipient lethal temperature for Atlantic salmon in freshwater streams (the temperature at which all salmon would exit a habitat if an opportunity were available). Elliott and Elliott (2010) state that in general, when water temperatures exceed 22 to 28^{0}C, Atlantic salmon will die unless they can move to cooler water.

Consequently, although an average water temperature range of 23 to 27^{0}C would prevent the survival of both AAS eggs and alevins; it would not necessarily prohibit AAS juveniles, smolts or adults from entering the lower-basin of the Chiriquí River watershed if an accidental breach of containment were to occur. In addition, AAS eggs or alevins that are accidentally released into the upper-basin could survive and develop into juveniles, smolts or adults with a greater ability to disperse from the area. However, the long-term survival of any AAS that enter the lower-basin will be limited by opportunities to move into cooler water before succumbing to the metabolic stress that is induced by the high temperatures.

Juvenile AAS that disperse down the Caldera River would likely stop moving downstream, or move back upstream, once water temperatures rise above 22^{0}C, the upper maximum for optimal feeding and growth (Danie et al. 1984, Elliott 1991, Elliott and Elliott 2010). This constraint could likely limit the spread of AAS parr to the upper reaches of the Caldera River. However, water temperatures along the lower reaches, and at the mouth of the Caldera River, before it enters the Chiriquí, are not known. Consequently, it cannot be stated with certainty that parr will not be able to spread downstream to the mouth of the Caldera River, where it joins to the Chiriquí River and water temperatures are known to reach 26^{0}C.

Regardless of this uncertainty, any AAS juveniles entering the Chiriquí River at this point would have few options for moving into cooler waters. Parr could move upstream, back into the cooler headwaters of the Caldera River, or possibly up into the headwaters of the Chiriquí River. Parr moving downstream, over the dam's spillway and into the lower section of the Chiriquí River would, in all likelihood, stop feeding and starve to death, or simply succumb to the high water temperatures in this section of the watershed and die. A third option available to parr that exit the Caldera River, would be to move down the diversion that leads from the top of the dam to Lake Esti, where cooler water temperatures might be available at greater depth. However, the canal that joins the Chiriquí River and Lake Esti is approximately 5 km long and exposed. The water in the canal is likely to exceed 26^{0}C and would not be an optimal environment for the dispersal of parr. Therefore, although there is a potential for juvenile AAS to spread into the lower section of the Caldera River and even enter the Chiriquí River, higher water temperatures in the lower section of the Chiriquí River would prevent further dispersal downstream. It is more likely that AAS parr will be restricted to the upper reaches of the Caldera River and its tributaries, possibly spreading to the upper reaches of the Chiriquí River and its tributaries.

It is not clear how the relatively constant water temperatures and photoperiods experienced near the equator will affect the timing of the parr-to-smolt transformation or the subsequent physiological and behavioural changes associated with this process in

Atlantic salmon (Bjornsson et al. 2011, Bjornsson and Bradley 2007, Saunders and Henderson 1970). There is a possibility, however, that smolts, though more sensitive to high water temperatures than parr (Alabaster 1967), may be physiologically compelled to migrate downstream (Thorstad et al. 2011, Ruggles 1980), into the Chiriquí River and over a dam's spillway, towards the sea. However, to reach the Pacific Ocean from the confluence of the Caldera and Chiriquí rivers, smolts would have to travel approximately 40 km through water for which temperatures are reported to remain above 26^0C throughout the year (ABT 2013, p.428). These temperatures are considered to be incipient lethal for Atlantic salmon smolts and death would be expected to occur within two hours (Elliott and Elliott 2010, Alabaster 1967). In their natural habitat, migration velocities for Atlantic salmon smolts have been observed to vary between 0.2 and 28 km per day (Ruggles 1980, Aarestrup et al. 2002). Consequently, it is highly unlikely that any AAS smolt would be able to survive the 40 km journey from the confluence of the Caldera and Chiriquí rivers to the Pacific Ocean if water temperatures in this section of the Chiriquí River remain above 26^0C.

As with smolts, it is difficult to predict the dispersal behaviour of an adult Atlantic salmon that either escapes from the production site or develops from a more juvenile stage in the freshwater environment of the upper-basin. Unlike smolt, for which known physiological and environmental cues initiate its downstream migration (Thorstad et al. 2011, Ruggles 1980) the proximate factors initiation the homeward migration of adult Atlantic salmon is still poorly understood (Thorstad et al. 2011, Hansen and Quinn 1998). Therefore, it is not clear whether escaping adults would disperse upstream or downstream. However, the tendency for adult Atlantic salmon to avoid water temperatures greater than 20^0C (Danie et al. 1984) suggests that, like juveniles, adults will likely restrict their movements to the upper-basin of the Caldera watershed, and may possibly disperse into the upper reaches of the Chiriquí River.

If adults were to enter the lower section of the Chiriquí River, they're larger size may confer to them greater resistance than smolts to the 26^0C plus water temperatures (Elliott and Elliott 2010). However, survival for a prolonged period in this section of the river is highly unlikely (Danie et al. 1984). Therefore, there may be a potential for adult AAS to spread into the lower section of the Caldera River and even enter the Chiriquí River, higher water temperatures in the lower section of the Chiriquí River would prevent further dispersal downstream. It is more likely that AAS adults will be restricted to the upper reaches of the Caldera River and its tributaries, possibly occurring to the upper reaches of the Chiriquí River and its tributaries.

Consequently, the potential exposure resulting from the survival and persistence of AAS that may be accidentally released from the facility in Panama may be high; the capacity for AAS to disperse to the lower section of the Chiriquí watershed is low to negligible.

The availability of peer reviewed data describing the physical requirements and tolerances of AAS and Atlantic salmon embryos, fry, parr, smolts, post-smolts and adults and detailed information about the physical parameters of the receiving environment, result in an assessment that is highly certain.

10.3.6 Potential for triploid AAS females to disperse beyond Panama and enter the territorial waters of Canada

The potential for AAS to enter the Canadian environment after an accidental release in Panama is negligible. The availability of peer reviewed data describing the physical requirements and tolerances of Atlantic salmon and AAS, as well as detailed information about the physical parameters of the regional environment, result in an assessment that is highly certain.

As indicated above, it is highly unlikely that any escaped transgenic Atlantic salmon that may be accidentally released from the facility in Panama would be capable of surviving the warm water temperatures that it would experience in the lower section of the Chiriquí River. The water temperatures along the 40 km stretch of river between the confluence of the Caldera and Chiriquí rivers and the Pacific Ocean are reported to remain above 26^0C throughout the year. Although such temperatures may not result in the immediate death of the organism, the ensuing deterioration in health brought about by the sub-optimal environment, would likely bring about its eventual demise.

Beyond the Chiriquí River, the environmental conditions in relation to the survival of Atlantic salmon, or the AAS, do not improve. The river eventually drains into the Pacific Ocean in a region (the Gulf of Chiriquí) where sub-surface water temperatures range between 25^{0}C and 30^{0}C throughout the year (Locarnini et al. 2010) and available dissolved oxygen remains below 5 ppm (Garcia et al. 2010). Stevens et al. (1998) found that at ~13^{0}C critical oxygen uptake levels (level at which oxygen uptake becomes limited by oxygen supply) for Atlantic salmon controls and AAS relatives was approximately 4 mg/L and 6 mg/L respectively. At temperatures above 25^{0}C, metabolic demand for oxygen is expected to be much higher. Thus, both high temperatures and low oxygen levels would be expected to have a detrimental effect on any AAS that may enter the Gulf of Chiriquí. At 75 meters below the ocean surface, water temperatures can cool to approximately 20^{0}C (Locarnini et al. 2010); however, at that depth, dissolved oxygen concentrations also drop to 3 mg/L (Garcia et al. 2010) which is well below the optimum of 6 mg/L for Atlantic salmon (Danie et al. 1984). Therefore, in the extremely unlikely event that an AAS managing to disperse from the point of entry in Panama to the Pacific Ocean, the likelihood of it surviving and swimming over 3000 km to reach suitable marine habitat is exceptionally remote.

Consequently, the potential for AAS to enter the Canadian environment after an accidental release in Panama is negligible. The availability of peer reviewed data describing the physical requirements and tolerances of Atlantic salmon and AAS, as well as detailed information about the physical parameters of the regional environment, result in an assessment that is highly certain.

10.3.7 Potential for triploid AAS female embryos to survive and disperse and persist during transport

The potential exposure resulting from the survival and persistence of AAS embryos that may be accidentally released during transport from the facility in PEI to the facility in Panama is expected to be negligible. The availability of peer reviewed data describing the physical requirements and tolerances of Atlantic salmon embryos and information about the physical parameters of the receiving environment, result in an assessment that is highly certain.

In the unlikely event of an unintentional release during transport, eggs would have to enter an environment in which physical and chemical factors such as temperature, dissolved oxygen, pH, salinity and mechanical stress are within acceptable limits for normal development. Given the proposed means of transport (see section 9.2.3) any eggs that are accidentally released during transport are most likely to enter a terrestrial or marine environment and die. In addition, to remain viable over the period of time needed to reach their destination, eggs will have to be shipped moist (but not wet) and at a temperature low enough to slow their metabolism without freezing. When released from this metabolic state, eggs must be slowly acclimated to the receiving environment in order to avoid high mortality. This further narrows the environmental conditions that would enable survival of AAS eyed-eggs should they be accidentally released. Consequently, the potential exposure resulting from the survival and persistence of AAS embryos that may be accidentally released during transport from the facility in PEI to the facility in Panama is expected to be negligible. The availability of peer reviewed data describing the physical requirements and tolerances of Atlantic salmon embryos and information about the physical parameters of the receiving environment, result in an assessment that is highly certain.

10.4 The Potential of AAS to Reproduce, Establish and Spread in the Canadian and Panamanian Environments

The capacity to reproduce, establish and spread in the receiving environment is limited to parr, smolt, post-smolt and adults that may enter the environment at the facility in PEI. All life-stages that may enter the environment in Panama will be sterile and female and will not be able to reproduce at the point of entry.

The assessment of the potential for AAS to reproduce and establish in the Canadian environment will consider the reproductive fitness of both AAS and AAS descendants. It will evaluate the likelihood of reproduction between failed triploids and diploid brood stock with either wild conspecifics or brown trout. The efficacy of triploid induction as a biological containment strategy will be evaluated (Devlin et al. 2010) keeping in mind that the majority of juvenile and adult fish held at the PEI facility will be diploid. The stability of the sex-determination systems used to generate all-female populations will also be considered as will the influence of propagule pressure.

The assessment of the potential for AAS to establish and spread in the environment will include measurement endpoints related to its overall fitness in the receiving environment, such as metabolic efficiency, growth rate, swimming performance, competitive ability for acquiring food resources, reproductive behavior, predator avoidance, capacity to adapt to environmental variability, breadth of habitat tolerance/preference, disease resistance, imprinting, migration, developmental rates and timing of critical life history stages.

10.4.1 Potential Effects of Triploidy, Gynogenesis and Sex-reversal on the Capacity of AAS to Reproduce and Establish and Spread In The Receiving Environment

Triploidy, combined with all-female populations produced through gynogenesis and sex-reversal, is expected to greatly decrease or remove the ability of the organism to reproduce in, establish in, and spread from the receiving environment.

Triploid fish are functionally sterile (Benfey 1999), and are therefore incapable of reproducing viable offspring in the receiving environment. If diploid individuals from failed triploidy escape, these are expected to be all-female and would therefore be incapable of reproduction in the absence of an existing mixed-sex population with which to breed with. If AAS brood stock with incomplete sex-reversal escaped, this phenotypically mixed-sex population could theoretically reproduce in appropriate conditions. However, sex-reversed salmon have poor gonad development (e.g. Johnstone and MacLachlan 1994, see Pandian and Koteeswaran 1998), and reproductive success is expected to be greatly diminished or absent. In addition, any offspring produced from such fish would be 100% genetically female and would therefore be unable to reproduce past a second generation. Sex determination in salmon is not entirely genetic, and can be influenced by environmental conditions (e.g. Craig et al. 1996, see McNair et al. 2012). Should fish be exposed to temperatures or other factors in culture or receiving environments that alter the phenotypic sex ratio, any diploid fish could theoretically reproduce in appropriate receiving conditions. However, offspring would be genetically all-female and would not persist in the absence of existing mixed-sex populations or unless continual environmental control of sex ratio is present.

10.4.2 Potential Effects of Domestication on the Capacity of AAS to Reproduce and Establish and Spread in the Receiving Environment

Domestication is expected to diminish the reproductive fitness of AAS, but is not expected to prevent it from reaching sexual maturity or ascending rivers to mate with appropriate con-specifics.

Though it has been established that escaped domesticated Atlantic salmon are capable of ascending natural river systems to successfully spawn with wild or naturalized con-specifics (Bourret et al. 2011, Thorstad et al. 2008, Morris et al. 2008, Ferguson et al. 2007, Skaala et al. 2006, Saegrov et al. 1997), their rate of success on the spawning ground and their ability to become established are questionable. The majority of studies investigating the capacity of domesticated Atlantic salmon to reproduce in the wild have looked at how behavioral changes brought about by selection (or adaptation) in the hatchery environment, or the conditions imposed by intensive aquaculture, can affect the reproductive fitness of farmed Atlantic salmon, relative to their wild counterpart (Moreau and Fleming 2012; Weir et al. 2005; Weir et al. 2004; Fleming et al. 1996). Fleming et al. (1996) and Weir et al. (2004) were able to demonstrate under experimental conditions that farmed adult males and females expressed several behavioral anomalies that diminished their ability to successfully reproduce. However, Weir et al. (2005) concluded that mature male parr of a domesticated line was able to adequately compete with wild mature parr and succeed in fertilizing eggs. The latter study illustrates how mature male parr may not only introduce domesticated genes into a wild population, but may also increase the rate of introgression by maturing earlier than adults and decreasing the time period between generations.

Consequently, even though domestication may have a negative effect on the reproductive fitness of Atlantic salmon adults, mature male parr may represent an alternative pathway by which domestic genes can be introduced into a wild population in a short period of time and prior to the removal of those genes by natural section during the marine phase.

Both Skaala et al. (2006) and Bourret et al. (2011) have found evidence of temporal changes in the genetic structure of wild Atlantic salmon populations that may have resulted from reproduction in the wild with domesticated lines.

10.4.3 Potential Effects of Growth Hormone Transgenesis on the Capacity of AAS to Reproduce and Establish and Spread in the Receiving Environment

Though growth hormone transgenesis may diminish the reproductive fitness of AAS, it is not expected to prevent it from reproducing successfully with an appropriate conspecific.

All studies investigating the reproductive performance of growth enhanced transgenic salmonids have been conducted in physically contained semi-natural arenas and illustrate the challenge of distinguishing between the effects of transgenesis, domestication and rearing environment on reproductive fitness. Bessey et al. (2004) found that both growth-enhanced transgenic and cultured non-transgenic coho salmon were reproductively inferior to a line of conspecifics that were reared in the wild, but spawned in a hatchery for stocking purposes, but could not separate the effects of domestication and transgenesis. Fitzpatrick et al. (2011) found the reproductive fitness of transgenic coho to be less than that of cultured coho, which was in turn, inferior to that of wild salmon. However, the author's stress that the response of wild-reared fish to a transgene may differ significantly from to that of cultured salmon and that a complete understanding of genotype-by-environment interactions for reproductive phenotypes is needed. Moreau et al. (2011) conducted a series of experiments comparing the reproductive success of AAS sexually mature adult males and sexually mature male parr (both from a cultured line) with wild adult males captured from the wild and wild mature parr that had been reared to maturity in a hatchery. The trials indicated that, with regards to reproductive success, non-transgenic males were superior to male AAS both as adults and parr. Again, it is difficult to separate the effects of the transgene and domestication on the performance of AAS; however the experiments do demonstrate that AAS males are capable of reproduction in the wild. The authors also acknowledge that the phenotypic expression of the opAFP-GHc2 construct in the natural environment and in a wild genetic background may be very different from that observed under experimental conditions.

Consequently, though growth enhanced transgenesis is likely to be a negative effect on the reproductive fitness of AAS, it does not preclude reproduction in the wild. Further, how the opAFP-GHc2 construct will affect the fitness of wild Atlantic salmon, should it be introduced to populations in the wild, is largely unknown.

10.4.4 Potential for Diploid AAS to Reproduce and Establish and Spread in the Receiving Environment in PEI

Though many of the rivers on PEI no longer support viable Atlantic salmon population, several rivers within the region do support sizable runs of Atlantic salmon and could provide suitable habitat for the reproduction, establishment and spread of AAS. However, the small number of AAS housed at the facility in PEI may be limiting.

In order for AAS to reproduce, establish and spread in the receiving environment, it must first survive long enough to reproduce; either as a sexually mature adult or as a sexually mature male parr. It must then be able to successfully reproduce, in a suitable freshwater habitat with a suitable mate. Establishment of the AAS genotype in the wild will depend partly on the fitness of AAS descendants in the wild (which is largely unknown) and partly on the propagule pressure or frequency of release from the point of entry; the latter being recognised as a predominant factor in the establishment and spread of invasive species (Lockwood et al. 2005; Colautti et al. 2006; Bennett et al. 2010).

[redacted] The number of triploid fish reared at the facility may vary from year to year depending on research needs. [redacted] Since these fish are triploid and functionally sterile, they pose no threat of exposure through reproduction or establishment. The majority of fish reared at the facility in PEI will be diploid and fertile and [redacted]

[redacted] will all be required to meet production needs in Panama, maintain brood stock and perform research and development. The estimated number of post-embryonic, fertile AAS to be reared at the facility in any given [redacted]

As indicated in section 9.3.4., AAS gametes, eggs, alevins and fry are not expected to survive in the saline environment of Bay Fortune and pose no threat of exposure through reproduction or establishment. Consequently, the potential for exposure resulting from reproduction and establishment will only be considered for AAS parr, smolts, post-smolts and adults.

10.4.4.1 Reproduction, Establishment and Spread of AAS Diploid Parr

Exposure resulting from the reproduction, establishment and spread of fertile AAS parr in the Fortune River is expected to be <u>negligible</u>. The availability of peer reviewed data describing the reproductive requirements of Atlantic salmon, information about the physical parameters of the receiving environment, history of Atlantic salmon introductions and detailed information regarding physical confinement and potential propagule pressure; result in an assessment that is <u>highly certain</u>.

As indicated in section 9.3.4.4., AAS parr entering Bay Fortune have the potential to survive, though any unintentional releases are expected to be very small and very infrequent (i.e. negligible). If any AAS parr are accidentally released, and are able to acquire the resources needed to persist for an extended period of time, there are two alternative life-history strategies it could follow to reproduce; it could remain in the Fortune River watershed and become sexually mature as either a parr (males only) or an adult, or it could migrate to the marine environment as a smolt, eventually returning to freshwater as a sexually mature adult (Moreau 2011; Saunders et al. 1998). The former strategy will be addressed in the current section. The latter strategy will be addressed in the following section (9.4.4.2.) which looks at the reproduction, establishment and spread of smolts, post-smolts and adult AAS.

If AAS were to remain in the Fortune River watershed, there is currently no established population of Atlantic salmon and past attempt to re-establish salmon in the river have failed (Cairns et al. 2010). Though populations of rainbow trout and brook trout (*Salvelinus fontinalis*) may be present in the watershed (p. 499), these species do not form viable hybrids with Atlantic salmon (Chevassus 1979). Chance meetings may occur between AAS and adult Atlantic salmon strays that are occasionally observes entering in the Fortune River from other systems (Cairns et al. 2010) however, such events are expected to be extremely rare. The co-occurrence of more than one AAS in breeding condition is also expected to be rare given that propagule pressure is expected to be greatly limited by physical confinement measures at the PEI facility. However, if such an event did occur, successful reproduction is unlikely given the behavioural anomalies associated with domestication (section 9.4.2) and a habitat that is no longer suited for the reproduction and establishment of Atlantic salmon (Cairns et al. 2010; Guignion 2009). To give the latter point some context, during the 1920s, 30s and 40s, nine separate introductions of Atlantic salmon, ranging from 15,000 to 60,000 fry, were made to the Fortune River without success (Cairns 2010). [redacted] in any given year; resulting in a potential propagule pressure that is relatively small.

Consequently, exposure resulting from the reproduction, establishment and spread of fertile AAS parr in the Fortune River is expected to be <u>negligible</u>. The availability of peer reviewed data describing the reproductive requirements of Atlantic salmon, information about the physical parameters of the receiving environment, history of Atlantic salmon introductions and detailed information regarding physical confinement and potential propagule pressure; result in an assessment that is <u>highly certain</u>.

An alternative to reproduction and establishment in the Fortune River would be to migrate into the marine environment as a smolt and return to a nearby freshwater system as a sexually mature adult. It is unknown if AAS that are accidentally released from the PEI facility will be able to sustain the rate of growth experienced while inside of the facility. Under conditions of low food availability, AAS growth may be restricted (Sundström et al. 2007) and parr may take longer to develop into smolt than has been observed for AAS relatives under hatchery conditions. Then again, uneaten food that exits from the facility as part of its effluent at the point of entry may provide sufficient nutrition to allow AAS parr to grow quickly and reach the smolt stage prior to the end of summer (Saunders et al. 1998). Consequently, there is a possibility the AAS parr entering the environment will be able to reach the smolt stage and migrate to the marine environment. The possible fate of AAS smolts that enter the marine environment is considered in the next section.

10.4.4.2 Reproduction, Establishment and Spread of AAS Diploid Smolts, Post-smolts and Adults

Exposure that could result from the reproduction, establishment and spread of fertile AAS smolts, post-smolts and adults that disperse from the Fortune River watershed into the marine environment is ranked as <u>moderate to high</u>. However, limited knowledge regarding the fate of AAS, AAS relatives and Atlantic salmon in the marine environment result in an assessment that is <u>reasonably uncertain</u>.

Factors contributing to the survival of Atlantic salmon in the marine environment are likely to be complex. In addition to influences within the marine environment, processes at play in the freshwater and estuarine (transitional) life-history stages may also have a consequence on marine mortality (Potter et al 2003; Jonsson and Jonsson 2004; Sheehan et al 2012). Hutchings and Jones (1998) found that average estimates of survival wild Atlantic salmon from the smolt stage to adults returning after a single winter at sea (grilse) to vary from 1% in Iceland to 7% in Newfoundland and 13% in the Maritimes. The likelihood of survival is expected to increase as fish become larger and are less susceptible to predation (Jonsson and Jonsson 2004).

