Fisheries and Oceans Canada | Pêches et Océans Canada
Ecosystems and Oceans Science | Sciences des écosystèmes et des océans

Canadian Science Advisory Secretariat (CSAS)

Research Document 2021/053

National Capital Region

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

Colin McGowan, Caroline Mimeault, Ivan Stefanov, Rosalind Leggatt and Jan Beardall

Fisheries and Oceans Canada
Aquaculture, Biotechnology and Aquatic Animal Health Science
200 Kent Street
Ottawa, ON K1A 0E6

November 2021

Canada

Foreword

This series documents the scientific basis for the evaluation of aquatic resources and ecosystems in Canada. As such, it addresses the issues of the day in the time frames required and the documents it contains are not intended as definitive statements on the subjects addressed but rather as progress reports on ongoing investigations.

Published by:

Fisheries and Oceans Canada
Canadian Science Advisory Secretariat
200 Kent Street
Ottawa ON K1A 0E6

http://www.dfo-mpo.gc.ca/csas-sccs/
csas-sccs@dfo-mpo.gc.ca

ISSN 1919-5044
ISBN 978-0-660-39870-9 Cat. No. Fs70-5/2021-053E-PDF

Correct citation for this publication:

McGowan, C., Mimeault, C., Stefanov, I., Leggatt, R., and Beardall, J. 2021. Environmental and Indirect Human Health Risk Assessment of the AquAdvantage® Salmon. DFO Can. Sci. Advis. Sec. Res. Doc. 2021/053. xiii + 134 p.

Aussi disponible en français :

McGowan, C., Mimeault, C., Stefanov, I., Leggatt, R., and Beardall, J. 2021. Évaluation des risques pour l'environnement et des risques indirects pour la santé humaine posés par le saumon AquAdvantage[MD]. Secr. can. de consult. sci. du MPO. Doc. de rech. 2021/053. xiii + 146 p .

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ACRONYMS

AAS: AquAdvantage® Salmon
ABC: AquaBounty Canada Inc.
ABP: AquaBounty Panama Inc.
ABT: AquaBounty Technologies Inc.
ASCU: Atlantic Salmon Conservation Unit
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
GH: Growth Hormone
GxE: Genotype X Environment interaction
IGF-1: Insulin-like growth factor
IMF: Integrated Modular Filtration
ISAV: Infectious Salmon Anaemia Virus
MPA: Marine protected areas
mRNA: Messenger RNA
MRNF: Ministère des Ressources Naturelles et de la Faune
NSNR(O): *New Substances Notification Regulations (Organisms)*
PEI: Prince Edward Island
PPT: Parts per thousand
rDNA: recombinant DNA
RNA: Ribonucleic acid
SARA: *Species at Risk Act*
SNAc: Significant new activity
SOP: Standard operating procedure

GLOSSARY

Note: Glossary terms may be specific to AquAdvantage® Salmon.

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

Abundance: the relative representation of a species in a particular ecosystem.

Anadromous: having a life-history which involves a migration to salt water followed by a return migration to fresh water to reproduce.

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 EO-1 founder animal.

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: that part of the genome separate from the transgene that is able to influence the phenotype under examination.

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

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.

Biotechnology: as defined in CEPA 1999, “biotechnology” means the application of science and engineering in the direct or indirect use of living organisms or parts or products of living organisms in their natural or modified forms.

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.

Comparator: something used as a standard for comparison.

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-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.

Fitness: the net survival and reproductive capacity of an organism, population, or genotype to generate reproductively competent progeny.

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

Genotype: the genetic constitution of an individual organism.

Genotype x Environment (GxE) interactions: how the genotype interacts with environmental fluctuations to differentially shape the observed phenotypes (morphological, physiological, or behavioural) of two or more genotypes to environmental fluctuations; differential phenotypic plasticity between genotypes.

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: the area or type of site where an individual or wildlife species occurs and depends on directly or indirectly to carry out its life processes; 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 exposure to AAS in the environment.

Introgression: 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 critical influence on ecosystem structure and function.

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.

Mosaic: an organism or part of an organism that is composed of cells with different genotypes.

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

Niche: the resources (in a broad sense) utilized by a population or species.

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.

Phenotype: the set of observable characteristics of an individual resulting from the interaction of its genotype with the environment.

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 prescribed.

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

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

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

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).

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 and the frequency of release events; the number of viable organisms that could arrive in a geographic area over a set time period.

Recombinant DNA (rDNA): DNA sequences that result from the use of laboratory methods to bring together genetic material from multiple sources, creating sequences that would not otherwise be found in biological organisms.

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 in response to environmental conditions differentially affecting the fitness of genotypes.

Significant New Activity (SNAc): in accordance with section 104 of CEPA 1999, “significant new activity” includes, in respect of a living organism, any activity that results or may result in (**a**) the entry or release of the living organism into the environment in a quantity or concentration that, in the Ministers’ opinion, is significantly greater than the quantity or concentration of the living organism that previously entered or was released into the environment; or (**b**) the entry or release of the living organism into the environment or the exposure or potential exposure of the environment to the living organism in a manner and circumstances that, in the Ministers’ opinion, are significantly different from the manner and circumstances in which the living organism previously entered or was released into the environment or of any previous exposure or potential exposure of the environment to the living organism.

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 or genotype 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.

Toxic: In accordance with section 64 of CEPA1999, a substance is toxic if it is entering or may enter the environment in a quantity or concentration or under conditions that (***a***) have or may have an immediate or long-term harmful effect on the environment or its biological diversity; (***b***) constitute or may constitute a danger to the environment on which life depends; or (***c***) constitute or may constitute a danger in Canada to human life or health.

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.

Sources used for the definitions in this glossary include: Anonymous 1996; Lincoln et al. 1998; CEPA 1999; Burgman 2005; Kapuscinski et al. 2007; Mair et al. 2007; Levin 2009; Aas et al. 2011, and NSN 16528.

ABSTRACT

Pursuant to the *Canadian Environmental Protection Act* (CEPA), a notification under the *New Substances Notification Regulations (Organisms)* (NSNR(O)) was submitted by AquaBounty Canada to Environment Canada (EC) for the commercial manufacture and export of the AquAdvantage™ Salmon (AAS), a transgenic Atlantic Salmon claimed to reach market size in half the time of a domesticated Atlantic Salmon. AquaBounty has indicated its intent to commercially produce all-female triploid, AAS eyed-eggs at their contained, land-based Prince Edward Island facility, for export to a contained, land-based grow-out facility in the highlands of Panama, where AAS will be grown to a commercial weight, and then processed for retail sale in approved markets as food. Fisheries and Oceans Canada (DFO) conducted an environmental and indirect human health risk assessment of AAS to support a regulatory decision by the Minister of the Environment and to underpin recommendations on measures necessary to manage risk.

The risk assessment analyzed potential hazards, likelihood of exposure and associated uncertainties, to reach a conclusion on risk. The exposure assessment was based on a Failure Mode Analysis that focused on the potential for AAS to enter the Canadian environment, with activities in Panama only relevant where they may have resulted in AAS exposure to the Canadian environment. The assessment concludes with reasonable certainty that the likelihood of AAS exposure to the Canadian environment is negligible and that AAS is manufactured at a location where AquaBounty is able to contain AAS in a manner that satisfactorily protects the Canadian environment and human health. The indirect human health hazard assessment characterized the potential for AAS to cause adverse effects to humans in Canada, relative to wild Atlantic Salmon, as a consequence of environmental exposure. Potential human health hazards associated with AAS, including potential toxicity and allergenicity and the capacity of AAS to act as a vector for human pathogens, were considered. The assessment concludes with reasonable certainty that the risk to human health in Canada as a consequence of environmental exposure is low. The environmental hazard assessment characterized the nature and severity of the potential harmful effects that AAS may cause to the Canadian environment. Potential hazards associated with AAS, including the potential to affect wild populations, wild Atlantic Salmon predators, prey, competitors, and habitat, were considered. The assessment concludes with reasonable certainty that the risk to the Canadian environment is low.

EXECUTIVE SUMMARY

BACKGROUND

On April 30, 2013, AquaBounty Canada Inc. submitted a regulatory package (NSN 16528) to Environment Canada (EC) under the *New Substances Notification Regulations (Organisms)* [NSNR (Organisms)] of the *Canadian Environmental Protection Act, 1999* (CEPA 1999) for the AquAdvantage® Salmon (AAS). Fisheries and Oceans Canada (DFO) conducted an environmental and indirect human health risk assessment of AAS to support a regulatory decision by the Minister of the Environment and to underpin recommendations on measures necessary to manage risk. The risk assessment was conducted on AquaBounty's proposed use scenario: to grow AAS under the containment conditions specified in the regulatory submission for the PEI and Panamanian facilities.

THE NOTIFIED ORGANISM

The AAS was developed by micro-injecting a gene construct (opAFP-GHc2) into the egg of a wild-type Atlantic Salmon (*Salmo salar*), followed by introgression of the transgene in the initial mosaic founder genotype into a non-transgenic genetic background. The opAFP-GHc2 gene construct consists 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. AquaBounty has indicated its intent to commercially produce all-female triploid, AAS eyed-eggs at their contained, land-based Prince Edward Island (PEI) facility, for export to a contained, land-based grow-out facility in the highlands of Panama. AAS will be grown to a commercial weight, and then processed for retail sale in approved markets for food consumption.

ASSESSMENTS

Exposure

The exposure assessment was based on a Failure Mode Analysis that focused on the potential for exposure of AAS to the Canadian environment; activities in Panama were only relevant where they may have resulted in AAS exposure to the Canadian environment (i.e., the potential for fish to be released in Panama and swim back to Canadian waters).

The assessment concludes with reasonable certainty that the likelihood of AAS exposure to the Canadian environment is negligible. AquaBounty provided well-defined parameters for the scope of their proposed activities in PEI and Panama, including the conditions under which AAS eggs will be produced, transported, and grown-out. Proposed containment measures (physical, biological, and geographical containment) at the PEI and Panamanian facilities were assessed and determined to result in a negligible likelihood of entry into the Canadian environment.

AquaBounty commits to ensuring that live eggs exported from the PEI facility to the facility in Panama will be reared only at the production site described in the regulatory submission and that no live fish of any life stage will be sold or given by AquaBounty Panama to a third party for grow-out. Based on the containment conditions and use scenario proposed by AquaBounty in its regulatory submission, the assessment concludes that AAS is manufactured at a location where AquaBounty is able to contain AAS in a manner that satisfactorily protects the Canadian environment and human health.

Indirect Human Health Risk

The indirect human health hazard assessment characterized the potential for AAS to cause adverse effects to humans in Canada relative to wild Atlantic Salmon as a consequence of environmental exposure to AAS (e.g., recreational swimming and fishing). Potential human health hazards associated with AAS, including potential toxicity and allergenicity and the capacity of AAS to act as a vector for human pathogens, were considered in the indirect human health hazard assessment. Human health hazards resulting from the consumption of AAS as food were not assessed as these are evaluated under the *Food and Drugs Act*.

The assessment concludes with reasonable certainty that the risk to human health in Canada as a consequence of environmental exposure is low. This conclusion was based on the finding that the likelihood of exposure of AAS to the Canadian environment was negligible with reasonable certainty and the finding that the hazards to human health associated with AAS as a consequence of environmental exposure were low with reasonable certainty.

Environmental Risk

The environmental hazard assessment characterized the nature and severity of the potential harmful effects that AAS may cause to the Canadian environment. Potential environmental hazards associated with AAS, including the potential to affect wild populations through impacts on aspects such as wild Atlantic Salmon predators, prey, competitors, and habitat were considered in the environmental hazard assessment. The risk assessment determined the likelihood that a harmful effect would be realized (the risk) based on the exposure and hazard assessments. Uncertainty associated with each element of the risk assessment was reported and was considered when drawing conclusions on the risk assessment and recommending measures to manage risk.

The assessment concludes with reasonable certainty that the risk to the Canadian environment is low. This conclusion was based on the finding that the likelihood of exposure of AAS to the Canadian environment was negligible with reasonable certainty and the finding that the hazard to the environment was high with reasonable uncertainty.

CONCLUSION

Changes to the use scenario or containment measures specified by AquaBounty in its regulatory submission may result in the entry or release of AAS into the environment in a quantity, manner, or under circumstances significantly different to the potential exposure of AAS that was evaluated in the risk assessment. Given the potential hazard of AAS to the environment, as well as the associated uncertainty in the risk of that hazard occurring, any significant new activity may result in an altered exposure and, consequently, in a different risk assessment conclusion than provided in this report.

The emphasis that has been placed on containment to prevent exposure to the Canadian environment—in particular physical containment of AAS, makes it imperative that the use scenario proposed by AquaBounty be maintained. This includes all physical, biological, geographical, and operational containment measures. Therefore, any activities outside of the well-defined parameters that have been described in the regulatory submission may be considered a significant new activity by EC, who is the regulatory authority, and could require a Significant New Activity (SNAc) notification.

1. INTRODUCTION

1.1. PURPOSE OF THIS DOCUMENT

This document comprises the environmental and indirect human health risk assessments conducted in 2013 under the *Canadian Environmental Protection Act, 1999* (CEPA 1999) with respect to the AquAdvantage® Salmon (AAS), a genetically engineered Atlantic Salmon notified by AquaBounty Technologies Inc. under the *New Substances Notification Regulations (Organisms)* [NSNR(O)].

Legislative environmental and human health protection goals are identified in CEPA 1999. This risk assessment identifies environmental and human health protection objectives as they pertain to AquaBounty's proposal. It also elaborates an appropriate scope and focus for the risk assessments, including assessment endpoints, based on the proposed use scenario and relevant hazards. The risk assessment explicitly addresses uncertainty throughout relevant areas of the document.

This environmental and indirect human health risk assessment of AquAdvantage® Salmon was scientifically peer reviewed and concluded within the 120-day legislative timeframe allowed by the NSNR(O) for notifications under Schedule 5.

Further information on CEPA 1999 and 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.

1.2. 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, a substance or organism is "toxic" if it is entering or may enter the environment in a quantity or concentration or under conditions that

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

Anyone proposing to import or manufacture a living animal product of biotechnology in Canada, including a genetically engineered fish, 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. 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 current risk assessment is limited only to the use scenario proposed by AquaBounty Canada Inc.

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 of the

organism, the genetic material of the organism, or material from the organism involved in toxicity into the environment.

Fisheries and Oceans Canada (DFO), Environment Canada, and Health Canada have a Memorandum of Understanding whereby DFO conducts the environmental and indirect human health risk assessments for fish products of biotechnology under the NSNR(O) of CEPA 1999. Based on these assessments, DFO 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, practical or feasible to obtain the test data necessary to generate the information. Under paragraph 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 1.3.6 of this report on the regulatory framework.

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.

1.3. RISK ASSESSMENT PROCESS

Regulatory decisions under CEPA 1999 are based on whether a living organism is toxic 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 incorporates 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 were conducted by DFO on the AquAdvantage® Salmon. The environmental risk assessment considered 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 considered the potential of the organism to pose a risk to human health in Canada from environmental exposure to the organism. The risk assessments followed 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 assessment of AAS conducted by DFO under the NSNR(O) was conducted in accordance with the following principles:

- The risk assessment was science-based and did not include considerations such as socio-economics, ethics, or harm/benefit ratios.
- A case-by-case approach was taken, in which the specific use scenario notified and elaborated by AquaBounty Canada in the regulatory submission, including any containment or mitigation measures, set the specific parameters around the risk assessments (e.g., possible exposure pathways).
- A comprehensive cradle-to-grave approach was taken, in which AAS was assessed from the time of manufacture, 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 were considered in the risk assessment.
- Out-crossing of the AAS with different commercial St. John River strains, as proposed by the notifier, was considered in the risk assessment.
- In assessing the potential environmental and indirect human health risks, the characteristics of AAS were compared to unmodified wild Atlantic Salmon individuals and populations.
- A predicted change to an assessment endpoint beyond the normal or historical range of variation was used as an indicator of a potential effect.
- All available relevant information (e.g., academic, aboriginal, governmental) in addition to that submitted by AquaBounty Canada under the NSNR(O) was used.

The scope of the AAS risk assessment and its conclusions were limited only to the production and grow-out scenario proposed by AquaBounty Canada. This scenario involves egg production and broodstock maintenance at the PEI production facility, egg transportation from the Canadian to the Panamanian facility, and commercial grow-out only at the Panamanian site described in the notification under the containment conditions specified for each facility and during transportation. Where a production range was notified, the highest proposed value in the range was used for the risk assessment. Activities and exposure events in Panama were only considered for their potential to result in 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 AAS to the Canadian environment and human health in Canada was conducted through an extensive assessment of exposure that detailed 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 associated with AAS and their potential direct, indirect, short-term, and long-term effects and severity (see Figure 1.1).

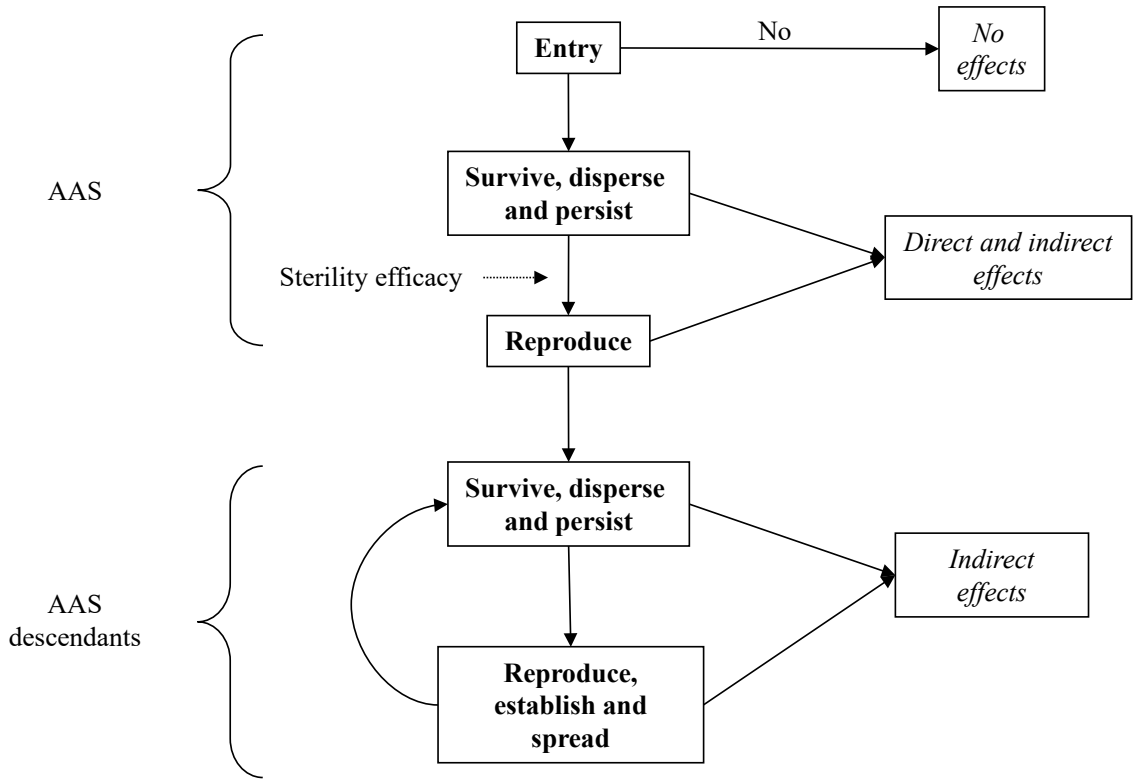


Figure 1.1: Logic model for the environmental risk assessment process (Adapted from Devlin et al. 2006).

1.3.1. Protection Goals

The goal of CEPA 1999 is to protect the Canadian environment and its biological diversity from immediate or long-term harmful effects, protect the environment on which life depends, and protect human life and health in Canada. For the AAS environmental assessment, the key to protecting the Canadian environment is maintaining, in a sustainable form, all of the components of the ecosystem that may interact directly or indirectly with AAS.

DFO's risk assessment conclusions and recommendations to Environment Canada regarding AAS risk management are aligned with these CEPA 1999 environmental and human health protection goals. In particular, DFO's conclusions are based on the likelihood, severity, and reversibility of harmful effects associated with AAS to the structure and function of the Canadian ecosystem or to indirect human health.

1.3.2. Exposure

The exposure assessment for AAS is focused on the Canadian environment. AquaBounty Canada submitted their regulatory package under Schedule 5 of the NSNR(O). On the basis that AAS is contained [see CEPA 1999, paragraph 106(8)(b)], AquaBounty also submitted a waiver request for regulatory information item 5(a) of Schedule 5 in respect of ecological effects of AAS (i.e., data from a test conducted to determine its invasiveness) (NSN 16528). The robustness of containment measures at both the Canadian and Panamanian facilities and during transportation was assessed to determine if a specific legislative test was met: that all life stages of AAS are contained so as to satisfactorily protect the Canadian environment and human health in Canada. DFO's conclusion was that this level of containment was demonstrated.

The assessment of exposure of AAS to the Canadian environment included 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 AAS, the exposure assessment focused 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) as well as the potential magnitude and frequency of dispersal (i.e., propagule pressure);
3. The potential of AAS to reproduce, establish, and spread in the Canadian and Panamanian environments (i.e., fate) as well as the magnitude and frequency of reproduction, establishment, and spread; 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 was examined, the assessment only considered the potential exposure of AAS to the Canadian environment. Consequently, assessment of potential exposure from activities in Panama focused primarily on the potential of AAS to return to Canadian waters, including the Atlantic and Pacific Oceans. Table 1.1 categorizes exposure of AAS in the Canadian environment based on entry and fate elements that were considered in the assessment.

The final ranking for exposure required consideration of multiple elements related to the biological, geographical, and physical containment of AAS, including a variety of pathways, to determine the potential for entry of AAS into the Canadian environment and its fate once there. In many cases, the significance of one element was limited by, or dependent on, another. For example, survival or reproduction in the Canadian environment is dependent on entry into the Canadian environment. Similarly, entry into the Canadian environment is dependent on the likelihood of physical containment failure. When considering physical containment alone, the likelihood of AAS bypassing a downstream barrier is dependent on the failure of all upstream barriers that exist along the same pathway to entry. In this latter example, the likelihood that AAS will bypass a particular barrier 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 1.1: Categorization for exposure of the AquAdvantage® Salmon (AAS) to 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 is capable of reproducing in the Canadian environment but is not able to enter it, 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 some other discrete pathway.

When events are independent from one another, the value of the highest ranked assessment element ultimately determines the exposure outcome and final ranking. For example, if AAS cannot enter the Canadian environment from Panama but could enter the Canadian environment from the PEI facility and survive in moderate numbers, then the final exposure ranking would be moderate.

1.3.3. Hazard

The hazard identification process considered the potential toxicity, allergenicity, and invasiveness of AAS and its capacity to act as a vector for pathogens. In addition, and as part of the invasiveness assessment, other potential ecological effects were identified by considering the AAS phenotypes that may result in a harmful effect.

Harmful effect refers to an immediate or long-term detrimental impact of environmental exposure of AAS on the structure or function of the ecosystem or on human health. 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 were assessed based on changes to the assessment endpoints. Table 1.2 categorizes the severity of potential environmental hazards based on the severity and reversibility of their biological consequences to the structure and function of the ecosystem.

Table 1.2: Categorization 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, e.g., 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.

Hazard would be ranked as negligible when no effects are expected (e.g., if a specific hormone is not expected to be bioactive between species). Table 1.3 categorizes the severity of the indirect human health hazards based on the severity of effects to individuals and the community, and on the availability of prophylactic treatments.

Table 1.3: 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, 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 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 requires consideration of multiple elements. The final ranking for either the environmental or human health hazard is that associated with the highest ranked assessment endpoint.