As indicated in sections 9.3.2 and 9.3.4.5., the process of domestication is likely to reduce the capacity of AAS to survive in the marine environment, but does not completely prevent them from surviving, dispersing over long distances and ascending rivers to spawn. Though the Fortune River may not be ideal for reproduction or establishment of AAS (section 9.4.4.1) other rivers that are nearby, such as the Morell and the Cardigan rivers on PEI, or the Miramichi River in New Brunswick and the

Margaree River in Nova Scotia, would provide suitable spawning habitat and plenty of Atlantic salmon to mate with.
As indicated in sections 9.4.2 and 9.4.3, domestication and growth enhanced transgenesis are likely to have a negative effect on the capacity of AAS to reproduce in the wild. However, several studies have also conclude that natural reproduction of domesticated or transgenic Atlantic salmon is possible and, in some cases, the genetic effects of introgression between domesticated and wild populations of Atlantic salmon have been observed. Given the robust physical containment at the PEI facility, propagule pressure from accidentally released AAS is expected to negligible. However, the result of a successful natural reproductive event between a wild Atlantic salmon and an AAS is, at this point, impossible to predict since the phenotypic expression of the opAFP-GHc2 gene construct has never been observed in the wild. The fitness of AAS conceived and reared in the wild will likely be significantly different from that of an AAS reared under hatchery conditions and its capacity to become established and spread in the wild cannot be predicted at this time. Therefore, just as the potential survival of wild Atlantic salmon in the marine environment is difficult to predict, exposure resulting from the reproduction, establishment and spread of fertile AAS smolts, post-smolts and adults that may enter the environment and disperse from the Fortune River watershed is also difficult to predict.

Consequently, exposure that could result from the reproduction, establishment and spread of fertile AAS smolts, post-smolts and adults that disperse from the Fortune River watershed into the marine environment is ranked as <u>moderate to high</u>. Limited knowledge regarding the fate of AAS, AAS relatives and Atlantic salmon in the marine environment result in an assessment that is <u>reasonably uncertain</u>.

10.4.5 Potential for Triploid AAS Females to Reproduce and Establish and Spread in the Receiving Environment in Panama

The likelihood of exposure resulting from the reproduction, establishment and spread of AAS in Panama is expected to be negligible. The availability of peer reviewed data describing the effectiveness of sterilization using induced triploidy and the effectiveness of generating all-female stocks, as well as detailed information about the physical parameters of the regional environment, result in an assessment that is highly certain.

If 100% of all AAS shipped to the facility are indeed female, there will be no opportunity for reproduction since no Atlantic salmon males will be present in the Caldera River or the Chiriquí watershed. Atlantic salmon females do not hybridize with rainbow trout or any other species that is endemic to, or has been introduced to the region. Only AAS eggs that are sterile and female will be shipped from the facility in Canada to the facility in Panama. Sterility is achieved through a standardized process of triploidy induction in which eggs are subjected to high pressure (9500 psi) shortly after fertilization, using a protocol that is 95 to 100% efficient. All-female stocks are achieved through the process of gynogenesis followed by indirect feminization, using a protocol that is 100% efficient.

Should AAS enter the environment in Panama, local conditions in the Caldera River are likely suitable for the survival of AAS (section 9.3.5). However, sterile individuals will not be able to reproduce and exposure will be limited to the lifetime of the organism. In the rare event of a fertile AAS being released (a fertile individual may result from failure of the sterilization process), it would still not be able to reproduce since there are no male Atlantic salmon present in either the Caldera River (or any other river in the region) with which it can mate. Rainbow trout (*Oncorhynchus mykiss*), a close relative of Atlantic salmon (*Salmo salar*) are known to have established populations in the Caldera River, but cannot form viable hybrids with Atlantic salmon. Consequently, exposure to fertile AAS females that may enter the environment in Panama will also be limited to the lifetime of the organism.

As indicated in section 9.3.6, opportunity for the dispersal of AAS away from AquaBounty Panama facility is also extremely limited. The dispersal of any AAS that are accidentally released from the facility in Panama will in all likelihood be restricted to the upper reaches of the Caldera River and there is no chance of dispersal to Canadian territorial waters. Consequently, the likelihood of exposure resulting from the reproduction, establishment and spread of AAS in Panama is expected to be negligible. The availability of peer reviewed data describing the effectiveness of sterilization using induced triploidy and the effectiveness of generating all-female stocks, as well as detailed information about the physical parameters of the regional environment, result in an assessment that is highly certain.

10.4.6 Potential for Triploid AAS Female Embryos to Reproduce and Establish and Spread in the Receiving Environment during Transport

The likelihood of exposure resulting from the reproduction, establishment and spread of AAS embryos that may enter the environment during transport from the facility in PEI to the facility in Panama is expected to be negligible. The availability of peer reviewed data describing the effectiveness of sterilization using induced triploidy and the physical requirements and tolerances of AAS and Atlantic salmon embryos, information about the physical parameters of the receiving environment and details regarding physical containment during transport result in an assessment that is highly certain.

As indicated in sections 9.2.4 and 9.3.7, the likelihoods of AAS embryos entering or surviving in the environment during transport are both negligible. This will preclude any chance of AAS eggs that may enter the environment from reproducing or establishing a viable population. The chances of this happening are further diminished by the process of induced triploid which will render a minimum of 95% of the eggs sterile. Consequently, the likelihood of exposure resulting from the reproduction, establishment and spread of AAS embryos that may enter the environment during transport from the facility in PEI to the facility in Panama is expected to be negligible. The availability of peer reviewed data describing the effectiveness of sterilization using induced triploidy and the physical requirements and tolerances of AAS and Atlantic salmon embryos, information about the physical parameters of the receiving environment and details regarding physical containment during transport result in an assessment that is highly certain.

10.5 The Potential for the Disposal of AAS Carcasses in Canada to Act as an Exposure Pathway

Based on the information provided in the regulatory submission for AAS, DFO is highly certain that the proposed methods of disposal of transgenic AAS eggs and carcasses will not allow the release in the environment of the organism, its genetic material, and material from the organism involved in toxicity.

In their submission, ABC have included a standard operating procedure (SOP) for the disposal of transgenic and / or bio-hazardous waste, which includes dead eggs, alevins, fry, parr, smolt, and adult fish [redacted]

[redacted]

The New Substances Notification Advisory Note 2010-02 explains the meaning of the word "organism" as referring to a living organism that is not a micro-organism. AAS meets this definition. The SOP is stipulating that no living transgenic or bio-hazardous waste will leave the facility (reference to "carcasses", whether or not the materials to be disposed have been frozen or not). Consequently, if disposal is undertaken [redacted], the transgenic waste, including AAS eggs and carcasses, to be transported and disposed of does not meet definition of "organism" and the condition in paragraph 2(4)(a) of the Regulations would be met.

The New Substances Notification Advisory Note 2010-02 explains that "the genetic material of the organism means:

i) nucleic acids that are contained within living cells capable of surviving long enough in the environment to come into contact with a sexually compatible cell that may result in the reproduction or propagation of the organism or a hybrid;

ii) nucleic acids, whether contained within living or dead cells, may autonomously increase the mobilization of a novel combination of genetic material or that have been genetically engineered to increase their potential for mobilization; or

iii) nucleic acids, whether contained within living or dead cells, are of unknown function and that are associated with a micro-organism strain known to be pathogenic, including a virus.

The DNA of AAS does not meet the criteria described under b(i), (ii) and (iii) and as a consequence do not require full containment and may be disposed of in compliance with municipal waste disposal standards and practices. Milt and eggs of freshly euthanized transgenic AAS might be partially meeting criterion b(i) for a short period; however, in order for the successful reproduction and propagation of AAS or AAS hybrids, mediated by these gametes, a series of sequential extremely low-probability events must occur

successfully, including survival of the gametes, contact and fertilization of sexually compatible gametes, survival of the fertilized egg(s) to hatching, survival of the alevins, and development into the mobile stages of the life cycle, and closing the cycle by reproduction. Although the likelihood of successful completion of all steps extremely low, it could not be ignored. However, as mentioned earlier, (ABT 2013, [redacted]), there will be no living transgenic organisms, including gametes that may result in the reproduction or propagation of the organism or a hybrid.
The New Substances Notification Advisory Note 2010-02 explains that "*material from the organism involved in toxicity*" refers to a substance that is produced by the organism at a concentration or in a quantity that is greater than that known to be produced naturally by the organism where the substance is:

i) released in an amount capable of causing death or harm when introduced into or absorbed by another organism; or

ii) released in an amount capable of interfering with biological processes when introduced into or absorbed by other organisms and capable of causing ecological effects at the population level.

The two disposal methods proposed by ABC and prescribed by [redacted] will adequately deactivate any biologically active substances that might be present in the carcasses of the AAS to be disposed.

Consequently, exposure resulting from the disposal of AAS carcasses in Canada is expected to be <u>negligible.</u> Detailed information provided in the regulatory submission regarding the proposed methods for disposal of transgenic AAS eggs and carcasses and definitions of "*living organism*" and "*material from the organism involved in toxicity*" provided under the NSNR (O) make this assessment <u>highly certain</u>.

10.6 Assessment of Exposure

A final ranking for exposure will require consideration of multiple elements related to the biological, geographical and physical containment of AAS, including a variety of pathways that determine the entry and fate of AAS in the Canadian environment. In many cases, the significance of one element will be limited by, or dependent on, another. For example, survival or reproduction in the Canadian environment will be dependent on entry into the Canadian environment. Similarly, entry into the Canadian environment will be dependent on the likelihood of physical containment failure. When elements are dependent, the final ranking for exposure is the ranking associated with the determining element. When events are independent from one another, it is value of the highest ranking element that ultimately determines the exposure outcome and final ranking. The overall uncertainty ranking associated with exposure is that associated with the element that determines the final ranking.

10.6.1 Expected exposure of AAS to the Canadian environment resulting from proposed activities at the facility in PEI, Canada

Exposure of AAS to the Canadian environment resulting from proposed activities at the facility in PEI, Canada is expected to be <u>negligible</u>, with <u>high certainty</u>.

The AquaBounty facility in PEI is located well within the natural range of Atlantic salmon, making it reasonable to assume that if AAS were to be released into the environment under favorable circumstances, there is a possibility for it to survive. Since the majority of fish housed at this facility will be fertile diploids, it is also reasonable to assume that any AAS surviving in the environment may be able to reproduce and establish viable populations in the wild. However, the likelihood of significant exposure resulting from the survival, reproduction and establishment of AAS that may enter the environment is highly speculative, given our limited knowledge regarding its invasiveness. Accordingly, AquaBounty has focused its efforts on ensuring that AAS raised in its facility on PEI do not enter the environment, effectively precluding their ability to be invasive in the Canadian environment. As summarized in Table 10-5, though the likelihood of exposure resulting from the release of AAS at later life-stages may be, with reasonable uncertainty, high, the likelihood of both entry and survival into the environment is negligible with high certainty for all life-stages present in the facility. AquaBounty has achieved this level of physical containment using redundant mechanical barriers (a [redacted]) and significant operational oversight to ensure that all aspects of physical containment are properly applied, maintained and monitored. Consequently, exposure of AAS to the Canadian environment resulting from proposed activities at the facility in PEI, Canada is expected to be <u>negligible</u>, with <u>high certainty</u>.

Table 10-5 Summary of multiple dependent and independent exposure estimates that were consolidated to provide an overall estimate for expected exposure of AAS to the Canadian environment resulting from proposed activities at the facility in PEI, Canada

Pathway to exposure		**likelihood of exposure (uncertainty)**	**overall exposure (uncertainty)**
Acute entry resulting from natural event		negligible (highly certain)	negligible (highly certain)
Acute entry resulting from security violation		negligible (highly certain)	negligible (highly certain)
Chronic exposure of gametes	entry	low (reasonably uncertain)	
	survival	negligible (highly certain)	negligible (highly certain)
Chronic exposure of embryos	entry	negligible (highly certain)	
	survival	negligible (highly certain)	negligible (highly certain)
Chronic exposure of fry	entry	negligible (highly certain)	
	survival	negligible (highly certain)	negligible (highly certain)
Chronic exposure of parr	entry	negligible (highly certain)	negligible (highly certain)
	survival and dispersal	high (highly certain)	
	reproduction (as parr)	negligible (highly certain)	
Chronic exposure of smolt	entry	negligible (highly certain)	negligible (highly certain)
	survival	high (highly certain)	

Pathway to exposure		**likelihood of exposure (uncertainty)**	**overall exposure (uncertainty)**
	dispersal	high (reasonably uncertain)	
	reproduction , establishment and spread	moderate to high (reasonably uncertain)	
Chronic exposure of post-smolts and adults	entry	negligible (highly certain)	negligible (highly certain)
	survival	high (reasonably certain)	
	dispersal	high (reasonably uncertain)	
	reproduction, establishment and spread	moderate to high (reasonably uncertain)	
Expected exposure of AAS to the Canadian environment resulting from proposed activities at the facility in PEI, Canada			**negligible (highly certain)**

10.6.2 Expected exposure of AAS to the Canadian environment resulting from proposed activities at the facility in Chiriquí, Panama

Exposure of AAS to the Canadian environment resulting from proposed activities at the facility in Chiriquí, Panama is expected to be <u>negligible</u>, with <u>high certainty</u>.

The AquaBounty facility in Chiriquí, Panama is located well outside the natural range of Atlantic salmon, near the equator and approximately 5900km from the territorial waters of Canada. In addition to this significant geographical barrier, AquaBounty has implemented extensive physical containment provisions in the form of redundant mechanical barriers ([redacted] and biological containment provisions in the form of female only, sterile triploid production stocks. Table 10-6 summarizes the multiple dependent and independent exposure estimates that were consolidated to provide an overall estimate for expected exposure of AAS to the Canadian environment resulting from proposed activities at the facility in Chiriquí, Panama. Though physical containment at the facility is robust, operational oversight may not be as thorough as practiced at the facility in PEI and it cannot be concluded with high certainty that AAS will not enter the environment in Panama.

Table 10-6 Summary of multiple dependent and independent exposure estimates that were consolidated to provide an overall estimate for expected exposure of AAS to the Canadian environment resulting from proposed activities at the facility in Chiriquí, Panama

Pathway to exposure	likelihood of exposure (uncertainty)
Acute release resulting from natural event	low (reasonably certain)
Acute release resulting from security violation	negligible (reasonably certain)
Chronic release	low (reasonably certain)
survival at the point of entry	high (high certainty)
dispersal from the point of entry	negligible (high certainty)
reproduction, establishment and spread	negligible (high certainty)
Expected exposure of AAS to the Canadian environment resulting from proposed activities at the facility in Chiriquí, Panama	**negligible (highly certain)**

Also, because of its remote location, there is only limited historical information regarding the possibility of natural events that may compromise physical containment. Regardless, regional water temperatures that are above the tolerance for survival of Atlantic salmon are expected to restrict any AAS that may be released, to the cooler headwaters of the watershed that are found only at higher elevations. Further, high water temperatures at the equatorial region of the Pacific Ocean will effectively prohibit any AAS from reaching the Canadian environment. Female only, sterile triploid stocks will limit exposure in the local, Panamanian environment to the natural lifetime of any fish that may escape. Consequently, exposure of AAS to the Canadian environment resulting from proposed activities at the facility in Chiriquí, Panama is expected to be negligible, with high certainty.

10.6.3 Expected exposure of AAS to the Canadian environment resulting from proposed transport between the

facility in PEI, Canada and the facility in Chiriquí, Panama

Exposure of AAS to the Canadian environment resulting proposed transport between the facility in PEI, Canada and the facility in Chiriquí, Panama is expected to be negligible, with high certainty.

During transport between AquaBounty facilities in PEI and Panama, sterile triploid, all-female AAS eyed eggs will be will be securely packaged and labeled and sealed for shipping and will travel a route that rarely intersects with habitat suited to the survival of Atlantic salmon embryos. The likelihoods of entry into the environment, survival in the receiving environment, and capacity of AAS to reproduce and establish in the receiving environment are all expected to be negligible with high certainty. Consequently, exposure of AAS to the Canadian environment resulting from proposed methods of disposal for the disposal of AAS carcasses is expected to be negligible, with high certainty.

10.6.4 Expected exposure of AAS to the Canadian environment resulting from proposed methods of disposal for the disposal of AAS carcasses

Exposure of AAS to the Canadian environment resulting from proposed methods of disposal for the disposal of AAS carcasses is expected to be negligible, with high certainty.

Since 1996, AquaBounty has been disposing of carcasses, eggs and gametes in a manner that is subject to provincial and federal regulations. The proposed methods of disposal of transgenic AAS eggs and carcasses will not allow the release in the environment of the organism, its genetic material, and material from the organism involved in toxicity. In addition, dead AAS eggs and carcasses do not meet the definitions of "living organism" and "material from the organism involved in toxicity" provided under the NSNR (O).

Consequently, exposure of AAS to the Canadian environment resulting from proposed methods of disposal for the disposal of AAS carcasses is expected to be negligible, with high certainty.

10.6.5 Summary and overall assessment of AAS exposure to the Canadian environment resulting from the specified activities

Since AAS are not expected to enter the Canadian environment or survive at the point of entry, exposure to the Canadian environment is expected to be negligible. This assessment is made with high certainty given the detailed information available on facility design, containment features, water treatment, SOPs, internal compliance documentation and information related to the frequency of past containment failures. In addition, the availability of peer reviewed data describing the physical requirements and tolerances of Atlantic salmon and detailed information about the physical parameters of potential receiving environments also contribute to an assessment that is highly certain.

The activity that has been proposed by AquaBounty, to commercially produce triploid female AAS eggs at its land-based aquaculture facility in PEI for export to a land-based, grow-out facility in the highlands of western Panama, will not result in the presence of AAS in the Canadian environment.
AAS will be restricted to only the facilities described in the notification, which have adequate and redundant mechanical barriers and operational procedures to ensure physical containment. Regulatory oversight is also in place to ensure that adequate provisions for physical containment of AAS are in place and will continue to be maintained. Both facilities are sited in locations and constructed to standards that prevent the unintentional release of AAS that may result from naturally occurring catastrophic events and reasonable security is in place to prevent unlawful entries that may result in theft or damage to property and could potentially result in an unintentional release of AAS. In the unlikely event of a physical containment failure in Panama, biological

containment measures (sterile, all-female stocks) and physiological barriers (lethal regional water temperatures) will restrict AAS to the upper reaches of a local watershed, prevent the establishment of a viable population (limiting exposure to the organism's lifetime) and prevent dispersal of AAS from the point of entry into the Canadian environment. In the unlikely event of a physical containment failure in PEI, physiological barriers (salinity) will prevent the survival of AAS at early stages of development.

AquaBounty has provided well-defined parameters for the scope of their activity, as described above. The proposed parameters (mechanical, physiological and reproductive confinement) are considered sufficient to minimize the potential for exposure.

Therefore, since AAS are not expected to enter the Canadian environment or survive at the point of entry, <u>exposure to the Canadian environment is expected to be negligible</u>. This <u>assessment is made with high certainty</u> given the detailed information available on facility design, containment features, water treatment, SOPs, internal compliance documentation and information related to the frequency of past containment failures. In addition, the availability of peer reviewed data describing the physical requirements and tolerances of Atlantic salmon and detailed information about the physical parameters of potential receiving environments also contribute to an assessment that is <u>highly certain</u>.

11 HAZARD ASSESSMENT

11.1 Indirect Human Health Hazard Assessment

The indirect human health hazard assessment considers only human health hazards that could result from environmental exposure to AAS such as through activities including recreational swimming or fishing. As such, human health hazards related to the consumption of fish as food are not the subject of the current indirect human health risk assessment. Human health hazards associated with occupational exposure to AAS are not considered in the indirect human health risk assessment either, however, the prevalence, nature and severity of adverse effects resulting from occupational exposure provide a valuable indicator of potential human health hazards from environmental exposure to AAS.

The objective of the indirect human hazard assessment is to characterize the nature, and severity of potential harmful effects that AAS may cause to humans in Canada if they were to be exposed as compared to wild Atlantic salmon. Although the indirect human health hazard assessment does not integrate exposure considerations per se (this is done in Section 12.1 Indirect Human Health Risk Assessment), the characterization of human health hazards is limited to those effects that would be realized as a consequence of dermal or aerosol exposure.

Three endpoints are addressed in this section:

- Potential human toxicity of AAS
- Potential human allergenicity of AAS
- Potential to act as vector for human pathogens

In general, the final hazard rank associated with these three endpoints is assigned in accordance with the prevalence, nature and severity of potential effects, the availability of prophylactic treatments and the potential for community-level effects as outlined in Table 8-3. Elements of uncertainty are elaborated throughout the human health hazard characterization for each endpoint with a final uncertainty ranking assigned in accordance with Table 8-7.

11.1.1 Indirect Human Health Hazard Characterization

11.1.1.1 Potential Human Toxicity of AAS

There have been no reports of adverse human health effects associated with toxins from AAS despite long-term human occupational exposure to AAS. Based on BLAST searches for nucleotide and amino acid sequence homology, the inserted sequence does not code for any known toxins or proteins other than the intended growth hormone. We are not aware of any endogenous toxins associated with Atlantic salmon. Triploidy, gynogenesis and sex-reversal are not expected to alter indirect human health hazards of AAS. We conclude with high certainty that the potential human health hazard associated with novel or endogenous toxins from AAS is negligible.

Under the NSNR(O) research and development Advisory Note, toxicity refers to "*a substance that is produced by the organism at a concentration or in a quantity that is greater than that known to be produced naturally by the organism where the substance is (1) released in an amount capable of causing death or harm when introduced into or absorbed by another organism or (2) released in an amount capable of interfering with biological processes when introduced into or absorbed by other organisms and capable of causing ecological effects at the population level*" (EC 2010).

AquaBounty has indicated that no adverse human health effects related to AAS have ever been observed in AquaBounty staff nor in individuals visiting the AquaBounty facilities (ABT Telephone call Report 2013 May 23). Since 2000, [redacted]

AquaBounty staff has been occupationally exposed to all life stages of AAS in the conduct of activities including handling, husbandry, facility maintenance, clinical and non-clinical studies, and the disposal of morbid and dead animals. In addition, a limited number of other individuals (e.g. researcher, visitors, inspectors, veterinarians) have also been exposed to various life stages of AAS (ABT 2013 p. 941).

To assess the potential for expression of a novel toxin resulting from the introduction of the opAFP-GHc2 construct, we performed BLASTn and BLASTx searches on the entire inserted sequence against the National Centre for Biotechnology Information (NCBI) database to identify known genes and proteins respectively. [redacted]

[redacted] We

conclude with high certainty that the indirect human health hazard associated with novel toxins in AAS is negligible.

There are no known unique indirect health hazards to humans posed by triploid, gynogenetic, or sex-reversed fish. Whether sex-reversed fish exposed to 17a-methyltestosterone could transfer methyltestosterone to humans through skin contact has not been addressed. The level of methyltestosterone in fish skin post treatment relative to normal levels has not been well reported, but in muscle of tilapia did not exceed maximum levels observed in control fish (Khalil et al. 2011). As well, exogenous steroid is generally absent by 10 days post exposure (Fagerlund and Dye 1979, Johnstone et al. 1983, Curtis et al. 1991), and therefore any potential indirect hazard is expected to be extremely transitory.

There is no experimental evidence to indicate whether transgenesis may have altered endogenous toxin production in AAS, however, a literature search did not reveal any reports of endogenous toxins in Atlantic salmon. We conclude with high certainty that the hazard associated with endogenous toxin production is negligible.