1.3.4. 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 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 AAS rather than a surrogate), and quality of peer-reviewed information and/or empirical data. Uncertainty in the risk assessment is also influenced by the prevalence of knowledge gaps, the inherent variability of biological systems and experimental data, and the strength of logical deduction and inferences from knowledge of the species.

While some forms of uncertainty can be reduced by filling knowledge gaps or using larger data sets, other forms of uncertainty cannot be reduced, owing to factors such as the inherent complexity and variability of biological systems or the occurrence of chance events. The following measures were employed while conducting the AAS environmental and indirect human health risk assessment to ensure an accurate understanding of uncertainty and to reduce uncertainty to the greatest extent possible:

- The risk assessment and development of recommended risk management measures were conducted using a comprehensive scientific peer-review process to ensure that expert advice was available in all key areas and that knowledge gaps and differences in scientific opinion were identified and adequately resolved wherever possible, and
- Uncertainty was explicitly estimated and stated separately for each element of the exposure and hazard assessments so that the overall risk was not over- or under-represented by the inclusion of cautionary assumptions.

1.3.4.1. Uncertainty in the Exposure Assessment

The exposure assessment required 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 relied on both the design and operational management of facilities, the evaluation of uncertainty relied upon the availability of accurate and detailed information that adequately demonstrated the efficacy and redundancy of mechanical barriers, and the efficacy of standard operating procedures (SOPs). This included diagrams of mechanical barriers and containment systems, incident reports, and training and compliance documentation. It also included information on the occurrence of chance events such as fires, floods, hurricanes, and earthquakes that could lead to a failure of containment (Table 1.4). In contrast, the evaluation of uncertainty associated with exposure that may result from the failure of biological and geographical containment depends on the availability and robustness of scientific information related to the biological and ecological parameters of AAS, valid surrogates, and the receiving environment. A lack of empirical data around the survival, fitness, and ability of AAS to reproduce in the natural environment (i.e., knowledge gaps) also contributes uncertainty to the exposure assessment (Table 1.5).

1.3.4.2. Uncertainty in the Hazard Assessment

Uncertainty in the hazard assessment of AAS is significant, owing to clear knowledge gaps and a lack of empirical data around the behaviour and effects of genetically engineered (GE) fish in general, and AAS in particular, in the natural environment. The knowledge gaps associated with disease susceptibility and the ability of AAS to act as a reservoir for the spread of infectious disease agents to other fish populations in the natural environment also contribute to uncertainty. Further uncertainty in the hazard assessment is added by the complex interactions of pathogens and hosts (behavioural and immune function) with environmental parameters in disease expression.

Table 1.4: Categorization of exposure uncertainty based on the assessment of physical containment (i.e., entry) of the AquAdvantage® (AAS) in the Canadian and Panamanian facilities.

Rank	Description
High certainty	Detailed information on facility design, containment structures, water treatment equipment, SOPs, internal compliance documentation, facility incident reports, and inspection reports are available. Long-term, reliable historical data on relevant chance events at or near the location of each facility are available.
Reasonable certainty	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 are available.
Reasonable uncertainty	Information on facility design, containment structures, and water treatment equipment is available; however, no SOPs or historical data on chance events are available.
High uncertainty	Limited information on facility design, containment structures, and water treatment equipment is available.

Table 1.5: Categorization of exposure uncertainty based on the assessment of effectiveness of biological and geographical containment (i.e., fate) of the AquAdvantage® Salmon (AAS). GxE: Genotype x Environment interaction.

Rank	Description
High certainty	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.
Reasonable certainty	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.
Reasonable uncertainty	Limited data on AAS, AAS-relatives or valid surrogate. Limited data on environmental parameters in the receiving environment. Knowledge gaps. Reliance on expert opinion.
High uncertainty	Significant knowledge gaps. Significant reliance on expert opinion.

The DFO Centre of Expertise for Aquatic Biotechnology Regulatory Research has conducted a significant amount of laboratory research on the fitness and behaviour of genetically engineered fishes to aid in estimating their fitness in the natural environment by comparing results of studies conducted in tanks, semi-natural streams, and mesocosms. Although this research was not conducted on AAS per se, it highlights 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 genetically engineered fishes would behave in the natural environment. These findings are as follows:

- The environment in which fish are reared can significantly affect the phenotypic expression of the transgene plasticity (Devlin et al. 2004; Sundström et al. 2007a). The influence of rearing environment limits our ability to extrapolate laboratory data as a reliable indicator of

how a genetically engineered 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 in the same way that wild-type fish do in the natural environment. In the absence of such control data, there is uncertainty around the extent to which laboratory data can be relied upon as an accurate indicator of behaviour 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). Therefore, 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 be a source of error when determining triploid efficacy induction rates (Devlin et al. 2010).

Given the lack of empirical data around the behaviour and fitness of AAS in the natural environment, significant attention to uncertainty considerations in the hazard assessment was required. Table 1.6 and Table 1.7, respectively, describe the ranking for uncertainty around the potential hazards of the AAS in the environment and to indirect human health. For each parameter being examined, as well as the integration of this information, quality of data refers to 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 among 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.

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

Table 1.6: Categorization of uncertainty related to environmental hazard. AAS: AquAdvantage Salmon; GxE: Genotype x Environment interactions.

Rank	Description
High certainty	High quality data on AAS. Demonstrated absence of GxE effects or complete understanding of GxE effects across relevant environmental conditions. Evidence of low variability.
Reasonable certainty	High quality data on AAS relatives or valid surrogate. Understanding of GxE effects across relevant environmental conditions. Some variability.
Reasonable uncertainty	Limited data on AAS, AAS relatives, or valid surrogate. Limited understanding of GxE effects across relevant environmental conditions. Knowledge gaps. Reliance on expert opinion.
High uncertainty	Significant knowledge gaps. Significant reliance on expert opinion.

Table 1.7: Categorization of uncertainty related to indirect human health hazard.

Rank	Description
High certainty	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.
Reasonable certainty	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.
Reasonable uncertainty	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.
High uncertainty	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).

1.3.5. Risk Estimation

DFO's conclusion of the environmental and indirect human health risk assessment was based on both the overall risk of AAS in the context of AquaBounty's proposed use scenario and the associated level of uncertainty. Figure 1.2 illustrates how the final exposure and hazard rankings were integrated to determine an overall estimate of risk. Each of the four rankings for both exposure and hazard were assigned a numerical value that increased (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 axis and Y axis were multiplied, creating a two-dimensional risk matrix in

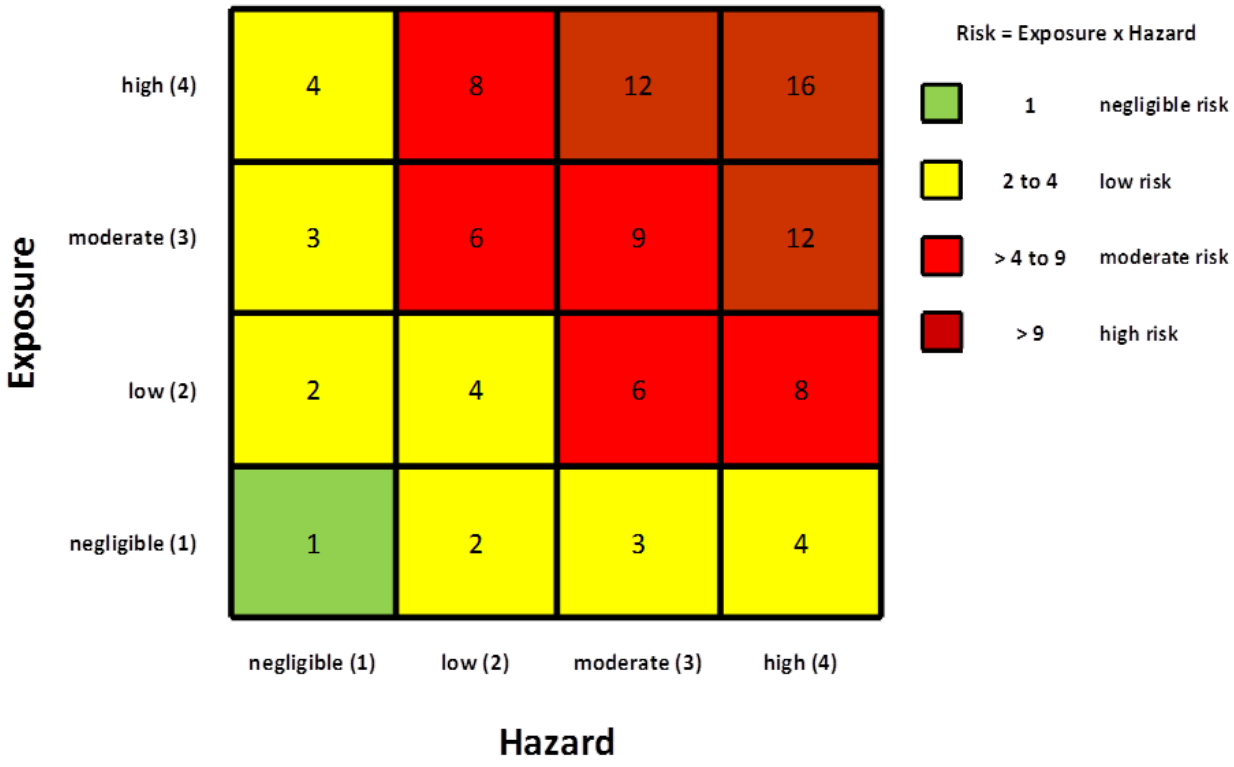


Figure 1.2: Risk matrix used in the integration of exposure and hazard to establish an overall estimate of risk.

which the increasing numerical value within each cell indicates an increasing level of risk. As indicated in the legend, each cell is assigned to one of four risk categories according to the severity of its numerical value.

Uncertainty associated with the risk assessment is explicitly communicated in DFO's conclusions. DFO will recommend that EC consider a conclusion of "CEPA toxic" if the risk is moderate or high. In general, DFO will recommend EC consider a conclusion of not "CEPA toxic" if the risk is negligible or low with reasonable certainty. If the rankings for uncertainty in the hazard and exposure assessments differ, the uncertainty for risk is that associated with the element that limits risk, either exposure or hazard, in the risk assessment paradigm (Risk = Hazard x Exposure). 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 conclusion of not "CEPA toxic" can be made.

1.3.6. Regulatory Framework

Under CEPA 1999, options for managing risks associated with an organism are available depending on the risk assessment outcome and are the responsibility of EC. As shown in Figure 1.3, 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;"

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;” or
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. a prohibition of the manufacture or import of the organism; or
   c. a prohibition pending submission and assessment of additional information determined to be required.

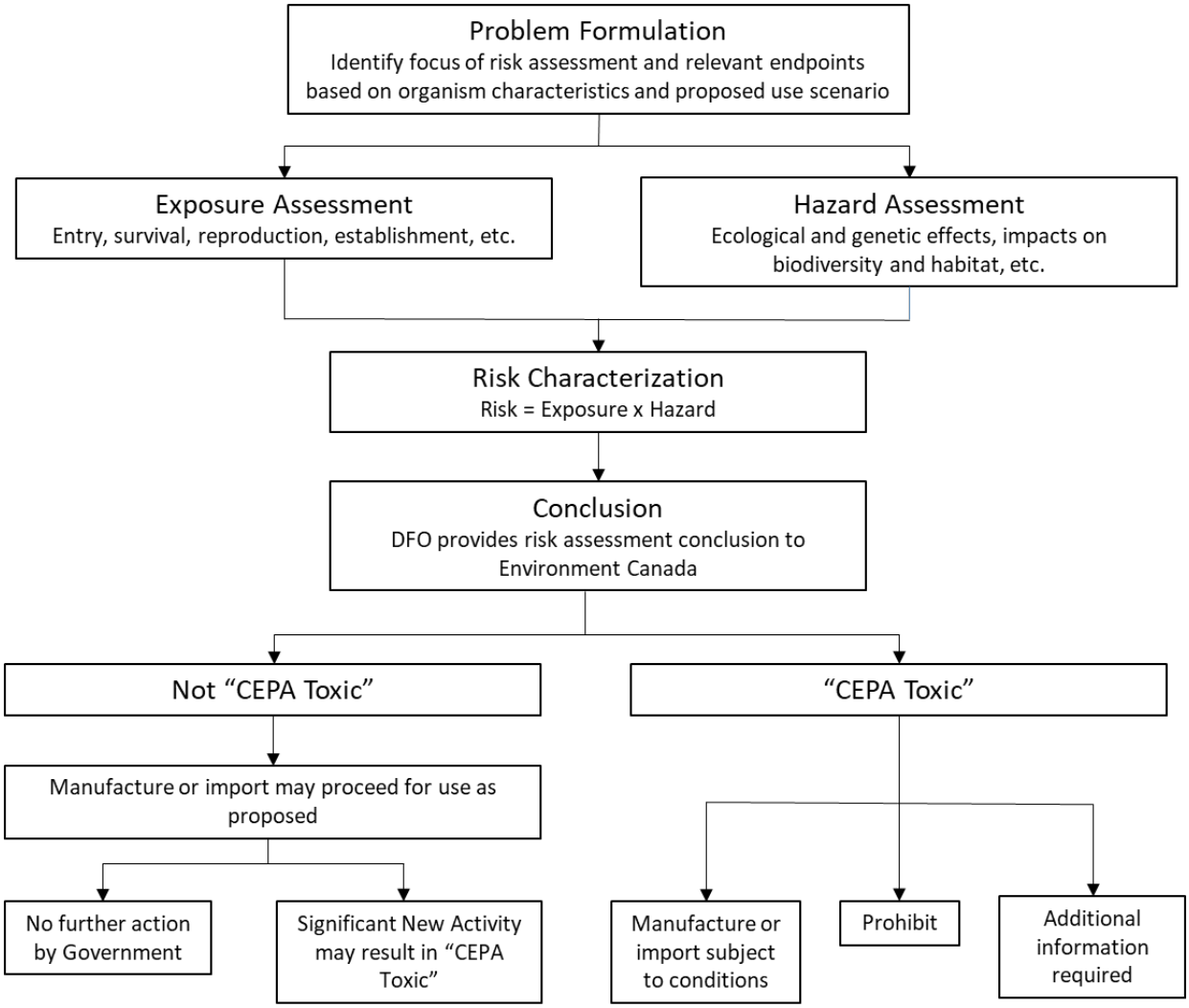


Figure 1.3: Regulatory framework for environmental and indirect human health risk assessments of fish products of biotechnology conducted at Fisheries and Oceans Canada (Adapted from Shahsavarani et al. 2008). CEPA: Canadian Environmental Protection Act, 1999.

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 enable the assessment of the organism under the circumstances of the Significant New Activity 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 (e.g., standards for containment of the organism).

2. BACKGROUND

2.1. AQUABOUNTY AND THE AQUADVANTAGE® SALMON

2.1.1. Company Structure

AquaBounty Technologies Inc. (ABT) is an American biotechnology company with a land-based, contained research and development facility in Prince Edward Island (PEI), Canada. ABT has genetically engineered an Atlantic Salmon (*Salmo salar*) referred to as AquAdvantage® Salmon (AAS) intended for human food consumption. The company claims that AAS grows faster than its non-genetically engineered counterpart. AquaBounty Canada Inc. (ABC) is a wholly-owned subsidiary of AquaBounty Technologies Inc. Corporate (ABT).

ABT has indicated its intention to manufacture triploid eyed-eggs at a single, land-based facility in PEI, Canada. The eggs are intended for the commercial production (i.e., grow-out) of triploid, all-female AAS at a single, land-based facility in Panama. ABC will be the manufacturer-seller of AAS eyed-eggs whereas AquaBounty Panama Inc. (ABP) will be the buyer of the eyed-eggs for commercial production. ABT is the sole owner and operator of both ABC and ABP and will exercise singular and direct control over the critical aspects of manufacture and production involving live animals (NSN 16528).

2.1.2. Proposed (Notified) Use Scenario

As described in section 1.3, the scope of AAS risk assessment and its conclusions are limited to the production and grow-out scenario proposed by ABT. This scenario consists of the following elements: egg production and broodstock maintenance at the PEI production facility, egg transportation from PEI to the Panamanian facility, and commercial grow-out only at the Panamanian site described in the notification. The production and grow-out scenario will be carried out under the containment conditions specified in the notification for each facility and during transportation. In addition, the risk assessment considers potential for the disposal of waste generated from the production of AAS to affect the Canadian environment.

2.1.2.1. Manufacture of AAS (Commercial Egg Production) in PEI

Female and neomale diploid broodstock are maintained at the PEI facility where AAS eggs and milt (sperm cells) are produced. The PEI facility is also where the eggs are fertilized to generate diploid broodstock and, using hydrostatic pressure shocking technology, triploid production fish. ABC has indicated its intention to commercially produce eggs for sterile female AAS at its land-based facility in PEI for export to a land-based grow-out facility in the highlands of western Panama. The planned maximum annual volume of eggs should meet the facility's maximum grow-out potential under its current configuration. ABC also commits to ensuring that live eggs exported from the facility in PEI will be reared only at the Panamanian production site described in the notification and that no live fish of any life stage would be sold or given by AquaBounty Panama Inc. to a third party for grow-out.

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 opAFP-GHc2 transgene. However, female and neomale AAS at all life stages (gametes through to sexually mature adults), as well as all genotypes (i.e., diploids, triploids, hemizygotes, homozygotes) would continue to be reared only at the PEI facility, as is currently the case.

2.1.2.2. Production and Processing of AAS in Panama

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

2.1.2.3. Transportation from Manufacturing Site to Production Site

AquaBounty Canada Inc. has proposed that packaged triploid, transgenic AAS eyed-eggs would be transported by ABC staff for air transport from either Charlottetown, PE (YYG) or Halifax, NS (YHZ) to the Panamanian grow-out facility in accordance with their standard operating procedure (SOP) for the transportation of live gametes (eggs and milt) and live fish (NSN16528). Air transport from Charlottetown or Halifax would be undertaken by a freight-forwarder to maintain chain-of-custody all the way through to arrival in Panama, where ABP staff would receive the shipment directly for transport to the production facility. Coordination of the effort via freight-forwarding will ensure compliance with permitting and other customs requirements.

2.1.2.4. Disposal of Waste

AquaBounty Canada Inc. has included an SOP for the disposal of transgenic fish and/or bio-hazardous waste, which includes dead eggs, alevins, fry, parr, smolt, and adult fish, as well as an operational protocol for the disposal of fish at the ABP facility.

2.2. CHARACTERIZATION OF AAS

2.2.1. Taxonomic Identification

The parental organism originated from Atlantic Salmon (*Salmo salar*) eggs and milt obtained from individuals captured from the Exploits and Colinet, Newfoundland Labrador, Canada (Du et al. 1992a, 1992b; NSN 16528). The opAFP-GHc2 transgene was assembled using genomic DNA isolated from Ocean Pout (*Zoarces americanus*) testes and mRNA isolated from Chinook Salmon (*Oncorhynchus tshawytscha*) pituitary glands (NSN 16528). Details of how the transgene was constructed are reviewed in section 2.2.3.

The AAS line can be distinguished from other Atlantic Salmon lines by detection of the opAFP-GHc2 transgene using one of two polymerase chain reaction (PCR) DNA amplification procedures. The notifier has provided all of the information necessary to perform these two procedures.

2.2.2. 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 and Labrador. 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.

In 1989, Atlantic Salmon eggs originating from the Exploits and Colinet rivers were microinjected with the transgenic constructs at the Ocean Sciences Centre (OSC) at Memorial University in Newfoundland and Labrador, Canada. In 1990, a single F_0 transgenic fry, referred to as the EO-1 female, with greater body weight than non-transgenic individuals, was selected for further development. By 1992 the EO-1 female had reached sexual maturity and her eggs were fertilized with milt of a wild Atlantic Salmon. Two rapidly-growing F_1 progenies were selected for further development.

Other lines of transgenic Atlantic Salmon that carry the opAFP-GHc2 construct, but are the result of other independent insertion events, have been the subject of numerous scientific investigations and published research (Saunders et al. 1998; Stevens et al. 1998; Stevens and Sutterlin 1999; Abrahams and Sutterlin 1999; Cook et al. 2000a, 2000b, 2000c). Although these transgenic Atlantic Salmon have not descended from the EO-1α line, and are, therefore, not a subject of the AAS risk assessment, information regarding the biology of these surrogate organisms (referred to in this report as AAS-relatives) has been included in the assessment.

2.2.3. Genetic Modifications

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

2.2.4. Characterization of the Transgene Construct

Developed in Canada during the late 1980s, the construction of the opAFP-GHc2 transgene involved standard molecular biology and cloning techniques in which the target sequences were first isolated from the source organisms (Chinook Salmon and Ocean Pout) then sequentially ligated into a plasmid vector to form a functional transcriptional unit (Du et al. 1992a, 1992b; NSN 16528). The opAFP-GHc2 construct consists of a 5'-flanking region (5'-FLANK) from the Ocean Pout (*Zoarces americanus*) antifreeze protein (opAFP) 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 opAFP gene, the coding region of the Chinook Salmon (*Oncorhynchus tshawytscha*) growth hormone (GH) gene, and a 3' regulatory sequence (3'OP) from the opAFP gene (see Figure 2.1).

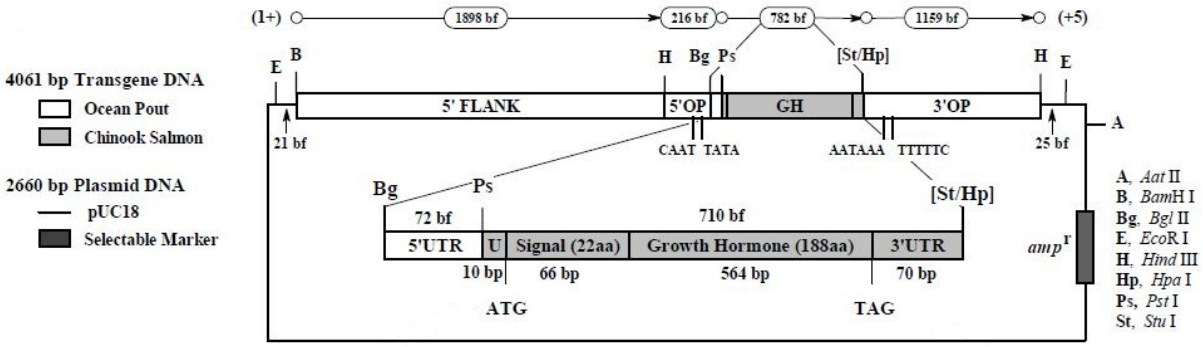


Figure 2.1: Physical characterization of the opAFP-GHc2 construct, ligated into a plasmid vector (NSN 16528).

The opAFP-GHc2 flanking regions and regulatory sequences originated from a Type III AFP gene clone that was isolated from a Charon 30 genomic library produced using a pUC18 vector and the genomic DNA from the testes of an Ocean Pout. Sub-clones in pUC9 were sequenced to identify a 5'-flanking sequence (from pUC18), the AFP gene, and the AFP gene regulatory-control elements. Sequencing also confirmed that the promoter contains the appropriate regulatory sequences including the CAAT and TATA boxes and a transcriptional start site. Evidence of the promoter's capacity to drive expression of a transgene in Rainbow Trout (*Oncorhynchus mykiss*) and Chinook Salmon cell lines was provided through *in-vitro* assays using the opAFP promoter and the bacterial chloramphenicol acetyltransferase (CAT) reporter gene (Du et al. 1992b). The opAFP promoter was also demonstrated to drive reporter gene expression *in vivo* when expressed in Medaka (*Oryzias latipes*) embryos (Du et al. 1992a) and in Atlantic Salmon (Hobbs and Fletcher, 2008). The broad tissue distribution of gene expression in both Atlantic Salmon and Ocean Pout indicates that the promoter lacks tissue specific control elements (Gong et al. 1992; Hobbs and Fletcher, 2008). AquaBounty has demonstrated the functionality of the promoter to drive gene expression in salmonids; since salmonids do not possess antifreeze proteins, the expression of genes driven by this promoter is not expected to be affected by the host genome (Du et al. 1992a). However, expression of the opAFP-GHc2 construct is reported to diminish once AAS reach adult size, suggesting some element of host genome control. The transcriptional terminator sequence for the opAFP gene was also isolated from the same genomic clone as the promoter sequence, but from a different sub-clone. It provides the opAFP-GHc2 construct with a polyadenylation site and its nature does not represent a concern for the risk assessment.

The growth hormone (GH) protein coding region of the opAFP-GHc2 construct was derived from a pUC13 cDNA library clone that was generated using the pituitary gland mRNA of a Chinook Salmon. Direct nucleotide sequencing of the clone confirmed the presence of a translational start codon (ATG) and a translational stop codon (TAG). Although the observed nucleotide sequence of the cDNA clone differed from what was expected by an insertion of two nucleotides in the 3' untranslated region (3'UTR) of the gene, and nucleotide substitutions at the third position of two codons in the protein coding region, these differences did not result in changes to the inferred amino acid sequence. Therefore, the clone was confirmed to contain a full-length cDNA sequence that encodes for a protein with an inferred amino acid sequence identical to that expressed by the endogenous GH-1 Chinook Salmon gene. The inferred amino acid sequences of Atlantic Salmon and Chinook Salmon growth hormone differ by 10 amino acids (95 per cent homology). The protein coding region of the transgene does not represent a concern for the risk assessment.

The step-wise assembly of the AFP regulatory sequences and the GH coding gene into the final opAFP-GHc2 construct was accomplished using standard molecular biology techniques (Du et al. 1992a, 1992b). 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. 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) using the NCBI database.

2.2.4.1. Insertion Methodology

Gene transfer of the opAFP-GHc2 insert into the genome of AAS was accomplished by microinjection of the construct through the micropyle of a recently fertilized, non-activated Atlantic Salmon egg, followed by random insertion of the construct into the genome (Du et al. 1992a). Microinjection was performed after digestion with a restriction enzyme to free the insert from the plasmid vector, but there was no further purification of the insert (NSN 16528). Two concerns are raised by this procedure.

First, although microinjection is a common transgene delivery method in fish (Nam et al. 2007), it often results in multiple copies of the transgene being integrated into different regions of the host genome (MacDonald and Ekker 2012). However, this concern can be alleviated by the appropriate characterization of the transgene and integration site in the host genome and evidence of a single integration event (see section 2.2.5). Second, microinjection of the unpurified digested construct could result in plasmid-vector sequence integration, including antibiotic resistance genes, into the host genome (Gong et al. 2007). This concern can also be alleviated with appropriate evidence to support the absence of vector in the host genome (see section 2.2.5.4).

Mobile genetic elements, such as viral vectors and transposons, are often used to improve the efficiency of transgene integration, but may also increase the risk of mobilization. They are, therefore, considered a concern in the context of an environmental risk assessment (Gong et al. 2007). No mobile genetic elements were used in the development of the opAFP-GHc2 construct and no nucleotide sequence from bacteriophage DNA used in the assembly of the final opAFP-GHc2 construct (Du et al. 1992a) were present in the final construct.

2.2.5. Additional Modifications

The manufacture of all-female, sterile triploid AAS for commercial production requires additional procedures to manipulate the reproductive biology of AAS and provide biological containment. Gynogenesis was used to establish an all-female line of AAS broodstock that are homozygous for the opAFP-GHc2 rDNA construct at the α-locus (bearing two copies of the transgene). Hormonal induction of sex-reversal is used to generate masculinized AAS females (genetic females with a male phenotype, referred to in this report as neomales) that are homozygous for the opAFP-GHc2 rDNA construct at the α-locus and can be used to fertilize non-transgenic Atlantic Salmon eggs to produce hemizygous female AAS (bearing one copy of the transgene). Pressure induced triploidy is used to render AAS production fish functionally sterile. The efficacy of these additional modifications and their potential for concern in the context of the AAS risk assessment are discussed below.

2.2.5.1. Gynogenesis

Gynogenesis was used by AquaBounty during the early development of its all-female commercial broodstock, but gynogenesis is no longer required or used for its maintenance (NSN 16528). Production of gynogenic Atlantic Salmon using irradiated milt from Atlantic Salmon, Rainbow Trout (*Oncorhynchus mykiss*) or Brook Trout (*Salvelinus fontinalis*), typically results in offspring that are 100 per cent female (Quillet and Gaignon 1990; Johnstone and Stet 1995; Pepper et al. 2004). However, phenotypic males have been observed among populations of gynogenic females (Pandian and Koteeswaran 1998). Gynogenesis in Rainbow Trout has occasionally resulted in production of phenotypic males due to a genetic mutation that overrides the sex determination gene in some individuals (Quillet et al. 2002). Through this mutation, phenotypic males can pass the male phenotype on to their offspring, resulting in an ”almost” all-female line having no Y chromosomes present, but with a proportion of male phenotypes that is greater than zero. Whether this mutation is present in other salmonid species has not been determined. Eisbrenner et al. (2013) identified phenotypic males among Tasmanian Atlantic Salmon populations that were predicted to be all-female, suggesting sex determination in Atlantic Salmon may not be solely genetic. King et al. (2012) have presented evidence that differentiation of sex is thermolabile in Atlantic Salmon and that elevated temperatures during embryo development may lead to male phenotypes in gynogenic populations. Thermolabile phenotypic sex determination has also been observed in Sockeye Salmon (*Oncorhynchus nerka*) and Rainbow Trout (Craig et al. 1996; Azuma et al. 2004; Magerhans et al. 2009). Consequently, though current gynogenic procedures are expected to produce populations that

are 100 per cent female, gynogenesis efficacy should still be confirmed when subject to different environmental conditions.

2.2.5.2. Sex-reversal

As described in section 2.2.4.1, propagation of the all-female line is accomplished by taking a portion of the population and inducing sex-reversal through hormonal treatments. Milt obtained from neomales can be used to fertilize normal eggs and produce AAS that are only female. This process, known as indirect feminization (Piferrer 2001), is common practice in the aquaculture industry and is also used in the commercial production of all-female triploid AAS. No complications in the production of AAS are expected from the described sex-reversal process.

2.2.5.3. Induction of Triploidy

The condition of triploidy (having three sets of homologous chromosomes) can be induced in salmonids by applying hydrostatic pressure shock to eggs shortly after they are fertilized, and is an additional genetic modification that is made to AAS prior to export. The hydrostatic shock causes retention of each egg's second polar body and results in an organism with two sets of maternal chromosomes and one set of paternal chromosomes. The process renders fish functionally sterile, providing a useful method of biological containment (Benfey 1999). Though male triploid salmonids can become sexually mature, their sperm cells are typically aneuploid and do not produce viable offspring (Benfey et al. 1986). Female triploid salmonids do not develop to sexual maturity (Benfey et al. 1989). Although the protocols for triploid induction are highly effective, triploidy induction rates of 100 per cent are not always achieved. Devlin et al. (2010) found that induction success was also close to 100 per cent (97 to 99.8 per cent). However, in these studies 39.4 per cent of the apparent diploids were found to contain the GH transgene. Therefore, unless it can be demonstrated otherwise, it should be assumed that a similar proportion of the AAS diploids that may result from failed triploid induction will be hemizygous for the EO-1α transgene.

AquaBounty adequately explains the process of triploid induction in AAS, and provides an acceptable methodology for the estimation of per cent triploidy success on a per batch basis. The process of generating triploids at a commercial scale is almost 100 per cent effective and the proposed sampling procedure to select eggs for export ensures a minimum efficacy of 95 per cent. However, it is not known if any diploid organisms that result from failed triploid induction will carry the opAFP-GHc2 construct transgene.

2.2.6. Characterization of the Transgene Integrant

Relevant genotypic changes are both related to the integration of the transgene and the triploidization of the eyed-eggs. Considerations about the integration of the transgene 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.

AquaBounty has 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). 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. The assessment concludes with reasonable certainty that the nature of the transgene integrant at the EO-1α locus is not of concern for the risk assessment.

2.2.6.1. Sequence of the Integrated Construct

Complete sequencing of the EO-1α locus provided solid evidence of a rearrangement to the opAFP-GHc2 construct upon insertion into the host genome (NSN 16528). The 4205 bp EO-1α integrant consists of the last 613 bp of the Ocean pout AFP 5'regulatory sequence (which, prior to insertion was 2186 bp and included the 5'-flanking region, the AFP promoter and the 5'-untranslated region; see section 2.2.3.1), 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 (see Figure 2.2).

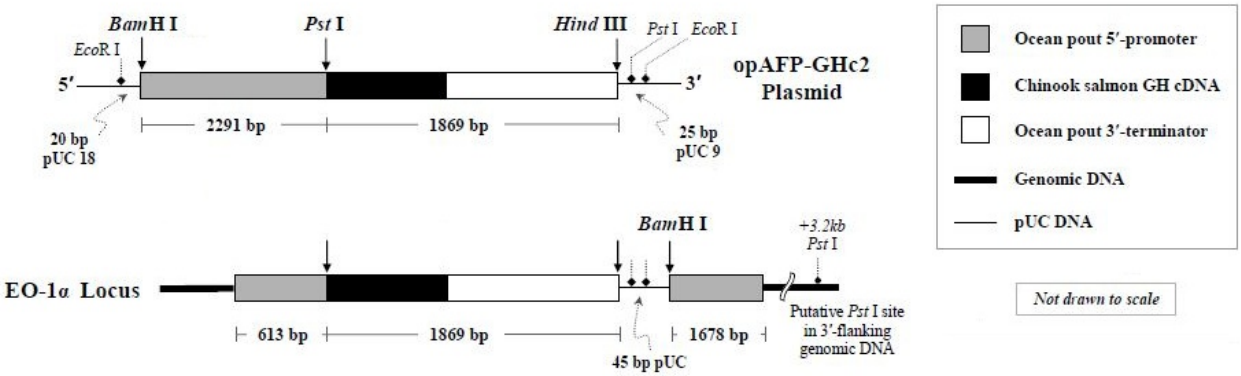


Figure 2.2: Physical structure of the microinjected plasmid construct (opAFPO-GHc2 Plasmid) and the integrated transgene (EO-1α locus) in the AAS genome (NSN 16528).

Nucleotide sequence analysis of the inserted transgene revealed a structural rearrangement of the integrated construct relative to the plasmid construct and the insertion of short, non-coding pUC sequences which are of no concern. Excluding the above differences, sequencing demonstrated complete identity of the integrant in the host genome and the construct.

2.2.6.2. Number of Integrants

The assessment considers both the number of integration sites (loci), and the number of copies integrated at each locus. Uh et al. (2006) reported concatemers of transgenes at single loci in transgenic Coho Salmon.

Results from Southern blot analysis suggest that there were two integration sites (the α- and β loci), in the founder animal (EO-1♀) and early generations (F_1 and F_2) of the AAS line (NSN 16528). Further study determined that these loci were segregating independently of one another and that only the α-integrant confers the enhanced growth phenotype. Through selective breeding, AquaBounty removed the non-functional β-integrant from the AAS EO-1α line and retained the functional α-integrant (NSN 16528).

AquaBounty provided results from Southern blot analysis over five generations of AAS as evidence that only the α-integrant is present, at a single locus, in the AAS line. Genomic DNA was digested with different restriction enzymes (*Pst*I, *Hind*III or *Bgl*II) prior to Southern blot hybridization, using probes designed to anneal at the opAFP promoter of the *opAFP-GHc2* rDNA construct. Differences in the molecular structure of α and β integrants were detected by differences in the *Pst*I digest (NSN 16528). Southern blot hybridization also demonstrated that the β-integrant is absent from later generations. In addition, multiplex PCR was used to confirm the absence of the β-integrant using primer sets that target the construct (NSN 16528).

Genomic Southern blot hybridization has been routinely used in plants (OECD 2010) and in fish (Du et al. 1992a, 1992b) to determine the number of integration sites. Although some uncertainty may remain regarding the sensitivity of the Southern blot detection method (Zhang et al. 2012), the combination of results from both Southern blot analyses and PCR analysis provide sufficient evidence that the β-integrant was successfully removed from the AAS EO-1α line. It is therefore concluded that all broodstock of the AAS EO-1α line contains the opAFP-GHc2 integrant at a single locus (NSN 16528).

2.2.6.3. Position of Integrants

The position of the integrant in the host genome is relevant to both its stability and its effect on the expression of the genes that may surround it (Phillips and Devlin 2009; Ohigashi et al. 2010). Information regarding the specific location of the EO-1α locus in the AAS genome is not required by the NSNR(O) and is not available.

Nucleotide sequencing data provided by AquaBounty to demonstrate the stability of the inserted transgene (reviewed in section 2.2.9) also indicates that the transgene did not disrupt endogenous genes, but is instead flanked by a 35 bp repeat sequence for at least 1136 bp upstream, and 730 bp downstream of the α-integration site (NSN 16528). The absence of coding sequence for endogenous genes in flanking regions alleviates some concern regarding the potential interactions with or disruption of surrounding genes, though some uncertainty regarding the stability of biological properties in nature remains (see section 2.2.7).

2.2.6.4. Vector Sequence in the Host Genome

AquaBounty provides sufficient evidence to conclude that no fragment larger than 161 bp, originating from the pUC vector that was co-injected with the transgene, was incorporated into the genome of AAS. To ensure that there is no expression of additional novel genes and no altered endogenous gene expression, it is important to ensure that no vector DNA has been integrated into the host genome (OECD 2010). AquaBounty provided appropriate controls to demonstrate the presence of genomic DNA on a membrane using the opAFP-GHc/*EcoR*I-*Pst*I digest as probe. AquaBounty has also conducted multiplex PCR analyses to verify that AAS had not incorporated the ampicillin resistance gene from the pUC vector (NSN16528). Results from these studies support the conclusion that neither vector nor ampicillin resistance genes have been incorporated into the genome of AAS.

2.2.7. Expression of the Transgene

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

2.2.7.1. Activity of the Truncated Promoter

Using *in vitro* analysis, AquaBounty tested a series of opAFP promoters of various sizes that were fused to the bacterial chloramphenicol acetyltransferase (CAT) gene (NSN 16528). The variable constructs were microinjected into the embryos of Medaka (*Oryzias latipes*) and transfected into the hematoma or embryonic cells of Rainbow Trout, Chinook Salmon, and Chum Salmon (*Oncorhynchus keta*). The experiments demonstrated that the basal activity of the promoter is retained despite differences at the 5’ end, including truncation of the CAAT sequence (NSN 16528).

Butler and Fletcher (2009) provide further evidence with the transfusion of eleven AFP constructs of various sizes that were fused to a luciferase reporter gene and transfected in salmon and human cell lines. The authors conclude that the expression of the EO-1α transgene in the AAS line was driven by nucleotide elements within the promoter, truncated upstream of the TATA box and, to a lesser degree, by the promoter sequence that was relocated downstream from the GH gene. 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's mechanism. Sequencing of the two regions that flank the integration site has identified a 35 bp repeat sequence that is likely to have limited biological function. Therefore, the relocated sequence is not expected to affect gene expression downstream from the EO-1α locus. Expression of the transgenic growth hormone in the AAS line is further evidence that the truncated promoter is functional.

2.2.7.2. Transgenic Growth Hormone Expression

Transcriptional evidence of the transgenic GH in AAS juveniles of approximately 400 g[1] is provided in 12 different tissues[2] but not in blood cells using reverse transcriptase PCR (Hobbs and Fletcher 2008). Primers used in the study targeted the 5'-junction between the promoter and the transgenic GH coding sequence, amplifying a 331 bp fragment in transgenic individuals, but failing to do so in non-transgenic controls. Northern blot analysis in the same study detected GH only in spleen; however, the sample size was small (n=2) and the study does not consider temporal changes.

Despite a significant difference in average weight (37.0 ± 10.2 g for AAS relatives and 5.94 ± 0.14 g for non-transgenic siblings), no statistical difference was observed between the plasma GH levels of AAS-relatives at the fry stage (39.9 ± 14.8 ng/mL), the 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. 1992a).

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 are limited to GH levels below detection limit in the muscle and skin of commercial size AAS and detectable levels in the plasma of AAS-relatives at the fry stage. Knowing that levels of plasma GH vary with life stages and environmental factors (Björnsson 1997; Ebbesson et al. 2008), it is therefore concluded that available information may not include the highest levels of GH in AAS.

2.2.8. Biological and Ecological Properties

Considerable uncertainty remains in the absence of data regarding the phenotype of AAS in the wild. It is difficult to predict the phenotype of AAS in the natural environment, using information from experiments performed under hatchery conditions. The following sections focus on the biological and ecological properties of the AAS. Information about AAS-relatives (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. Transgene expression and physiological effects are strain specific and depend on the integration site(s). For example, Devlin et al. (2004) observed that different strains of transgenic

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

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

growth-enhanced salmon, injected with the same OnMTGH1 construct, but descended from independent insertion events, have different survival rates and different fry and juvenile growth rates. These findings suggest that the insertion site and transgene structure can affect transgene expression. Therefore, it cannot be assumed that the reported phenotypes for AAS-relatives will be the same for AAS. Consequently, AAS-relatives are included in this section; however, less weight is attributed to this information. Similarly, the biological and ecological properties of other transgenic growth-enhanced salmonids from numerous studies are also incorporated into the hazard assessment, but with an appropriate consideration of uncertainty.

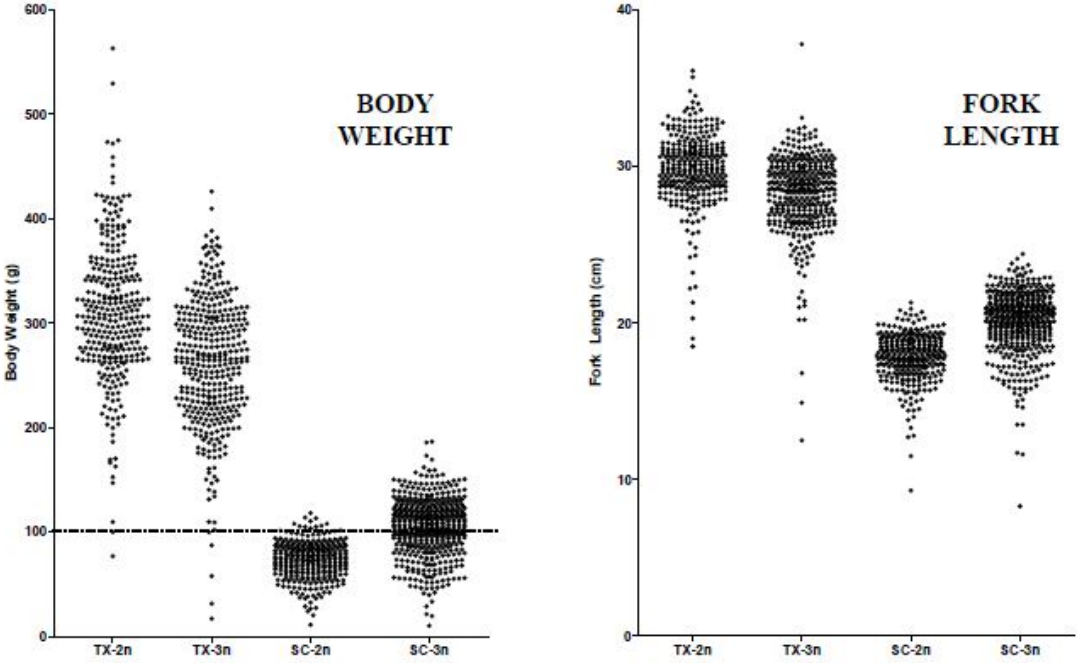
2.2.8.1. Size and Growth

Growth rates reported in studies provided by AquaBounty and published in the scientific literature provide evidence of an enhanced growth phenotype in AAS under hatchery conditions (Figure 2.3). Varying results have been reported under naturalized environmental conditions, highlighting the importance to consider gene–environment interactions.

Moreau and Fleming (2012a) observed that AAS continued to outgrow non-transgenic siblings when raised in hatchery tanks and under food limited conditions for the first year of life (Figure 2.4). However, the size of AAS also varied with feed levels, suggesting that AAS growth is limited by feed availability. When food was limited in an artificial stream microcosm, AAS and control siblings had similar growth rates (Moreau et al. 2011b), though all groups were reported to have lost weight over the 37-day trial period. In these experiments, AAS were derived from an outcross with wild Atlantic Salmon from the Exploits River, rather than St. John River stock. Unpublished results indicated that AAS outgrew non-transgenic controls under hatchery conditions and under artificial stream conditions when food levels remained high (D. Moreau, personal communication, 2013). Variation in the magnitude of growth response under different rearing environments is indicative of gene–environment interactions that can affect expression of the AAS growth phenotype.

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	1.55	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 2.3: Body measuration data for diploid and triploid AAS and control siblings at 2,700 degree-days (NSN 16528).

Though delayed, the phenotypic response to the transgene is observed within the first three months of exogenous feeding (D. Moreau, personal communication, 2013). In addition, the growth rates of AAS and non-transgenic controls in a naturalized stream are lower than under hatchery conditions.

Oke et al. (2013) found that AAS had higher growth rates than the non-transgenic controls under hatchery conditions (approximately 1.8 per cent and 1.4 per cent growth per day, respectively), whereas AAS had lower growth rates than the non-transgenic controls (approximately 0.65 per cent and 1 per cent growth per day, respectively) when reared in an artificial stream microcosm.

Other evidence supporting an increased growth rate include significantly higher mass and length for AAS (14.33 ± 3.32 g and 11.75 ± 0.81 cm) compared to their non-transgenic siblings (2.83 ± 0.75 g and 6.57 ± 0.49 cm) at four months of age (Levesque et al. 2008) and a 3.6 times faster growth rate in F_5 AAS compared to non-transgenic comparators from the St. John River strain (not siblings) (Deitch et al. 2006).

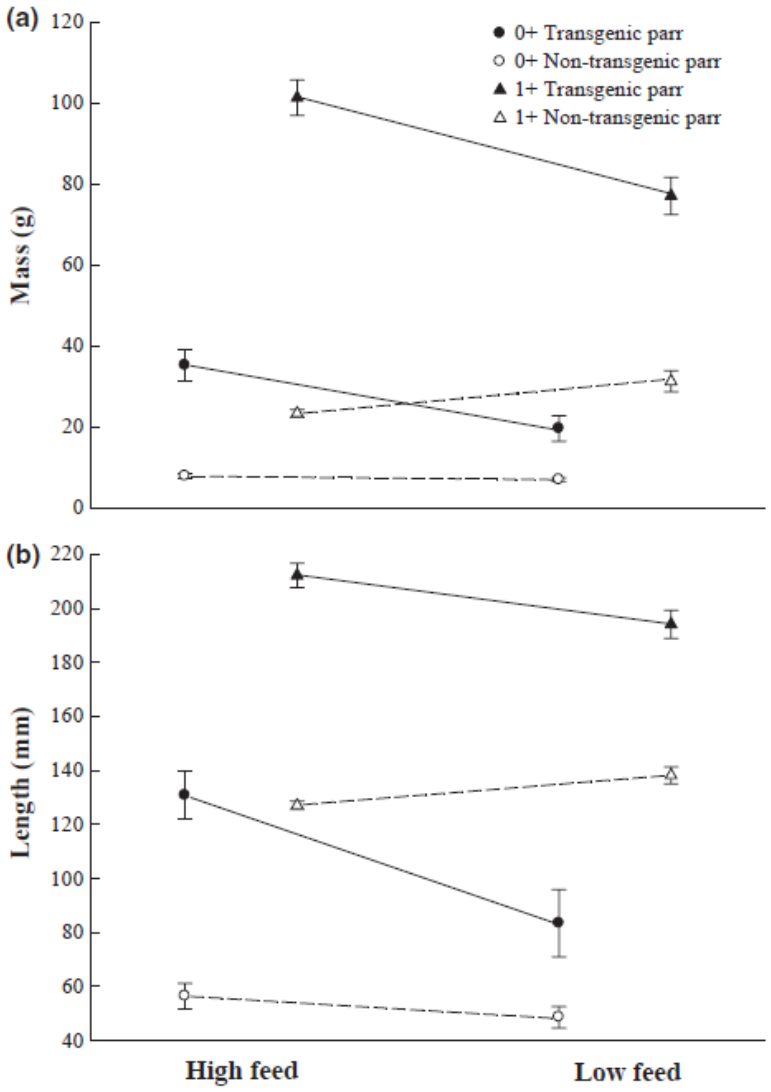


Figure 2.4: Mass and fork length of transgenic (AAS) and non-transgenic precocious male Atlantic Salmon during the first (0+) and second (1+) years of life. High and low feed levels were applied only during the first year of life (from Moreau and Fleming 2012a).