There is no evidence to suggest that the enhanced growth phenotype of AAS per se would pose a toxicity hazard to humans from environmental exposure to AAS. No altered behavioural characteristics in AAS as compared to non-transgenics have been reported by AquaBounty that would pose a toxicity hazard to humans.

Given the evidence that there are no novel toxins and a lack of evidence for any toxins associated with Atlantic salmon, we conclude with high certainty that the incremental indirect human health hazard of AAS as compared to wild type is negligible.

11.1.1.2 Potential Human Allergenicity of AAS

Experimental evidence is highly uncertain as to whether endogenous allergen production is altered in diploid or triploid AAS as compared to wild type Atlantic salmon. However, even if endogenous allergen production were increased in AAS, based on the prevalence, nature and severity of allergic responses to dermal and aerosol exposure reported in the scientific literature, we conclude with reasonable certainty that the potential increased hazard to human health related to endogenous allergens in AAS triploids is negligible and that related to AAS diploids is low. This conclusion is supported by the fact that there have been no reported adverse human health effects associated with AAS, despite long-term human occupational exposure. Based on BLAST searches for nucleotide and amino acid sequence homology with known allergens, we conclude with high certainty that the potential indirect human health hazard associated novel allergens is negligible. Taken together, the potential indirect human health hazard related to allergenicity is low with reasonable certainty.

Fish is a common food allergy and is estimated to affect 0.4% of the population in the United States (Taylor et al. 2004) with reactions usually occurring after consumption (Onesimo et al. 2012). Seafood allergy is now a leading cause of anaphylaxis (Turner et al. 2011). The three most common allergenic fish in allergy patients in a clinic in Texas were reported to be tuna, catfish and salmon (Khan et al. 2011). Sensitization through aerosol and dermal exposure to fish protein allergens (Porcel et al. 2001) has been reported in occupational settings (Jeebhay et al. 2001, Onesimo et al. 2012) as well as in individuals with food allergy to fish (Turner et al. 2011, Pitsios et al. 2010). The prevalence of occupational asthma in fish processors due to aerosols from salmon was reported to be 8% (Douglas et al. 1996). Rodriguez et al. 1997 report inducing asthma due to specific bronchial challenge of salmon aerosol. Other occupational allergic reactions to seafood can manifest as rhinitis, conjunctivitis, asthma, urticarial, protein contact dermatitis and occasionally systemic anaphylactic reactions (Jeebhay et al. 2001). The prevalence of occupational dermatological allergy to bony fish (cod, coalfish, haddock) in workers in the fish processing industry was reported to range from 3% to 11% (Jeebhay et al. 2001) but no data was available for occupational exposure to salmon. The majority of skin reactions associated with dermatological allergy to seafood are contact urticaria (hives) and eczematous contact dermatitis although in the more severe form, local skin contact with seafood may result in generalised uriticaria or system angioedema or wheezing (Jeebhay et al. 2001). The development of contact dermatitis usually requires disruption of the intact skin barrier and repeated skin contact (Jeebhay et al. 2001). Thus, nature and severity of adverse effects in humans related to dermal and aerosol exposure of fish allergens reported in the literature are generally mild, consistent, self-resolving and are without potential for community-level effects.

As indicated in Section 11.1.1. of this document AquaBounty has indicated that no adverse human health effects, including allergencity, related to AAS have ever been observed in AquaBounty staff nor in individuals visiting the AquaBounty facilities (ABT Telephone call Report 2013 May 23).

AquaBounty has solicited external opinion from the Johns Hopkins University School of Medicine Reference Laboratory for Dermatology, Allergy and Clinical Immunology on a BLAST analysis and immunochemical studies performed by a third Party to assess the relative allergenicity of edible tissue from diploid and triploid AAS compared to non-transgenic Atlantic salmon (Hamilton 2010).

As indicated in Section 11.1.1of this document, we ran a BLASTx search which confirmed that the insert does not code for any protein other than the intended growth hormone. Fish growth hormone protein (GH-1) occurs naturally in native salmon and is not known to be a human allergen (Nakamura et al. 2009; Hamilton 2010). Hamilton 2010 concurs with the conclusions of the U.S Food and Drug Administration (USFDA 2010) that there are no potential allergen concerns identified with the salmon growth hormone based on a search of the AllergenOnline Structural Database of Allergenic Proteins for amino acid sequence homology. Sequence homology of greater than 35% in a segment of 80 amino acids or more indicates that the protein is likely to be an allergen, whereas a negative sequence homology indicates that the protein is not a known allergen (Codex, 2003). Thus, with the negative results, we conclude with high certainty that the indirect human health hazard associated with the expression of novel allergens in AAS is negligible.

It is possible that transgensis could alter the expression levels of endogenous allergens (Hill et al. 2000) although it has recently been suggested that the likelihood of upregulating an endogenous allergen due to transgenesis is no greater than from traditional breeding which has a history of safety and is largely unregulated (Herman and Ladics, 2011).

Parvalbumin is reported to be a major allergen in Atlantic salmon (Lindstroem et al. 1996). Fish type-I collagen has also been determined to be an allergen present in bigeye tuna and is suggested to be commonly allergic regardless of fish species (Hamada et al, 2001). A qualitative SDS-PAGE and Western analysis of allergen extracts from AAS identified a single band (M_r ~11-12 kD) responsive to antibody against parvalbumin, however, the potential presence of fish type-I collagen in AAS was not addressed.

A radioallergosorbent test (RAST) inhibition (RI) analysis of allergen extracts from AAS muscle-skin from 6 diploid AAS, 6 triploid AAS and 6 non-transgenic domesticated Atlantic salmon was commissioned by AquaBounty to determine relative allergenic potency between these groups. AAS used in these treatment groups were not balanced for gender or sexual maturity. This has no consequence for the purposes of the current indirect human health risk assessment because environmental exposure to AAS, if they were to escape, could be to either mature or immature AAS of either gender reared at the PEI facility.

When normalized vis-à-vis the mean potency for the non-transgenic control, relative allergenic potencies for diploids and triploids AAS was 1.52 and 1.2 respectively. The difference in mean potency as compared to domesticated non-transgenic controls was statistically significant for diploids but not for triploids. No data was presented on the comparative allergenic potency of AAS compared to wild Atlantic salmon.

AquaBounty asserts that the 52% (i.e. 1.5 fold) increase in allergenic potency observed in diploid AAS compared to non-transgenic controls lies within the bounds of an equivalent response for batch-wise, clinical safety testing for manufactured dust mite and grass allergen vaccines for humans (0.5- to 2.0-fold) (CBER 2000) and thus present no change in risk to sensitising previously non-allergic consumers. However, in consultation Situ and Lefebvre 2013, it was noted that the FDA determined that the 0.5-2.0 range is inappropriate for the interpretation of finfish allergen potency as this range is standardised to dust mite and grass allergen vaccine lots (CBER 2000). Health Canada and the DFO are in agreement with the FDA's opinion. It is uncertain whether the 52% increase in endogenous allergens in diploid female AAS will be biologically relevant in the absence of historical control data (e.g. RI analysis) from non-transgenic salmon

DFO and others have identified a number of uncertainties that limit our ability to interpret the significance of potential human health implications related to allergenicity of AAS:

1. There were only 6 fish per test group employed in the RI assay. It is not clear to what extent this limited number of domesticated control fish that were employed would adequately represent the natural variability of allergen levels in controls. It is known that the parvalbumin content in most commonly consumed fish species (salmon, trout, cod, carp, mackerel, herring, redfish and tuna) may vary from several fold to one hundred fold (Kuehn et al. 2010);
2. To assess indirect human health hazard from environmental exposure to AAS, the appropriate comparator would be wild salmon. It is not clear to what extent the domesticated salmon controls that were employed would be representative of endogenous allergen levels in wild Atlantic salmon;
3. According to Hamilton 2010, the only definitive method for confirming no heightened allergenicity is with prospective monitoring of individuals who have been exposed to AAS;
4. There is no consensus in the scientific and medical communities regarding the magnitude of increase in endogenous allergens in an allergic food that would present an additional risk to the public. It is known that the parvalbumin content in most commonly consumed fish species (salmon, trout, cod, carp, mackerel, herring, redfish and tuna) may vary from several fold to one hundred fold Kuehn et al. 2010).

Based on mRNA analysis, isolelectric focusing and spectrophotometric analysis in Coho salmon, Rehbein and Devlin (2009) found no evidence for enhanced parvalbumin content in the light muscle of growth hormone transgenic coho salmon as compared to non-transgenic coho salmon. However, this result is inconsistent with the results of Hill et al. 2000 who found a significant increase in the expression of cDNA fragments similar to the parvalbumin gene in fast muscle from transgenic coho salmon as compared to non-transgenic coho salmon. Nakamura et al. 2009 reported no increase in parvalbumin nor fish type-1collagen in growth hormone transgenic amago salmon (*Oncorhynchus masou ishikawae*) as compared to non-transgenic amago salmon. Preliminary results from a representative western blot comparing relative allergenicity of growth hormone transgenic coho salmon and farmed salmon showed a 2-3 fold increase in IgE binding material for 4 of 10 atopic sera from salmon allergic individuals (Bondy and Curran, 2007). The inconsistencies in the results of the foregoing studies indicate that the effect of transgensis on the level of expression of endogenous allergens may be complex. Furthermore, without robust data that represents the variability in background levels of endogenous allergens in the appropriate comparators (wild or farmed fish), it is difficult to interpret the significance of the results.

Hence, we conclude with reasonable uncertainty, based on the data submitted by AquaBounty, that triploid AAS have approximately equal allergenic potency to diploid domesticated controls and diploid AAS have a higher allergenic potency. The relative potency of endogenous allergens in AAS compared to wild Atlantic salmon has not been addressed but it is likely that the upregulation of endogenous allergens due to transgenesis is no greater than from traditional breeding (Herman and Ladics 2011). Thus, we will assume that relative allergenic potency of diploid AAS and triploid AAS is the same whether compared to domesticated or wild Atlantic salmon. In short, we will assume that triploid AAS have approximately equal allergenic potency compared to wild Atlantic salmon and diploid AAS have a higher allergenic potency. The application of this assumption will lower the certainty level to highly uncertain. The biological and human health significance of higher allergenic potency in diploids is also highly uncertain, particularly in the context of food consumption. However, given the prevalence, nature and severity associated with dermal- and aerosol-related allergic reactions reported in the scientific literature, we conclude with reasonable certainty that the potential increased hazard to human health related to endogenous allergens in AAS triploids is negligible and that related to AAS diploids is low, yielding a final human health hazard rank related to endogenous allergens of low with reasonable certainty. Based on sequence homology analysis, we conclude with high certainty that the indirect human health hazard associated with the expression of novel allergens in AAS is negligible.

11.1.1.3 Potential to Act as a Vector for Human Pathogens

Based on the fact no pathogens of human significance have ever been detected in the PEI facility, and the fact that no adverse human health impacts associated with AAS exposure have ever been reported in AquaBounty staff over almost two decades, we conclude with high certainty, that the indirect human health hazard associated with AAS acting as a vector for the introduction of human pathogens into the environment from the PEI facility is negligible. Given significant uncertainties related to the relative susceptibility of AAS to fish zoonotics as compared to wild Atlantic salmon, and the relative fitness of AAS in the wild, we are unable to conclude on whether AAS would have an increased capacity to act as a reservoir for the transmission of disease agents to humans. However, even if AAS were to have increased capacity to act as reservoir for human pathogens, based on the prevalence, nature and severity of adverse effects related to topically acquired zoonosis reported in the scientific literature, we conclude with high certainty that the hazard to human health related to AAS acting as a reservoir for human pathogens is low. In summary, the potential indirect human health hazard related to AAC acting as a vector for human pathogens is <u>low with high certainty.</u>

Many bacterial and parasitic fish pathogens are known to be zoonotic (Roberts et al. 2009) and transmitted to humans primarily through consumption of infected fish (Lima dos Santos and Howgate 2011; Curtis et al. 1988). Humans are also exposed to zoonotic pathogens through handling fish. There are no reports of fungal, parasite or viral zoonoses in humans transmitted through topical exposure (Lowry and Smith 2007;

Boylan 2011). There have been occasional reports of topically acquired bacterial zoonoses from fish occurring in recreational fishers and swimmers (Lehane and Rawlin 2000) but these infections are unusual. Bacterial zoonoses arising through contact with mucus and tissues from infected carrier fish are generally considered to infect humans opportunistically, with human disease occurring only sporadically or in immune-compromised individuals (Lowry and Smith 2007). *Aeromonas hydrophila, Edwardsiella tarda, Erysipelothrix rhusiopathiae, Mycobacterium marinum, Streptoccocus iniae, Vibrio vulnificus* and *Vibrio damsel* are the main bacteria acquired by humans through topical exposure including puncture wounds and open skin (Lehane and Rawlin 2000). Humans tend to have good natural immunity to marine bacteria (Lehane and Rawlin 2000). Symptoms from infections of these organisms are generally localized or self-limiting (Lehane and Rawlin 2000). In rare cases, severe illness, including meningitis, septicemia with endocarditis, severe cellulitis or myositis, and death have been reported but tend to be associated with highly virulent strains, deep penetration of the skin, or immune impairment particularly in individuals infected with vibrios (generally associated with marine species) or aeromonads (generally associated with freshwater species) (Lowry and Smith 2007; Lehane and Rawlin 2000). We conclude with high certainty that, in general, the severity of indirect human health hazards related to topically acquired fish zoonoses is low.

To our knowledge, there are no reports in the literature of transmission of zoonotics specifically from Atlantic salmon to humans through environmental exposure such as recreational fishing or swimming.

AAS may act as a vector for human pathogens either by direct introduction into the environment of pathogens associated with escaped AAS from the PEI facility or by acting as a reservoir in the environment for diseases of human health significance. Altered resistance to pathogens is known to occur in other GH transgenic salmonids (Jhingan et al. 2003). Increased disease resistance coupled with enhanced fitness may heighten the capacity of transgenics to act as a reservoir for the transmission of disease agents to other organisms (Jhingan et al. 2003). However, if AAS were to have increased disease susceptibility but succumb to the disease quickly then AAS may actually be less likely to act as a reservoir for the transmission of diseases than domesticated or wild Atlantic salmon in the natural environment.

We know from the data presented in Section 9.2.7.8 that AAS is more susceptible [redacted] There is strong evidence that selectively breeding Atlantic salmon for disease resistance can be highly successful (Kjoglum et al. 2008). In addition, it is unlikely that the disease susceptibility of AAS will remain contain with subsequent generations as AAS will continue to be crossed with the St. John River strain which is itself undergoing selective breeding, perhaps also for disease resistance and performance.

The significance of the apparent increased susceptibility of AAS to [redacted] is further complicated as pathogen susceptibility may vary depending on life stage, ploidy, background genetics, the pathogen in question as well as other environmental factors that influence overall health and fitness (Jhingan et al., 2003, Sundström et al., 2007). However, as there have been no reports of *A. salmonicida* infections in humans (Lowry and Smith 2007), increased susceptibility to this pathogen would not present a human health hazard.

We also concluded in Section 9.2.7.8 that AAS and domesticated comparators have comparable susceptibility [redacted]. However, because there is no data on the relative susceptibility of AAS compared to wild Atlantic salmon [redacted], we are unable to conclude on whether AAS is likely to be more or less susceptible to these disease agents than wild Atlantic salmon. In addition, we have no data on the relative susceptibility of AAS to disease agents of human significance. To add further uncertainty, disease resistance may continue to be altered in subsequent generations of

AAS as a consequence of ongoing crossing to St. John River stock that is subject to continued selective breeding.

Several studies report triploid salmonids, including GH transgenic coho salmon, to have increased susceptibility and/or decreased resistance to a number of infectious organisms (Parsons et al. 1986, Yamamoto and Iida 1994, Ojolick et al. 1995, Cotter et al. 2002, Jhingan et al. 2003, Ozerov et al. 2010), although others do not (e.g. Yamamoto and Iida 1995). As such, AAS, particularly 3N AAS, may have increased disease susceptibility in some circumstances. However, what impact this may have, if any, on vector capability of AAS has not been examined. The disease resistance and vector capability of gynogenetic and sex-reversed fish has not been examined.

Thus, while we have some data to indicate that AAS is more susceptible than domesticated Atlantic salmon to [redacted], it is highly uncertain how this may translate to other human disease agents. In addition, as indicated in Section 10.4.4 it is possible that diploid AAS could become established in the waters surrounding PEI but this is reasonably uncertain. Thus, given the uncertainty elaborated above we are unable to conclude whether AAS would have an increased capacity to act as a reservoir for the transmission of disease agents to humans compared to wild Atlantic salmon.

[redacted]

[redacted] This virus is reported to be broadly distributed among trout populations in the western United States (Batts et al. 2011). The type species for the [redacted] which is well known as the causative agent of hepatitis in humans. However, nucleotide sequences of this virus are sufficiently different from hepeviruses isolated from mammals and birds to justify the creation of a novel genus within the *Hepeviridae* family for this virus (Batts et al. 2011). [redacted] leads us to conclude that Hepeviridae does not constitute a human health hazard under these circumstances.

Diseased fish having high bacterial loads are more likely to transmit bacterial infections to humans (Lehane and Rawlin 2000). Land-based aquaculture provides opportunity to implement specific management practices and to monitor and manage fish disease and monitor the transmission of zoonotics to humans. The absence of disease outbreaks in fish and humans, [redacted], provides a good indication that disease risk at the AquaBounty PEI facility is well managed. Consequently, AAS would be very unlikely to carry any new pathogens of human health significance if they were to escape.

The development of antibiotic resistance has been reported in fish pathogens, however, there is no epidemiological evidence to indicate the transfer of antibiotic resistance genes from fish pathogens to human pathogens (Lehane and Rawlin 2000). Ampicillin resistance was used as a selectable marker in the cloning process to derive the opAFP-GHc2 construct. If the ampicillin resistance gene (*amp*$^{R)}$ had remained in the integrant, horizontal gene transfer of the *amp*^{R} gene to pathogenic bacteria of human health significance may pose a risk to the therapeutic use of ampicillin in the treatment of human diseases. However, [redacted] We conclude that the use of the ampicillin resistance gene in the development of AAS does not represent an indirect health hazard.

11.1.2 Outcome of Indirect Human Health Hazard Assessment

The current indirect human health hazard assessment has characterized the potential for AAS to cause adverse effects to humans in Canada as compared to wild Atlantic salmon as a consequence of environmental exposure (e.g. recreational swimming and fishing) through dermal and aerosol exposure. We have considered the potential toxin-, allergen- and pathogen-related human health hazards associated with AAS resulting from:
1) the potential expression of a novel gene products coded for by the opAFP-GHc2 insert;
2) potential altered level of expression of an endogenous gene product or toxin; and
3) pleitropic effects (e.g. altered disease susceptibility).

[redacted], it is highly certain that the inserted sequence does not code for any known toxins, allergens or proteins other than the intended growth hormone. We conclude that the indirect human health hazard related to novel toxin or allergen production is negligible with high certainty.

Atlantic salmon is generally considered safe and wholesome for consumption as a food except to individuals who may suffer from fish allergies. We are not aware of any endogenous toxins associated with Atlantic salmon. We conclude that the indirect human health hazard related to altered production of endogenous toxins is negligible with high certainty.

Information is available in the scientific literature related to prevalence, nature and severity of allergic response in humans to occupational dermal and aerosol exposure to endogenous fish allergens. The nature and severity of adverse effects in humans are generally mild and consistently reported in the literature, and do not pose a community-level risk. Data from AquaBounty indicates that diploid AAS have roughly 50% higher allergenic potency than non-transgenic comparators. Despite the uncertainty associated with this data, based on the nature and severity of allergic response to endogenous allergens, we conclude with reasonable certainty that the indirect human health hazard related to altered expression of endogenous allergens is low.

Based on the fact that AquaBounty [redacted] in almost 20 years and the fact that no adverse human health associated with AAS have been reported by AquaBounty staff and visitors in the same timeframe we conclude that the human health hazard related to AAS acting as a vector for new pathogens is negligible with high certainty.

A significant amount of information is also available relating to the etiology, prevalence, nature and severity of adverse effects in humans resulting from topically acquired fish zoonoses. The nature and severity of adverse effects in humans are generally mild and consistently reported in the literature, and do not pose a community-level risk. Thus, despite the limited data provided by AquaBounty related to potential increased capacity to act as a reservoir for human pathogens as compared to wild Atlantic salmon and the uncertainties associated with that data, we conclude with high certainty that the indirect human health hazard related to the ability of AAS to act as vector for human pathogens is low.

In general, knowledge gaps and uncertainties related to human health hazard endpoints include:

- Those outlined in section 11.1.1.2 pertaining to allergenicity;
- A lack of experimental data on AAS (e.g. altered susceptibility to pathogens of human importance) necessitating the extrapolation from the literature;
- Where data was generated, the use of a non-transgenic comparator of domesticated origin was often inappropriate for the purposes of indirect human health hazard. The appropriate comparator would have been wild Atlantic salmon as this is what human would encounter in nature; and
- The phenotype of AAS will be continually evolving in subsequent generations of AAS as St. John River strain broodstock is subject to ongoing selective breeding.

Consequently, phenotypic characteristics related to background genetics of AAS which may have pleitropic effects (e.g. altered disease susceptibility).

Table 11-1 Summary of indirect human health hazards from AAS

Endpoint	Rank	Certainty
Toxin – novel	Negligible	Highly certain
Toxin – endogenous	Negligible	Highly certain
Final – Toxin	**Negligible**	**Highly certain**
Allergen – novel	Negligible	Highly certain
Allergen – endogenous (diploid AAS)	Low	Reasonably certain
Final – Allergen	**Low**	**Reasonably certain**
Vector for human pathogens - new	Negligible	Highly certain
Vector for human pathogens - reservoir	Low	Highly certain
Final - Vector for human Pathogens	**Low**	**Highly certain**

11.2 Environmental Hazard Assessment

The objective of the environmental hazard assessment is to characterize the nature, and severity of potential harmful effects that AAS may cause to the Canadian environment. The potential hazards of the following assessment endpoints are considered: (1) wild populations of Atlantic salmon, (2) prey of Atlantic salmon, (3) predators of Atlantic salmon, (4) competitors of Atlantic salmon, (5) habitat and (6) biodiversity. The potential toxicity, capacity to act as a vector for diseases/parasites and horizontal gene transfer are characterized to determine their potential effects on hazard assessment endpoints. The magnitude of biological consequences of environmental hazards is categorized in accordance with Table 8-2. Elements of uncertainty are elaborated with a final uncertainty ranking assigned in accordance with Table 8-6. Hazard considerations are not limited to all female eyed-eggs triploid AAS hence also includes all life stages and genotypes of the AAS maintained at the PEI facility.

11.2.1 Environmental Hazard Characterization

11.2.1.1 Potential Environmental Toxicity of AAS

We conclude with reasonable uncertainty that there is a negligible toxicological hazard to potential predators resulting from consumption of AAS containing potentially elevated levels of GH and IGF-1 or resulting from gynogenesis, sex reversal and triploidization processe. [redacted]

We previously concluded that no new toxic sequences had been inserted in the genome of the AAS (see section 11.2.1.1). The environmental toxicity hence refers to both endogenous and new substances produced by the AAS compared to wild Atlantic salmon. Toxicological concerns for the AAS are the oral exposure of potential AAS predators to Atlantic salmon and chinook salmon growth hormone, two proteins with long history of safe consumption in the human population and in the environment, making classic acute toxicological studies unnecessary. Finally, although not produced by the AAS, the potential unintended toxicological effects of gynogenesis, sex reversal and triploidization processes are considered.

Although there is solid evidence of an enhanced growth rate for AAS (see section 9.2.7.1), information about GH concentration has not been reported throughout its life cycle. A study reports that the GH levels are all under detection limit (6.24 ng/ml) in the muscle of commercial size AAS (Erisman 2004). Due to difficulty in developing assays, relatively few authors attempted to determine the GH levels in GH-enhanced transgenic fish (Devlin 2011). Nevertheless, studies conducted on AAS-relatives and other GH transgenic salmonids provide sufficient evidence that GH concentration can be significantly elevated in GH transgenic salmonids compared to non-transgenic counterparts. Information about plasma GH levels is available in AAS-relatives fry (n = 5 to 7) in which there was no statistical difference between the plasma GH levels in the transgenic (39.9 ± 14.8 ng/ml), five biggest aged-matched non-transgenic siblings (28.2 ± 8.8 ng/ml) and other non-transgenic siblings (20.5 ± 7.8 ng/ml) (Du et al. 1992). Overall, plasma GH concentrations in GH transgenic salmonids range from 0 to 40-fold higher compared to non-transgenic counterparts (Du et al. 1992; Devlin et al. 1994; Raven et al. 2008, 2012; Higgs et al. 2009; Leggatt et al. 2012) and to reach average levels over 60 ng/ml in an F_1 generation of coho salmon bearing an opAFP-GHc construct compared to less than 5ng/ml in non-transgenic fish (Devlin et al. 2000). Circulating GH concentration varies is response to internal and external stimuli and consequently varies between life stages (Björnsson 1997, 2000, Ebbesson et al 2008). Consequently, and based on the above studies, we conclude that the characterization of GH levels in AAS is insufficient to conclude that GH levels do not increase above normal range for non-transgenic or wild counterparts throughout lifespan, hence we cannot conclude that potential predators consuming AAS in the environment would not be exposed to increased levels of GH compared wild conspecifics. This raises the question of what are the potential effects to predators upon consumption of prey with higher GH content.

The ability for the GH to bind to the growth hormone receptor and induce somatotropic effects are not universal among GH source and recipient treatment organisms among vertebrates (USFDA, 2010, p. 77 and Appendix C). Previous literature suggests that non-primate GH (e.g. salmon GH-1) cannot activate human GHR due to evolutionary divergence in amino acid sequence (Juskevich and Guyer, 1990; Liu *et al.* 2001; Behncken *et al.* 1997; Souza *et al.* 1995). Results from both *in vivo* studies and amino acids sequence comparisons provide evidence that chinook and Atlantic salmon GH would not likely elicit a biological response in higher vertebrates including human, other mammals and birds (USFDA, 2010). Nevertheless, the Atlantic salmon is known to be preyed upon by several fish species, including the Atlantic salmon itself, and GH has been shown to be bioactive across fish species (Duan and Hirano 1991, Moriyama 1993, 1995, Xu et al. 2001, Liu et al. 2012).

Experimental digestibility data of the chinook salmon growth hormone protein was not provided by AquaBounty. We conducted an *in-silico* analysis of the chinook salmon growth hormone protein translated from the inserted sequence reported in the AAS fourth generation (Yaskowiak et al. 2006) using the ExPASY Peptide Cutter tool (http://web.expasy.org/peptide_cutter/) which reported the GH protein to be cleaved by 20 different enzymes, with many cleavage sites per enzymes, including chymotrypsin, pepsin and trypsins which have been reported fish (Dabrowski and Glogowski 1977, Hidalgo et al. 1999, German et al. 2004, Santigosa et al. 2008). Digestive processes are less known in fish compared to mammals but appear to be similar (Hidalgo et al. 1999). The above *in-silico* analysis provides supporting evidence of the digestibility of the chinook salmon growth hormone.

Evidence of gastric uptake of growth hormone in fish includes the detection of human GH in rainbow trout serum 30 minutes after intubation (Habibi et al. 2004) and the increase in plasma GH in Japanese eel one hour post intra-intestinal injection of recombinant eel GH through catheter (Duan and Hirano 1991). Plasma GH levels were increased up to four fold with a dose of 1 µg of recombinant GH per mg of wet pellet, hence providing evidence for its transport as an intact and biologically active hormone circulating in blood (Moriyama 1993, 1995). However, as only a small portion of orally administered hormone reaches the circulation, indicating GH appears to be destroyed in the stomach under acidic conditions and/or digested by proteolytic enzymes (Moriyama et al. 1993). Research has been conducted to develop efficient delivery mechanisms of GH for potential aquaculture applications. Delivery mechanisms include coating GH with gelatin, sodium alginate or hydroxypropylmethyl cellulose phalate or protecting GH in yeast cells (Xu et al. 2001, Kim et al. 2002, Liu et al. 2012). Oral administration of coatedor protected recombinant eel GH and recombinant chinook salmon GH promotes the growth of red sea bream (Xu et al. 2001), oral administration of coated recombinant salmon GH increases plasma GH and promotes growth in rainbow trout (Moriyama et al. 1993), and oral administration of protected recombinant Japanese flounder growth hormone promotes the growth of juvenile Japanese flounders (Liu et al. 2012).

High doses of orally administered unprotected GH can also elicit a biological response in fish (Duan and Hirano 1991, Moriyama 1993, 1995, Xu et al. 2001, Liu et al. 2012). However, the maximum potential concentration of GH in AAS is unlikely to reach high enough concentrations to elicit a biological effect. GH levels are generally higher during early stages of Atlantic salmon development (1 to 20 ng/ml) compared to sexual maturation (2-5 ng/ml) and adulthood (1 ng/ml) (Björnsson et al. 1997). Average plasma GH levels in AAS-relative fry were reported to be 39.9 ± 14.8 ng/ml (Du et al. 1992). Since levels of plasma GH vary with life stages and environmental factors (Björnsson 1997, Ebbersson et al 2008) we concluded that there was no evidence to demonstrate that GH levels could not reach higher levels. Based on other GH transgenic salmonids, the maximum average plasma GH level reported past the G_0 stage (approximately 65 ng/ml) (Devlin et al. 2000) would translate to approximately 3.6 ng GH per gram of total fish[33]. The highest plasma GH concentration ever reported in GH transgenic fish (425 ng/ml) in a founder population (G_0)[34] would translate to approximately 26 ng GH per gram of total fish.We are reasonably certain that the maximum concentration of GH in AAS would not reach hazardous levels for predators as a 2% body weight weekly oral administration of 5,000 ng of unprotected GH per gram of feed does not promote growth of juvenile rainbow trout over 6 weeks (Moriyama et al. 1993), a 6% body weight daily oral administration of 40,000 ng of unprotected GH per gram of diet fails to stimulate growth of red sea bream over 42 days (Xu et al. 2001), and diet-elevated plasma GH and IGF-1 levels decline after cessation of consumption within days (Moriyama 1995). Based on the above, we conclude with reasonable certainty that GH levels in AAS represent a negligible hazard to predators.

No differences were reported for IGF-1in the muscle-skin samples from the commercial sized AAS (ABT 2013). Several studies reported up to 4-fold increases in IGF-1 levels in GH transgenic salmonids compared to non-transgenic controls (Raven et al. 2008; Devlin et al. 2009; Higgs et al. 2009; Leggatt et al. 2012). IGF-1 is reported to be more resistant to gastric digestion than GH-1 (Kimura et al. 1997), however, the oral activity of salmon

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[33] Approximation of the concentration of GH in the total body of fish was based on the average plasma GH levels (65 ng/ml), average weight (241.1 g) (Devlin et al. 2000) and blood volume of coho salmon (6.1% of body volume) (Randall and Wright, 1995).

[34] Note the construct used in Devlin et al. 2000 is opAFPGHc (same as in AAS) where very high GH was observed, whereas the construct used in other GH transgenic coho salmon were OnMTGH1 and OnH3GH1 constructs which generated lower GH levels.

IGF-1 in fish and birds species has not been assessed. Recombinant bovine IGF-1 was concluded to be orally inactive at doses up to 2 mg per kg per day in rats (Juskevich and Guyer, 1990). [redacted] Based on a 20,000-fold difference between the maximum potential daily intake for fish and a no observed effect concentration in rats, we conclude with reasonable uncertainty that potential increased levels of IGF-1 in AAS would not affect potential predators.

No studies examined the relative potential for AAS to accumulate toxicants compared to domesticated or wild conspecifics. However, oxygen consumption rates in the AAS appear to be similar to non-transgenic wild siblings during early life stages (Moreau 2011) and to be up to 25% higher in adult fish (Deitch et al. 2006). Larger differences have been reported in AAS-relative fry, reaching 1.70-fold increase while feeding and 2.30-fold increase after 24 hours starvation compared to non-transgenic controls (Cook et al. 2000b). Considering the positive correlation between waterborne toxicant uptake and oxygen consumption in fish (Rodgers and Beamish 1981, Yang et al. 2000), the reported increased oxygen consumption in AAS could lead to an increased uptake and subsequently to higher bioconcentration factors of waterborne contaminants in AAS compared to wild conspecifics. We conclude with reasonable certainty that increased oxygen consumption could increase bioconcentration of waterborne contaminants in AAS. However, it is not possible to conclude on the magnitude of the hazard which would depend on the status of the predator population as well as on the mode of action, effect and concentration of the contaminants in the natural environment. It is also not

[35] Approximation of the concentration of IGF-1 in the total body of fish based on IGF-1 plasma levels of 27 ng/ml in a group of fish having a 55 g average weight (Raven et al. 2008). Final approximate concentration was calculated assuming the blood volume of coho salmon to be 6.1% of body volume (Randall and Wright, 1995).

possible to conclude on the relative importance of this accumulation compared to the potential accumulation of organic toxicants in domesticated Atlantic salmon compared to wild conspecifics and/or to the potential accumulation of heavy metals in wild Atlantic salmon (reviewed in ABT 2013).

No toxicological concerns are associated with the gynogenesis and triploidization processes used in the production of AAS. Sex-reversal through 17α-methyltestosterone exposure increases whole body levels of methyltestosterone in treated fish, which could potentially impact predator fish if consumed in significant quantities. However, experiments in other fish models demonstrate that increase in 17α-methyltestosterone in treated fish is transient and exogenous methyltestosterone is removed by 10 days post treatment (Cravedi et al. 1989, Fagerlund and Dye 1979, Johnstone et al. 1983, Curtis et al. 1991). As such, any potential hazards to predators of escaped treated fish would be over an extremely limited time frame. We therefore conclude with reasonable certainty in negligible hazard associated with the use of 17-methyl-testosterone to produce sex reversed males in the production of AAS.

A preliminary whole food toxicological assessment of the effects of growth enhanced transgenic coho salmon on rodent development was conducted by Health Canada (Curran et al. 2007). However, the experimental design precluded attribution of observed effects to the transgene and we therefore concluded not to consider this study in our toxicological assessment of the ASS.

Based on the above considerations, we conclude with reasonable certainty that consumption of AAS with potentially increased levels of GH would present a negligible hazard for predators. We also conclude with reasonable uncertainty that consumption of AAS with potentially increased IGF-1 would present a negligible hazard for predators. We also conclude there is a negligible hazard from gynogenesis, sex reversal and triploidization processes used to produce the AAS. We are reasonably certain that an increase in bioconcentration of waterborne contaminants could result in AAS, but cannot conclude on the magnitude of this hazard.

11.2.1.2 Potential to Act as a Vector for Native or Introduced Pathogens

Based on long-term, historical data on the lack of occurrence of reportable fish diseases at the AquaBounty PEI facility, it is reasonably certain that AAS, if there were to escape, will not act as a vector for the introduction of new fish pathogens. In addition, given the lack of data on pathogen and other uncertainties, we are unable to conclude whether AAS would have an increased capacity compared to wild Atlantic salmon to act as a reservoir for the transmission of pathogens including those that may affect wild Atlantic salmon as well as predators, prey and competitors of Atlantic salmon.

AAS may act as a vector for pathogens either by direct introduction into the environment of pathogens associated with escaped AAS from the PEI facility or by acting as a reservoir in the environment for diseases of significance to wildlife including other fishes. Altered resistance to pathogens is known to occur in GH transgenic coho salmon (Jhingan et al. 2003). Increased disease resistance coupled with enhanced fitness may heighten the capacity of transgenics to act as a reservoir for the transmission of disease agents to other organisms (Jhingan et al. 2003). However, if AAS were to have increased disease susceptibility but succumb to the disease quickly then AAS may actually be less likely to act as a reservoir for the transmission of diseases than domesticated or wild Atlantic salmon in the natural environment.

[redacted], however we do not know the relative disease susceptibility of AAS compared to wild Atlantic salmon. In addition we do not know to what extent disease resistance has been selected for in the St. John River stock to which AAS neomales are crossed. There is strong evidence that selectively breeding Atlantic salmon for disease resistance can be highly successful (Kjoglum et al. 2008). In addition, it is unlikely that the disease susceptibility of AAS will remain constant with subsequent generations as AAS will continue to be crossed with the St. John River strain which is itself subject to selective breeding.

[redacted] or other diseases is further complicated as pathogen susceptibility may vary depending on life stage, ploidy, pathogen dose, fish species, background genetics, the pathogen in question as well as other environmental factors that influence overall health and fitness (Jhingan et al., 2003, Sundström et al., 2007). Kim et al. 2013 observed higher susceptibility in two year classes of growth hormone transgenic coho salmon (*Oncorhynchus kisutch*) challenged with *A. salmonicida* as compared to wild-type. Similarly, Jhingan et al. 2003 reported that growth hormone transgenic diploid coho salmon smolts displayed higher cumulative mortality when exposed to *Vibrio anguillarum* than did non-transgenic smolts. However, diploid transgenic and non-transgenic coho fry were roughly equally susceptible to high doses of *V. anguillarum* but the transgenic triploids were more susceptible than non-transgenic triploids. In contrast, at a lower pathogen dose, transgenic diploid and triploid coho salmon fry were less susceptible than their non-transgenic counterparts. The foregoing suggests complex interactions of ploidy, transgenesis, and pathogen dose on disease susceptibility.

[redacted]. However, because there is no data on the relative susceptibility of AAS compared to wild Atlantic salmon [redacted], we are unable to conclude on whether AAS is likely to be more or less susceptible to these disease agents than wild Atlantic salmon. In addition, we have no data on the relative susceptibility of AAS to other disease agents of environmental significance. To add further uncertainty, disease resistance may continue to be altered in subsequent generations of AAS as a consequence of ongoing crossing to St. John River stock that is subject to continued selective breeding, perhaps also for disease resistance and performance.

Several studies report triploid salmonids, including GH transgenic coho salmon, to have increased susceptibility and/or decreased resistance to a number of infectious organisms (Parsons et al. 1986, Yamamoto and Iida 1994, Ojolick et al. 1995, Cotter et al. 2002, Jhingan et al. 2003, Ozerov et al. 2010), although others do not (e.g. Yamamoto and Iida 1995). As such, AAS, particularly 3N AAS, may have increased disease susceptibility in some circumstances. However, what impact this may have, if any, on vector capability of AAS has not been examined. The disease resistance and vector capability of gynogenetic and sex-reversed fish has not been examined.

Thus, while we have some data to indicate that AAS is more susceptible than domesticated Atlantic salmon [redacted], it is highly uncertain how this may translate to other disease agents. In addition, as indicated in Section 10.4.4 it is possible that diploid AAS could become established in the waters surrounding PEI but this is reasonably uncertain. Thus, given the uncertainty elaborated above we are unable to conclude whether AAS would have an increased capacity compared to wild Atlantic salmon to act as a reservoir for the transmission of disease agents to wildlife including those that may affect wild Atlantic salmon as well as predators, prey and competitors of Atlantic salmon.

[redacted] It is therefore reasonably certain that AAS would not introduce new pathogens into the surrounding area in the event that they were to escape from the PEI facility.

[redacted]

[redacted]

Land-based aquaculture provides opportunity to implement specific management practices and to monitor and manage fish disease and monitor the transmission of pathogens. The absence of disease outbreaks in fish, [redacted], provides a good indication that disease risk at the AquaBounty PEI facility is well managed. Consequently, AAS would be very unlikely to carry any new pathogens if they were to escape.

11.2.1.3 Potential Horizontal Gene Transfer

Horizontal gene transfer between higher eukaryotes is considered to be rare and usually involves mobile genetic element. Given that no characteristics of the transgene suggest potential changes in mobility, the potential for horizontal gene transfer (HGT) of the EO-1α transgene is expected to be similar to that of naturally occurring HGT in Atlantic salmon. Were HGT to occur, it would most likely be to prokaryotes. Although possible, this is not considered a concern since the GH gene is naturally occurring and the transgene integrant does not contain any sequences that confer toxicity or pathogenicity. In addition, the notifier demonstrated the absence of a complete ampicillin resistance gene in AAS genome.

Horizontal gene transfer (HGT) is the non-sexual exchange of genetic material between organisms of the same or different species (DFO 2006). Horizontal gene transfer is a rare event, often measured on an evolutionary time frame, and is more frequent among prokaryotes than eukaryotes (see ESFA 2013). Genetic analyses suggests HGT events may have taken place repeatedly in vertebrate evolution, including in fish (e.g. Uh et al. 2006; Thomas et al. 2010; Kuraku et al. 2012), although definitive evidence of HGT from eukaryotes to either eukaryotes or prokaryotes is currently lacking. DFO (2006) recommended the transfer potential and selective advantages of HGT be evaluated on a case-by-case basis in novel organisms. In order for HGT of a specified transgene to take place on a biologically relevant scale, the following steps must occur: *Exposure* and *Uptake* of the free transgene to a novel organism, *Stability* and *Expression* of the gene within the novel organism, and neutral or positive *Selection* of the novel organism expressing the transferred gene (see DFO 2006). In general, the EO-1α transgene is expected to have similar (i.e. highly unlikely) probability of HGT to a new organism as native Atlantic salmon genes. Were HST to occur, it would most likely be to prokaryotic organisms, and consequently the following examination of the potential HGT pathway of EO-1α focuses on HGT to prokaryotes.

Exposure: The transgene in free DNA form must be available to a novel organism. DNA released from an organism is rapidly degraded in most environments, although can persist for weeks or longer (see DFO 2006). Persistence of DNA is more likely in sediments or soil than the water column, and can be influenced by many factors including temperature, substrate composition, etc. (see DFO 2006). [redacted] (see DFO 2012), [redacted]. Bacteria could also be exposed to free DNA containing the EO-1α within the AAS fish's gut, or through feces, mucus and other waste sloughed off by the fish into the water. However, these pathways of exposure are not expected to differ from that of native Atlantic salmon genes.

Uptake: A novel organism must take up the DNA intact. Prokaryotes are more competent than eukaryotes at uptake, and some bacteria are more competent than others. EFSA (2013) suggested that increased transfer mobility of transgenes above that of host genes should be the main focus when determining potential for HGT. The EO-1α transgene does not contain viral vectors, transposable elements (ABT 2013) or other known factors that may increase the potential for DNA uptake/mobility to a new organism. DFO (2006) listed the transgene type of EO-1α as being third least likely to have increased mobility of nine different classifications of transgenes. As such, the EO-1α gene is not expected to have increased uptake relative to native Atlantic salmon genes.

Stability: The DNA must be stable in the new host. DFO (2006) identified stability as the most significant barrier to HGT by natural transformation as there is often a lack of homology between the transgene and bacteria recipient DNA. The EO-1α transgene is constructed of fish sequences or partial non-sense artificial cloning vector sequences (ABT 2012) that do not share homology to any known bacterial sequences. Consequently EO-1α is expected to have similar stability to native Atlantic salmon genes.

Expression: In order for the transgene to be expressed resulting in phenotypic change, it requires co-transfer of regulatory elements. The EO-1α transgene would have an increased probability of expression once HGT takes place, as the close proximity of the ocean pout antifreeze promoter to the GH gene could increase the likelihood of them being co-transferred. However, vertebrate promoters commonly used in transgenesis have low activity in prokaryotic hosts (see DFO 2006), although this has not been directly addressed for AFP promoter.

Selection: Neutral or positive selection for the organism with the novel phenotype is necessary for the transferred gene to result in biological changes in a population. Should all previous steps occur, it is unknown whether the EO-1α gene could confer a selective advantage to any new organisms it is transferred to. While close proximity of the promoter and GH gene could increase potential for expression of the EO-1α post-transfer, the lack of mobile elements and lack of homology between EO-1α and bacterial sequences indicate HGT of the EO-1α gene to be highly unlikely.

11.2.1.4 Potential for AAS to Affect Wild Populations of Atlantic salmon

The potential hazards of AAS to wild populations of Atlantic salmon are concluded to be high with reasonable uncertainty. Highest hazards are expected from competition or through potential genetic introgression of fertile broodstock with wild populations. Magnitude of the hazard is enhanced at low population sizes of wild Atlantic salmon. The reasonable degree of uncertainty is attributable to the lack of information on phenotypic characteristics of AAS in the natural environment, ecological interactions of AAS life stages and the reproductive capacity of AAS.

The hazard assessment of the AAS includes hazards associated with domesticated fish and considers the effects of the environment

The potential hazard of AAS to wild populations of Atlantic salmon is related to the relative fitness of the two genotypes in nature (Devlin 2011). Relevant phenotypes to consider include competitive, predatory, reproductive and migratory behaviours, fecundity, potential to act as a vector for pathogens/parasites and ability of the AAS to reproduce with wild Atlantic salmon. Factors to consider related to the assessment of the hazards of AAS on wild populations of Atlantic salmon include that (1) AAS is bred with the domesticated SJR strain of Atlantic salmon (ABT 2013) hence the consideration of hazard concerns related to effects of escaped farmed salmon on wild populations; (2) the same fitness traits that are to be compared between AAS and wild conspecifics are also affected by rearing and experimental conditions (Sundström et al. 2007; Devlin 2011) hence the importance of considering genotype-by-environment (G x E) effects, and (3) although the species specific results from experiments conducted using other GH transgenic fish species do not directly apply to the environmental risk assessment of the AAS, several of their conclusions do. Research on GH transgenic salmonids provides evidence that resource levels, background genetics, early rearing conditions, life stages and predation levels have critical effects on the ecological consequences of transgenic fish in the environment (Devlin et al. 2004; Sundström et al. 2009, 2011).