AAS-relatives were also reported to have growth rates two to six times greater than non-transgenic controls during the first year of life (Du et al. 1992a), 2.62 to 2.85 times greater as pre-smolts fed to satiation (Cook et al. 2000a), and 1.57 to 2.1 times in juveniles (Stevens and Sutterlin 1999; Stevens et al. 1999). Abrahams and Sutterlin (1999) also demonstrated that the growth rate of AAS-relatives (1.53 per cent per day) was significantly greater than control fish (not siblings, but from the same strain), over a weight interval of 1 to 10 g.

Based on the above data and studies, as well as scientific publications, the assessment concludes that there is sufficient evidence for the enhanced growth phenotype of the AAS under hatchery conditions. Varying results have been reported under naturalized environmental conditions, highlighting the importance of considerations of gene–environment interactions. The assessment also concludes that there is no evidence to suggest that AAS could not grow to a larger maximum size than non-transgenic Atlantic Salmon.

2.2.8.2. Morphology

Based on several studies and observations, ABC asserts that there are no significant differences between AAS and non-transgenic comparators and that the morphological irregularities in some AAS specimens were of low magnitude and of non-debilitating nature. AquaBounty has provided a study report in which the gross and microscopic morphology and the clinical and histological pathologies of diploid and triploid AAS, and 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 at the same time 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 triploidy in AAS as an indication of general health and welfare.

No obvious or remarkable difference in relative organ weights (gastrointestinal tract, heart, liver, gall bladder, and spleen) between the Treated and Sponsor Control animal subjects, or between the diploid and triploid fish of either treatment, was noted. The authors of this study concluded that abnormalities in gross and microscopic morphology for AAS were of low magnitude, limited distribution, and of a non-debilitating nature (NSN 16528).

Several morphological features were also reported for F_5 AAS (eight 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, opercula 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 bulbous 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 per cent increase in maximum cardiac output as compared to the controls.

Morphological differences were also reported in the gills and gastrointestinal tracts of AAS-relatives compared to their non-transgenic comparators[3]. Growth hormone 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 larger surface area in the pyloric caeca (Stevens et al. 1999). In addition, in contrast to adult AAS (Deitch et al. 2006), pre-smolts of 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. (2006) attributed the reported differences to the higher mass-specific oxygen requirements of the freshwater pre-smolts.

2.2.8.3. Life-history

AquaBounty reports to have never observed any acceleration or delay in time to hatch for diploid, triploid, hemizygous or homozygous growth hormone transgenic Atlantic Salmon relative

[3] 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.

to their non-transgenic counterparts at the research and development (R&D) facility on PEI (NSN 16528).

Moreau (2011) crossed hemizygous AAS males with non-transgenic wild Atlantic Salmon females from the Exploit River. The cross resulted in approximately half of the offspring inheriting the transgene and the other half developing as non-transgenic siblings with a neutral genetic background and an absence of maternal effects. Over 60 per cent of the fish in each family of transgenic and non-transgenic Atlantic Salmon hatched over a period of three to four days (Figure 2.5) (Moreau 2011). AAS 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 2.5 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 (D. Moreau, personal communication, 2013). In the same study, the amount of yolk remaining near emergence time in the transgenic fish (13.38 ± 0.27 mm^2) was slightly greater than in the non-transgenic fish (12.99 ± 0.26 mm^2). Finally, the transgenic fish weigh less than their non-transgenic counterparts (0.148 ± 0.001 g vs. 0.151 ± 0.001 g) and were smaller (25.08 ± 0.09 mm vs. 25.26 ± 0.12 mm) at time of emergence.

The sexual maturation rate of AAS male parr was the same as their non-transgenic siblings, but had slowed to half that of the controls during year two (Figure 2.6; Moreau and Fleming 2012a). Although not quantified, observations of secondary smolt characteristics (silver colouration and loss of parr marks) in the immature transgenic parr (not observed in non-transgenic salmon), suggest that the transgene may influence physiological pathways related to smoltification and to early sexual maturation (Moreau and Fleming 2012a).

AAS-relatives can reach smolt size and completed smoltification at six months of age (Saunders et al. 1998) and AAS-relatives appeared to undergo precocious smoltification, developing silver colouration and a loss of dark vertical parr marks at a smaller size than non-transgenic controls (Cook et al. 2000b).

2.2.8.4. 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 AAS.

Serum analysis revealed significant lower serum glucose and cholesterol in AAS compared to their non-transgenic comparators from common parentage (NSN 16528)[4]. Several other parameters (including chloride, aspartate aminotransferase, bilirubin, total protein, albumin, globulin, calcium, and phosphorous), though different, remained within the normal ranges observed in Atlantic Salmon. No differences were reported for sodium, potassium, and alanine aminotransferase and creatine phosphokinase.

[4] 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.

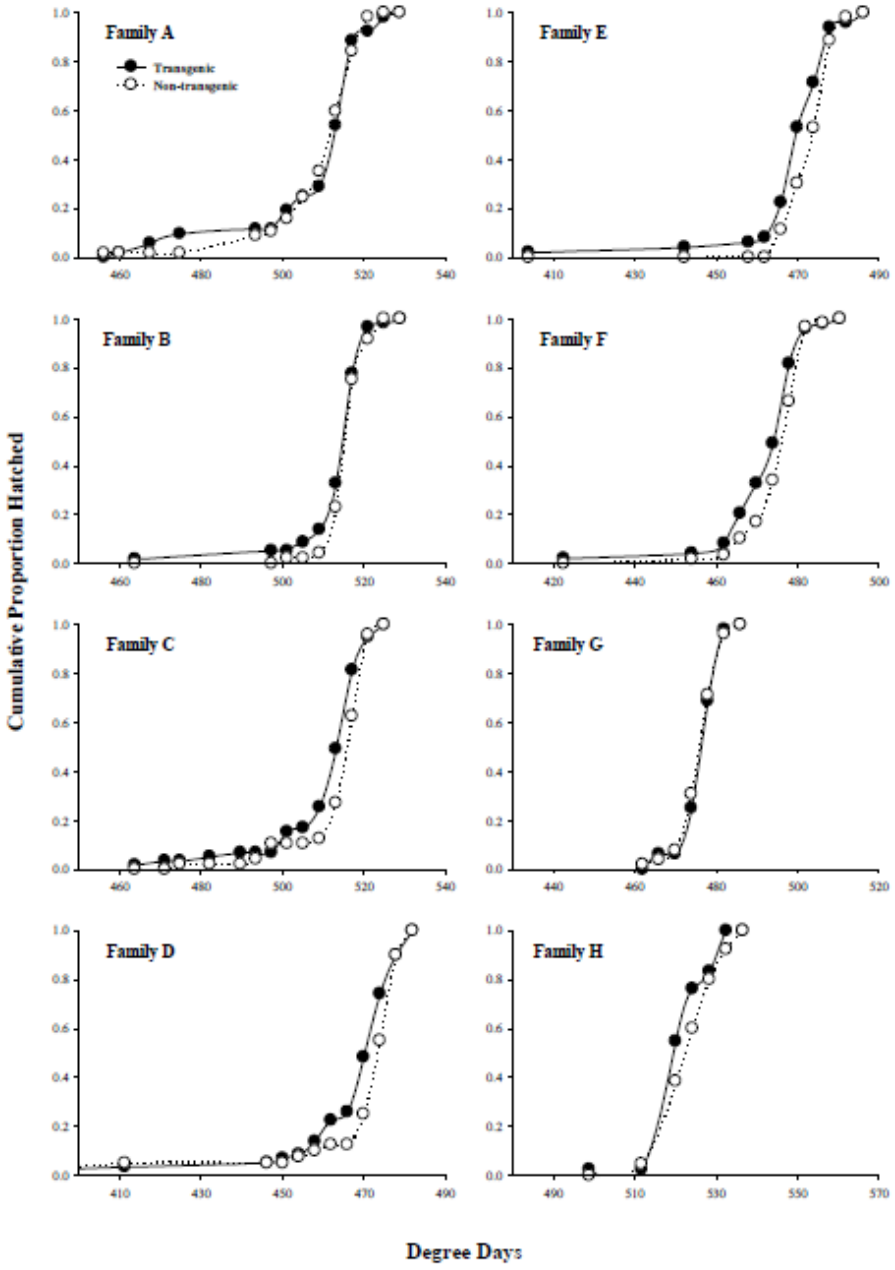


Figure 2.5: Time of hatch (degree days) of full sibling GH-enhanced transgenic and non-transgenic Atlantic Salmon (Salmo salar) from eight families (n=100 eyed-eggs for each family) (from Moreau 2011).

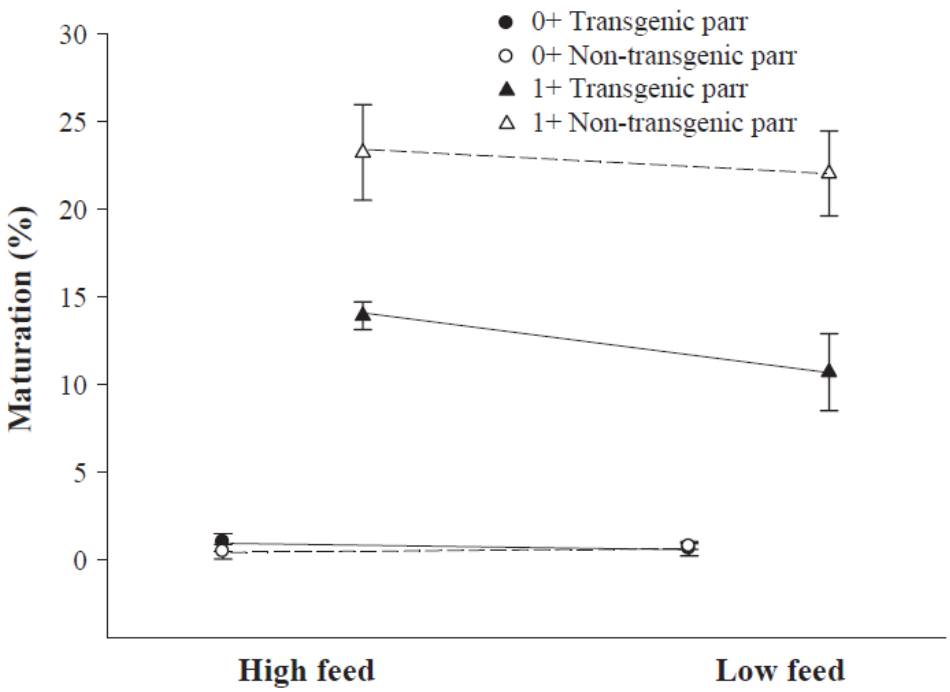


Figure 2.6: Incidence of mature male transgenic and non-transgenic Atlantic Salmon parr during the first (0+) and second (1+) years of life. High and low feed levels were applied in the first year of life only. Transgenic fish are AAS in the Exploit River genetic background (from Moreau and Fleming 2012a).

The respiratory metabolism of AAS during early embryonic development has been examined in several families resulting from crosses between AAS and wild Atlantic Salmon from the Exploit River, Newfoundland and Labrador (Moreau 2011). The oxygen consumption in AAS eyed-eggs and alevins was not significantly different from full sibling controls (Figure 2.7) (Moreau 2011). In addition, mean oxygen consumption in first feeding 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}) was not significantly affected by the transgene. This study suggests the oxygen consumption differences between AAS and non-transgenic Atlantic Salmon to be minimal at this critical early life stage. Moreau (2011) has suggested that fitness of AAS may not be affected during its early ontogeny, possibly the result of the observed delay in the phenotypic expression of the transgene.

Adult AAS have increased oxygen consumption requirements as demonstrated by a 21 to 25 per cent higher oxygen consumption rate in F_5 AAS (828 ± 40 g) compared to their size-matched non-transgenic comparators (884 ± 86 g) from the St. John River strain (not siblings). However, they do not appear to have different maximum oxygen consumption rates (Deitch et al. 2006).

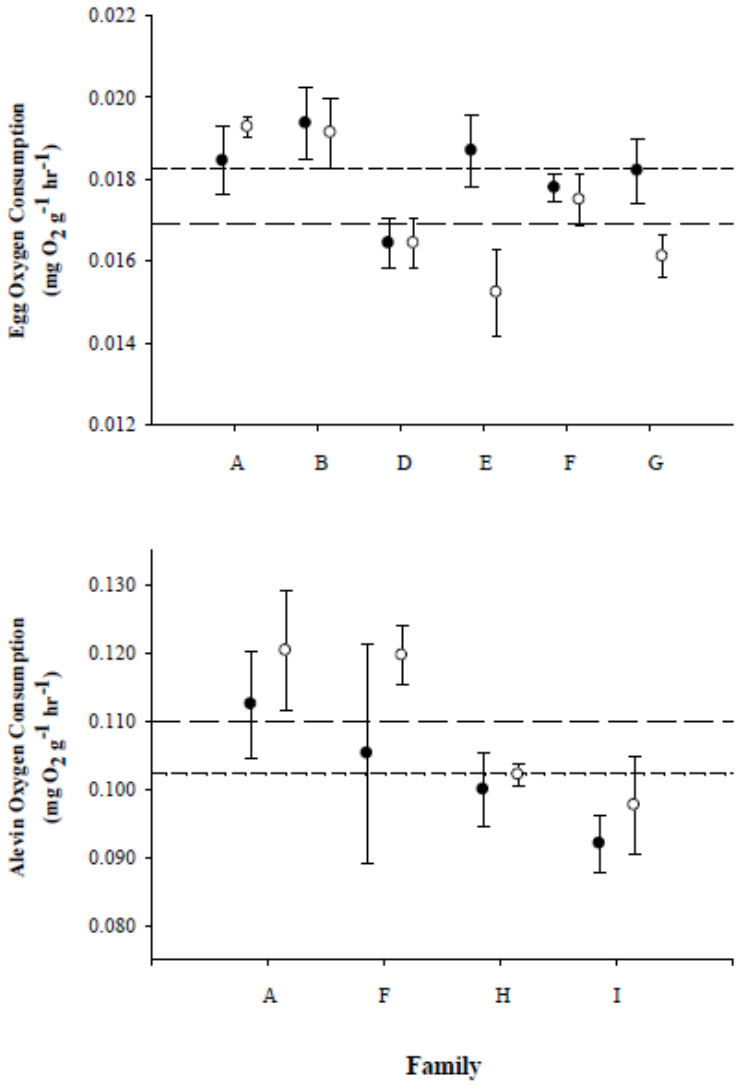


Figure 2.7: Oxygen consumption in transgenic (black circles) and non-transgenic (white circles) full-sibling Atlantic Salmon eggs (top panel) and alevins (bottom panel). Data represent mean values within different families. The overall transgenic and non-transgenic means are represented by the short and long dashed lines, respectively. Transgenic fish are AAS with an Exploit River genetic background (from Moreau 2011).

Other metabolic and physiological differences include an 18 per cent lower metabolic scope, 9 per cent lower critical swimming speed, 29 per cent larger hearts, 18 per cent greater mass-specific *in situ* maximum cardiac output, 14 per cent higher post-stress blood hemoglobin concentrations, and 5 to 10 per cent higher aerobic enzyme activities (Deitch et al. 2006). The authors concluded the limited ability of the adult AAS to elevate its maximum metabolic rate and swimming performance to be related to their findings of no changes in the gill surface area in AAS compared to non-transgenic counterparts. Together, the above studies demonstrate physiological and metabolic differences between different life stages of AAS and highlight the importance of considering the entire life cycle of the organism in risk assessment.

Metabolic and physiological differences are also reported in AAS-relatives. Abrahams and Sutterlin (1999) demonstrated the rates of feed consumption for AAS-relatives to be approximately five times that of non-transgenic controls (fish from the same strain but not siblings) over a weight interval of 1 to 10 g. Cook et al. (2000a) observed feed consumption rates 2.14 to 2.62 times greater for AAS-relatives than for controls over a pre-smolt body weight interval of 8 to 55 g. They also observed increased appetite and a 10 per cent improvement in gross feed conversion efficiency.[5] Routine oxygen consumption rates were 1.54 to 1.70 times higher in AAS-relatives than non-transgenic controls over the same weight interval (Cook et al. 2000b). Oxygen consumption remained 1.58 to 2.30 higher in transgenic Atlantic Salmon than in non-transgenic controls after 24 hours of starvation (Cook et al. 2000b). As starvation progressed over 8 weeks, transgenic fish exhibited a more rapid decline in oxygen consumption, body protein, lipid, and energy reserves than controls (Cook et al. 2000c). Oxygen uptake in AAS-relatives was 1.7 times higher than in control 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 controls during forced swimming activity. Critical swimming speed (speed at which a fish will exhaust itself within a set time period) was not different between groups (Stevens et al. 1998).

2.2.8.5. Endocrinology

Available data for the GH concentrations in AAS are scarce. Plasma GH concentrations across the life cycle of AAS have not been reported. Muscle-skin of commercial size AAS and non-transgenic counterparts have GH levels below detection limit, suggesting that there is 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.

AquaBounty conducted a blind comparative muscle-skin hormonal composition study of domesticated Atlantic Salmon and AAS (NSN 16528). In this study, muscle-skin samples were collected from F_4 AAS individuals[6] (n=30), non-transgenic controls[7] (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), but the size of the farmed commercial fish is not reported. Samples were analyzed for growth hormone (GH), insulin-like growth factor-1 (IGF), triiodothyronine (T3), tetraiodothyronine (T4), estradiol (EST), testosterone (TT), and 11-keto-testosterone (11kT). These analyses were conducted by a private laboratory in accordance with good laboratory practices (GLP) regulations under approved SOPs using radio-immunoassays (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 (NSN 16528). It should be noted that the farmed comparators were not included in the statistical analysis and that a sub-sample of the non-transgenic Sponsor Control (SC) was selected for the analysis. The analysis concluded that there were no significant differences in the hormone levels of muscle-skin samples taken from

[5] The authors calculated “feed conversion efficiencies” as the wet weight of gain by fish over the dry weight of feed consumed by fish (Cook et al. 2000a).

[6] Specific position in the genealogy of sampled fish is not specified in the study report (NSN 16528). Nevertheless, summary genealogy in the notification suggests that the fish are from individuals transferred to the PEI facility from which the AAS line descends.

[7] Sponsor Control animal subjects were the progeny of wild-type male and female Atlantic Salmon maintained at the facility (NSN 16528).

the AAS and the SC, at commercial size (NSN 16528). However, GH and IGF-I levels may also be influenced by feed intake (Raven et al. 2008).

Deitch et al. (2006) reported eight-month-old F_5 AAS to have similar resting cortisol levels than size-matched, twenty-month-old non-transgenic comparators[8] (Table 2.1). Plasma levels of epinephrine, norephinephrine, and total cathecholamines were significantly higher in transgenic fish compared to controls, with the exception of epinephrine in the rested fish. Results suggest a potential impaired cortisol response in AAS compared to Atlantic Salmon, suggesting a potential impact on the hypothalamic-pituitary-adrenal axis. In addition, results suggest a different catecholamine response in AAS and their non-transgenic counterparts.

		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.

Figure 2.8: Plasma cortisol and catecholamine levels in rested and stressed GH transgenic and control Atlantic Salmon (from Deitch et al. 2006).

Du et al. (1992a) observed that plasma T_3 levels in the five biggest aged-matched non-transgenic Atlantic Salmon parr (2.8 ± 0.5 ng/mL) were significantly higher than that of AAS-relatives (1.1 ± 0.5 ng/mL) and smaller non-transgenic siblings (1.9 ± 0.1 ng/mL).

2.2.8.6. Behaviour

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

Under hatchery conditions, AAS display no abnormal postures or abnormal feeding and avoidance behaviour relative to hatchery reared domesticated Atlantic Salmon. Territorial defence of natural habitats at the fry stage was investigated by Moreau et al. (2011a) in experimental stream mesocosms using AAS crossed with wild fish from the Exploit River, Newfoundland and Labrador. These experiments determined that territorial dominance benefits from residency rather than the AAS genotype. There have been no studies to investigate behavioural changes in AAS that might have an effect on prey selection, food preference, schooling tendency, predator avoidance, and migration.

Abrahams and Sutterlin (1999) found that AAS-relatives spent significantly more time feeding in the presence of a predator than did the non-transgenic controls. They also found that the AAS-

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

relatives had a significantly higher average speed of movement (328 cm/min) than controls (96 cm/min). In these experiments AAS-relatives were more willing to risk predation while foraging, than non-transgenic Atlantic Salmon.

2.2.8.7. Reproduction

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

The fecundity and fertility of AAS females relative to wild conspecifics has not been examined. However, studies conducted under a naturalized stream mesocosm reported that wild anadromous males outperform hatchery-reared AAS anadromous males in terms of nest fidelity, quivering frequency, and spawn participation (Moreau et al. 2011a). In addition, despite displaying less aggression, hatchery reared Atlantic Salmon mature parr were superior competitors relative to AAS parr in terms of nest fidelity and spawn participation (Moreau et al. 2011a). 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 2012a). 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.

Data supplied by AquaBounty suggest that similar sized AAS and Atlantic Salmon females have similar fecundity; however, the reproductive success of AAS females in the wild has never been addressed. This knowledge gap significantly limits any prediction of overall reproductive fitness of AAS in the natural environment, since females must allocate more energy in offspring production than males (Fleming 1996).

Reproductive performance ultimately depends on the survival rates of offspring. Under simulated natural rearing conditions, the GH transgene did not influence survival or growth, up to the onset of exogenous feeding (Moreau et al. 2011b). However, beyond first feeding, both groups lost weight, hence the results require cautious interpretation. Nevertheless, the study provides evidence that AAS, after hatching under naturalized conditions, can survive past the first feeding stage under food-limited conditions.

Reproduction of triploid AAS is also relevant as AquaBounty proposes to export triploid female eyed-eggs (NSN 16528). As reviewed in the section on triploidy (section 2.2.4.3), triploid AAS, like other triploid fish, are not expected to mature sexually and are, therefore, considered to be functionally sterile.

2.2.8.8. Health Status

2.2.8.8.1. Disease susceptibility

In disease challenge experiments using GH transgenic Coho Salmon, Kim et al. (2013) concluded that the transgenics were more susceptible to *A. salmonicida* challenge than non-transgenic (wild) controls. For the purposes of the AAS risk assessment, the relative disease susceptibility of AAS compared to wild Atlantic Salmon and the ability of AAS to act as a vector for pathogens compared to wild Atlantic Salmon is of interest. Based on data provided by the company, the assessment concludes with reasonable certainty that AAS is susceptible to *A. salmonicida* and infectious salmon anaemia virus (ISAV); however, available data cannot conclude with reasonable certainty the relative disease susceptibility of AAS and wild Atlantic Salmon.

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 ISAV susceptibility.

Based on Fish Health Certificate data, it is concluded that disease risk at the AquaBounty facility in PEI is well managed.

2.2.8.8.2. Morphological irregularities

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, soft tissue mineralization, hepatic vacuolization, and cardiac shape abnormalities, were associated with triploidy.

Data regarding the gross morphology of AAS (see section 2.2.7.2) were reviewed by a Doctor of Veterinary Medicine (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 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 AAS line in commercial production" was a reasonable conclusion based on the findings that were presented. However, this conclusion is less certain given the shortcomings of the study design and the 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 AAS (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". 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, the inflammatory lesions in the transgenic fish were deemed incidental. This conclusion is not challenged, based on the results and diagnostic materials considered in the study. However, the study was restricted to 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).
- AquaBounty states: "In the aggregate, these findings were generally of low magnitude, limited distribution, and a non-debilitating nature that would be unlikely to compromise the overall health of AAS in commercial production." Presumably, this alludes to at least a part of the client's study objective that involved determining whether the AAS triploid fish are

healthy enough to withstand the rigors of commercial production. The study design was too restrictive in scope to provide a satisfactory answer to this question.

Based on studies and observations of gross morphological and clinical pathologies, the assessment concludes with reasonable uncertainty that morphological irregularities derived from the transgene do not represent serious fish health issues, and are unlikely to compromise the overall health of these fish during commercial production.

2.2.8.8.3. Tolerance to physical factors

There are no studies comparing the biological tolerances of AAS with that of a non-transgenic comparator. Physical factors such as temperature, salinity, oxygen, and pH can have significant influence over the survival and persistence of Atlantic Salmon in the wild (see section 2.3.3). In early life, the low oxygen conditions that can occur beneath gravel stream beds is unlikely to affect AAS eyed-eggs, alevins, and young fry any differently than wild salmon (Moreau 2011). However, the increased oxygen requirements of AAS, without an increase in gill surface area (Deitch et al. 2006), provides indirect evidence of reduced metabolic scope in AAS and reduced capacity to survive up to adulthood under lower dissolved oxygen concentration or higher water temperatures than are tolerated by non-transgenic Atlantic Salmon.

Observations of secondary smolt characteristics in immature AAS parr, as opposed to the non-transgenic controls, suggest preferential physiological pathways towards smoltification (Moreau and Fleming 2012a). AAS-relatives can become smolts at six months of age and, therefore, tolerate direct transfer from fresh water to 35‰ salinity and survive over 96 hours, which contrasts with their aged-matched non-transgenic counterparts that all died within 24 hours[9] (Saunders et al. 1998).[10] 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.

2.2.8.9. Body Composition

AquaBounty provided information about the body composition of AAS at market size (2.0 to 7.5 kg) (NSN 16528). Although this information is mainly relevant to safety for human consumption, extreme deviations from the body composition of wild Atlantic Salmon could potentially affect predators of Atlantic Salmon.

AAS and controls enrolled in the study were raised on a commercial salmon diet (Moore-Clarke, 1140 Industrial Way, Longview, WA, United States) consisting of three different proteins (ranging from 37 to 46 per cent) and fat (25 to 36 per cent) content, depending on the stage (NSN 16528; USFDA 2010). AAS and non-transgenic controls were fed similar diets during the three months prior to sample collection (USFDA 2010). Samples from AAS contained 71 per cent higher total fat, 6 per cent lower protein, 13 per cent lower pantothenic acid, 21 per cent lower vitamin B1, and 30 per cent lower vitamin C compared to the non-transgenic control samples. Despite other small differences (less than 10 per cent) between AAS compared and non-transgenic controls, all reported values were similar to farmed salmon (NSN 16528). Considering the remote potential of any hazard to predators resulting from the body composition of AAS, a full analysis of the compositional and nutritional raw data provided by ABC was not

[9] Fish were transferred to seawater when they had reached (for transgenic) or were approaching (for non-transgenic) smolt size (14 to 16 cm).

[10] The genotype of fish in this study is determined 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.

performed. Based on the data provided by ABC and the USFDA, it was concluded that market size AAS has a similar body composition to other commercial Atlantic Salmon strains when fed the identified commercial diets. 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, remains unknown.

Juvenile AAS-relatives (ranging from 8 to 55 g) have less body fat than their non-transgenic comparators, which is reported to be a function of their elevated metabolic rates (Cook et al. 2000a). Uncertainty remains about the effects of opAFP-GHc2 on the fat content in Atlantic Salmon. Several factors are known to influence lipid content in Atlantic Salmon, including size (Shearer et al. 1994) and ration levels (R. Devlin, personal communication, 2013).

2.2.9. Inheritance of the Transgene

The determination of the inheritance mechanism is based on ratios of non-transgenic to transgenic individuals as determined by PCR.[11] The notifier provided inheritance ratios for 80 different crosses over five generations of AAS in both families of AAS. Progenies of crosses of males from the F_1 generation with non-transgenic females resulted in 73 per cent and 67 per cent of transgenic fry, respectively, suggesting a Mendelian inheritance of two independently segregated integrants (named α and β) at chromosomally distinct loci (NSN 16528). Selective breeding was applied to increase growth performance and lead to the establishment of an AAS broodstock that only bears the α-integrant. Progenies of crosses of males from the F_2 generation, derived from 3482αβ, with wild-type females all resulted in 50 per cent 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 (NSN 16528). The notifier also provided evidence of a transgene inheritance percentage of 0 per cent and 100 per cent for crosses between wild-type fish and involving transgenic homozygous fish, respectively (NSN 16528). The evidence is considered adequate to conclude that there is a Mendelian inheritance of the opAFP-GHc2 transgene across generations in the AAS.

2.2.10. Stability of the Transgene

There is sufficient evidence over five generations to conclude that there is 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.

2.2.10.1. Genotypic Stability

There is sufficient evidence, based on multi-generational sequencing and multiplex PCR, to conclude that there is molecular stability of the opAFP-GHc2 transgene at the EO-1α locus. Broodstock 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 (NSN 16528). Additional demonstration of 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 (NSN 16528). It should be noted that the insertion of the transgene in a simple

[11] 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

sequence repeat region of the genome has the potential to alter locus structure, but only over evolutionary timeframes (Grechko 2011).

2.2.10.2. Phenotypic Stability

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 ABC and in numerous published papers on AAS and relatives (see section 2.2.7.1). However, there are 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 ABC. 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. The effects of different standard culture conditions on accelerated growth of AAS fish would provide valuable information on the phenotypic stability, but have not been directly assessed. AAS fish maintained high growth rate and body size at the PEI facility, at the Ocean Sciences Centre, and at the grow-out site in Panama, However, 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 and four or more times greater in size at 15 to 18 months when grown at the PEI facility. 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.

Of particular interest is whether AAS would maintain high growth rates if released into natural environments. Oke et al. (2013) found AAS fry grown in a hatchery had a 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 lost their phenotypic high growth rate, to the point of having lower growth than that of non-transgenic fish. Moreau (2011) also found the AAS did not have increased growth or size above non-transgenic fish for two weeks post-emergence in an artificial stream with limited live food and low or high density. This latter experiment should be interpreted with caution, as all fish had a 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 (2012a) 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 that 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.

2.3. BIOLOGY OF WILD ATLANTIC SALMON

The Atlantic Salmon (*Salmo salar*) is world renowned for both its spectacular life-history and its economic importance to recreational and commercial fisheries. It has been exploited for

centuries and, in Canada as elsewhere, has experienced significant declines due to a combination of factors that include habitat destruction, pollution, over-exploitation, climate change, and invasive species. Concern for the ongoing, sustainable exploitation of Atlantic Salmon has resulted in tens of thousands of scholarly papers and monographs on the ecology, distribution, behaviour, physiology, genetics, taxonomy, and all other aspects of Atlantic Salmon life. There are also numerous policies, position papers, popular science, and media articles related to its utilization, management, cultivation, and preservation. Not surprisingly, Atlantic Salmon is one of the most studied fish species in the world.

Comprehensive reviews of Atlantic Salmon ecology and genetics can be found in Aas et al. (2011) and Verspoor et al. (2007), respectively. Here, a brief overview of Atlantic Salmon biology is provided with an emphasis on domesticated Atlantic Salmon.

2.3.1. Taxonomic Status of Atlantic Salmon

Atlantic Salmon has been classified as a distinct species for over 250 years. Linnaeus classified the Atlantic Salmon as the species *Salmo salar* in 1758. It is one of approximately 20 species in the sub-family Salmoninae, of the Salmonidae family. The genus *Salmo* consists of two species—the Atlantic Salmon and Brown Trout (*Salmo trutta*). In the past, these species have been viewed to be composed of a number of distinct evolutionary lineages (polytypic origin); however, most contemporary researchers consider these species to be monotypic, with a high degree of phenotypic plasticity (King et al. 2007; Webb et al. 2007).

2.3.2. Distribution

The native distribution of Atlantic Salmon is throughout the North Atlantic Ocean and its associated freshwater drainage basins (Scott and Crossman 1973; MacCrimmon and Gots 1979; Webb et al. 2007; Thorstad et al. 2011). They are 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 phase of their life cycle, the freshwater spawning and rearing habitat forms highly structured and population specific groups.

2.3.3. Physical, Chemical and Biological Requirements

Atlantic Salmon populations have complex and flexible life-histories that begin in fresh water and may involve extensive migrations through marine and freshwater environments. Transitions between the various life-history stages are accompanied by profound hormonal, physiological, and morphological changes. 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. 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, although non-spawning adults can tolerate levels as low as 5-6.5 mg/L (Hendry and Cragg-Hine 2003). Lower temperature limit for the survival of Atlantic Salmon is around 0°C, while estimates of upper incipient and lethal temperature limits tend to vary between 22°C and 33°C depending on the strain of salmon, the life stage, and the methodology used to obtain critical values (reviewed by Elliott and Elliott 2010). Tolerance of either the marine or freshwater environment is highly dependent on life-history stage, with early life stages (embryos, alevins, fry, and parr) restricted to fresh water.

2.3.4. Life-history

Atlantic Salmon are, for the most part, anadromous, spending their embryonic (egg and alevin) and juvenile (fry and parr) life stages in freshwater streams before migrating as smolts to the

Atlantic Ocean where they grow to the adult stage (reviewed by Thorstad et al. 2011). After a period of growth at sea, sexually mature adults migrate back to their natal streams where they spawn, depositing fertilized eggs into the river's gravely substrate. They display considerable phenotypic plasticity and variability in life-history characteristics ranging from fully freshwater resident forms to anadromous populations characterized by one to five sea-winter salmon. Their life cycle includes a series of anatomical, physiological, and behavioural changes that enable life in both the fresh water and marine environments (Hutchings and Jones 1998).

2.3.5. Changes in GH Levels over the Lifetime 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, skeletal and soft tissue growth, reproduction, and immune function, as well as, lipid, protein, and carbohydrate metabolism. Recent studies have indicated that GH affects several aspects of behaviour, including appetite, foraging behaviour, aggression, and predator avoidance (for reviews, see Peter and Marchant 1995; Björnsson 1997; Reinecke et al. 2005).

Growth hormone levels vary throughout the animal's life cycle. Plasma growth hormone levels in hatchery reared juveniles vary depending on the light regime and other clues, but, in general, the levels stay between 0 and 4 ng/mL, with the higher values appearing in April, May, and June and the lower values during the winter photoperiod (Ágústsson et al. 2001; Ebbesson et al. 2008). Smoltification brings about growth hormone increases in the range of 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, Björnsson et al. (1994) measured plasma growth hormone levels around 1 ng/mL from February through September, rising to about 2 ng/mL in October.

2.3.6. Background Genetics

Although the migratory ranges of many Atlantic Salmon populations overlap during the marine stage of their life cycle, the freshwater spawning and rearing habitat is highly structured and subdivides the species into many distinct populations (King et al. 2007). A strong tendency to return to their natal streams to spawn has resulted in a considerable level of evolutionary diversity and genetic structuring; although morphological diversity has remained narrow (King et al. 2007). Atlantic Salmon in the Western and Eastern Atlantic Ocean belong to two distinct, deeply divergent phylogeographic groups that have experienced limited gene flow for approximately 500,000 years (King et al. 2007; COSEWIC 2010). DFO has proposed 28 different Atlantic Salmon Conservation Units (ASCUs) based on both genetic and non-genetic criteria (DFO and MRNF 2008). COSEWIC (2010) has proposed to characterize Canadian Atlantic Salmon populations into 16 Designatable Units (DUs) that recognize populations or groups of populations having attributes that make them discrete and evolutionarily significant relative to other populations. These 16 DUs deviate in health of populations from Not at Risk (4 DUs), Special Concern (4 DUs), Threatened (1 DU), Endangered (5 DUs), Extinct (1 DU), and not defined due to deficient data (1 DU, COSEWIC 2010).

2.3.7. History of Invasiveness

In contrast to its close relative, the Brown Trout (*Salmo trutta*), Atlantic Salmon are not predisposed to colonizing territories outside their native range. With a few struggling exceptions, attempts to establish Atlantic Salmon populations outside the North Atlantic Ocean have failed. When compared with some other salmonid species, such as Brown Trout, Rainbow Trout (*Oncorhynchus mykiss*), or Brook Trout (*Salvelinus fontinalis*), Atlantic Salmon is considered a

poor colonizer 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, 2013), although 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).

2.4. BIOLOGY OF DOMESTICATED ATLANTIC SALMON

The environmental and selective pressures in hatcheries and fish farms differ drastically from those in the natural habitat of Atlantic Salmon. As a result, cultivated fish are subject to morphological, physiological, ecological, and behavioural changes. In salmon farming (or salmon aquaculture), the entire life cycle of the fish, from fertilization to harvesting or gamete production is carried out under controlled conditions. Rearing in artificial environments exposes domesticated fish to a variety of new selective forces (e.g., absence of natural habitat, high density, daylight manipulation, handling, vaccination), while other pressures are alleviated (e.g., high food availability, no predators, disease resistance, artificial reproduction). Through generations, these forces have led to significant morphological, physiological, behavioural, and life-history changes (Reviewed by Gross 1998; Jonsson and Jonsson 2006; Cross et al. 2007; Ferguson et al. 2007). As EO-1α Salmon have a domestic strain genetic background (St. John River domestic), are produced in culture and presumably have continuing selective pressure for performance in a culture environment in both the EO-1α broodstock and St. John River broodstock used to produce AAS, the effects of domestication and culture rearing should be understood and taken into account.

2.4.1. Morphology and Anatomy

Phenotypic divergences can be shaped by environmental conditions early in life. Under artificial conditions, the protective environment of the hatchery allows fish to allocate more energy into protein growth and lipid deposition, and less energy into the mobilization of carbohydrates. Several morphological changes may occur in response to changes in selective pressure. For example, farmed Atlantic Salmon differ from wild counterparts at the parr and mature stages in head shape, jaw distortions, smaller rayed fins, larger adipose fins, and horizontal trusses in the trunk region, and some of these differences are maintained when farmed salmon are sea-ranched (Fleming et al. 1994). Changes that have been noted in other cultured salmonids include smaller brain size in cultured Rainbow Trout and Coho Salmon (Lema et al. 2005; Jonsson and Jonsson 2006), abnormal heart shape in cultured Atlantic Salmon and Rainbow Trout (Poppe et al. 2003), and higher concentrations of mucous cells in both skin and gills of cultured Atlantic Salmon smolts (Poole et al. 2003).

2.4.2. Physiology and Biochemistry

In order to optimize commercial operations, most fish farms not only select broodstock with traits that suit fast growth and good health under intensive culture conditions, but also manipulate many of the environmental variables that cue life stage transitions. These deviations from the “natural” environment bring forth not only changes in the morphology and anatomy of cultured fish, but also changes in their physiological functions and biochemical characteristics. For example, Fleming et al. (2002) found higher levels of growth hormone in domestic Atlantic Salmon relative to their wild counterpart. Additional differences in serum glucose levels, gill Na/K ATPase activity, plasma chloride levels, and growth hormone levels may underlie

observed differences in the survival of smolts when transferred to full-strength seawater at different temperatures (Handeland et al. 2003; Jonsson and Jonsson 2006).

2.4.3. Behaviour and Life-history

Wild and domesticated Atlantic Salmon can often differ in behaviour and life-history. In addition to the genetic effects of selection, differences in the rearing environment experienced by cultured and wild salmon will influence behavioural traits such as territorial dominance, feeding, predator avoidance, migration, reproductive behaviour, and life-history (Ferguson et al. 2007), all of which play a critical role in survival.

2.4.3.1. Aggression and Dominance

Einum and Fleming (1997) observed that farmed Atlantic Salmon parr dominated wild fish in one-on-one challenges, with hybrids exhibiting intermediate success. Similar dominance of cultured fish over wild conspecifics has been observed in Coho Salmon (Rhodes and Quinn 1998; Jonsson and Jonsson 2006). Riley et al. (2005) found no evidence to suggest rearing environment causes more aggression in cultured and wild Rainbow Trout fry.

2.4.3.2. Predator Avoidance

In predator-response experiments, Jonsson and Jonsson (2006) reported that domesticated Atlantic Salmon parr had a lower heart rate and less pronounced flight response when exposed to a model predator. Multiple studies have demonstrated that domesticated Atlantic Salmon accepted a greater risk of predation relative to wild Atlantic Salmon, with hybrids being intermediate to parental populations (Einum and Fleming 1997; Jonsson and Jonsson 2006; Houde et al. 2010a; Fleming and Einum 2011).

2.4.3.3. Feeding

In the marine environment, Jacobsen and Hansen (2001) observed that the diet of wild and cultured salmon was similar, indicating that at least some cultured fish can adapt to life in the ocean. In the North Atlantic, cultured Atlantic Salmon post-smolts were often sampled in the wild with considerably more food items in their stomachs than wild post-smolts (Jonsson and Jonsson 2006). Amphipods were the most abundant item in the stomachs of cultured post-smolts, whereas krill was the most abundant food item of wild post-smolts. Sand Lances (Ammodytidae), the largest prey item consumed by both types, were almost twice as abundant in the diet of cultured post-smolts relative to their wild counterparts, demonstrating differences in feeding preferences between cultured and wild Atlantic Salmon.

2.4.3.4. Smolt Emigration

Wild smolts usually move to the sea over a long period, starting in cool temperature and moving downstream by night (Thorpe et al. 1994). When released into rivers, cultured Atlantic Salmon smolts move quickly to the sea, even when released in daylight. In Norway, juvenile Atlantic Salmon actively migrate through fjords and into the ocean (Finstad et al. 2005), whereas sexually maturing cultured post-smolts are more inclined to stay in coastal areas and may enter rivers as they migrate (Hansen et al. 1987; Jonsson et al. 1993).

2.4.3.5. Reproduction

Experimental evidence suggests that the reproductive success of farmed Atlantic Salmon males is low compared to wild males (Fleming et al. 1996; Weir et al. 2004, 2005). Cultured female Atlantic Salmon also have reduced reproductive fitness relative to wild conspecifics; a result of morphological maladaptation, smaller metabolic scope, inferior breeding behaviour, and greater mass of unreleased eggs post-spawning (Fleming et al. 1994; Fleming et al. 1996; Gross 1998).

Studies in North America and Europe have indicated that genetic exchange between domesticated and wild populations of Atlantic Salmon can lead to a temporary reduction in fitness of wild populations or a permanent reduction in fitness if wild populations are small or exposed to repeat escape events (Fleming et al. 2000; McGinnity et al. 2003; Houde et al. 2010b, a; Fraser et al. 2010a; Fraser et al. 2010b). Outbreeding between domestic and wild Atlantic Salmon can lead to the disruption of co-adapted genotype-phenotype complexes, such as maternal and genetic effects on the early development of offspring (Debes et al. 2013).

2.4.4. History of Invasiveness

The invasiveness and potential detrimental impact of domesticated Atlantic Salmon has received considerable attention (McGinnity et al. 1997; Youngson and Verspoor 1998; McGinnity et al. 2003; Naylor et al. 2005; Hindar et al. 2006; Morris et al. 2008). Accidental releases resulting from activities in the aquaculture industry have been implicated in the spread of disease and parasites (Naylor et al. 2005; Amundrud and Murray 2009) and increased competition for resources (Volpe et al. 2001; Fiske et al. 2006). Reproduction of escaped domestic Atlantic Salmon within its native range, and hybridization with wild Atlantic Salmon populations is well documented, with noted changes in the life history characteristics and genetic integrity of wild Atlantic Salmon populations (Skaala et al. 2006; Bourret et al. 2011). However, frequent accidental and deliberate releases of Atlantic Salmon outside of their natural range over a period of many years have not resulted in any known established populations. Follow-up studies on a reported successful spawning of adults and early rearing of juveniles in the Tsitika River, British Columbia (Volpe et al. 2000) did not document the presence of either adult or juvenile Atlantic Salmon (Piccolo and Orlikowska 2012).

3. EXPOSURE

3.1. EXPOSURE CHARACTERIZATION

The assessment of exposure of AAS to the Canadian environment includes both its potential to enter the environment and its fate once there. It examines the physical, geographical, and biological containment strategies that are proposed for all life stages of AAS. Specifically, the exposure assessment considers:

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). Where applicable, the magnitude and frequency of dispersal (i.e., propagule pressure) is also considered.
3. The potential of AAS to reproduce, establish, and spread in the Canadian and Panamanian environments (i.e., fate). Where applicable, the magnitude and frequency of reproduction, establishment, and spread is also considered.
4. The potential for disposed AAS carcasses in Canada to act as an exposure pathway.

Although containment at both the Canadian and Panamanian facilities is examined, the assessment only considers the exposure of AAS to the Canadian environment. Consequently, assessment of potential exposure from activities in Panama is focused primarily on the potential of AAS to enter Canadian waters following an unintentional release at the AquaBounty Panama facility. Measurement endpoints 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 are also 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 extreme circumstances.

3.1.1. Entry Scenarios

Although land-based hatcheries and grow-out facilities offer the potential for a high level of aquatic organism confinement, the prevention of accidental releases from such facilities requires considerable forethought regarding 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, security violations, and chronic failure of physical containment.

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 it has the potential to release a large number of organisms. It is a common experience when farming salmon in 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 located close to streams and there have been breaches associated with the flooding of outdoor commercial fish rearing ponds in Nova Scotia (G. Chaput, personal communication, 2013). Although it is difficult to predict events of this nature, it is important to consider their potential when assessing the geographic location of a facility and its siting, construction, and emergency procedures in place to prevent the possibility of containment failure under such scenarios.

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 all measures that AquaBounty has put in place to prevent security violations, especially given the contentious stance that several private interest groups have taken towards this product.

Finally, chronic failure of physical containment is commonly recognized 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, the standard operating procedures (SOPs), and oversight in place to ensure that physical barriers are properly used and maintained.

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 (Skilbrei et al. 2009; Skilbrei and Jørgensen 2010; Chittenden et al. 2011). Therefore, recapture is not considered to be an acceptable mitigation measure for the accidental release of AAS.

3.1.2. Standards and Methodologies

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) emphasize the importance of having

mechanical barriers, security, and operational procedures in place to maintain physical containment and mitigate catastrophic events. The document suggests that three to five 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 (New Brunswick 2013), which advocates a minimum of three barriers.

To facilitate the assessment of the physical containment in both the Canadian and Panamanian facilities, a Failure Modes Analysis (FMA) was conducted following guidance from Stamatis (2003) and McDermott et al. (2009). Failure Modes Analysis (also known as Failure Modes and Effects Analysis) was first adopted by the automotive industry for use 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 suppliers to the automotive industry to conduct product design and process FMAs in an effort to prevent failures before they happen (McDermott et al. 2009). Failure Modes Analysis has also been extended to the interface of mechanical and biological systems by Hayes (2002), who used it to assess the potential spread of marine organisms via human vectors.