Wild populations of Atlantic salmon are in a state of deterioration:

Of the 16 designatable units (DUs) of Atlantic salmon in Canadian waters, 11 have COSEWIC status demonstrating the deteriorating state of wild populations (COSEWIC 2010). At proximity to the PEI facility are the Gaspé-Southern Gulf of St. Lawrence DU of special concern status, the endangered Anticosti Island and Eastern Cape Breton DUs and the threatened South Coast Newfoundland DU (COSEWIC 2010). The biological characteristics of Atlantic salmon specifically on PEI have been modified by intensive stocking since 1880. As with original PEI salmon populations, small rivers are dominated by fall runs of large (over 63 cm fork length) fish, while large rivers, where stocking has been intense, are now dominated by early-run of small (less than 63 cm) salmon (Cairns et al. 2010). Threats to salmon populations in PEI include stream sedimentation, physical blockages (created by beaver dams, artificial impoundments, and improperly installed culverts), pesticides, and competition with rainbow trout. Populations in many rivers are very small and face the likelihood of extirpation if current trends continue (Cairns et al. 2010). Average ages at smoltification, sex ratios, and sizes of Atlantic salmon have been characterized for several rivers of eastern Canada (Chaput et al. 2006). Mean age at smoltification in the Gaspé-Southern Gulf of St. Lawrence DU, the closest to the PEI facility are 2 to 3 years old. Large salmon are mainly females, as in all sampled rivers in eastern Canada, while small salmon being mainly males (less than 25% females) . Mean fork length ranges from 54 to 58 cm and 70 to 90 cm for small and large salmon respectively.

Escaped domesticated fish can affect wild populations of Atlantic salmon:
Transgenic fish, being domesticated fish, are expected to result in at least the same genetic effects on wild conspecifics as non-transgenic domesticated fish (Ferguson et al. 2007). The effects of domestication are especially relevant to GH transgenic fish as domestication and GH transgenesis modify similar genetic pathways including genes involved in growth (Devlin et al. 2009). Nevertheless, additional hazards in the transgenic GH enhanced fish may be expected compared to domesticated fish as the number of genes and magnitude of effects are greater in transgenic than in domesticated animals (Devlin et al. 2009). For that reason we also consider the incremental hazards of AAS compared to domesticated fish to capture the overall potential hazard of AAS compared to wild conspecifics (DFO 2012).

The potential impacts of escaped farmed salmon on wild populations of Atlantic salmon have been addressed and extensively reviewed in the literature (Jonsson 1997; Fleming et al. 2000; Ferguson et al. 2007; Leggatt et al. 2010; Cote et al. 2013). The degree of impact of farmed salmon is dependent on several factors including the scale and frequency of the escapes, the status of the native wild population and the fate and relative fitness of the escapes compared to wild conspecifics in the natural environment (reviewed in Cote et al. 2013). The fitness of the escapes also depends on several factors such as the stage of release, level of domestication, area and season of release, and presence of competitors and predators (reviewed in Cote et al. 2013). In general, there is consensus that Atlantic salmon escapees have poor survival, poor foraging, irregular migration behaviour, and poor reproductive capacity relative to wild conspecifics (Ferguson et al. 2007; Leggatt et al. 2010). Despite reduced fitness, farmed Atlantic salmon decrease productivity of wild juveniles, reduce effective population size of wild populations and have direct genetic effects through successful reproduction with wild conspecifics leading to interbreeding, transfer of cultured phenotypes to wild population, backcrossing with subsequent generations and ultimately a reduction in the adaptive potential of the species (Ferguson et al. 2007; Leggatt et al. 2010).

Growth rate of AAS in the natural environment will depend on several factors:
In Atlantic salmon, body size is the phenotype most related to overall fitness, being positively correlated with freshwater and marine survival, fecundity, egg size, reproductive success and offspring survival (Garcia de Leaniz et al. 2007). Although there is sufficient evidence of an enhanced growth rate phenotype for the AAS under hatchery conditions, there is also evidence of changes in the magnitude of the growth-enhanced phenotype of AAS under different food abundance and environmental conditions (Oakes et al. 2007; ABT 2013; Oke et al. 2013). Reduction or prevention of increased growth rates in naturalized environments compared to hatchery conditions have also reported in GH transgenic coho salmon (Devlin et al. 2004; Eales et al. 2004; Tymchuk et al. 2005; Sundström et al. 2004, 2005, 2007, 2009, in preparation a; Sundström and Devlin 2011). Numerous factors in artificial streams have been reported to decrease growth rates of transgenic coho salmon to equal to or lower than that of non-transgenic conspecifics. These include low food level (Sundström et al. 2004, 2005; Sundström and Devlin 2011), presence of predators (Sundström et al. 2004), early arrival of predators (Sundström et al. 2005), presence of resident competitors (Sundström et al. in preparation a), prior culture in the hatchery (Sundström in preparation a), and increased complexity of habitat (Sundström et al. in preparation a). In addition, strong

genotype by environmental interactions have been noted in numerous experiments, where transgenic and non-transgenic fish differ in their response in growth to different environmental factors (e.g. Devlin et al. 2004; Tymchuk et al. 2005; Sundström et al. 2004, 2007, in preparation a). Overall, high growth of AAS juveniles observed under hatchery conditions is expected to be diminished, prevented, or reversed in natural environments, depending on numerous environmental and biological factors.

Although the natural environment is generally assumed to provide limited and stochastic food abundance (see Moreau 2011), we cannot conclude that high food abundance can never been encounter in the natural environment. The effect of environmental conditions on growth rates of adult GH transgenic salmon has not been examined past the juvenile stage and proximity to hatchery outlets or to open net pens could provide additional constant food resources (Carss 1990) in the natural environment benefiting marine life stages of AAS, although the magnitude of the effect on the growth rate and size are unknown. Taken together, the above suggest the ability to predict whether AAS fish may maintain high growth phenotype in natural environments to be highly problematic, although current studies suggest accelerated growth may be limited in many circumstances especially in juvenile stages.

AAS have potential to reproduce with wild Atlantic salmon, although at a reduced rate presenting a remote but existing potential for genetic introgression:

Evidence of the successful participation of the AAS in natural spawning events in presence of wild conspecifics provides solid evidence for the potential introgression of the domesticated genetic background and transgene from AAS into subsequent generations of wild populations. GH transgenic coho salmon have also been demonstrated to successfully spawn with hatchery reared wild conspecifics under simulated natural environment resulting in viable offspring (Bessey et al. 2004; Fitzpatrick et al. 2011). However, due to reduced reproductive breeding performance, GH transgenic salmonids sire very low percentages of the offspring (Fitzpatrick et al. 2011; Moreau et al. 2011a). Reduced success in the competitive behaviour of AAS has been demonstrated to account for its reduced overall breeding performance (Moreau et al.

2011a). The postovulatory competitive ability of the AAS has not been assessed; however reduced sperm quality was demonstrated in GH transgenic coho salmon relative to wild conspecifics, contributing to its overall reduced breeding performance (Fitzpatrick et al. 2011). Alternative male phenotypes contribute to the overall reproductive fitness of Atlantic salmon. Individual parr fertilize up to 44% of the eggs in a redd (Hutchings and Myers, 1988; Richard et al. 2013), thereby increasing their probability of reproduction and gene transfer to subsequent generations (Hutchings and Myers 1994; Moreau and Fleming 2012). The reduced occurrence of sexually mature AAS parr (Moreau et al. 2011a) could result in a diminished chance to contribute to the gene pool at an early stage compared to wild conspecifics. However, AAS, and other GH transgenic salmonids, have also been reported to have accelerated smoltification and adult maturation (Devlin et al. 1995, 2000, 2004; Moreau et al. 2011a; Moreau and Fleming, 2012; ABT 2013). Such phenotypes could, under nutrient rich conditions, shorten the life-cycle of AAS allowing reaching the behaviourally dominant strategy of anadromous males faster than wild conspecifics hence providing a potential reproductive advantage to AAS. Successful reproduction of anadromous AAS would also depend on its survival rates at sea which has not been examined nor has the migratory behaviour of AAS. However studies conducted on GH transgenic coho salmon concluded that early rearing conditions have a stronger effect on the migratory behaviour of coho salmon than the GH transgene with earlier timing of migration onset when reared in hatchery conditions but not when reared in stream conditions (Sundström et al. 2010). The overall reproductive performance also depends on the survival rates of the offspring. Results that require cautious interpretation suggest that genotype does not influence the offspring survival and growth at the onset of exogenous feeding (Moreau et al. 2011a). However, offspring survival was reported to be lower in GH transgenic fry coho salmon compared to non-transgenic controls in tanks (Bessey et al. 2004) and to be lower or similar under naturalized stream conditions (Sundström et al. 2005, 2010).

Based on the above studies, it is difficult to predict the overall reproductive fitness of fertile AAS in the natural environment. Variations relative to wild conspecifics in several phenotypes such as reproductive behaviour, reproductive physiology, life stages, survival rates at sea and survival of offspring all contribute to the overall fitness of AAS. In addition, complicating factors such as the effects of genetic background and rearing conditions as well as knowledge gaps about the reproductive fitness of female AAS make predictions even more difficult. However, reports of successful reproduction of escaped farmed Atlantic salmon with wild conspecifics in the environment (Jonsson et al. 1997), despite their general lower reproductive success, provides evidence that AAS could also reproduce in the natural environment, despite its reported reduced breeding performance. The combination of the effects of domestication and transgenesis on reproductive success have not been assessed in AAS, nevertheless due to important knowledge gaps we cannot conclude that sexually mature life stages of AAS would not reproduce in the natural environment. We therefore conclude with reasonable uncertainty that sexually mature AAS could reproduce in the natural environment but they would likely have reduced reproductive success compared to wild conspecifics. Introgression of the domesticated genetic background of AAS and of the GH transgene into wild populations of AAS is therefore possible. The transfer of domesticated phenotypes to subsequent generations is expected to reduce the adaptive potential of wild populations (see Leggatt et al. 2010). The effects of the transfer of the GH transgene on the phenotypes of any subsequent generations of AAS remain unknown although are expected to differ from phenotypes expressed under hatchery conditions and to depend on available resources and other environmental and biological factors (Bessey et al. 2004, Sundström et al. 2007, 2009).

Under high food environments AAS could cause a moderate to high genetic hazard to wild Atlantic salmon with reasonable uncertainty:

Based on experiments conducted under high feed conditions, if AAS escaped into an environment in which food abundance was not limited, AAS could be expected to consume more prey, have a higher feed conversion, higher metabolism and to grow faster than non-transgenic conspecifics (Deitch et al. 2006; Levesque et al. 2008; Moreau and Fleming 2013; ABT 2013). High food environments would likely result in increased survival of AAS, potentially increasing the number of AAS reaching spawning age. Combined with the potential, albeit reduced ability, of AAS to spawn with wild Atlantic salmon, AAS could cause genetic hazards to wild Atlantic populations through transgene introgression in high food environments. Phenotypes of subsequent generations of AAS in the natural environment can be expected to differ from the founder generation due to phenotypic plasticity and impact of early rearing conditions. However, no evidence suggests that the transgene would provide a fitness advantage to the early life stages of the offspring as it does not significantly affect territorial dominance, time for hatching, and reduces sizes near emergence (Moreau 2011; Moreau et al. 2011b). Studies in GH coho salmon find transgenic fish are more similar in phenotype to non-transgenic fish when reared under naturalized conditions from early stages (Sundström et al. 2007; Sundström et al. 2009; Sundström et al. 2010). As such, it is reasonable to expect phenotypes of subsequent generations containing the EO-1α to be more similar to wild phenotypes. Based on the available information, we conclude with reasonable uncertainty that in a normal food abundant environment, AAS and wild populations of Atlantic salmon could cohabit (Devlin et al. 2004) and a low incidence of gene flow into the wild Atlantic salmon populations of the transgene could occur. It is difficult to determine if the transgene would be purged from the wild population, and if so, over how many generations.

Due to potential introgression of the transgene, and consequently of the associated domesticated genetic background, in wild populations, we conclude that under high food environments, AAS could cause a moderate to high hazard with reasonable uncertainty. The size of the wild population compared to the size of the invading AAS population is expected to influence the magnitude of this hazard, with small, or threatened and endangered, wild populations being more at-risk than large healthy wild populations.

Under low food environments AAS could cause moderate to high competitive hazards to wild Atlantic salmon with reasonable uncertainty:

Less information is available to predict the phenotype and competition ability of AAS under low food conditions. Increased feeding motivation and appetite of AAS and AAS-relatives under hatchery conditions (Abrahams and Sutterlin, 1999, ABT 2013) might translate into increased foraging, and consequent competitive behaviour, in food limited natural environments (Devlin 2011), but no studies have been conducted to confirm the feeding motivation of AAS under low food abundance. As growth hormone is known to stimulate appetite (Björnsson 1997; Lõhmus et al. 2008), one could reasonably expect that under the control of the anti-freeze protein promoter, which is detected year round with increased levels in the winter in the ocean pout (Fletcher et al. 1985), AAS would have increased appetite all year round compared to its wild conspecifics and could thereby compete for food resources in the natural environment (Tymchuk et al. 2005). However, despite an increased appetite, AAS would not be expected to have an increased growth rate under low food conditions due to limitation of food resources and potential increased mortality due to increased tolerance for predation risk (Abrahams and Sutterlin, 1999).

Survival and growth of first-feeding AAS in food limited naturalized streams was reported not to be affected by transgenesis (Moreau et al. 2011), but these results are difficult to interpret due to the loss of weight of all fish, including controls, during the experiment. Overall, information about the phenotypes and potential pleiotropic effects of AAS under low food resources is limited and does not include any evidence for a potential increased fitness of the AAS as compared to non-transgenic fish.

In stream environments with limited food, the survival of AAS first-feeding fry did not differ from that of non-transgenic siblings over 37 days (Moreau et al. 2011b). However, it should be noted that AAS development data suggest a delayed phenotypic response of the transgene (Moreau 2011). In contrast, interactions of GH transgenic coho salmon and wild conspecifics fry in tanks resulted in population collapse under low food conditions within three months while populations without transgenic fish maintained survival and biomass over the same time period (Devlin et al. 2004). Populations including newly emerged transgenic and non-transgenic coho salmon in presence of natural predators did not collapse as in the previous study under naturalized conditions providing unpredictable low food abundance and potential to hide and escape from predators (Sundström and Devlin 2011). Nevertheless, the fate of the populations over a longer time period than two months remains undetermined but suggests potential collapse based on the low survival rates. We therefore conclude with reasonable uncertainty AAS could significantly reduce the size of wild populations of Atlantic salmon under low food abundance through ecological interactions.

Potential for AAS to affect wild populations of Atlantic salmon through a Trojan gene effect cannot be eliminated representing high hazard to wild populations with high uncertainty:
Models examining introgression of a transgene into a wild population suggest that increased reproductive success combined with decreased viability of offspring of transgenic organisms can cause a Trojan gene effect, crashing the wild population (Muir and Howard 1999). Potential Atlantic salmon population extinction through the Trojan gene effect is unlikely as AAS and other GH transgenic salmon have reduced breeding performance and that transgenesis does not appear to have a considerable effect on the fitness at first-feeding stages (Moreau 2011, Moreau et al. 2011a). However, significant gaps in knowledge remain about the reproductive capacity of female AAS and naturally reared AAS and studies examining post first-feeding stage survival of GH transgenic coho salmon reported both similar and reduced survival relative to wild conspecifics under naturalized stream conditions (Sundström et al. 2005, 2010). Based on the above considerations, we conclude with reasonable uncertainty that it would be imprudent to claim that conditions for a potential Trojan gene effect scenario could never be meet in the natural environment.

Gynogenetic and/or sex-reversed AAS broodstock are expected to have similar or decreased genetic hazards to wild Atlantic populations in most circumstances, and are expected to have equal competitive hazards:
The potential genetic hazards of monosex broodstock populations through gynogenesis without sex-reversal have not been examined. Quillet (1984) reported gynogenetic fish had decreased absolute fecundity and delayed maturation, indicating gynogenesis may decrease potential genetic hazards of AAS broodstock to wild populations. Models examining the release of sex-reversed fish for stocking found release of such fish could theoretically exterminate the sex determination system of a wild population (Kanaiwa and Harada 2002, 2008). However, these models assume sex-reversed individuals have

normal reproductive success. While the reproductive success of sex-reversed fish has not been directly addressed, sex-reversed salmon have poor gonad development (e.g. Johnstone and MacLachlan 1994, see Pandian and Koteeswaran 1998). As such, the potential for genetic hazards to wild populations through reproductive interactions with sex-reversed fish may be limited. These techniques are also not expected to increase or result in new competitive hazards to wild populations.

Overall, gynogenesis and sex-reversal are expected to decrease or have no effect on genetic and competitive hazards to wild Atlantic salmon populations in most circumstances.

Triploid AAS are expected to have reduced hazards compared to fertile broodstock:

Triploidy is expected to greatly decrease or eliminate genetic hazards to wild populations of Atlantic salmon. As triploid fish are functionally sterile (Benfey 1999), there are no associated hazards of genetic contamination of wild populations due to reproduction with AAS. Consequently, all-female production combined with triploidy is expected to decrease or eliminate genetic hazards of notified sponsor product.

Triploidy is expected to decrease or have no effect on hazards to wild populations of Atlantic salmon through competition in most circumstances. While the potential effects of triploidy on competitive ability in AAS have not been assessed, triploid AAS grow at a slower rate than diploid AAS (Buchanan and Runghan 2009, Plouffe et al. 2013), indicating decreased overall performance. Studies examining triploidy in other salmonid models demonstrate equal or lower competitive hazards relative to diploid counterparts. In laboratory experiments triploid fish have been found to have lower or equal aggressive behaviour and food consumption relative to diploid fish (see Fraser et al. 2012). O'Keefe and Benfey (1997), found one strain of triploid brook trout had lower competitive ability than diploid fish, but triploid Atlantic salmon and two other strains of brook trout did not. Kozfkay et al. (2006) found stocked triploid trout had decreased survival in systems with low productivity, indicating triploid fish competed poorly for limiting resources. However, triploid adult female salmon could theoretically pose increased competitive hazards in some circumstances. As triploid female fish do not have decreased growth and increased mortality associated with spawning (Chatterji et al. 2008, Sumpter et al. 1991; Sheehan et al. 1999; Teuscher et al. 2003; Poontawee et al. 2007), they could theoretically obtain a larger size than diploid counterparts, potentially becoming better competitors. There is an anecdotal report of triploid female rainbow trout obtaining unusually large sizes after escape from an aquaculture facility (e.g. www.trophytroutguide.com/articles/diefenbaker.htm), but this has not been reported in other aquaculture, stocking, or laboratory programs. As such, the potential for triploid female salmon to reach large size, and their competitive ability is not known.

In summary, we are reasonably uncertain that triploid female adult AAS could pose increased competitive hazards to wild Atlantic salmon in some circumstances. However, triploidy is expected to decrease or have no effect of competitive hazards during other life stages, and decrease or prevent genetic hazards to wild Atlantic salmon with reasonable uncertainty.

It is reasonably uncertain that AAS will not carry more diseases than domesticated Atlantic salmon:

As reviewed under section 11.2.1.2, it is reasonably certain that AAS would not act as a vector for the introduction of new fish pathogens in the natural environment. However, we are unable to conclude whether, relative to wild Atlantic salmon, AAS would have an increased capacity to act as a reservoir for the transmission of pathogens, including those that may affect wild populations of Atlantic salmon.

Overall, AAS are expected to pose low to high competitive and genetic hazards to wild populations with high uncertainty:

Most ecological studies about potential effects of GH transgenic salmonids have been conducted on juvenile stages hence an existing gap in the knowledge of potential impacts of the adult life stage AAS and during life at sea. In addition, predictions about the overall impact of AAS on wild populations of Atlantic salmon are complicated by the effects of food resource levels, background genetics, early rearing conditions, life stages and predation levels. Nevertheless, based on the current status of Atlantic salmon populations in Canada, and on above studies, we conclude with high uncertainty that AAS could pose moderate to high hazards on wild populations of Atlantic salmon. Highest hazards are expected from potential introgression of fertile broodstock with wild populations, or through competition under food limiting environments. Triploid AAS are expected to have reduced hazards compared to fertile broodstock.

11.2.1.5 Potential for AAS to Affect the Prey of Wild Atlantic salmon

The potential hazards of AAS to prey of wild Atlantic salmon are concluded to be moderate with high uncertainty. In the natural environment, AAS are expected to have similar or increased feeding motivation. However, it is not possible to predict the fitness or the numbers of AAS in the natural environment, making it difficult to foresee the magnitude of potential pressure on prey. The high degree of uncertainty is attributable to the lack of information on AAS feeding behavior and ability to avoid predators in the natural environment.

The impact of AAS on prey of wild Atlantic salmon will depend on the feeding motivation of AAS in the environment and the ability of AAS to escape predation during foraging. The magnitude of the impact will depend on the prey resources available in the environment which will partially determine their growth rate. Other relevant phenotypes include the maximum attainable size of AAS and its capacity to act as a vector for diseases in nature.

There has been no study conducted to determine the potential foraging behaviour of AAS in natural environments in the presence or absence of predators. However, behaviours of AAS in hatchery conditions, as well as behaviour of other GH transgenic salmonids under experimental conditions, indicate they have potential for increased feeding motivation in natural environments. AAS have increased feeding motivation and appetite under hatchery conditions (ABT 2013), which might translate into increased foraging behaviour in the natural environment (Devlin 2011). Greatly increased feeding motivation was demonstrated by AAS-relatives compared to control fish, both in the presence or absence of predators (Abrahams and Sutterlin, 1999). In addition, as AAS-relatives maintain high metabolic rates over at least 24 hours of starvation (Cook et al. 2000c), AAS could be expected, with reasonable certainty, to have increased feeding motivation. GH transgenic coho salmon attacked prey more often and more rapidly in aquarium conditions compared to non-transgenic controls (Sundström et al. 2004), although preliminary results suggest that feeding and risk taking by GH transgenic coho salmon are more related to environmental food resources and presence of predation than to genotype (Sundström et al. in preparation b). Growth hormone is known to stimulate appetite and to be reduced during winter (Björnsson 1997; Lõhmus et al. 2008). Under the control of the promoter for the anti-freeze protein, which is detected year round with increased levels in the winter (Fletcher et al. 1985), AAS would be expected to have increased appetite all year round compared to its wild conspecifics. However, decreased food intake in concert with winter temperatures have been observed in AAS (Darek Moreau 2013, personal communication) as opposed to GH transgenic coho salmon which, in contrast also to wild salmon, do not reduce their food intake during the winter (Lõhmus et al. 2008). Finally, even if GH transgenic fish can eventually become satiated, they return to active feeding more rapidly than non-transgenic fish even when their guts are filled (reviewed in Devlin 2011). Overall, based on the reported information in AAS, AAS-relatives and other growth enhanced transgenic salmonids; we conclude with reasonable uncertainty that the AAS would have similar or increased feeding motivation compared to wild conspecifics in the natural environment, hence similar or increasing pressure on potential prey.

Increased feeding motivation in the natural environment could be expected to translate either into increased growth rates for AAS, hence increased pressure on prey; or into higher mortality rates for AAS during foraging activities, hence alleviating predatory pressure on prey. As previously reviewed under section about the potential for AAS to affect wild populations of Atlantic salmon (see section 11.2.1.4), predicting whether AAS fish may maintain high growth phenotype in natural environments is highly problematic, although current studies suggest accelerated growth may be limited in many circumstances. Nevertheless, as reviewed under section11.2.1.4, we cannot conclude that the natural environment would never provide sufficient amount of food to sustain a growth enhanced phenotype. Although some studies report behaviours that may suggest higher mortality during feeding in presence of predators for AAS-relatives and GH transgenic coho salmon (Abrahams and Sutterlin, 1999, Sundström et al. 2003; Sundström et al. 2004), studies assessing mortality due to predation provide inconsistent results. Increased mortality due to predation was reported in GH transgenic fry coho salmon in absence of safe habitat (Sundström et al. 2004) but not when transgenic and non-transgenic fry and parr coho salmon, that had previously been reared under hatchery conditions, had the option to hide from predators in a safe habitat (Tymchuk et al. 2005). The authors reported the early first feeding rearing conditions to differ between the two experiments partially explaining the contrasting results. Sundström et al. (2005) reported transgenic fry to have higher mortality than non-transgenic fish if a predator was present when fry emerged, but not if the predator was introduced after emergence. In another experiment, newly emerged GH transgenic coho salmon had higher survival than non-transgenic fish in naturalized streams in the presence of predators and access to refuges (Sundström and Devlin 2011). The above experiments demonstrate the complexity in assessing the potential predation pressure that AAS could have on prey in the natural environment. In addition, inconsistent results among growth enhanced transgenic salmonids about swimming speed (Farrell et al. 1997; Abrahams and Sutterlin, 1999; Lee et al. 2003; Deitch et al. 2006) renders prediction on how AAS could escape predators difficult. An important difference to note between the AAS and GH transgenic coho salmon used in the above studies, other than the species and transgene, is the background genetics of the transgenic animals. While the AAS has been crossed with domesticated strains of Atlantic salmon for over 12 generations (ABT 2013), the GH transgenic coho salmon are backcrossed with wild fish at each generation to minimize the differences in the genetic background of the transgenic and control animals (Sundström et al. 2005, Tymchuk et al. 2005, Sundström and Devlin 2011). The reported differences can therefore more confidently be attributed to the presence of the transgene in studies conducted with GH transgenic coho salmon studies than AAS to which the effects of domestication might be expected (Devlin et al. 2001). As survival rates of escaped farmed Atlantic salmon are reported to be lower than in wild fish due to higher risk taking, local conditions and genetic basis (Jonsson 1997, Houde et al. 2010), we conclude with high uncertainty that AAS would also be affected by the reported effects of domestication. Based on the multiple interactions between the rearing and receiving environment, available resources, access to refuges and predation pressure, and based on the additional effects of domestication, we conclude with reasonable uncertainty that it is not possible to conclude on whether AAS would suffer from more or less predation in the natural environment, and are therefore unable to make a conclusion on whether to expect an increase or decrease pressure of Atlantic salmon prey.

Determining if the prey selection of the AAS is likely to be different from wild conspecifics is also of consideration when assessing the potential impact of AAS on prey populations. Atlantic salmon is already known to be an opportunistic feeder with a broad diet that varies depending on several factors such as the life stage, size, resource availability, location and season (reviewed in Johansen et al. 2011; Rikardsen and Dempson, 2011). To our knowledge, there are no studies to assess prey selection of AAS under hatchery or naturalized conditions. Nevertheless, studies on GH transgenic coho salmon can provide some information that can be transposed to AAS with reasonable uncertainty. GH transgenic coho salmon, previously fed the same amount of food as satiated wild controls, attacked edible and non-edible prey under aquarium conditions at the same frequency as control fish (Sundström et al. 2004). However, GH transgenic coho salmon fry have different dispersal behaviour than their non-transgenic wild comparators being more dispersed and more likely to explore habitats previously not used (Sundström et al. 2007b). This raises the possibility that AAS would prey on additional species compared to wild conspecifics. In addition, there is a some uncertainty on whether AAS will not reach a larger maximum size than wild conspecifics, particularly considering observations that GH transgenic coho salmon grow larger than non-transgenic fish when raised in mesocosms under high food abundance (Robert Devlin 2013, personal communication) and GH transgenic rainbow trout mature at a much larger size than their wild counterparts (Devlin et al. 2001). Should AAS reach a larger size than its wild conspecifics, they could potentially predate upon larger species not normally preyed upon by wild Atlantic salmon. Based on the above studies, we conclude with reasonable uncertainty that AAS would be likely to feed on additional prey compared to wild conspecifics, hence increasing pressure on potential prey compared to wild conspecifics.

As reviewed under section 11.2.1.2, it is reasonably certain that AAS would not act as a vector for the introduction of new fish pathogens in the natural environment. [redacted]

The effects of triploidy, sex reversal and gynogenesis on potential to influence the effects of AAS on prey species have not been directly assessed. However, based on the lower growth rate of triploid AAS, and equal or lower feeding and competitive behaviour of other triploid salmonids, there is a reasonable degree of uncertainty that triploidy would decrease or have no effect on the predation hazard of AAS on Atlantic salmon prey in most circumstances. There is a theoretical chance that triploid female AAS could reach a larger size than diploid fish after maturation age (see section 11.2.1.4), thereby increasing range of prey sizes or types that it could prey upon. However, there is a high degree of uncertainty to this, as the size of triploids salmonids past maturity has been poorly examined in laboratory or culture conditions. The predatory ability of gynogenetic or sex-reversed fish has not been assessed, but these techniques are not expected to increase or result in new predatory hazards of released fish.

Phenotypic plasticity combined with the wide range of environmental conditions makes specific predictions about the impact of AAS inconclusive for the different prey species. The magnitude of the hazard associated with an overall increased pressure on prey in presence of AAS in the natural environment will depend on several factors including early rearing conditions, type of AAS released, environment and available resources that will affect the growth rate and size of AAS over time. In an environment with high food resources, as in hatchery conditions, AAS would be expected to be able to fulfill its metabolic requirements and to have an enhanced growth phenotype. In such an environment, AAS would consume more fish that wild conspecifics and could result in low to high hazard, depending on the consumed prey, with reasonable uncertainty. In contrast, in environments with limited resources, AAS would not be expected to have enough food to express an enhanced growth rate phenotype compared to wild conspecifics (Sundström et al. 2007, Oke et al. 2013). In such case, either enough food would be available to maintain a wild conspecific equivalent growth rate, or increased metabolic rates would deplete protein, lipid and energy reserves (Cook et al. 2000c; Sundström et al. 2010) and result in mortality of the AAS. In such a limiting environment, any increase in predators could decrease prey populations, so the predation hazard of AAS could be expected to be lower, equal or higher than non-transgenic Atlantic salmon depending on the circumstances.

Based on the above studies reported in the AAS, AAS-relatives and other GH transgenic salmonids, we conclude that the overall potential for AAS to affect prey of Atlantic salmon to be low to moderate with reasonable uncertainty.

11.2.1.6 Potential for AAS to Affect Predators of Wild Atlantic salmon

The potential hazards of AAS to predators of wild Atlantic salmon are concluded to be low with high uncertainty. This assessment was mainly based on conclusions that toxicological impacts to predators through consumption are expected to be low with reasonable uncertainty. However, the overall high degree of uncertainty is attributable to the lack of information about the hormones concentrations, allergens levels and nutritional value of AAS throughout its life cycle, and to inconclusive evidence about the ability of AAS to avoid predators in the natural environment.

AAS, as with escaped farmed Atlantic salmon, are expected to use the same resources as wild conspecifics and to be preyed upon by the same predators. The impact of AAS on these predators will depend on the predator avoidance behaviour of AAS, the toxicity, allergenicity and nutrition value of AAS and its capacity to act as a vector for pathogens and parasites in nature.

The predatory avoidance behaviour of AAS has not been examined. However, AAS-relatives have increased tolerance to predator exposure risk under hatchery conditions (Abrahams and Sutterlin, 1999). The feeding motivation and risk tolerance in the presence of predators in naturalized environments have not been assessed for AAS-relatives but studies were performed on other GH transgenic salmonids and are reviewed under the potential for AAS to affect the prey of Atlantic salmon section (see section 11.2.1.4). Briefly, studies assessing mortality of GH transgenic salmonids due to predation provide inconsistent results. Considering the effect of domestication, we concluded with reasonable uncertainty that it is not possible to predict if AAS would be more or less prone to predation in the natural environment, hence the impossibility to predict the prevalence of wild predators to feed on AAS compared to wild conspecifics.

Consumption of AAS with potential increase in plasma GH, IGF-1, and T_3 is not expected to be hazardous to predators as reviewed under potential toxicity section (see section 11.2.1.1). Uncertainty remains around the consumption of diploid AAS that could potentially have increased steroid levels, but is expected to be remote. Triploidization and gynogenesis are not expected to alter hazards to predators of AAS. Sex-reversal through 17α-methyltestosterone exposure increases whole body levels of methyltestosterone in treated fish, which could potentially impact predator fish if consumed in significant quantities. However, experiments in other fish models demonstrate that increase in 17α-methyltestosterone in treated fish is transient and exogenous methyltestosterone is removed by 10 days post treatment (Fagerlund and Dye 1979, Johnstone et al. 1983, Curtis et al. 1991). As such, any potential hazards to predators of escaped treated fish would be over an extremely limited time frame.

Experimental evidence is highly uncertain as to whether endogenous allergen production is altered in AAS as compared to wild type Atlantic salmon. In addition, the potential allergenic reaction of Atlantic salmon wild predators to AAS has not been examined. It is therefore not possible to conclude on the allergenic impact of the AAS on potential predators

Body composition of Atlantic salmon varies with life stage, size, and quality and quantity of nutrition affecting particularly lipid and moisture content (Reinitz, 1983, Shearer et al. 1994, Anderston et al. 1996). Evidence suggest protein content to be endogenously controlled by fish size while lipid level is affected by both endogenous and exogenous factors and whole body moisture is inversely related to body lipid (Shearer 1994). ABT report that the muscle and skin composition of market-sized AAS has higher fat content than sponsor, but similar fat content to farmed fish controls (Erisman 2004). [redacted] Whether AAS differs from non-transgenic fish in body composition during other life stages, or under different environmental conditions or diets has not been assessed. However, Higgs et al. (2009) found GH transgenic coho salmon differed from non-transgenics in body composition in response to diets of low lipid or low protein content. It is difficult to determine the potential impact of a change in body composition in the AAS compared to wild conspecifics for the potential predators considering gap of knowledge about the changes in body composition of the AAS throughout its life cycle and the numerous potential predators and their nutrient requirements. Body composition of AAS in the environment would be expected to change with time and diet. Effects on predators are expected to be minimal, if any, and of short duration.

As reviewed under section 11.1.1.3, it is reasonably certain that AAS would not act as a vector for the introduction of new fish pathogens in the natural environment. However, we are unable to conclude whether, relative to wild Atlantic salmon, AAS would have an increased capacity to act as a reservoir for the transmission of pathogens, including those that may affect predators of Atlantic salmon.

Based on the above information, we conclude with high uncertainty that AAS consumption would have negligible to low hazards to potential predators.

11.2.1.7 Potential for AAS to Affect the Competitors of Wild Atlantic salmon

The potential hazards of AAS on competitors of wild Atlantic salmon are concluded to be moderate with reasonable uncertainty. Effects of AAS on competitors of Atlantic salmon are expected to result from ecological interactions rather than from genetic introgression through interspecies hybridization with non-native brown trout. The associated reasonable degree of uncertainty is attributable to the lack of information on phenotypic characteristics of AAS and on the relative competitive ability of AAS with coexisting species in the natural environment.

The impact of AAS on competitors of wild Atlantic salmon will depend on the competitive behaviour of AAS for food and habitat, reproductive interference of AAS with other species and potential of AAS to transmit diseases to competitors.

Atlantic salmon are known to compete with brook trout (*Salvelinus fontinalis*), rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*) for habitats in freshwaters and can also compete with percids and cyprinids in slow waters (Cairns 2006, DFO and MNRF 2008, Nislow et al. 2011). The interspecies competitive behaviour of AAS has only been studied in relation to transgenic and non-transgenic hybrids between AAS and brown trout (*Salmo trutta*) (Oke et al. 2013). Both AAS and transgenic hybrids grow faster than their wild-type counterparts under hatchery conditions. However, wild-type Atlantic salmon and AAS have reduced growth rates in presence of transgenic and non-transgenic hybrids in food-limited stream mesocosms suggesting a competitive dominance of the hybrids (Oke et al. 2013). Furthermore, complex interactions between hybridization and transgenesis are suggested as AAS had a greater mass in growth than wild conspecifics in the presence of the hybrids (Oke et al. 2013). Several parameters could have contributed to the competitive advantage of a population of transgenic and non-transgenic hybrids over the AAS and Atlantic salmon including the increased foraging motivation of the transgenic individuals (Abrahams and Sutterlin, 1999, Sundström et al. 2004) and the competitive dominance of juvenile brown trout over juvenile Atlantic salmon (Van Zwol et al. 2012) potentially making hybrids of intermediate dominance (Oke et al. 2013). Rearing fish under hatchery conditions prior to the observations reported in the stream mesocosm could also have impacted competitiveness as suggested in interspecific competition study with GH transgenic coho salmon (Sundström et al. in preparation a). GH transgenic coho salmon were reported to have similar impacts as non-transgenic coho on steelhead trout and chinook salmon fry growth and survival in an artificial stream, when reared in the stream. However, if invading coho salmon were first reared in hatchery conditions, GH transgenic coho had greater impact than non-transgenic fish. The reported effect could partially be due to the increased size of hatchery reared fish but is not likely to be the only determining factor as the wild fish reared under hatchery were also bigger than the invaded Chinook population but did not impact survival and growth (Sundström et al. in preparation a). As natural hybridization would only occur in nature, hybrids would not have been previously exposed to hatchery conditions hence affecting their expected body size and behaviour (Oke et al. 2013, Sundström et al. in preparation b), making it difficult to predict their competitive dominance. Based on the above studies, we conclude that competitiveness of AAS is affected by previous rearing conditions and it likely to be reduced under food limiting conditions.

Hybridization with other species in the natural environment is another pathway through which AAS could impact competitors of Atlantic salmon. Atlantic salmon are known hybridize naturally with brown trout both in America and in Europe though the causes behind the breakdown of pre-reproductive isolating mechanisms may vary (Verspoor 1988, McGowan and Davidson 1992a, McGowan and Davidson 1992b, Youngson et al. 1993, Castillo et al. 2008). Oke et al. (2013) have recently demonstrated that the opAFP-GHc2 transgene can successfully be transmitted into interspecific hybrid through artificial hybridization. Although the above results suggest a competitive advantage of the hybrids over the AAS, predictions of the competitive advantage of the different genotypes in the natural environment are further complicated by hybridization considerations. First, there is no evidence for natural hybridization of AAS and brown trout as the reproductive behaviour has not been examined. Successful artificial hybridization does not necessary translate into successful natural hybridization (McGowan and Davidson 1992a, McGowan and Davidson 1992b). Second, the potential for hybrids in Canada is remote and their survival depends on the nature of variable pre-reproductive mechanisms. Reciprocal natural hybridization has been reported (Youngson et al. 1993, Castillo et al. 2008) but appears to be unidirectional between brown trout females and Atlantic salmon males in Canada (McGowan and Davidson1992, Gephard et al. 2000). The expected all sterile female product form of AAS would prevent potential interspecies hybridization, however a remote possibility remains from the maintenance of neomales in the facility on PEI in Canada or from diploid individuals present in pressure-shocked groups. However, if mature diploid AAS were to escape, their potential to hybridize with brown trout would be diminished compared to wild conspecifics considering their reduced overall reproductive performance relative non-transgenic wild siblings in naturalized stream ecosystems (Moreau et al. 2011a). Furthermore, the occurrence of BT hybrids appears to be dependent on the abundance of sexually mature parr (McGowan and Davidson 1992, Youngson et al 1993, Wirtz 1999, Gephard et al. 2000) of which the occurrence was reported to be reduced in AAS (Moreau and Fleming, 2012). If despite the above obstacles AAS was to hybridize with brown trout in the environment, a low survival of transgenic hybrids could be expected as hybrids from brown trout mothers (BT hybrid) were reported to have higher mortality rates than salmon, trout and hybrids from Atlantic salmon mothers (AS hybrid) (Oke et al. 2013). Nevertheless, the survival rates could also differ if the hybrids were the results of natural hybridization (McGowan and Davidson 1992a, 1992b). Third, the potential for introgression is questionable. Some evidence suggests introgression between the genomes of Atlantic salmon and brown trout via hybridization to be effectively blocked (Galbreat and Thorgaard 1995, Garvia-Vazquez et al. (2004) suggesting any hybridization between AAS and natural populations of brown trout would only last one generation. However, introgression of hybrids back into Atlantic salmon has been demonstrated under artificial conditions (Castillo et al. 2008) and hybridization could thereby continue in theory past the founder generation in the presence of Atlantic salmon. Based on the above studies, despite the competitive advantage of artificially-produced hybrids between AAS and brown trout over non-transgenic fish, it is expected with reasonable certainty, that potential natural hybridization of AAS with brown trout would be lower than for wild conspecifics in natural environment. Hazard to Atlantic salmon competitors through introgression and subsequent impacts on other competitors is therefore considered to be negligible to low with reasonable uncertainty.

Studies examining the competitiveness of GH transgenic salmonids with other species, including potential competitors, are limited to the ones described above. Other studies reporting on phenotypes that are known to affect relative competitiveness, such as growth rates, dominance and swimming speed are also relevant. As previously reviewed under section about the potential for AAS to affect wild populations of Atlantic salmon (see section 11.2.1.4), predicting whether AAS fish may maintain high growth phenotype in natural environments is highly problematic, although current studies suggest accelerated growth may be limited in many circumstances. The competitive ability and performance of first-feeding AAS and non-transgenic siblings are equally dominant under low food conditions (Moreau et al. 2011b). AAS fry have reduced growth rates compared to non-transgenic siblings when live prey were provided in stream mesocosm environment (Oke et al. 2013) suggesting a reduced ability to catch prey, hence reduced competitiveness. GH transgenic coho salmon have increased ability to compete for food under hatchery conditions (Devlin et al. 1999, Devlin et al. 2004), are better at seizing prey in tanks (Sundström et al. 2004) and are equally competitive as sized-matched non-transgenic controls under limited environmental conditions (Tymchuk et al. 2005). The above studies suggest the competitive advantage of GH transgenic salmonids under hatchery conditions is lost in naturalized environment. Results on swimming speed of GH transgenic salmonids are inconsistent (Farrell et al. 1997; Abrahams and Sutterlin, 1999; Lee et al. 2003; Deitch et al. 2006) and renders prediction on how fast AAS could forage for common prey difficult.

As reviewed under section 11.1.1.3, it is reasonably certain that AAS would not act as a vector for the introduction of new fish pathogens in the natural environment. However, we are unable to conclude whether, relative to wild Atlantic salmon, AAS would have an increased capacity to act as a reservoir for the transmission of pathogens, including those that may affect prey of Atlantic salmon.

The effects of triploidy, sex reversal and gynogenesis on potential to influence the effects of AAS on competitor species of Atlantic salmon have not been directly assessed. However, based on the lower growth rate of triploid AAS, and equal or lower feeding and competitive behaviour of other triploid salmonids, there is a reasonable degree of uncertainty that triploidy would decrease or have no effect on the competition hazard of in most circumstances. The predatory ability of gynogenetic or sex-reversed fish has not been assessed, but these techniques are not expected to increase or result in new predatory hazards of released fish.

Based on the above studies, we conclude that the limited potential for AAS to affect the competitors of Atlantic salmon would results from ecological interactions with competitors rather than from introgression through interspecies hybridization. Potential interspecific competitiveness of juvenile stages of AAS with competitors is expected to be lower or similar to wild conspecifics while effects of adult stages have not been remain undetermined. Although accelerated growth may be limited in many circumstances, we cannot conclude that AAS would never expressed increased growth rates in the environment providing AAS a size advantage and theoretical increased interspecies competitiveness relative to wild conspecifics. Overall, we conclude the hazard of AAS to potential competitors of Atlantic salmon to be negligible to moderate with reasonable uncertainty resulting from important knowledge gap and limited understanding of demonstrated genotype x environment effects.

11.2.1.8 Potential for AAS to Affect Habitat

The potential hazards of AAS to habitat are concluded to be low with high uncertainty on the basis of expert opinion. The high degree of uncertainty is attributable to the lack of information on fitness, population size, migration and spawning behaviors, [redacted] propensity for spawning and overall longevity of repeat AAS spawners..

In order to determine the potential for AAS to affect habitat, DFO examined their relevant phenotypic characteristics, focusing on behaviour and size of AAS and the potential to affect habitat attributes.

Potential of salmonids to affect habitat

Ecosystem engineers are organisms that directly or indirectly change the availability of resources to other species by substantially modifying the physical structure (i.e. biotic and/or abiotic materials) of their habitat (Jones et al. 1994; Meysman et al. 2006). The first factor determining the role of an animal as an ecosystem engineer is its behaviour (Moore 2006). The potential for AAS to influence habitat during four essential activities is addressed: reproductive behaviour, foraging and predatory behaviour, anti-predator behaviour, and migratory (trophic and reproductive) behaviour. Salmonid behaviour during foraging, predator avoidance, and migration has not been associated with significant effects on habitat, and no known phenotypic characteristic of AAS is expected to alter this lack of effect. However, reproductive behaviour of salmonids, including Atlantic salmon, has been shown to influence habitat through ecosystem engineering and bioturbation (Scott and Crossman 1973; Grant and Lee 2004; Verspoor et al. 2007; Gottesfeld et al. 2008).

Redd construction and excavation in stream gravel by spawning salmonids, when spawning at high densities, can significantly disturb the streambed (Gottesfeld et al. 2004; Hassan et al. 2008). In constructing redds, salmonids can move large quantities of coarse sediments short distances downstream, and can consequently influence habitat attributes in a number of ways. Redd excavation affects substrate composition by disturbing and sorting the substrate, and remove various amount of the finer sediments through interaction with water current (Moore 2006; Gottesfeld et al. 2008), as well as increases concentration of suspended particular matter (i.e. turbidity, Moore 2006). Reported secondary effects of salmonid redd construction include decreased stream macrophyte, algae and moss biomass, and decreased or altered insect communities (Field-Dodgson 1987; Minakawa and Gara 2003; Moore and Schindler 2008). Redd construction can increase the interstitial flow within the site (De Vries 2008) and modify pool-riffle characteristics (Field-Dodgson 1987), but does not influence the overall flow rate of a stream (De Vries 2008). Redd construction or other behaviour in salmonids has not been associated with alterations in other habitat attributes (i.e. temperature, dissolved oxygen, pH).