For the AAS assessment, an FMA was conducted on the mechanical and operational processes of physical containment at both ABC and ABP, and during the transportation of eggs between the two facilities. The FMA was intended to identify potential weaknesses along all potential pathways of entry into the environment. The FMA also provide a systematic method to examine and assess each and every element of physical containment. Therefore, the effectiveness of each barrier, the operational procedures in place to maintain and ensure the proper use of each barrier, and the potential consequences of a failure at each barrier were all taken into consideration.

The methodology used to conduct the FMA is simple and straightforward. 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), and the mitigation measures 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 as shown in Table 3.1, Table 3.2, and Table 3.3. 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 entry pathways, and indicate where a recommendation of additional mitigation may be required (Table 3.4).

Table 3.8: 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; 1 downstream barrier still present
3	High; entry possible; no downstream barrier present

Table 3.9: Rankings 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 ≤ O < 5 recorded incidents per year or no recorded incidences
3	High; O ≥ 5 recorded incidents per year or no records available

Table 3.10: Rankings 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; SOPs include daily inspection or compliance documentation
3	Low; SOPs do not include daily inspections or compliance documentation

Table 3.11: 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 provides a qualitative estimate of the likelihood of an unintentional release through the examination of every element of physical containment for each life stage of AAS along all pathways of 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 recommended. Since uncertainty regarding the assessment of a particular pathway is likely

to increase in the absence of data, the assessment of physical containment takes into consideration both the redundancy of mechanical barriers for a particular pathway to entry and 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 two years (Occurrence <1) if there is no downstream barrier (Severity = 3) but excellent operational procedures are in place (Mitigation = 1). In contrast, the FMA does not account for improvements made to the system over time that are directed at lowering the incidence of failure. Consequently, the FMA is predominantly used as methodology to assess the efficacy of physical containment and identify weaknesses, not as an absolute standard.

3.2. UNINTENTIONAL RELEASE OF AAS INTO THE RECEIVING ENVIRONMENT

The likelihood and potential magnitude of AAS exposure to the Canadian aquatic environment resulting from a failure of physical containment at the Canadian and Panamanian facilities, as well as during transportation, was assessed. All life-history stages and all pathways of entry into the environment for both sterile triploid (3n) and fertile diploid (2n) AAS were considered.

3.2.1. Canadian Facility

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. The location is approximately 50 metres from the Bay Fortune Estuary and approximately 1,000 metres from a spit of land that extends into Rollo Bay and the Northumberland Straight.

The facility is entirely land-based. All organisms are maintained within the confines of a two-story main building, and all life stages of AAS are housed in various locations within the building. A variety of mechanical and chemical barriers designed to prevent the accidental release of AAS into the environment are in place. These are supported by 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 research and development (R&D) involving transgenic organisms.

3.2.1.1. Natural Events

An acute release of AAS resulting from natural disasters, 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 (Public Safety Canada 2013). Though several tornados have been reported in New Brunswick, Nova Scotia and Quebec over the past 100 years, none have been reported on PEI (Public Safety Canada 2013).

The most likely natural disaster to challenge the facility's 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 (Environment Canada 2013). 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/hour).

The building itself is structurally sound, built to local building codes by professional contractors. Infrastructure has withstood several severe storms, including the 120 km/hour winds of Hurricane Juan in September of 2003. This challenge was followed shortly afterwards by the "White Juan" blizzard of February 2004, which dropped approximately one metre of snow on the region without damaging the facility. In addition to a sturdy above-ground construction, 90 per cent of the main building's ground floor is below grade, surrounded by a cement foundation. 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.

Although PEI has a history of flooding, the effect of tidal surges tends to be at its worst around Charlottetown diminishing 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 metres 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 metres above chart datum, the highest levels recorded on the island in 100 years (Public Safety Canada 2013). 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 metres (Atlantic Climate Adaptation Solutions Association 2012).

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. Although a hand held GPS unit places the facility at approximately 12 metres (39 feet) above the high water line of the Fortune River, 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 metres above the mean 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 these physical limitations, employees are trained on emergency procedures and they follow SOPs designed to limit the effects of catastrophic events or a loss of operational capacity (NSN 16528).

The assessment concludes with reasonable certainty that the likelihood of an accidental release of AAS resulting from a natural event such as a hurricane or a tidal surge is negligible. This conclusion is based on information about the facility's siting, structural integrity, construction and design, SOPs, and emergency procedures currently in place. It is also based on knowledge of extreme natural events that may occur in the region and may challenge facility's physical containment of AAS.

3.2.1.2. Security Violations

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

- An eight-foot high, galvanized chain-linked perimeter fence, with locked gates, that encloses the facility's main building;
- Exterior lighting throughout the premises at night;
- Steel exterior doors, key control, and entry logs;

- Intercoms and a remote unlock mechanism used to confirm the identity of approved visitors and enable their access to the facility; and
- Steel bars on all ground floor windows.

These provisions have been put in place despite the very limited number of threats to the facility that have occurred without incident (several small and peaceful protests). The facility has never experienced an unauthorized entry of the building 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 no history of security violations, the assessment concludes with reasonable certainty that the likelihood of an accidental release of AAS resulting from a security violation is negligible.

3.2.1.3. Chronic Failure of Physical Containment

Housing and husbandry for all life stages of Atlantic Salmon require a broad variety of tank sizes, incubators, water flow rates, operational procedures, and mechanical barriers to prevent accidental releases. The following assessment examines all mechanical and chemical barriers for each pathway and for all AAS life-history stages. AquaBounty has provided detailed information regarding the facility's floor plan, drainage system, operational procedures, and redundant barriers designed to contain AAS. It has also provided written SOPs that instruct staff on how to employ and maintain the various elements of the containment system, how to identify, report, and troubleshoot problems, and how to ensure that adequate containment is not lost during maintenance procedures.

3.2.1.3.1. Physical containment of AAS gametes

Failure Modes Analysis (FMA) for spawning stage of development identifies physical containment components and six potential failure modes. The majority of these failure modes are likely to be the result of human error such as the accidental spilling of gametes on the floor during collection or a failure to properly secure floor drain covers.

Despite mitigation measures, the risk priority numbers (RPNs) associated with the various failure modes are in the high range (9 to 12). This is a result of limited redundancy in physical containment during this activity, as well as uncertainty with regard to the frequency and occurrence of failure modes. SOPs and internal compliance oversight will likely limit the incidence of release to a very low frequency; however, there is still a chance for AAS gametes to 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 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 (see section 3.3.4.1 on the survival, dispersal, and persistence of AAS gametes).

3.2.1.3.2. Physical containment of AAS embryos (eggs and alevins)

Atlantic Salmon egg diameters 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, New Brunswick 2013). 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.

There are a total of six distinct pathways by which AAS embryos that are under physical containment at the PEI facility can enter the environment. This includes containment during pressure shocking procedures to induce triploidy. Each pathway is considered, in turn, below.

3.2.1.3.2.1. Containment during fertilization and pressure shocking

Eggs that are expected to be exported from the hatchery to the commercial grow-out facility in Panama must undergo pressure shocking to induce triploid sterilization. During these activities, there is a potential for both diploid and triploid AAS to spill onto the floor and enter the environment via the floor drainage system. Should fertilized eggs enter the 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.

The FMA for these activities identifies several physical (and chemical) containment components and 10 potential failure modes. The majority of these failure modes are likely to be the result of human error such as the accidental spilling of eggs onto the floor during transfers between egg containers and pressure shocking cylinders, or a failure to inspect and confirm the correct placement of all containment elements. AquaBounty appropriately recognizes the inevitability of spilled eggs and addresses this issue in SOPs. 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. Written compliance documentation in the form of a checklist to ensure that all elements of containment are in place at the appropriate time is used. The RPNs associated with potential failure modes during these activities range from low to medium (3 to 9). This is primarily the result of a lack of information regarding the occurrence of failures.

Given the limited time frame for this activity, the redundant mechanical and chemical containment, and the operational oversight, the likelihood viable AAS embryos entering the environment by means of this pathway is negligible. Detailed information available on facility design, containment features, water treatment, SOPs, and internal compliance documentation result in this conclusion being made with high certainty.

3.2.1.3.2.2. Containment in upwelling incubation units

The upwelling units will be used to incubate AquaBounty eggs that are destined for commercial sales. Consequently, all eggs in these units are expected to be triploid, all-female, hemizygous AAS and, depending on water temperatures and shipping schedules, may be contained at this location for a period of up to five months.

For a fertilized AAS egg to enter the drainage system from the upwelling incubation units, several mechanical barriers, the majority of which are subject to daily oversight (inspections) and internal compliance documentation in the form of a checklist, must simultaneously fail.

The FMA for this pathway identifies several physical containment components and 21 potential failure modes that may result from material failures, electrical failures, human error, or a combination of these. In addition to multiple and redundant containment features , written SOPs (daily inspection documentation) promote compliance and enable staff to immediately detect the majority of failure modes and correct them. The RPNs associated with potential failure modes along this pathway range from low to medium (2 to 6), although the majority of failure modes are ranked as low.

Given the redundant mechanical containment and the operational oversight, the likelihood of viable AAS embryos entering into the environment 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 allow this conclusion to be made with high certainty.

3.2.1.3.2.3. Containment in Heath stacks (area 1)

Heath stack incubators in area 1 receive fresh water directly from a freshwater header-tank. Water drains from the system, after a single pass, into a shallow sump that filters the effluent as it enters the floor drains. The effluent then passes through a containment sump before exiting the facility via the exterior drainage. Embryos may be removed from an incubation unit prior to hatching, or as alevins, just prior to egg-sac absorption.

For a fertilized AAS egg to enter the downstream drainage system from the Heath stacks in area 1, seven mechanical barriers must simultaneously fail. Five of these barriers are subject to daily oversight (inspections) and internal compliance documentation in the form of a checklist and one (Heath tray screens) is subject to an annual inspection prior to use.

The FMA for this pathway identifies 22 potential failure modes that may result from material failures, electrical failures, human error, or a combination of these. In addition to multiple and redundant containment features (barriers), written SOPs in the form of daily inspection documentation, promote compliance and enable staff to immediately detect the majority of failure modes and correct them. The RPNs associated with potential failure modes along this pathway range from low to medium (2 to 6), although the majority of failure modes are ranked as low. The severity of a failure such as torn screens are ranked as low since there are six additional barriers downstream to maintain containment.

Given the redundant mechanical containment and the operational oversight, the likelihood of viable AAS embryos entering into the environment by means of this pathway is negligible. Detailed information available on facility design, containment features, water treatment, SOPs, internal compliance documentation, and information related to the frequency of past containment failures allows this conclusion to be made with high certainty.

3.2.1.3.2.4. Containment in individual egg trays (area 1)

Depending on the circumstances, individual egg trays may be set up within holding tanks that are located in area 1. Embryos may be removed from the unit prior to hatching, or as alevins, prior to egg-sac absorption. These incubation units receive water from a freshwater header-tank and drain into water recirculating system, which is essentially closed. Water displaced from the recirculating system must exit the facility via the area 1 floor drains, a containment sump, and the facility’s exterior drainage.

The FMA for this pathway identifies 20 potential failure modes that may result from material failures, electrical failures, human error, or a combination of these. In addition to multiple and redundant containment features (the four barriers), written SOPs in the form of daily inspection documentation, promote compliance and enable staff to immediately detect the majority of failure modes and correct them. The RPNs associated with potential failure modes along this pathway range from low to medium (1 to 6), although the majority of failure modes are ranked as low.

Given the redundant mechanical containment and the operational oversight, the likelihood of viable AAS embryos entering the environment by means of this pathway is negligible. Detailed information available on facility design, containment features, water treatment, SOPs, internal compliance documentation, and information related to the frequency of past containment failures allow this conclusion to be made with high certainty.

3.2.1.3.2.5. Containment in individual egg trays (area 2)

Individual egg trays can also be set up in area 2. As with the individual egg trays in area 1, these units may be used to incubate diploid or triploid AAS embryos.

The tanks receive water directly from a freshwater header. After a single pass through the tanks, the effluent drains into the drainage system, exiting the facility via a containment sump and exterior drainage. Embryos may be removed from the unit prior to hatching, or as alevins, prior to egg-sac absorption.

The FMA for this pathway identifies 13 potential failure modes that may result from material failures, electrical failures, human error, or a combination of these. In addition to multiple and redundant containment features, written SOPs in the form of daily inspection documentation, promote compliance and enable staff to immediately detect the majority of failure modes and correct them. The RPNs associated with potential failure modes along this pathway range from low to medium (1 to 6), although the majority of failure modes are ranked as low.

Given the redundant mechanical containment and the operational oversight, the likelihood of viable AAS embryos entering into the environment 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 allow this conclusion to be made with high certainty.

3.2.1.3.2.6. Containment in Heath stacks (area 2)

When located in area 2, Heath stacks may operate using water that is recirculated in a closed loop to enable temperature control using a water-chilling unit. Water drains from the bottom Heath stack into a sump similar to that used for the upwelling units. Replacement water is added to the system from a freshwater header-tank, while displaced water drains from the system through the floor drains and a containment sump, before exiting the facility via the exterior drainage.

The FMA for this pathway identifies 16 potential failure modes that may result from material failures, electrical failures, human error, or a combination of these. In addition to multiple and redundant containment features, written SOPs in the form of daily inspection documentation, promote compliance and enable staff to immediately detect the majority of failure modes and correct them. The RPNs associated with potential failure modes during these activities ranged from low to medium (1 to 6), although the majority of failure modes are ranked as low.

Given the redundant mechanical containment and the operational oversight, the likelihood of viable AAS embryos entering the environment by means of this pathway is negligible. Detailed information available on facility design, containment features, water treatment, SOPs, internal compliance documentation, and information related to the frequency of past containment failures allow this conclusion to be made with high certainty.

3.2.1.3.3. Physical containment of AAS fry

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. The small size of fry makes it possible for them to pass through containment screens if the pore size is too big. The pathways to entry for AAS fry that are under physical containment at the PEI facility are considered below.

3.2.1.3.3.1. Containment of fry – pathway 1

Tanks are part of a water recirculation system, receiving water from the recirculation header that drains back to a BIO filter. Although the recirculation system is essentially closed, fresh water is constantly added to the system from a freshwater header-tank. It is displaced from the system at the BIO filter via the floor drain, a containment sump, and the facility's exterior drainage. Water displaced from the BIO filter must also pass through a containment sump before entering the facility's exterior drainage. If any water is diverted from the BIO filter as a result of a drum filter failure, it must first pass through a sock filter covering the emergency bypass before entering the floor drains and containment sump. As fry grow into parr, they can continue to be held in these tanks or transferred into larger tanks where appropriate biomass concentrations are more easily maintained.

The FMA for this pathway identifies 23 potential failure modes that may result from material failures, electrical failures, human error, or a combination of these. In addition to multiple and redundant containment features (barriers), written SOPs in the form of daily inspection documentation, promote compliance and enable staff to immediately detect the majority of failure modes and correct them. The RPNs associated with potential failure modes along this pathway are ranked as low (1 to 3).

Given the redundant mechanical containment and the operational oversight, the likelihood of AAS fry entering the environment by means of this pathway is negligible. Detailed information available on facility design, containment features, water treatment, SOPs, internal compliance documentation, and information related to the frequency of past containment failures allow this conclusion to be made with high certainty.

3.2.1.3.3.2. Containment of fry – pathway 2

Tanks along this pathway are part of a water recirculation system, receiving water from the recirculation header that drains back to the BIO filter. Although the water recirculation system is essentially closed, fresh water is constantly added to the system from a freshwater header and displaced from the system at the BIO filter, via the floor drain, a containment sump, and the facility's exterior drainage.

The FMA for this pathway identifies 21 potential failure modes that may result from material failures, electrical failures, human error, or a combination of these. In addition to multiple, redundant containment features (the barriers), written SOPs, in the form of daily inspection documentation, promote compliance and enable staff to immediately detect the majority of failure modes and correct them. The RPNs associated with potential failure modes along this pathway are ranked as low (1 to 3).

Given the redundant mechanical containment and the operational oversight, the likelihood of AAS fry entering the environment by means of this pathway is negligible. Detailed information available on facility design, containment features, water treatment, SOPs, internal compliance documentation, and information related to the frequency of past containment failures allow this conclusion to be made with high certainty.

3.2.1.3.3.3. Containment of fry – pathway 3

Tanks along this pathway are not part of a water recirculation system. Instead, they receive fresh water directly from the facility's freshwater header-tank. After a single pass, water drains from the system through the floor drains, a containment sump, and the exterior drainage. As fry grow into parr, they can continue to be held in these tanks or are transferred into larger tanks to maintain appropriate biomass concentrations.

The FMA for this pathway identifies 16 potential failure modes that may result from material failures, electrical failure, human error, or a combination of these. In addition to multiple and redundant containment features (the barriers), written SOPs, in the form of daily inspection documentation, promote compliance and enable staff to immediately detect the majority of failure modes and correct them. The RPNs associated with potential failure modes along this pathway are ranked as low (1 to 3).

Given the redundant mechanical containment and the operational oversight, the likelihood of AAS fry entering the environment by means of this pathway is negligible. Detailed information available on facility design, containment features, water treatment, SOPs, internal compliance documentation, and information related to the frequency of past containment failures allow this conclusion to be made with high certainty.

3.2.1.3.4. Physical containment of AAS parr

There are a total of six distinct pathways for AAS parr that are under physical containment at the PEI facility to enter the environment. Each pathway is considered below.

3.2.1.3.4.1. Containment of parr – pathway 1

The FMA for this pathway examined 23 potential failure modes that may result from material failures, electrical failures, human error, or a combination of these. In addition to multiple and redundant containment features (the barriers), written SOPs in the form of daily inspection documentation, promote compliance and enable staff to immediately detect the majority of failure modes and correct them. The RPNs associated with potential failure modes along this pathway are all ranked as low (1 to 3).

Given the redundant mechanical containment and operational oversight, the likelihood of AAS parr entering the environment by means of this pathway is negligible. Detailed information available on facility design, containment features, water treatment, SOPs, internal compliance documentation, and information related to the frequency of past containment failures allow this conclusion to be made with high certainty.

3.2.1.3.4.2. Containment of parr – pathway 2

The FMA for this pathway examined 27 potential failure modes that may result from material failures, electrical failures, human error, or a combination of these. In addition to multiple, redundant containment features (the barriers), written SOPs in the form of daily inspection documentation, promote compliance and enable staff to immediately detect the majority of failure modes and correct them. The RPNs associated with potential failure modes along this pathway are all ranked as low (1 to 3).

Given the redundant mechanical containment and the operational oversight, the likelihood of AAS parr entering the environment by means of this pathway is negligible. Detailed information available on facility design, containment features, water treatment, SOPs, internal compliance documentation, and information related to the frequency of past containment failures allow this conclusion to be made with high certainty.

3.2.1.3.4.3. Containment of parr – pathway 3

The FMA for this pathway identifies 16 potential failure modes that may result from material failures, electrical failures, human error, or a combination of these. In addition to multiple and redundant containment features (the four barriers), written SOPs in the form of daily inspection documentation, promote compliance and enable staff to immediately detect the majority of

failure modes and correct them. The RPNs associated with potential failure modes along this pathway are all ranked as low (1 to 3).

Given the redundant mechanical containment and the operational oversight, the likelihood of AAS parr entering the environment by means of this pathway is negligible. Detailed information available on facility design, containment features, water treatment, SOPs, internal compliance documentation, and information related to the frequency of past containment failures allow this conclusion to be made with high certainty.

3.2.1.3.4.4. Containment of parr – pathway 4

Tanks along this pathway are deep and are typically used for rearing parr, smolts, and post-smolts. Fresh water is supplied directly from the facility's main header-tank. After a single pass, the water drains into a common drain pipe that releases effluent into the floor drains. As parr grow into smolt, they can continue to be held in these tanks or transferred into larger tanks to maintain appropriate biomass concentrations.

The FMA for this pathway identifies 25 potential failure modes that may result from material failures, human error, or a combination of these. In addition to multiple, redundant containment features (the five barriers), written SOPs in the form of daily inspection documentation, promote compliance and enable staff to immediately detect the majority of failure modes and correct them. The RPNs associated with potential failure modes along this pathway are all ranked as low (1 to 3).

Given the redundant mechanical containment and the operational oversight, the likelihood of AAS fry entering the environment by means of this pathway is negligible. Detailed information available on facility design, containment features, water treatment, SOPs, internal compliance documentation, and information related to the frequency of past containment failures allow this conclusion to be made with high certainty.

3.2.1.3.4.5. Containment of parr – pathway 5

Tanks along this pathway receive water that is recirculated, but supplemented with freshwater.

The recirculated water is pumped directly to the tanks from the recirculation system, while the fresh water is supplied directly to the tanks from a freshwater header-tank. Water is displaced from the system at the BIO filter after passing through the drum filter; it is discharged into the exterior drainage and facility containment sump.

The FMA for this pathway identifies 19 potential failure modes that may result from material failures, human error, or a combination of these. In addition to multiple, redundant containment features, written SOPs in the form of daily inspection documentation, promote compliance and enable staff to immediately detect the majority of failure modes and correct them. The RPNs associated with potential failure modes along this pathway range from low to medium (1 to 4), although the majority of failure modes are ranked as low. Downstream containment provided by the facility containment sump limit the severity of a potential failure at this element to a rating of low.

Given the redundant mechanical containment and the operational oversight, the likelihood of AAS parr entering the environment by means of this pathway is negligible. Detailed information available on facility design, containment features, water treatment, SOPs, internal compliance documentation, and information related to the frequency of past containment failures allow this conclusion to be made with high certainty.

3.2.1.3.4.6. Containment of parr – pathway 6

The tanks along this pathway are a Swedish design with external standpipes. Fresh water is supplied directly from a freshwater header tank. After a single pass, the water drains into a common drain pipe that releases effluent into the floor drains. As parr grow into smolts, they can continue to be held in these tanks or transferred into larger tanks to maintain appropriate biomass concentrations.

The FMA for this pathway identifies 16 potential failure modes that may result from material failures, human error, or a combination of these. In addition to multiple, redundant containment features, written SOPs in the form of daily inspection documentation, promote compliance and enable staff to immediately detect the majority of failure modes and correct them. The RPNs associated with potential failure modes along this pathway are all ranked as low (1 to 3).

Given the redundant mechanical containment and the operational oversight, the likelihood of AAS fry entering the environment by means of this pathway is negligible. Detailed information available on facility design, containment features, water treatment, SOPs, internal compliance documentation, and information related to the frequency of past containment failures allow this conclusion to be made with high certainty.

3.2.1.3.5. Physical containment of AAS smolts, post-smolt juveniles, and adults

After completing the smolt stage (in their first year of life), AAS will continue to be reared in fresh water throughout the post-smolt juvenile stage and into their development as sexually mature adults. All AAS will continue to be maintained in fresh water throughout this stage of their life cycle. Since AAS smolts at the PEI facility are kept in the same locations and the same tanks as AAS parr, the analysis of physical containment and conclusions regarding the likelihood of entry into the environment are the same (i.e., negligible with high certainty).

All diploid and triploid post-smolt and adult AAS will be physically contained in the GOA of the PEI facility Since AAS post-smolt and adults are kept in the same locations and the same tanks as AAS parr and smolt, the analysis of physical containment and conclusions regarding the likelihood of entry into the environment are also the same (i.e., negligible with high certainty). Details of physical containment for the tanks used to house smolts, post-smolts, and adult AAS at the PEI facility are provided for parr in section 3.2.1.3.4.