Potential of Atlantic salmon to affect habitat

The scale of streambed bioturbation during redd construction depends on the species, female size, number and density of spawning salmon, and the spatial extent of the spawning beds in the stream. In particular, Moore (2006) identified body size and population density as the two most important factors after behaviour influencing the ability of ecosystem engineers to affect habitat. The role of large densities of large-sized Pacific salmon as environmental engineers during spawning has been well identified, and the majority of the studies listed above examine Pacific salmon. The size and behaviour of spawning Atlantic salmon indicate they have the potential for ecosystem engineering roles in forming aquatic habitats. The majority of natural mature Atlantic salmon females are expected to be 55 to 75 cm in fork length (reviewed by Hutchings and Jones 1998). As well, Atlantic salmon generally construct redds ranging in size between 2.3 and 5.7 m^2 (Gaudemar et al. 2000), burying eggs between 15 to 35cm deep in the gravel (De Vries 1997; Amiro 2006), indicating large potential to impact substrate composition within a stream. It is important to note that bioturbation can also enhances production of the stream by mobilizing nutrients, and changing interstitial flows within the sediment that promotes survival of intermediate life stages (Gérald Chaput 2013, personal communication). However, return estimates (Jones et al. 2004; Lanteigne 2012; Reddin and Veinot 2010; Reddin 2010), as well as COSEWIC reports, indicate that historically, and even more so currently, Atlantic salmon spawn in numbers and densities that are relatively modest compared to the densities of Pacific salmon species (Scott and Crossman 1973; Murota 2003; Schoonmaker et al. 2003). The bioturbation and habitat modification performed by spawning Atlantic salmon populations (including wild, hatchery raised, or escaped farmed Atlantic salmon) in Eastern North America does not appear as important in the geomorphic processes that shape stream habitat in the Pacific northwest (Gottesfeld et al. 2008). The current state of Atlantic salmon populations does not indicate that a drastic increase in the number of spawning Atlantic salmon, and a resulting increase in their importance as ecosystem engineers, can be expected in the foreseeable future.

Potential of AAS to affect habitat

[redacted] However, the reported reduced fitness and decreased tolerance to low oxygen levels and higher water temperature (ABT 2013) could potentially decrease the capability of the spawning individuals to excavate redds, thus diminishing their bioturbation effects on the habitat. Experiments with growth-enhanced transgenic coho salmon indicated that transgenic females had a lower rate of spawning and less redd digging and covering than hatchery and cultured non-transgenic controls (Bessey et al. 2004). As well, any triploid AAS escapes would be sterile and consequently not have associated habitat alterations through redd construction. As such, AAS are expected to have a diminished effect on habitat through redd construction than wild-type Atlantic salmon.

[redacted] (ABT 2013) and GH transgenic rainbow trout mature at a much larger size than their wild counterparts (Devlin et al. 2001). However, the effect of the EO-1α transgene on longevity and size over successive reproductive periods has not been address, particularly in the context of phenotype x environmental interactions. As such, AAS are expected to attain maximum species-specific size of Atlantic salmon, but it is uncertain whether they may exceed this size, and if so what affect this may have on reed building behaviour. Increased male or female spawning size could potentially influence substrate and stream position preference in salmon in some circumstances (Roni and Quinn 1995; cited in Beechie et al. 2008), potentially altering the sections of stream habitat affected by spawning salmon. As such, whether size of AAS could influence its effects on habitat are unclear. Whether sufficient number of AAS could influence Atlantic salmon population density and hence impact as ecosystem engineers, will depend on this exposure. However, considering low numbers of fish involved, negligible effects are expected.

Due to the limited role of Atlantic salmon in habitat alteration, the potential for decreased nest building of AAS, and the sterility of triploid AAS, AAS are expected to have limited or negligible ability to affect habitat in terms of substrate construction and turbidity. There is a high degree of uncertainty to this, due to the lack of information on spawning behaviour, longevity, and maximum size of repeat AAS spawners. In addition, AAS are expected to have negligible effects on other aspects of habitat structure (i.e. stream flow rates, temperature, oxygen, pH).

11.2.1.9 Potential for AAS to Affect Biodiversity

The potential hazards of AAS to biodiversity in Canada are unknown. AAS are expected to have low effects on nutrient cycles in rivers unless AAS adults have a greater propensity to semelparity (die after spawning) than is expressed in wild Atlantic salmon populations. Effects of AAS to biodiversity through displacement/exclusion from habitat of non-salmonid species or to feed on biota in rivers not consumed by juvenile or adult non-transgenic salmon are unknown. Potential hazards of escaped farmed fish to biodiversity are largely unknown (Leggatt et al. 2010) making reliable prediction of the effects of AAS on the overall community dynamics, ecosystem functions and biodiversity very difficult.

Biodiversity is the variability among living organisms from all sources, including terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they form a part and includes the diversity within species, between species, and of ecosystems (CEPA 1999). Direct pathways through which AAS could affect biodiversity include genetic alteration of wild populations of Atlantic salmon through introgression and hybridization with other salmonids species. Indirect pathways through which AAS could affect biodiversity include changes in the abundance and distribution of one wild species, either through ecological interactions or transfer of diseases or parasites, thereby potentially resulting in altered food-web dynamics and local community biodiversity (Leggatt et al. 2010, Diana 2009). The above potential direct and indirect pathways have been addressed under previous sections (see sections 11.2.1.4 to 11.2.1.7).

The potential for altered biodiversity from repeated exposure of sensitive ecosystems to escaped farmed fish has been poorly addressed (Leggatt et al. 2010). However, the effects of nutrients on ecosystems are widely recognized being the limiting factor on primary production (DeAngelis et al. 1989). Atlantic salmon have potential to influence stream and river nutrient cycles through emigration and immigration. Salmon can export river nutrients through marine migration as smolts, and import marine nutrients through spawning migration to and mortality in rivers. The role of Pacific salmon in river nutrient cycles has been well examined, and spawning mortality of Pacific salmon is reported to have inconsistent, but positive effects on river nutrients (see Jonsson and Jonsson 2003; Janetski et al. 2009). This can result in multiple direct and indirect effects on river nutrients and ecosystems such as increased periphytoplankton biomass, increased litter mass loss rate (Yoder et al. 2006), increased biofilms of bacteria and eukarya (Schuldt 1998), and increased food resources and productivity of juvenile salmon (Shaff and Compton 2009). As well, introduction of Atlantic salmon carcasses to upland streams resulted in increased juvenile salmon biomass up to 2 times that of reference streams (Williams et al. 2009).

Unlike semelparous Pacific salmon, Atlantic salmon are iteroparous and may return to the ocean after spawning. Reported survival rates of Atlantic salmon after spawning vary between systems and years, and range from 9% to 74% (Fleming 1996). As well, Atlantic salmon spawning numbers in Canada are much lower than that of Pacific salmon (see Section 11.2.1.8), indicating Atlantic salmon have much lower potential to impact nutrient cycles than Pacific salmon. The few studies examining the influence of Atlantic salmon on river nutrient cycling have found inconsistent results, but indicate Atlantic salmon can have a small influence on river nutrient cycling in some circumstances. A small run of spawning Atlantic salmon (less than 300 fish per year) was reported to contribute significant amounts of phosphorous (5% of total river phosphorous) to a low nutrient system in France, but minimal amounts of nitrogen (0.2%, Jonsson and Jonsson 2003). The annual import of carbon, nitrogen, and phosphorous in seven rivers in England accounted for only a fraction of the annual river export (0.09-0.24%, Elliott et al. 1997; Lyle and Elliott 1998), although Lyle and Elliott (1998) postulated the impact on localized areas in the upper reaches of the river where the salmon spawned and died could be much greater. In contrast, Nislow et al. (2004) found Atlantic salmon migrations resulted in a net export of phosphorous in a river in Scotland, due to poor return of adult spawners. Jardine et al. (2009) found no input of marine isotopes from Atlantic salmon spawners in resident sculpins in a river in New Brunswick. They postulated than river carbon and nitrogen input from Atlantic salmon in Canada may be minimal due to low numbers of spawning salmon, although Jonsson and Jonsson (2003) found a small number of spawning salmon can have a significant nutrient input in low nutrient systems.

The potential for AAS to affect river nutrient cycles through migration and spawning mortality has not been examined. Whether a GH transgene affects spawning mortality in AAS or other iteroparous fish has not been examined, and we cannot determine if AAS would differ in from wild-type Atlantic salmon in river nutrient flux due to spawning mortality. However, triploid all-female AAS are not expected to mature, and would consequently have much lower spawning migrations than diploid Atlantic salmon (Warrillow et al. 1997; Cotter et al. 2000; Wilkins et al. 2001; Chatterji et al. 2008). As such, the sponsored product form of AAS would not import significant marine nutrients to river systems, although could potentially export river nutrients if large numbers were reared in the river until smolt. While the potential impact of AAS on river nutrient cycling is not known, Atlantic salmon are expected to have limited roles in nutrient cycling in most systems in Canada. As such, the impact of AAS on river nutrient cycling is expected to be negligible, unless released in sufficient numbers to systems with low nutrient levels.

We conclude that the impact of migrating Atlantic salmon on nutrient cycles in Canadian rivers is expected to vary between systems from negligible to small impacts. If introduced into the North American environment, whether AAS and their progeny would differ from wild-type Atlantic salmon in the magnitude or direction of impact on river nutrient cycling is not known, although triploid AAS could potentially increase net export of nutrients from rivers systems in some circumstances. Nevertheless, since effects escaped fish may have on overall community dynamics or ecosystem function are not yet known (Leggatt et al. 2010) it is not possible to make reliable predictions of the potential effects of AAS on the overall community dynamics, ecosystem functions or biodiversity.

11.2.2 Outcome of the Environmental Hazard Assessment

The current environmental hazard assessment characterized the potential adverse effects of AAS to the Canadian environment, assuming entry of AAS into the natural environment. The following assessment endpoints, representing legislative protection goals and selected based on potential and most relevant interactions of AAS with the ecosystem components (Devlin et al. 2007c), were determined to be (1) wild populations of Atlantic salmon, (2) prey of Atlantic salmon, (3) predators of Atlantic salmon, (4) competitors of Atlantic salmon, (5) habitat and (6) biodiversity (DFO 2013). Hazard considerations included the potential toxicity of AAS, the capacity of AAS to act as a vector of diseases/pathogens, the potential for horizontal gene transfer of the transgene to other organisms and the potential ecological and genetic interactions of AAS with each assessment endpoint. As much as possible, the relative magnitudes of the potential hazards of AAS compared to wild conspecifics are reported. Prediction of the effects of AAS on the assessment endpoints are summarized in Table 11-2.

Based on the thorough molecular characterization of the inserted construct in the AAS and on supporting evidence from basic alignment sequence analyses, we conclude with high certainty that the inserted construct at the EO-1α locus does not contain coding sequence for any known toxins, allergens or proteins other than the intended growth hormone. We also concluded with reasonable certainty that no other coding sequence were inserted in the AAS genome in proximity of the EO-1α locus. Gynogenesis, sex reversal and triploidization processes used in the manufacture of the AAS were concluded to be of negligible toxicological hazard. Consumption of potentially elevated

GH levels in different life stage of AAS was also determined with reasonable certainty to be of negligible hazard to potential predators based on evidence of proteolytic digestion and differences between maximum potential concentration in salmonids and doses required to elicit a biological response. Consumption of potentially elevated IGF-1 and thyroid hormones, as well as steroid hormones in triploid AAS, were concluded to be of negligible hazard to predators with reasonable uncertainty while uncertainty remains around the hazard associated with the potential increase in other hormone levels. Finally, we conclude with reasonable certainty that bioconcentration factor of waterborne contaminants could be relatively higher in AAS compared to wild conspecifics, but we cannot predict the magnitude of potential associated hazard. Overall, we conclude that the **environmental hazard related to the potential toxicity of AAS to be low with reasonable uncertainty**.

Given considerable knowledge gap related to pathogens, we were unable to determine whether AAS would have an increased capacity to act as a reservoir for the transmission of pathogens compared to wild Atlantic salmon. However, based on long-term, historical data on the lack of occurrence of reportable fish diseases at the AquaBounty PEI facility, we concluded with reasonably certainty that AAS would not act as a vector for the introduction of new fish pathogens into the natural environment. We therefore **cannot conclude on the environmental hazard related to the capacity of AAS to act as a vector of diseases/pathogens.**

The potential for horizontal gene transfer (HGT) of the EO-1α transgene from AAS is expected to be similar to that of naturally occurring HGT in Atlantic salmon. EO-1α may have increased potential for expression once transferred, although is not expected to differ from native genes in potential for HGT via exposure, uptake, stability and selection. We therefore conclude that the **environmental hazard related to the potential HGT to be negligible with reasonable uncertainty.**

Most ecological studies about potential effects of GH transgenic salmonids have been conducted on juvenile stages hence an existing gap in the knowledge of potential impacts of the adult life stage AAS and during life at sea. In addition, predictions about the overall impact of AAS on wild populations of Atlantic salmon are complicated by the effects of food resource levels, background genetics, early rearing conditions, life stages and predation levels. Nevertheless, based on the current status of Atlantic salmon populations in Canada, and on studies conducted on AAS, AAS-relatives and other GH transgenic salmonids, we conclude with high uncertainty that AAS could pose moderate to high hazards on wild populations of Atlantic salmon. Highest hazards are expected to be from potential introgression of fertile broodstock with wild populations, or through competition under food limiting environments. We therefore conclude the **overall environmental hazard of AAS to wild populations of Atlantic salmon to be high with reasonable uncertainty.**

In assessing the potential effects of AAS on potential prey of Atlantic populations we considered the predatory pressure and selection of AAS. We cannot conclude on the potential predatory pressure that AAS would present in the natural environment as it is not possible to determine whether AAS would suffer from more or less predation and as the phenotype of AAS will be dependent on environmental conditions, especially food resources. However, we conclude with reasonable uncertainty that AAS would be likely to feed on additional prey compared to wild conspecifics, hence potentially increasing pressure on prey compared to wild conspecifics. We therefore conclude the **overall environmental hazard of AAS to prey of Atlantic salmon to be moderate with high uncertainty.**

The impact of AAS on potential predators of Atlantic salmon would depend on several factors. The relative ability of AAS to avoid predators compared to wild conspecifics is difficult to predict due to inconclusive evidence under naturalized conditions. Toxicological impacts through predation are expected to be negligible to low with reasonable uncertainty. Despite further uncertainties revolving around the potential allergenicity and nutrition value of AAS and its capacity to act as a vector for pathogens, we conclude that any potential hazards to predators are expected to be minimal, if any, and of short duration, as effects would require high and continuous consumption rates of

AAS. We therefore conclude the **overall environmental hazard of AAS to predators of Atlantic salmon to be low with high uncertainty.**

Based on available studies, we conclude that the limited potential for AAS to affect the competitors of Atlantic salmon would results from ecological interactions with competitors rather than from introgression through interspecies hybridization. Potential interspecific competitiveness of juvenile stages of AAS with competitors is expected to be lower or similar to wild conspecifics while effects of adult stages remain undetermined. Although accelerated growth may be limited in many circumstances, we cannot concludeAAS would never express increased growth rates in the environment providing AAS a size advantage and theoretical increased interspecies competitiveness relative to wild conspecifics. Hazard to Atlantic salmon competitors through introgression and subsequent impacts on other competitors is considered to be negligible to low with reasonable uncertainty. We therefore conclude the **overall environmental hazard of AAS to competitors of Atlantic salmon to be moderate with reasonable uncertainty.**

Due to the limited role of Atlantic salmon in habitat alteration, the potential for decreased nest building of AAS, and the sterility of triploid AAS, we conclude the **overall environmental hazard of AAS to the habitat to be low with high uncertainty.**

The potential hazards of AAS to the Canadian biodiversity, as for the potential hazards of escaped farmed fish, have been poorly addressed. Nutrient load being the limiting factor on primary production, we assessed the potential for AAS to affect nutrient cycle in rivers and concluded that it was expected to be negligible unless sufficient numbers of AAS were to enter a system with low nutrient levels. Excluding the potential genetics hazards of AAS to wild populations of Atlantic salmon and competitors which were addressed in the above sections, it was not possible to make reliable predictions of the effects of AAS on the overall community dynamics, ecosystem functions and biodiversity. We therefore **cannot conclude on overall environmental hazard of AAS to biodiversity.**

Table 11-2 Summary of the environmental hazard assessment. The magnitude of the hazard and its related uncertainty are indicated for each hazard assessment endpoint.

Assessment endpoints	Hazard	Uncertainty
Wild populations of Atlantic salmon	High	Reasonable uncertainty
Prey of Atlantic salmon	Moderate	High uncertainty
Predators of Atlantic salmon	Low	High uncertainty
Competitors of Atlantic salmon	Moderate	Reasonable uncertainty
Habitat	Low	High uncertainty
Biodiversity	Unknown	
Overall	**High**	**High uncertainty**

The overall potential hazards of AAS to the Canadian environment are concluded to be high with high uncertainty. If AAS was to enter the natural environment, we expect that the highest potential hazards would be to wild populations of Atlantic salmon, followed by prey and competitors of Atlantic salmon. Hazards are expected to be low for predators and habitat. We cannot conclude on the potential hazard to biodiversity.

There is an overall high degree of uncertainty associated with the environmental hazard assessment. The high degree of uncertainty results from the lack of information on phenotypic characteristics of AAS in the natural environment, genotype x environment interactions and effects of background genetics. Predictions regarding the potential ecological and genetic effects of GH transgenic fish in variable natural environments are complex as the rearing and experimental conditions affect the same fitness traits under investigation in studies assessing the effects of transgenesis. Studies over the last two decades provide solid evidence of the effects of resource levels, backgrounds genetics, early rearing conditions, life stages, and predation levels on the potential ecological consequences of GH transgenic salmonids. For the above reasons, the magnitude of the potential environmental hazards of AAS is difficult to predict and remains highly uncertain.

12 RISK ASSESSMENT

Reiterate that we are only assessing the scenario where eggs are produced in PEI and shipped to Panama. Contaiment just so, production just so. No other manufacturing facitilities in Canada.

12.1 Indirect Human Health Risk Assessment

The indirect human health hazard assessment has characterized and ranked the incremental human health hazards that could result from environmental exposure to AAS as compared to wild Atlantic salmon based on the potential toxicity and allergenicity of AAS and the capacity of AAS to act as a vector for human pathogens.

The outcome of the indirect human hazard assessment is summarized in Table 12-1and suggests that the effects to human health resulting from environmental exposure to AAS range from negligible to low. This reflects the fact that there are no known toxins associated with AAS and also the fact that even if the allergenic potency or capacity of AAS to act as a vector for human pathogens were elevated as compared to wild Atlantic salmon, the nature and severity of adverse effects in humans from dermal or aerosol exposure is generally mild and without potential for community-level effects (see Table 8-3).

There is reasonable to high certainty associated the indirect human hazard assessment which reflects the number of reports in the scientific literature pertaining to adverse effects from dermal and aerosol exposure to fish allergens and zoonoses and the consistency with which these adverse effects are reported (see Table 8-7).

The exposure assessment has examined the potential for AAS to enter the Canadian environment given the redundant physical, geographical and biological containment provisions that AquaBounty has proposed. The findings of the exposure assessment are also summarized in Table 12-1, and suggest that for the specific activities that have been notified, exposure of AAS to the Canadian environment is expected to be negligible. That is to say, AAS are sufficiently contained and are not expected to enter or survive in the Canadian environment. The exposure assessment is made with high certainty, and is based on detailed information on facility design, containment structures, SOPs, internal compliance documentation, incident reports, and long term reliable historical data on chance events at or near the location of each facility. It is also based on high quality data available for AAS and valid surrogates and data on the environmental parameters of the potential receiving environments.

For the purposes of the indirect human health risk assessment, the potential exposure of humans in Canada to escaped AAS, if that were to occur, would be further limited to incidental encounters during swimming and recreational fishing. While human contact with Atlantic salmon during swimming is expected to be extremely rare, the recreational Atlantic salmon fishery, limited as it is, does provide opportunity for dermal exposure through handling fish that are caught on a line. This eventuality is even remote given the determination of negligible exposure with high certainty mentioned above.

Table 12-1 Indirect human health risk assessment

	Hazard		Exposure to humans in Canada via								Risk
			PEI		Panama		Transport		Disposal		
	Rank	Uncertainty	Rank	Uncertainty	Rank	Uncertainty	Rank	Uncertainty	Rank	Uncertainty	Rank
Toxicity	N	HC	N	HC	N	HC	N	HC	N	HC	N
Allergenicity	L	RC	N	HC	N	HC	N	HC	N	HC	L
Vector for human pathogens	L	HC	N	HC	N	HC	N	HC	N	HC	L
FINAL	**N-L**	**RC**	**N**	**HC**	**N**	**HC**	**N**	**HC**	**N**	**HC**	**L**

The indirect human health risk assessment is conducted in accordance with the classical risk assessment paradigm where risk is directly related to the exposure and hazard of the organism, or R = H x E (see section 8.3.7). In accordance with the provided risk matrix (Figure 8.2), the indirect human health risk is expected to be low; a product of low hazard multiplied by negligible exposure (Table 12-1). The uncertainty associated with the risk assessment is derived from those associated with both the exposure and hazard assessment and cannot be easily summarized. As indicated in section 8.3.7, when rankings for uncertainty in the hazard and exposure assessments differ, the higher uncertainty ranking is generally assigned to the risk

Therefore, given the exposure assessment of negligible with high certainty, the indirect human health risk associated with the manufacture and production of AAS is expected to be low with high certainty under the proposed use scenario specified in the notification by AquaBounty. In addition, given the low rank for indirect human health through environmental exposure, and the further limitation to human exposure to AAS through recreational fishing even if AAS were to enter the environment, we do not suspect that a significant new activity in relation to AAS would result in AAS becoming toxic.

12.2 Environmental Risk Assessment

The environmental hazard assessment has examined the potential for AAS to impact: (1) wild populations of Atlantic salmon; (2) Atlantic salmon prey, predators and competitors; (3) habitat; and (4) biological diversity. The findings of the hazard assessment are summarized in Table 12-2and suggest that impacts to the environment resulting from exposure to AAS may range from low to high (with the exception of biodiversity which is unknown) depending on which component of the ecosystem is under consideration. Uncertainty associated with the hazard assessment is high and reflects the limited availability of data on the expression and behavior of the AAS phenotype in the wild, our limited understanding of AAS phenotypic plasticity across relevant environmental conditions and significant knowledge gaps regarding the phenotypic expression of the opAFP-GHc2 construct across different genetic backgrounds.

The exposure assessment has examined the potential for AAS to enter the Canadian environment given the redundant physical, geographical and biological containment provisions that AquaBounty has proposed. The findings of the exposure assessment are also summarized in Table 12-2, and suggest that for the specific activities that have been notified, exposure of AAS to the Canadian environment is expected to be negligible. That is to say, AAS are sufficiently contained and are not expected to enter or survive in the Canadian environment. The exposure assessment is made with reasonable certainty, and is based on detailed information on facility design, containment structures, SOPs, internal compliance documentation, incident reports, and long term reliable historical data on chance events at or near the location of each facility. It is also based on high quality data available for AAS and valid surrogates and data on the environmental parameters of the potential receiving environments.