3.2.2. Panamanian Facility

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. 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.

To prevent the accidental release of AAS into the environment, a variety of mechanical barriers are in place along all potential pathways to entry. Standard operating procedures (SOPs) and internal compliance documentation are also in place to ensure that all containment and security 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. These provisions prevent 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 and will be pressure-treated to induce triploidy. Also, with the exception of high-altitude sections of the watershed, 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 of these factors are considered in the assessment with respect to the likelihood of AAS entering Canadian waters via the Pacific Ocean.

3.2.2.1. Natural Events

A catastrophic release of fish resulting from natural disasters 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 as a result of its proximity to borders between the Cocos, Nazca, and Caribbean tectonic plates (Benz et al. 2011). However, the majority of significant earthquakes 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 noted 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. 2008). 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 any significant damage to any property in the region and the aquaculture facility has never been affected by them. 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. 2008). Tsunamis and tidal surges are highly unlikely to affect the facility given its elevation. While 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 to 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 effect 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. Since 2008, there have been additional reports of flooding and mud slides in the province of Chiriquí with no reported impact to the facility. The facility's siting, at a high altitude and near the headwaters of the watershed should effectively mitigate any potential damage from such an event; however, the lack of long-term historical data on their frequency and magnitude at this location make it difficult to predict the consequences with high certainty. 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.

3.2.2.2. Security Violations

Regardless of its remote and peaceable location, AquaBounty Panama has put in place several security measures to protect both its property and personnel. Given its remote location, and information regarding the measures that are 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 concluded, with reasonable certainty, to be negligible.

3.2.2.3. Chronic Failure of Physical Containment

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. AquaBounty has provided detailed information regarding the facility's floor plan, drainage system, operational procedures, and redundant barriers designed to contain AAS. It has also provided written SOPs that instruct staff on how to employ and maintain the various elements of the containment system, how to identify, report, and troubleshoot problems, and how to ensure that adequate containment is not lost during maintenance procedures. Therefore, in addition to providing a critical review of the suitability and redundancy of the physical containment, the assessment includes a failure modes analysis (FMA) for all life stages present and for all potential pathways to entry. They provide a detailed look at all containment provisions and all measures in place to mitigate or prevent potential failures.

3.2.2.3.1. Physical containment of AAS embryos

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

The FMA for this pathway identified 16 potential failure modes that may result from material failures, human error, or a combination of both. In addition to multiple, redundant containment features (the five barriers), 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 checklist that must be signed by the attending staff member.

The RPNs associated with potential failure modes along this pathway range from medium to high (4 to 12). Despite redundant mechanical barriers, moderate and high ranking result from 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. The absence of internal compliance documentation, such as a daily checklist to ensure that all relevant mechanical barriers are in place and functioning properly, also contributes to the moderate and high RPNs.

Consequently, given the redundant mechanical containment, but the absence of operational oversight documentation, the assessment concludes that the likelihood of entry into the environment of viable AAS embryos by means of this pathway is low. Detailed information available on facility design, containment features, water treatment, and SOPs result in a conclusion that is reasonably certain.

3.2.2.3.2. Physical containment of AAS fry

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 1.5 grams and can be effectively retained by mechanical barriers with a mesh size smaller than or equal to 6 mm.

The FMA for this pathway identifies 12 potential failure modes that may result from material failures, human error, or a combination of both. In addition to multiple, redundant containment

features (barriers), 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 checklist that must be signed by the attending staff member. The RPNs associated with potential failure modes along this pathway range from low to high (2 to 12). Despite redundant mechanical barriers, moderate and high rankings result from the absence of internal compliance documentation, such as a daily checklist to ensure that all relevant mechanical barriers are in place and functioning properly.

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, water treatment, and SOPs result in a conclusion that is reasonably certain.

3.2.2.3.3. Physical containment of AAS parr

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 1.5 grams (i.e., 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.

The FMA for this pathway identifies 34 potential failure modes that may result from material failures, human error, or a combination of both. The majority of potential failure modes are mitigated by written SOPs in the form of daily inspection. The RPNs associated with potential failure modes along this pathway range from low to high (2 to 18). Despite redundant mechanical barriers, moderate and high rankings result from the absence of internal compliance documentation, such as a daily checklist 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 viable AAS parr entering the environment by means of this pathway is considered to be low. Detailed information available on facility design, containment features, water treatment, and SOPs allow this conclusion to be made with reasonable certainty.

3.2.2.3.4. Physical containment of AAS juveniles and adults

The FMA for this pathway identifies 46 potential failure modes that may result from material failures, human error, or a combination of both. The majority of potential failure modes are mitigated by written SOPs; however, there is no internal compliance documentation such as a daily checklist that must be signed by the attending staff member.

The RPNs associated with potential failure modes along this pathway range from low to high (2 to 6). Despite redundant mechanical barriers, moderate and high rankings result from the absence of internal compliance documentation, such as a daily checklist 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, water treatment, and SOPs result in a conclusion that is reasonably certain.

3.2.3. Transport Between Facilities

The shipping and handling of eyed triploid all-female AAS eggs from the hatchery to the grow-out site represents an additional pathway by which AAS may enter the environment. It is also a

period when, in addition to human error and material failure, unauthorized entry and significant impact may lead to containment failure. Still, transportation is restricted to the embryonic stage, which has low tolerance for physical or chemical conditions outside of its narrow ranges for survival (see sections 3.3.4.2 and 3.3.5).

The primary concern is an accident on land or in the air, over suitable habitat, that results in the introduction of diploid AAS into a freshwater system where they may survive, smolt, and subsequently return to a river possessing mature Atlantic Salmon. However, in addition to experiencing an accident that is severe enough to break open the packaging and expose live eggs to fresh water, the conditions of the freshwater environment would still have to be such that the eggs can survive (the water would have to be at temperatures between 2°C and 5°C and well-oxygenated).

During ground transport from the PEI facility to the Charlottetown airport or the Halifax airport (see section 2.1.2.3), 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 in Panama. 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). All AAS eggs received at ABP will be acclimated and disinfected according to SOPs.

The FMA for this pathway identifies 17 potential failure modes that may result from material failures, human error, or a combination of both. The RPNs associated with potential failure modes along this pathway range from low to medium (3 to 6), though rankings are difficult to make given the unpredictable nature of incidents involving significant impact or the severity of a containment failure, which will depend 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 SOPs that are as stringent as those for 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 safety. 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 concluded to be 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 reasonably certain.

3.3. POTENTIAL FOR SURVIVAL, DISPERSAL, AND PERSISTENCE OF AAS IN THE RECEIVING ENVIRONMENTS

In the unlikely event of an unintentional release, the principle factors limiting the survival, dispersal, and persistence of AAS will be the chemical and physical conditions of the receiving environment. Though the conditions of triploidy, gynogenesis, sex-reversal, domestication, and growth hormone transgenesis may have an effect on the overall fitness of AAS, they are not expected to prevent AAS from reaching the adult life stage given a favourable environment.

Assessment of the potential for AAS to survive, disperse, and persist in the environment relies heavily on available fitness data. Relevant measurement endpoints include the physical tolerance of AAS, or valid comparators, at different life stages, to environmental parameters such as temperature and salinity at the potential points of entry. 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 is considered for all relevant life stages. The potential effects of triploidy, gynogenesis, sex-reversal, domestication, and transgenesis on the ability of AAS to survive, disperse, and persist are also taken into consideration.

3.3.1. Effects of Triploidy, Gynogenesis, and Sex-reversal

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 (Johnson et al. 1986; Galbreath and Thorgaard 1995a; Withler et al. 1995; McCarthy et al. 1996; O'Flynn et al. 1997; Benfey 2001; Taylor et al. 2007), and during exposure to 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; Koenig et al. 2011). As well, Berrill et al. (2012) found triploid Rainbow Trout had overall greater survival than diploid fish in aquaculture within the UK. Overall, triploidy is expected to decrease the 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; Komen 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 the spawning season.

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). The effect of gynogenesis or sex-reversal on dispersal has not been examined, but is not expected to increase potential dispersal of the notified organism.

3.3.2. Effects of Domestication

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 it 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 behaviour 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 (DFO 2006; Jonsson and Jonsson 2006; Moreau and Fleming 2012b). For example, faster growth rates and aggressive behaviour 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; Houde et al. 2010b). 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 (DFO 2006). However, it should be noted that many of these studies cannot ascribe effects to domestication alone, as fish in many cases were grown under culture conditions that are known to affect fish performance.

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 to (McGinnity et al. 1997) 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 strong migratory behaviour and dispersed quickly from fiords whereas smolts released in the fall demonstrated little or no migratory or dispersal behaviour. When released as adults, domesticated Atlantic Salmon tend to follow prevailing currents, demonstrate little homing ability and enter non-natal streams to spawn. Hansen and Youngson (2010) found that domesticated Atlantic Salmon released at a site in Norway during the spring, remained within 150 km of the release site, where they eventually entered local freshwater 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. Hansen (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.

3.3.3. Effects of Growth Hormone Transgenesis

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 it 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) 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 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 complemented by similarities in gene expression (Devlin 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 laboratory environments (Moreau et al. 2011b).

Few studies have investigated the effects of growth-enhanced transgenesis on dispersal or the migratory behaviour of salmonids. Sundström et al. (2007b) 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 et al. 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).

3.3.4. Effect of Environmental Conditions in PEI

At the ABC facility in PEI, 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).

AquaBounty states that local waters (the Bay Fortune Estuary) are quite saline (approximately 20 to 30 ppt), which makes the local environs inhospitable year-round to early salmonid life stages. 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 28 ppt during the summer months and 30 ppt during the winter (Petrie et al. 1996).

AquaBounty also states that during the winter months, the Bay Fortune Estuary is covered with ice and that water temperatures range between -2°C and 2°C, making local conditions in the area of the facility inhospitable to salmonids at all life stages during the coldest months of winter (NSN 16528). Average sea surface temperatures in this part of the Northumberland Straight fall below 0°C during the winter (Petrie et al. 1996).

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.

3.3.4.1. Survival, Dispersal, and Persistence of AAS Gametes

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°C and 16°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, 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 concluded 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 a conclusion that is highly certain.

3.3.4.2. Survival, Dispersal, and Persistence of AAS Diploid and Triploid Embryos

Throughout the embryonic stage of development, Atlantic Salmon is restricted to fresh water and 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. 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 hours 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, water temperatures at the point of entry are expected to range below 2°C and would severely limit the survival of both AAS eggs and alevins which have lower temperature limits of 0°C to 2°C (Elliott and Elliott 2010).

Consequently, exposure resulting from the survival, dispersal, and persistence of AAS embryos is concluded to be negligible. The availability of peer reviewed data describing the physical requirements and tolerances of Atlantic Salmon embryos and detailed information about the physical parameters of the receiving environment, result in a conclusion that is highly certain.

3.3.4.3. Survival, Dispersal, and Persistence of AAS Diploid and Triploid Fry

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.

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 months 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 fry 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, water temperatures at the point of entry are expected to range below 2°C and would severely limit the survival of AAS fry which have a lower temperature limit of 0°C to 2°C (Elliott and Elliott 2010).

Consequently, exposure resulting from the survival, dispersal, and persistence of AAS fry is concluded to be negligible. 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.

3.3.4.4. Survival, Dispersal, and Persistence of AAS Diploid and Triploid Parr

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 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 (Hutchings 1986; McCormick et al. 1998), 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).

During the summer, mean surface water temperatures in the Northumberland Straight range from 5°C to 18°C (Petrie et al. 1996). 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 is concluded to 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 a conclusion that is highly certain.

3.3.4.5. Survival, Dispersal, and Persistence of AAS Diploid and Triploid Smolt, Post–smolts, and Adults

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 (McCormick et al. 1998; 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 water temperatures and diurnal cycle; it 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 one and seven years of age or at a size of 12 to 14 cm in length (O'Connell et al. 2006). AquaBounty states that AAS reach an average fork length of greater than 25 cm within 2700 degree days of hatching (NSN 16528).

From May to July, mean surface water temperatures in the Northumberland Straight range from 5°C to 18°C (Petrie et al. 1996). 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 (NSN 16528). 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 are accidentally released from the facility in PEI. In winter, mean surface water temperatures in the Northumberland Straight range below 1°C (Petrie et al. 1996) and could have a limiting effect on the survival of Atlantic Salmon (Elliott and Elliott 2010). However, during the summer, mean surface temperatures range between 5°C and 18°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 sea water, a partial re-adaptation to fresh water, 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 (Hoar 1988; Stefansson et al. 1998). It is generally believed 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 Damsgård (1998) suggest that the period of diminished salt water tolerance is short lived and that Atlantic Salmon smolts and post-smolts held in fresh water 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 to one or two smolt production facilities at the eastern end of Prince Edward Island, and there is no adult grow out. 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 freshwater 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.

What is known about escaped domesticated Atlantic Salmon is that they are able to disperse long distances (Hansen 2006; Whoriskey et al. 2006; Hansen and Youngson 2010) and are capable of ascending natural river systems to successfully spawn with wild or naturalized conspecifics (Saegrov et al. 1997; Skaala et al. 2006; Ferguson et al. 2007; Morris et al. 2008; Thorstad et al. 2008; Bourret et al. 2011). 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 populations) could be exposed to AAS 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 release, the life stage of the escapee, and prevailing ocean currents at the point of release. Jensen et al. (2013) demonstrated that escaped domesticated Atlantic Salmon smolts and early post-smolts migrated with wild salmon and displayed similar behaviour. If dispersal is more random in nature, individuals could remain close to the site of release (within 150 km), or drift away from the area on prevailing ocean currents that move eastward past PEI, then push north along the western shore of Cape Breton Island before exiting the region through the Cabot Straight, near the southern shore of Newfoundland (Drinkwater and Gilbert 2004).

Consequently, the potential exposure resulting from the survival, dispersal, and persistence of AAS parr that are accidentally released into the environment is concluded to 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 a conclusion that is highly certain.

3.3.5. Effect of Environmental Conditions in Panama

Environmental conditions at the potential point of entry in Panama will likely permit the survival of any AAS that are unintentionally released. However, regional freshwater temperatures at lower altitudes and regional temperatures in the ocean will prevent AAS from dispersing to lower sections of the watershed, or from surviving long enough to reach a suitable marine environment.

The point of entry for any AAS that are accidentally released from the facility in Panama is the upper reaches of the Caldera River, a tributary within the Chiriquí River watershed that drains into the Pacific Ocean at the Gulf of Chiriquí. AquaBounty states that “the upper basin of the Caldera River has conditions that favour the establishment of salmonids” (NSN 16528). 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 in NSN 16528) 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.

Water temperatures in the lower basin of the Chiriquí watershed are reported to range between 23.6°C and 25.8°C (NSN 16528) and are expected to remain constant throughout the year, given the watershed's proximity to the equator. Where the Caldera River enters the Chiriquí River, there is a hydro dam above which water temperatures of 26.0°C or higher have been recorded (NSN 16528). AquaBounty suggests that these water temperatures exceed the lethal limit of ~23°C for Atlantic Salmon at all life stages. 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 affects both the survival and production of most freshwater fish populations, and is a pervasive determinant of habitat suitability (Magnuson et al. 1979; Jobling 1981; Amiro 2006, Elliott and Elliott 2010). Still, it is difficult to predict whether Atlantic Salmon can tolerate an environment where temperatures may range between 23°C and 27°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°C for prolonged periods of time and have been recorded at temperatures above 29°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°C and alevins, or sac-fry, will not survive above 25°C (Elliott and Elliott 2010). Garside (1973) estimated 27.5°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°C, but determined that prolonged survival (over 7 days) was limited by an upper temperature of 27.8°C and that feeding only occurred at temperatures below 22.5°C. According to Elliott and Elliott (2010), estimates of upper incipient and lethal water temperatures for parr range between 22°C and 33°C, but feeding will not occur above 28°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°C. Smolts are most sensitive to temperature when making the transition from fresh to salt water, but improve slightly once acclimated (Alabaster 1967). As post-smolt juveniles increase in size, they are expected to become more sensitive to high water temperatures (DFO 2012b), though 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°C and mortality is expected at temperatures above 28°C. Temperatures of 20°C to 27°C reduce resistance to disease and are therefore considered to be indirectly lethal (Danie et al. 1984). Amiro (2006) has proposed 27.8°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°C to 28°C, Atlantic Salmon will die unless they can move to cooler water. Consequently, although an average water

temperature range of 23°C to 27°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°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°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 a 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 five kilometres long and exposed. The water in the canal is likely to exceed 26°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 likely 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 (Saunders and Henderson 1970; Björnsson and Bradley 2007; Björnsson et al. 2011). 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 (Ruggles 1980; Thorstad et al. 2011), 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 kilometres through water for which temperatures are reported to remain above 26°C below the power dam throughout the year (NSN 16528). These temperatures are considered to be incipient lethal for Atlantic Salmon smolts and death would be expected to occur within two hours (Alabaster 1967; Elliott and Elliott 2010). In their natural habitat, migration velocities for Atlantic Salmon smolts have been observed to vary between 0.2 and 28 kilometres per day (Ruggles 1980; Aarestrup et al. 2002). Consequently, it is highly unlikely that any AAS smolt would be able to survive the 40-kilometre 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°C.

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 smolts, for which known physiological and environmental cues initiate its downstream migration (Ruggles 1980; Thorstad et al. 2011) the proximate factors initiating the homeward migration of adult Atlantic Salmon is still poorly understood (Hansen and Quinn 1998; Thorstad et al. 2011). 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°C (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, their larger size may confer to them greater resistance than smolts to the 26°C plus water temperatures (Elliott and Elliott 2010), but as post-smolt juveniles grow into adults, their resistance to high water temperatures is expected to decrease with size (DFO 2012b). Consequently, survival for a prolonged period in this section of the river is highly unlikely (Danie et al. 1984) and although there is 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 likely 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 spreading to the upper reaches of the Chiriquí River and its tributaries.

Therefore, although the potential for exposure resulting from the survival and persistence of AAS that are accidentally released from the facility in Panama is concluded to be high, the capacity for AAS to disperse to the lower section of the Chiriquí watershed is concluded to be low. 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 conclusions that are highly certain.

3.3.6. Potential for AAS to Disperse from Panama to Canada

High water temperature is the principal factor limiting the dispersal of AAS from the potential point of entry in Panama to the Canadian environment. Though environmental conditions at the potential point of entry will likely permit the survival of any AAS that are unintentionally released, regional freshwater temperatures at lower altitudes and regional temperatures in the ocean will prevent AAS from dispersing to lower sections of the watershed, or from surviving long enough to reach a suitable marine environment.

As indicated above, it is highly unlikely that any AAS unintentionally 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-kilometre stretch of river between the confluence of the Caldera and Chiriquí rivers and the Pacific Ocean are reported to remain above 26°C 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°C and 30°C throughout the year (Locarnini et al. 2010) and available dissolved oxygen ranges between 4 and 5 ppm (Garcia et al. 2010). Stevens et al. (1998) found that at ~13°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°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 metres below the ocean surface, water temperatures can range between 15°C and 20°C (Locarnini et al. 2010); however, at that depth, dissolved oxygen concentrations also drop to a range of 1 to 3 ppm (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 circumstance that AAS are able to disperse from the point of entry in Panama to the Pacific Ocean, the likelihood of it surviving and swimming over 3,000 km to reach suitable marine habitat or the 8,000 km needed to reach the Canadian environment is exceptionally remote.

Consequently, the potential for AAS to enter the Canadian environment after an unintentional 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.

3.3.7. Effect of Environmental Conditions during Transport

In the unlikely event that AAS eggs are unintentionally released from containment during transport, they would most likely enter a terrestrial or marine environment and die. AAS eggs will be shipped from the facility in Canada to the facility in Panama by a combination of ground and air transport (see sections 2.1.2.3 and 3.2.3). To survive an unintentional release during transport, AAS 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. 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. Given the proposed means of transport, any AAS eggs that are accidentally released during transport will most likely enter a terrestrial or marine environment and die or will enter an inappropriate freshwater environment and perish.

Therefore, the potential exposure resulting from the survival and persistence of AAS embryos that are accidentally released during transport from the facility in PEI to the facility in Panama is concluded to be negligible. The availability of peer reviewed data describing the physical requirements and tolerances of Atlantic Salmon embryos and information about the shipping route and physical parameters of potential receiving environments result in a conclusion that is reasonably certain.

3.4. POTENTIAL FOR REPRODUCTION, ESTABLISHMENT, AND SPREAD

In the unlikely event of an unintentional release, the principle factors limiting the reproduction, establishment, and spread of AAS will be its reproductive fitness, the availability of suitable spawning habitat, and the availability of suitable mates. The characterization of exposure takes into consideration the efficacy of sterilization procedures and the stability of female-only populations, as well as the effects of domestication and growth hormone transgenic on reproductive fitness. The influence of propagule pressure is also considered.

3.4.1. Effects of Triploidy, Gynogenesis, and Sex-reversal

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 producing viable offspring in the receiving environment. However, the process of generating triploid populations at a commercial scale is not absolute (Devlin et al. 2010), and may leave some individuals fertile (0 to 5 per cent based on statistical sampling procedures to select batches of eggs for export, NSN 16528). Regardless, commercial broodstock held at the PEI facility will be diploid (homozygous females and homozygous neomales) and physiologically capable of reproducing if unintentionally released into the environment.

Sex-reversed AAS (neomales) that are used to generate female-only populations of AAS, have poor gonad development (e.g., Johnstone and MacLachlan 1994; see Pandian and Koteeswaran 1998) and are expected to have a reproductive capacity that is greatly diminished or absent. The populations of AAS that are 100 per cent genetically female are unable to reproduce or establish populations in the absence of male conspecifics, though it should be noted that 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 the receiving environment, that alter the phenotypic sex ratio, any diploid fish could theoretically reproduce under the appropriate conditions. However, offspring would be genetically all-female and the resulting populations would not persist in the absence of existing mixed-sex populations or unless continual environmental control of sex ratio is present.

3.4.2. Effects of Domestication

Although it has been established that escaped domesticated Atlantic Salmon are capable of ascending natural river systems to successfully spawn with wild or naturalized conspecifics (Saegrov et al. 1997; Skaala et al. 2006; Ferguson et al. 2007; Thorstad et al. 2008; Morris et al. 2008; Bourret et al. 2011), 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 behavioural 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 (Fleming et al. 1996; Weir et al. 2004; Weir et al. 2005; Moreau and Fleming 2012b). Fleming et al. (1996) and Weir et al. (2004) were able to demonstrate under experimental conditions that farmed adult males and females expressed several behavioural anomalies that diminished their ability to successfully reproduce. However, Weir et al. (2005) concluded that mature male parr of a domesticated line are 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 selection 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.

3.4.3. Effects of Growth Hormone Transgenesis

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 laboratory culture and transgenesis. Fitzpatrick et al. (2011) found the reproductive fitness of transgenic Coho Salmon to be less than that of cultured Coho, which was in turn, inferior to that of wild salmon. However, the authors stress that the response of wild-reared fish to a transgene may differ significantly from that of cultured salmon, and that a complete understanding of genotype-by-environment interactions for reproductive phenotypes is needed. Moreau et al. (2011a) 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 regard 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 have a negative effect on the reproductive fitness of AAS, it does not preclude reproduction in the wild. Still, there are no data to demonstrate that transgenic salmon are inferior spawners or how the opAFP-GHc2 construct will affect the fitness of wild Atlantic Salmon, should it be introduced to populations in the wild.

3.4.4. Capacity to Reproduce, Establish, and Spread in PEI

While many of the rivers on PEI no longer support viable Atlantic Salmon populations, 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. The small number of AAS housed at the facility in PEI is 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).