Table 12-2 Environmental risk assessment

	EXPOSURE to Canada via								HAZARD		RISK
	PEI		Panama		Transport		Disposal				
	Rank	Uncertainty	Rank	Uncertainty	Rank	Uncertainty	Rank	Uncertainty	Rank	Uncertainty	
Wild Atlantic salmon	N	RC	N	HC	N	RC	N	HC	H	RU	L
Prey	N	RC	N	HC	N	RC	N	HC	M	HU	L
Predators	N	RC	N	HC	N	RC	N	HC	L	HU	L
Competitors	N	RC	N	HC	N	RC	N	HC	M	RU	L
Habitat	N	RC	N	HC	N	RC	N	HC	L	HU	L
Biodiversity	N	RC	N	HC	N	RC	N	HC	U		L
TOTAL	**N**	**RC**	**N**	**HC**	**N**	**RC**	**N**	**HC**	**H**	**HU**	**L**

Abbreviations for rank: N: negligible; L: low; M: moderate; H: high and U: unknown. Abbreviations for uncertainty: HC: highly certain; RC: reasonably certain; RU: reasonably uncertain; and HU: highly uncertain.

The environmental risk assessment is conducted in accordance with the classical risk assessment paradigm where risk is directly related to the exposure and hazard of the organism, or R = H x E (see section 8.3.7). The uncertainty assigned to risk is that associated with the element that limits risk, either exposure or hazard, in the risk assessment paradigm (Risk = Hazard X Exposure). Therefore, given the exposure assessment of negligible with reasonable certainty and the hazard assessment of high with high uncertainty, the environmental risk associated with the manufacture and production of AAS is expected to be low with reasonable certainty under the proposed use scenario specified in the notification by AquaBounty, including all physical, biological, geographical and operational containment measures. However, the emphasis that has been placed on containment to prevent exposure to the Canadian environment and in particular on physical containment of AAS, makes it imperative that the use scenario proposed by AquaBounty be maintained including all physical, biological, geographical and operational containment measures.

Given the potential hazards to the environment, and the uncertainties associated with the invasiveness of AAS any alterations to the proposed use scenario or to the proposed containment measures may result in the entry or release of AAS into the environment in a manner and circumstances significantly different to any previous exposure or potential exposure of the environment to AAS. Consequently, a significant new activity in relation to AAS could result in AAS becoming toxic..

12.3 Final Recommendations for Regulatory Decision-Making

(To be completed after peer review process).

13 RECOMMENDATIONS FOR RISK MANAGEMENT

The company (AquaBounty Canada) has indicated its intent to commercially produce sterile female AAS eggs at its land-based aquaculture facility in PEI for export to a land-based, grow-out facility in the highlands of western Panama. No more than 100,000 eggs will be exported to Panama in any given year. In Panama, AAS will be grown to a commercial weight of 1 to 3 kg, then harvested, euthanized and transported to a processing plant in close proximity to the Panamanian grow-out facility where they will be processed and shipped to the United States for human food consumption.

AquaBounty has also committed to ensuring that live eggs exported from the facility in PEI to the facility in Panama, will be reared only at the production site described in the notification and that no live fish of any life stage will be sold or given by AquaBounty Panama to a third party for grow-out. This is also the basis of the application made to the US FDA and a condition of sale as outlined on the formal label that can be found on p. 579 of the notification (ABT 2013).

Rational for SNAc

The AAS is intended for use under strictly controlled conditions that include physical confinement in two clearly defined facilities. AquaBounty has provided well-defined parameters for the scope of their activity, as outlined above. The proposed parameters, which include physical, biological and geographical containment provisions, have been deemed sufficient to preclude the potential for escape and the possibility of entry into the Canadian environment.

Given the potential hazards to the environment, and the uncertainties associated with the invasiveness of AAS, any alterations to the proposed use scenario or to the proposed containment measures may result in the entry or release of AAS into the environment in a manner and circumstances significantly different to any previous exposure or potential exposure of the environment to AAS. Consequently, a significant new activity in relation to AAS could result in AAS becoming toxic..

The emphasis that has been placed on containment to prevent exposure to the Canadian environment and in particular on physical containment of AAS, makes it imperative that the use scenario proposed by AquaBounty be maintained including all physical, biological, geographical and operational containment measures. Therefore, any activities outside of the well-defined parameters that have been described in the notification would be considered a significant new activity and would require a Significant New Activity Notice.

In relation to the AquAdvantage salmon, a significant new activity would be any activity other than the following:

1. Commercial production at the AquaBounty Canada facility, near Souris, PEI that has been described in the notification and is under the singular and direct control of AquaBounty Technologies, of hemizygous triploid female AAS eyed-eggs using milt from homozygous masculinized AAS females (neo-males) and eggs from normal Atlantic salmon females that are derived from the domesticated St. John River strain;

2. Export of no more than 100,000 hemizygous triploid female AAS eyed-eggs from the AquaBounty Canada facility, near Souris, PEI, to the AquaBounty Panama facility near Boquete, Chiriquí Province, Panama that has been described in the notification and is under the singular and direct control of AquaBounty Technologies, for commercial grow-out and human consumption; and

3. Physical containment of AAS at all life-stages at the facility in PEI, Canada, and the facility in Chiriquí, Panama and while in transport between the two facilities as described in the notification.

If a significant new activity in relation to the AquAdvantage salmon is proposed, AquaBounty shall provide to the Minister of the Environment, at least 120 days prior to the commencement of the proposed significant new activity, the following information:

1. a description of the proposed significant new activity in relation to the living organism;

2. a detailed description of all physical, biological and geographic containment measures proposed to be used;

3. the information specified in paragraph 5(b) of Schedule 5 of the *New Substances Notification Regulations (Organisms)*; and

4. any other information or data in respect of this living organism in AquaBounty's possession or to which they have access, that is relevant in order to determine whether the living organism is invasive or capable of becoming invasive.

The above information will be assessed within 120 days after the day on which it is received by the Minister of the Environment.

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15 APPENDICES

Appendix A. Waiver Request (Proposed)

Information Requirements:

Data from tests conducted to determine the pathogenicity, toxicity, or invasiveness of the notified substance (a genetically modified Atlantic salmon or the AquAdvantage® salmon) as requested in paragraph 5(a) of Schedule 5 of the *New Substances Notification Regulations (Organisms)*. Fisheries and Oceans Canada has indicated to AquaBounty that invasiveness would be the appropriate endpoint to which to direct this testing.

Basis of Waiver Request: Paragraph 106(8)(a) of the Environmental Protection Act, 1999 (CEPA 1999)

(8) On the request of any person to whom subsection (1), (2), (3) or (4) applies, the Minister may waive any of the requirements to provide information under that subsection if

(a) in the opinion of the Ministers, the information is not needed in order to determine whether the living organism is toxic or capable of becoming toxic;

(b) a living organism is to be used for a prescribed purpose or manufactured at a location where, in the opinion of the Ministers, the person requesting the waiver is able to contain the living organism so as to satisfactorily protect the environment and human health; or

(c) it is not, in the opinion of the Minister, practicable or feasible to obtain the test data necessary to generate the information.

Use Scenario:

The notified substance is a genetically engineered Atlantic Salmon (*Salmo salar*) referred to as the *AquAdvantage*® salmon (AAS hereinafter) that is claimed to grow faster than its non-genetically engineered counterpart and is intended for human food consumption.

The company (AquaBounty Canada) has indicated its intent to commercially produce sterile female AAS eggs at its land-based aquaculture facility in PEI for export to a land-based, grow-out facility in the highlands of western Panama. No more than 100,000 eggs will be exported to Panama in any given year. In Panama, AAS will be grown to a commercial weight of 1 to 3 kg, then harvested, euthanized and transported to a processing plant in close proximity to the Panamanian grow-out facility where they will be processed and shipped to the United States for human food consumption. AquaBounty has also committed to ensuring that live eggs exported from the facility in PEI to the facility in Panama, will be reared only at the production site described in the notification and that no live fish of any life stage will be sold or given by AquaBounty Panama to a third party for grow-out. This is also a condition of sale as outlined on the formal label that can be found on p. 579 of the notification (ABT 2013).

Although the product for export to Panama will be sterilized female AAS eggs, both fertile and sterilized male and female AAS at all life-stages (gametes through to sexually mature adults) will continue to be reared at the PEI facility as broodstock for egg production and for research and development purposes.

AquaBounty's Justification:

AquaBounty has requested a waiver for information required under information element 5(b) of Schedule 5 of the *New Substances Notification Regulations (Organisms)* [NSNR(Organism)] in accordance with Section 106(8) of CEPA 1999. This information element requires data from tests conducted to determine invasiveness of the AAS. The request is based on AquaBounty's assertion that the organism is *manufactured at a location where the person requesting the waiver is able to contain the living organism so as to satisfactorily protect the environment and human health.*

In the opinion of AquaBounty, the waiver request is based on their rational that the living organism is physically contained within land-based facilities and, in the unlikely event of an accidental release, does not have the capacity to become established in the wild. Land-based containment of AAS significantly mitigates any material risk that may be associated with their potential pathogenicity, toxicity and invasiveness in the wild. Therefore, invasiveness will not be a factor in determining toxicity for the proposed use. AquaBounty has provided the following information as justification for the waiver request:

1. Regulatory oversight

Containment at the facility in PEI has been subject to oversight by Fisheries and Oceans Canada and Environment Canada, pursuant to its use for R&D involving transgenic aquatic organisms, since 1996. It has also been subject to assessments of operations by the U.S. Food and Drug Administration with regards to containment practice, adherence to Good Laboratory Practice regulations, Good Clinical Practice guidelines and for acceptability as a manufacturing establishment.

The facility in Panama is subject to oversight by a number of Panamanian authorities including the National Environmental Authority, the Ministry of Agriculture and the National Biosecurity Commission.

Summary: regulatory oversight is in place to ensure that adequate provisions for physical containment of AAS are in place and will continue to be maintained.

2. Security to prevent unlawful entry to facilities or access to AAS during transport

The facility in PEI has in place several security measures to protect both its property and personnel including: an 8 foot high, galvanized chain-linked perimeter fence with locked gates; [redacted]

In addition to its remote location, security at the facility in Panama includes: [redacted]

Steps are also taken to prevent unlawful access to AAS live eggs while in transport between the two facilities. During ground transport from the PEI facility to either the Halifax or Charlottetown airport, the eggs will be in the possession of AquaBounty Canada staff. Air transport from Canada to Panama will be facilitated by a commercial freight-forward company to maintain a chain-of-custody through to its arrival [redacted]. The AAS live eggs will be received in Panama and transported to the grow-out facility under the supervision of an official from the Ministry of Agriculture's (MIDA) Quarantine Department and will be unpacked and inspected at the facility under the supervision of an official from the National Animal Health Authority (DINASA, also a division of MIDA).

<u>Summary:</u> reasonable security and oversight is in place at both facilities to prevent unlawful entries that may result in theft or damage to property and could potentially result in an unintentional release of AAS.

3. Facility siting and construction to mitigate the effects of natural catastrophic events

The facility in PEI is located in a region of the country that is not prone to natural disasters. The most likely natural disaster to challenge the facility's infrastructure and the physical containment of AAS would be a hurricane or the flooding that may result from a tidal surge. To mitigate this threat, the building is built to modern standards of construction for the region and has withstood several incidents of extreme wind, rain and snowfall. [redacted] In addition, the building is sited over 4 meters above the highest sea levels recorded for the region over the past 100 years and is located on a part of the island that is less vulnerable to the effects of tidal or storm surges.

The facility in Panama is located in a region of the country where historical accounts of natural disasters are rare. The most significant threat to the facility would be from flooding of the adjacent Caldera River. Significant floods experienced along the course of this river in 2008 had no effect on the facility. It is believed that the siting of the facility 5 meters above the Caldera River's normal water level is sufficient to avoid flooding in the future.

Summary: both facilities are sited in locations and constructed to standards that prevent the unintentional release of AAS that may result from naturally occurring catastrophic events.

4. Physical containment of AAS in land-based facilities with acceptable confinement procedures and management practices

There will be no intentional release of AAS into the environment. The use of AAS will be restricted to the AquaBounty Canada, Incorporated, land-based facility in Bay

Fortune, PEI and the AquaBounty Panama, SA, (a wholly-own subsidiary of AquaBounty Technologies) land-based facility, near the town of Boquete, Chiriquí Province, Panama. Both facilities are adequately equipped to contain AAS at all life-stages and prevent entry of AAS into the environment. [redacted]

Summary: restricting the production of AAS to only the facilities described in the notification, which have adequate and redundant mechanical barriers and operational procedures to ensure physical containment, the potential for accidental release of AAS into the environment will be minimized. AquaBounty has also committed to ensuring that live eggs exported from the facility in PEI to the facility in Panama, will be reared only at the production site described in the notification and that no live fish of any life stage will be sold or given by AquaBounty Panama to a third party for grow-out. This is also a condition of sale as outlined on the formal label that can be found on p. 579 of the notification (ABT 2013).

5. Incapacity of AAS to become established in the wild

Only AAS eggs that are sterile and female will be shipped from the facility in Canada to the facility in Panama. Sterility is achieved through a standardized process of triploidy induction in which eggs are subjected to high pressure (9500 psi) shortly after fertilization, using a protocol that is 95 to 100% efficient. All-female stocks are achieved through the process of gynogenesis followed by indirect feminization, using a protocol that is 100% efficient.

Should AAS enter the environment in Panama, local conditions in the Caldera River are likely suitable for the survival of AAS. However, sterile individuals will not be able to reproduce and exposure will be limited to the lifetime of the organism. In the rare event of a fertile AAS being released (a fertile individual may result from failure of the sterilization process), it would still not be able to reproduce since there are no male Atlantic salmon present in either the Caldera River (or any other river in region) with which it can mate. Rainbow trout (*Oncorhynchus mykiss*), a close relative of Atlantic salmon (*Salmo salar*) are known to have established populations in the Caldera River, but cannot form viable hybrids with Atlantic salmon. Consequently, exposure to fertile AAS females that may enter the environment in Panama will also be limited to the lifetime of the organism.

Opportunity for the dispersal of AAS away from the AquaBounty Panama facility is also extremely limited. Though conditions in the upper reaches of the Caldera River may be favorable for the survival of salmonids, water temperatures in the lower section of the watershed and the surrounding Pacific Ocean are above the upper range of incipient lethal tolerance for Atlantic salmon. The temperature tolerance range for AAS is not known, but given its reduced metabolic scope relative to Atlantic salmon, it is not expected to have greater upper temperature tolerance. Consequently, the dispersal of any AAS that are accidentally released from the facility in Panama will in all likelihood be restricted to the upper reaches of the Caldera River and there is no chance of dispersal to Canadian territorial waters.

If AAS were to enter the environment in PEI, local conditions in the Bay Fortune Estuary are not suitable for the survival of AAS during its early life-stages (eggs, alevins and fry). Salinities at the point of entry are above the range of incipient lethal tolerance for the early life-stages of Atlantic salmon, which is restricted to freshwater for reproduction and early rearing. In addition, during the coldest months of winter, water temperatures in Bay Fortune range below the lower range of incipient lethal tolerance for Atlantic salmon. Consequently, survival of AAS that are accidentally released from the facility in PEI will be restricted to the spring, summer or fall and to older life-stages (juveniles and adults) that can tolerate the marine environment.

Though the majority of juvenile and adult AAS maintained at the facility in PEI will be fertile males and females that are capable of reproduction, their ability to survive, disperse and reproduce in the wild will be constrained by the behavioral and physiological changes imposed by domestication; changes that have resulted in an overall reduction of fitness, relative to wild Atlantic salmon, in the natural environment.

Summary: in the unlikely event of a physical containment failure in Panama, biological containment measures (sterile, all-female stocks) and physiological barriers (lethal regional water temperatures) will restrict AAS to the upper reaches of a local watershed, prevent the establishment of a viable population (limiting exposure to the organism's lifetime) and prevent dispersal of AAS from the point of entry into the Canadian environment. In the unlikely event of a physical containment failure in PEI, physiological barriers (salinity) will prevent the survival of AAS at early stages of development. Inferior reproductive fitness will limit is ability to reproduce in the wild and establish a viable population.

Fisheries and Oceans Canada is of the opinion that until data from a test conducted to determine the potential invasiveness of AAS has been assessed, there remains considerable uncertainty with respect to the potential risk that AAS may pose to the environment, for the following reasons:

- The environment in which fish are reared can significantly affect the phenotypic expression of the transgene. The influence of rearing environment limits our ability to extrapolate laboratory data as a reliable indicator of how a GE fish may behave (e.g. survive, disperse, compete, reproduce) in the natural environment unless it can be demonstrated that wild-type controls reared in the laboratory environment behave the same way as wild-type fish in the natural environment. In the absence of such control data, there is uncertainty around the extent to which we can rely upon laboratory data as an accurate indicator of behavior in the natural environment;

- The phenotypic effects of the transgene can vary significantly with the genetic background of the parent (e.g. wild-type vs. domesticated, species). For example, the performance of a wild-type fish with an inserted growth hormone gene construct may be very different from the performance of a domesticated fish of the same species into which the same construct has been inserted. Consequently, regulators must scrutinize the background genetics of experimental controls when evaluating the scientific validity of experimental data to assess whether the phenotype is durable across multiple genotypes as would be encountered in nature. Experimental data on transgene expression in one species or strain should be interpreted with caution as it may or may not be representative of the expression of the same transgene in a different species or strain;
- A single transgene may result in several phenotypic expressions, termed pleiotropic effects. For example, some empirical data demonstrates that increased growth in some fish species may also affect disease resistance. Thus, unless the investigator has specifically directed attention towards an unintended effect, it may go undetected; and
- The efficacy of the proposed sterilization procedure is not absolute.

Therefore, the waiver should only be granted if it can be demonstrated with certainty that the notified organism is physically and geographically contained such that it cannot enter the Canadian environment.

Assessment:

The substance, a transgenic Atlantic salmon (*Salmo salar*) bearing the *opAFP-GHc2* construct at the α-locus in the EO-1α lineage and given the common name AquAdvantage® salmon (AAS), is intended to be commercially produced as sterile (triploid) female eggs, at a contained, land-based facility is PEI, for export to a contained, land-based, grow-out facility in the highlands of Panama where they will be grown to a commercial weight of 1 to 3 kg, then harvested, euthanized and transported to a processing plant in close proximity to the Panamanian grow-out facility where they will be processed and exported to the United States for human food consumption. No live AAS are intended to enter the environment outside of the confined, land-based aquaculture facilities that are specified in the current notification.

The AAS was developed by micro-injecting a gene construct (*opAFP-GHc2*) into the egg of a wild-type Atlantic salmon, followed by introgression of the transgene in the initial mosaic founder genotype into a non-transgenic genetic background. The *opAFP-GHc2* gene construct is comprised of a Chinook salmon (*Oncorhynchus tshawytscha*) growth hormone (GH) gene under the control of an ocean pout (*Macrozoarces americanus*) anti-freeze protein (AFP) promoter. The most relevant phenotypic difference between the AAS and non-transgenic Atlantic salmon is the intended increase in growth rate. After 2,700 degree-days, the weight and the length of both diploid and triploid AAS are significantly greater than the diploid and triploid non-transgenic counterparts. However, the accelerated growth-rate of the AAS is not sustained over later stages of development. The organism has been in development since 1992 and reared under research and development conditions at the Ocean Sciences Center at Memorial University of Newfoundland, the Huntsman Marine Science Center in New Brunswick, the AquaBounty Technologies facility near Fortune, PEI and the AquaBounty Technologies facility in Chiriquí province, Panama. It is claimed that, under the described rearing conditions, the AAS reaches market size (1 to 3 kg) faster than their non-transgenic counterparts. The rapid-growth phenotype is intended to create benefit by significantly reducing time-to market.

The AAS is intended for use under strictly controlled conditions that include physical confinement in two clearly defined facilities. Standards for the physical containment of genetically modified fish are currently not available. The U.S. Department of Agriculture's 'Performance Standards for Safely Conducting Research with Genetically Modified Fish and Shellfish' (ABRAC 1995) emphasizes the importance of mechanical barriers, security and the operational procedures that are in place to maintain physical containment and mitigate catastrophic events. It has suggested that 3 to 5 independent barriers along a single pathway are sufficiently redundant to effectively contain an organism. However, it acknowledges that an adequate level of redundancy may depend on the specific location of the facility or the nature of the proposed research. To facilitate the assessment of the physical containment of AAS in both Canada and Panama, a Failure Modes Analyses (FMA) was conducted following guidance from Stamatis 2003 and McDermott et al., 2009. The FMA provided a systematic method for the examination and assessment of each and every element of physical containment. Both the mechanical barriers and the operational procedures in place to maintain and ensure the efficacy of each barrier were considered along with the potential consequences of a failure at each barrier.

At the Canadian facility, there are 16 independent pathways to entry for all life-stages of AAS.
To prevent an accidental release from the facility, there is a minimum of 3 (and as many as 6) independent mechanical barriers along each pathway. The FMA identified a total of 120 independent elements of containment and 328 potential failure modes for all pathways. In all cases, suitable operational measures and oversight are in place to avert or mitigate potential failures and prevent living AAS at all life-stages from entering the Canadian environment. In addition, the facility is sited in locations and constructed to standards that effectively prevent the unintentional release of AAS that may result from naturally occurring catastrophic events. Finally, extensive security measures are in place to prevent any unlawful entry that may result in theft or damage to property.

During transport from the facility in Canada to the facility in Panama, AAS eggs will be securely packed and labelled for shipment by air and chain-of-custody will be maintained through to its arrival at the Panama using a commercial freight–forward company. The AAS eggs will be received and transported to the facility in Panama under the supervision of an official from the Ministry of Agriculture's (MIDA) Quarantine Department and will be unpacked and inspected at the facility under the supervision of an official from the National Animal health Authority (DINASA, also a division of MIDA).

At the Panamanian facility, there are 4 independent pathways to entry for all life-stages of AAS. To prevent an accidental release from the facility, there is a minimum of 4 (and as many as 12) independent mechanical barriers along each pathway. The FMA identified a total of 32 independent elements of containment and 108 potential failure modes for all pathways. In most cases, suitable operational measures are in place to avert or mitigate potential failures and prevent living AAS at all life-stages from entering the Panamanian environment. Further, in the unlikely event of AAS escaping from the facility in Panama, geographical isolation will prohibit AAS from entering the Canadian environment since water temperatures in the region are above the range of tolerance for Atlantic salmon and are in all likelihood above the range of tolerance for AAS. AquaBounty has provided well-defined parameters for the scope of their activity, as outlined above. Proposed parameters (mechanical and geographical containment) have been deemed sufficient to preclude the potential for escape and the possibility of entry into the Canadian environment.

Recommendation:

Fisheries and Oceans Canada evaluators recommend that the waiver request be granted under paragraph 106(8) (b) of the Act. Given the use scenario and that the information provided as rationale for the waiver was considered satisfactory, data on pathogenicity, toxicity or invasiveness as required under paragraph 5(a) of schedule 5 is not needed to determine whether the organism is toxic as defined by S. 64 of CEPA 1999 for the intended and specified use. Any activities outside of the well-defined parameters described above would be considered a significant new activity and would require a Significant New Activity Notice.

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