As indicated in section 3.3.4, AAS gametes, eggs, alevins, and fry are not expected to survive in the saline environment of Bay Fortune and therefore pose no threat of exposure through reproduction or establishment. Consequently, the potential for exposure resulting from reproduction and establishment is only considered for AAS parr, smolts, post-smolts and adults.

3.4.4.1. Reproduction, Establishment, and Spread of AAS Diploid Parr

Successful reproduction is likely to be limited given the behavioural anomalies associated with domestication and the low frequency of conspecifics. However, despite a general decline in the

regional abundance of wild salmon, the local habitat may still be capable of supporting viable salmon populations.

As indicated in section 3.3.4.4, 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 AAS parr are unintentionally 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 fresh water as a sexually mature adult (Saunders et al. 1998; Moreau 2011). The former strategy will be addressed in the current section. The latter strategy will be addressed in the following section (section 3.4.4.2), 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, opportunities to reproduce are likely to be rare. There is currently no established population of Atlantic Salmon in the watershed (Cairns et al. 2010). Though populations of Rainbow Trout and Brook Trout (*Salvelinus fontinalis*) may be present (NSN 16528), 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 observed entering in the Fortune River from other systems (Cairns et al. 2010).

Even without the extensive physical containment provisions described in section 3.2.1.3.4, the maximum propagule pressure of AAS that can be expected from unintentional releases at the PEI facility will limit the possibility of establishment. For example, failed attempts to re-establish Atlantic Salmon in the Fortune River have involved the introduction of 15,000 to 60,000 fry at a time (Cairns et al. 2010). Therefore, the maximum propagule pressure that can result from an unintentional release is likely to be very small and short-lived. Successful reproduction is also likely to be limited by the behavioural anomalies associated with domestication (section 3.4.2) and a habitat that is in decline (Guignion 2009; Cairns et al. 2010). However, despite a general decline in the regional abundance of wild salmon, the local habitat may still be capable of supporting viable salmon populations. Also, there is a possibility the AAS parr entering the Fortune watershed will be able to survive to the smolt stage, migrate to the marine environment and return to a nearby freshwater system as a sexually mature adult. For this reason, the exposure expected to result from the reproduction, establishment, and spread of fertile AAS parr that are unintentionally released from the Canadian facility, but are restricted to the Fortune River, is concluded to be high with reasonable uncertainty. The possible fate of AAS smolts that enter the marine environment is considered in the next section.

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

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 fresh water 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 for wild Atlantic Salmon from the smolt stage to adults returning after a single winter at sea (grilse) to vary from 1 per cent in Iceland to 7 per cent in Newfoundland and 13 per cent 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 3.3.2 and 3.3.4.5, 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 and dispersing over long distances, and ascending rivers to spawn. Although the Fortune River may not be ideal for reproduction or establishment of AAS, 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 and the Mulls River in Cape Breton, Nova Scotia, would provide suitable spawning habitat and plenty of Atlantic Salmon to mate with.

As indicated in sections 3.4.2 and 3.4.3, domestication and growth-enhanced transgenics are likely to have a negative effect on the capacity of AAS to reproduce in the wild. However, several studies have also concluded 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 be negligible. Still, the result of a single 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 concluded as high, though limited knowledge regarding the fate of AAS, AAS relatives, and Atlantic Salmon in the marine environment result in an assessment that is reasonably uncertain.

3.4.5. Capacity to Reproduce, Establish, and Spread in Panama

AquaBounty has stated that only sterile female AAS eggs 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 shortly after fertilization, using a protocol that is highly efficient, but not absolute.

Any diploid AAS females that result from a failure of the sterilization process and are unintentionally released in Panama will not have opportunity for reproduction since no Atlantic Salmon males will be present in the Caldera River or the Chiriquí watershed. In addition, Atlantic Salmon cannot hybridize with Rainbow Trout or any other species that is endemic to, or has been introduced to the region. Environmental factors, such as temperature, may influence the sex-determining process in salmonids and offers a remote possibility of genetic females expressing a male phenotype (see section 3.4.1). However, all offspring would have a female genotype and environmental conditions would have to be maintained to enable reproduction past a single generation. Therefore, although all AAS shipped to Panama will have been pressure treated and will all be genetic females, there still exists a very remote possibility of limited reproduction in Panama. Regardless, the capacity for AAS to disperse away from AquaBounty Panama facility is also extremely limited such that any AAS accidentally released from the facility in Panama will, in all likelihood, be restricted to the upper reaches of the Caldera River. Consequently, the likelihood of exposure to the Canadian environment resulting from the reproduction, establishment, and spread of AAS in Panama is concluded 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 a conclusion that is highly certain.

3.4.6. Capacity During Transport

During transport between the two facilities, the possibility of AAS embryos developing, reproducing, and establishing viable populations is limited by their narrow range of tolerance to physical and chemical parameters in the freshwater environment and no capacity to survive in the terrestrial or marine environment. Their reproductive capacity is further diminished by the process of induced triploidy. 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 concluded to be negligible. This conclusion is made with high certainty, given the availability of peer reviewed data describing the effectiveness of sterilization using induced triploidy, the physical requirements and tolerances of AAS and Atlantic Salmon embryos, and information about the physical parameters of potential receiving environments.

3.5. POTENTIAL FOR THE DISPOSAL OF AAS CARCASSES IN CANADA TO ACT AS AN EXPOSURE PATHWAY

In its submission, ABC has 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. The proposed methods for disposal (incineration or private landfill) are in compliance with municipal waste disposal standards and practices and will not result in the release of live AAS, its genetic material or material from AAS involved in toxicity into the environment.

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 stipulates that no living transgenic or bio-hazardous waste will leave the facility (reference to “carcasses,” whether the materials to be disposed have been frozen or not). Consequently, if disposal is undertaken, 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 “the genetic material of the organism” means:

1. 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;
2. 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
3. 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 does 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. The likelihood of successful completion of all steps is extremely low and, as mentioned above, no living transgenic organisms or bio-hazardous waste will leave the facility, 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:

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.

Consequently, exposure resulting from the disposal of AAS carcasses in Canada is expected to be negligible. 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 highly certain.

3.6. EXPOSURE ASSESSMENT

The final ranking for exposure requires 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 some cases, the likelihood of one element is limited by the likelihood of other elements. For example, the likelihood of reproduction, establishment, and spread will be limited by the likelihood of survival, dispersal, and persistence, which are in turn limited by the likelihood of entry. In such cases, the overall exposure will be determined by the lowest ranked element. In other cases, the likelihood of one element is not limited by the likelihood of another element. For example, the likelihood of entry into the Canadian environment for one pathway, such as entry from the facility in PEI, will not influence the likelihood of entry for a different pathway, such as dispersal from the facility in Panama. In this case, the final exposure is determined by the highest ranked element. The certainty of the final exposure ranking is determined by the certainty associated with the exposure element that limits the overall exposure.

AAS is intended for use under strictly controlled conditions that include physical containment at two clearly defined facilities. At the Canadian facility, all potential pathways of entry into the environment have been considered for all life stages of AAS. To prevent an unintentional release from the facility, there are mechanical barriers along each pathway. 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 facilities are 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 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 labeled for shipment by air and chain-of-custody will be maintained through to its arrival in Panama using a commercial freight-forward company. AAS eggs will be received and

transported to the facility in Panama under the supervision of an official from the Ministry of Agriculture's Quarantine Department and will be unpacked and inspected at the facility under the supervision of an official from the National Animal Health Authority.

At the Panamanian facility, all potential pathways of entry into the environment have been considered for all life stages of AAS. To prevent an unintentional release from the facility, there are mechanical barriers along each pathway. 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. Furthermore, 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.

The various elements of exposure are consolidated in Table 3.5 to obtain a final exposure ranking. The certainty of the final exposure ranking is determined by the certainty associated with the exposure element that limits the overall exposure. Consequently, the assessment concludes with reasonable certainty that the likelihood of AAS exposure to the Canadian environment is negligible.

The high degree of certainty associated with the physical containment (i.e., entry) of AAS results from available information that adequately demonstrates the efficacy and redundancy of mechanical barriers, and the efficacy of standard operating procedures and operational oversight. It includes detailed diagrams of facility design, mechanical barriers and containment systems, incident reports, and training and compliance documentation. It also includes information on the occurrence of chance events such as fires, floods, hurricanes, earthquakes, and security violations that could lead to a failure of containment.

In contrast, uncertainty associated with the biological and geographical containment of AAS that may enter the environment is derived largely from the limited availability of empirical data regarding the survival, fitness, and ability of AAS to reproduce in the natural environment. However, robust scientific information related to the biological tolerances of valid surrogates and the ecological parameters of the receiving environment moderate this aspect of uncertainty.

Table 3.12: Summary of the exposure assessment of AAS to the Canadian environment.

Pathways of entry into the Canadian environment	Entry	Survival, dispersal, persistence	Reproduction, establishment, spread	Overall
Acute failure in PEI	Negligible (reasonable certainty)	High (high certainty)	High (reasonable uncertainty)	Negligible (reasonable certainty)
Chronic failure in PEI	Negligible (high certainty)			Negligible (high certainty)
Return from Panama	Negligible (high certainty)			Negligible (high certainty)
Acute failure in Transit	Negligible (reasonable certainty)	Negligible (reasonable certainty)	Negligible (high certainty)	Negligible (reasonable certainty)
Disposal in Canada	Negligible (high certainty)	n/a	n/a	Negligible (high certainty)
Final				**Negligible (reasonable certainty)**

4. HAZARD

4.1. INDIRECT HUMAN HEALTH HAZARD

The indirect human health hazard assessment considers only the human health hazards that could result from environmental exposure to AAS through activities such as 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, as it is considered by Health Canada under the Food and Drugs Act. Also, 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 5.1

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; and
- Potential of AAS 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 1.3. Elements of uncertainty are elaborated throughout the human health hazard characterization for each endpoint, with a final uncertainty ranking assigned in accordance with Table 1.7.

4.1.1. Indirect Human Health Hazard Characterization

4.1.1.1. Potential Human Toxicity of AAS

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*" (Environment Canada 2010).

AquaBounty has indicated that no adverse human health effects related to AAS have ever been observed in AquaBounty staff or in individuals visiting the AquaBounty facilities (ABC personal communication, 2013). Since 2000, AquaBounty staff have been occupationally exposed to all life stages and genotypes of AAS in the conduct of activities that include handling, husbandry, facility maintenance, clinical and non-clinical studies (e.g., weighing, grading, dissecting), and the disposal of morbid and dead animals. In addition, a limited number of other individuals (e.g., researchers, visitors, inspectors, veterinarians) have also been exposed to various life stages and genotypes of AAS (NSN 16528). However, it is important to note that for the majority of time, staff or researchers have worn gloves to protect fish health during handling, or to insulate their skin from cold water. Also, times when handling of AAS is expected to be highest (spawning, harvesting) may not coincide with times of elevated transgene expression.

Several studies suggest that topical GH therapy may be effective in humans (Waago 1987; Monafo et al. 2000). However, these studies typically involve the application of 1.5 to 3 mg of mammalian GH to open wounds using a vehicle to protect against peptide degradation (4–8 IU in 50 mL ointment applied topically). Though large volumes of GH might be encountered during slaughter and processing, the ability for the GH to bind to the growth hormone receptor and induce somatotropic effects are not universal among vertebrates (USFDA 2010). 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; Souza et al. 1995; Behncken et al. 1997). Humans also have a history of safe exposure to GH-1 in Chinook Salmon, which is a commercially harvested finfish. Consequently, the assessment concludes with high certainty that the indirect human health hazard associated with known exogenous toxins in AAS is negligible.

There are no known unique indirect health hazards to humans posed by triploid, gynogenetic, or sex-reversed fish. Indeed, all three conditions are known to occur naturally in some fish

populations, such as trout, carp, and Pink Salmon, following changes in climate (Dunham 2011). Whether sex-reversed fish exposed to 17α-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 Tilaplia it 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. In addition, only homozygous AAS broodstock in the previous generation (i.e., not AAS for grow-out) would have been treated with methyltestosterone.

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. The assessment concludes with reasonable certainty that the hazard associated with endogenous toxin production is negligible. Also, 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 behavioural characteristics with the potential to pose a toxicity hazard to humans have been reported in AAS. The lack of statistically significant or biologically relevant differences between transgenic AAS and non-transgenic comparators (see section 2.2.7) make the likelihood of increased toxicity in AAS very small.

Given the evidence that there are no known exogenous toxins and a lack of evidence for any endogenous toxins associated with Atlantic Salmon, the assessment concludes with reasonable certainty that the incremental indirect human health hazard of AAS as compared to wild type Atlantic Salmon is negligible.

4.1.1.2. Potential to Act as a Vector for Human Pathogens

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 (Curtis et al. 1988; Lima dos Santos and Howgate 2011). Humans are also exposed to zoonotic pathogens through the handling of fish. There are no reports of fungal, parasite, or viral zoonoses in fish transmitted to humans 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) (Lehane and Rawlin 2000; Lowry and Smith 2007). Given the prevalence of human health reports and the rarity with which severe effects are reported, the assessment concludes 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.

There is strong evidence that selective breeding of Atlantic Salmon for disease resistance can be highly successful (Kjøglum 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 undergoing selective breeding, perhaps also for disease resistance and performance.

The significance of any altered susceptibility of AAS 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. 2007a). However, as there have been no reports of *A. salmonicida* infections in humans (Lowry and Smith 2007), any potential increased susceptibility of AAS to this pathogen would not represent a human health hazard. It was also concluded in section 2.2.7.8 that, like all Atlantic Salmon, AAS is highly susceptible to certain disease agents. However, because there are no data on the relative susceptibility of AAS compared to wild Atlantic Salmon, 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, there are 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 domesticated 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 the potential for AAS to act as a vector for pathogens has not been examined. The disease resistance and vector potential of gynogenic and sex-reversed fish has not been examined.

Given the uncertainty elaborated above we are unable to conclude whether AAS would have an increased potential to act as a reservoir for the transmission of disease agents to humans compared to wild Atlantic Salmon. 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 near-absence of disease outbreaks in fish and absence in humans 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, the assessment concludes that the use of the ampicillin resistance gene in the development of AAS does not represent an indirect health hazard since there is no evidence of its presence in AAS.

Based on the fact that no pathogens of human significance have ever been detected in the PEI facility, and the fact that adverse human health impacts associated with AAS exposure have never been reported in AquaBounty staff over almost two decades, the assessment concludes 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 the 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, the assessment is unable to conclude whether AAS would have an increased capacity to act as a reservoir for the transmission of disease agents to humans. However, if AAS were to have increased capacity to act as a reservoir for human pathogens, based on the prevalence, nature, and severity of adverse effects related to topically acquired zoonosis reported in the scientific literature, the assessment concludes 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 AAS acting as a vector for human pathogens is low with high certainty.

4.1.2. 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 aerosol and dermal contact. The assessment has considered the potential toxin-, allergen-, and pathogen-related indirect human health hazards associated with AAS resulting from:

1. the potential expression of known exogenous gene products coded for by the opAFP-GHc2 insert;
2. the potential altered level of expression of an endogenous gene product or toxin; and
3. pleiotropic effects (e.g., altered disease susceptibility).

It is highly certain that the inserted sequence does not code for any known toxins, allergens, or proteins other than the intended growth hormone. The assessment concludes with high certainty that the indirect human health hazard related to known exogenous toxin or allergen production is negligible.

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. The assessment concludes with high certainty that the indirect human health hazard related to altered production of endogenous toxins is negligible.

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. Although serious health consequences have been reported in a small proportion of individuals occupationally exposed to 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 per cent 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. However, it should be noted that individuals already allergic to fish protein may also be highly likely to have allergic responses if exposed to AAS and that severe allergic responses are possible in a small minority of the population.

In almost 20 years of operations, no observed adverse human health effects associated with AAS have been reported by AquaBounty staff or visitors. Based of this fact, the assessment concludes 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 susceptibility to pathogens as compared to wild Atlantic Salmon and the uncertainties associated with that data, the assessment concludes with high certainty that the indirect human health hazard related to the ability of AAS to act as a vector for human pathogens is low.

In general, knowledge gaps and uncertainties related to human health hazard endpoints include:

- Those outlined in section 4.2.1 pertaining to allergenicity.
- A lack of experimental data on AAS (e.g., altered susceptibility to pathogens) necessitating the extrapolation from the literature.
- Where data were generated, the use of a non-transgenic comparator of domesticated origin was often inappropriate for the purposes of an indirect human health hazard assessment. The appropriate comparator would have been wild Atlantic Salmon as this is what humans would encounter in nature.
- 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 may also evolve and have pleiotropic effects (e.g., altered disease susceptibility).

Table 4.13: Summary of indirect human health hazards for AAS

Endpoint	Rank	Certainty
Toxin – novel	Negligible	High certainty
Toxin – endogenous	Negligible	Reasonable certainty
Final – Toxin	**Negligible**	**Reasonable certainty**
Allergen – novel	Negligible	High certainty
Allergen – endogenous (diploid AAS)	Low	Reasonable certainty
Final – Allergen	**Low**	**Reasonable certainty**
Vector for human pathogens – new	Negligible	High certainty
Vector for human pathogens - reservoir	Low	High certainty
Final - Vector for Human Pathogens	**Low**	**High certainty**
Overall	**Low**	**Reasonable certainty**

4.2. ENVIRONMENTAL HAZARD

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 1.2. Elements of uncertainty are elaborated with a final uncertainty ranking assigned in accordance with Table 1.6. Hazard considerations are not limited to all female triploid AAS at the eyed-egg stage, hence also includes all life stages and genotypes of the AAS maintained at the PEI facility.

4.2.1. Environmental Hazard Characterization

4.2.1.1. Potential Environmental Toxicity of AAS

Assessment of the potential environmental toxicity of AAS is concerned with both new substances produced by the AAS and relative production of endogenous compared to wild Atlantic Salmon. It was previously concluded that no new known toxic sequences had been inserted in the genome of the AAS (see section 4.1.2). 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 2.2.7.1), information about growth hormone (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 (NSN 16528). 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. 1992a). Overall, plasma GH concentrations in GH transgenic salmonids range from 0 to 40-fold higher compared to non-transgenic counterparts (Du et al. 1992a; 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 5 ng/mL in non-transgenic fish (Devlin et al. 2000). Circulating GH concentration varies in response to internal and external stimuli and consequently varies between life stages (Björnsson 1997; Ebbesson et al. 2008). Consequently, and based on the above studies, the assessment concludes that the characterization of GH levels in AAS is insufficient to determine whether GH levels do not increase above normal range for non-transgenic or wild counterparts throughout their lifespan; therefore, a conclusion cannot be made 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). 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; Souza et al. 1995; Behncken et al. 1997). 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 et al. 1993; Moriyama 1995; Xu et al. 2001; Liu et al. 2012).

Experimental digestibility data of the Chinook Salmon growth hormone protein were not provided by AquaBounty. An *in-silico* analysis of the Chinook Salmon growth hormone protein translated from the inserted sequence was conducted for the AAS fourth generation using the ExPASY Peptide Cutter tool that reported the GH protein to be cleaved by 20 different enzymes, with many cleavage sites per enzyme, including chymotrypsin, pepsin, and trypsins which have been reported in 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 et al. 1993; Moriyama 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. Available delivery mechanisms include coating GH with gelatin, sodium alginate, or hydroxypropylmethyl cellulose phalate or protecting GH into yeast cells (Xu et al. 2001; Kim et al. 2002; Liu et al. 2012). Oral administration of coated or 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 Flounder (Liu et al. 2012).

High doses of orally administered unprotected GH can also elicit a biological response in fish (Duan and Hirano 1991; Moriyama et al. 1993; Moriyama 1995; Xu et al. 2001; Liu et al. 2012). However, the maximum potential concentration of GH in AAS is unlikely to reach concentrations high enough 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 1997). Average plasma GH levels in AAS-relative fry were reported to be 39.9 ± 14.8 ng/mL (Du et al. 1992a). Since levels of plasma GH vary with life stages and environmental factors (Björnsson 1997; Ebbersson et al. 2008), it was concluded that the potentially highest GH concentration in AAS remains unknown. Surrogate information based on other GH transgenic salmonids indicate that the maximum average plasma GH level reported past the G_0 stage to be approximately 65 ng/mL (Devlin et al. 2000), which would translate to approximately 3.6 ng GH per gram of total fish[12]. The highest plasma GH concentration ever reported in GH transgenic fish (425 ng/mL) in a founder population (G_0)[13] would represent the worst case scenario and translates to approximately 26 ng GH per gram of total fish. It is reasonably certain that the maximum concentration of GH in AAS would not reach hazardous levels for predators as a 2 per cent 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 per cent body weight daily oral administration of 40,000 ng of unprotected GH per gram of diet failed 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). Fine et al. (1993) found that growth hormone (GH) from Carp (*Cyprinus carpio*) is 6-10 per cent as active as bovine GH on mammalian cells with GH receptors present. Based on the above, the assessment concludes with reasonable certainty that GH levels in AAS represent a negligible hazard to predators.

No differences were reported for other hormones, including IGF-1, T3, T4, estradiol, testosterone, and 11-keto-testosterone (11kT) in the muscle-skin samples from the commercial sized AAS (NSN 16528). However, 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

[12] 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 per cent of body volume) (Randall and Wright 1995).

[13] 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.

to gastric digestion than GH-1 (Kimura et al. 1997); however, the oral activity of salmon 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). Based on an approximate 1,774-fold difference between the maximum potential daily intake for fish and a no observed effect concentration in rats, the assessment concludes with reasonable uncertainty that potential increased levels of IGF-1 in AAS would not affect potential predators.

The thyroid hormone levels of AAS have not been examined, but Coho Salmon containing the opAFPGHc or OnMTGH1 transgene did not have elevated thyroxine (T_4) plasma levels (Devlin et al. 2000; Eales et al. 2004). However, Coho Salmon containing the OnMTGH1 transgene had over 3-fold greater 3,5,3'-triiodothyronine (T_3) plasma levels compared to non-transgenic individuals (Eales et al. 2004). Oral doses of T_3 were concluded to have no effect on rats and primates at doses up to 0.015-0.02 mg/kg (Atterwill et al. 1988). The highest plasma T_3 concentration reported in transgenic fish would roughly approximate to 0.04 ng T_3 per gram of body weight[14], which consumed at 6 per cent body weight per day would result in a daily intake of approximately 2.7 µg T_3 per kg body weight. Based on a 135 to 180-fold difference between the maximum potential daily intake for fish and the reported no effect dose in rats, the assessment concludes with reasonable uncertainty that potential increased levels of T_3 in AAS would not affect potential predators.

The assessment concludes with reasonable uncertainty that effects of consumption of AAS would not have an impact on potential predators. Although it is reasonably certain that sex hormone levels in triploid all-female AAS would not pose a hazard to potential predators, it cannot be concluded if the magnitude of the potential effect, if any, of consumption of increased steroid concentration of diploid AAS.

No studies have examined the relative potential for AAS to bioaccumulate toxicants compared to domesticated or wild conspecifics. Bioconcentration is one mechanism through which bioaccumulation can occur where the accumulation of waterborne chemicals occurs through non-dietary routes (Barron 1990). 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 per cent 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. The assessment concludes 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. In addition, it is also not possible to conclude on the overall 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. Potential higher bioaccumulation through food consumption in AAS compared to non-transgenic counterparts could be expected for age-matched fish based on its reported increased food consumption. Considering the above, and

[14] Approximation of the concentration of T_3 in the total body of fish was based on the average plasma T_3 levels (0.73 ng/mL), average weight (55 g) (Eales et al. 2004) and blood volume of Coho Salmon (6.1 per cent of body volume) (Randall and Wright 1995).

that lipid content in fish highly depends on diet and feeding levels, it is difficult to make any conclusions about the potential bioaccumulation with confidence.

Based on the above considerations, the assessment concludes with reasonable certainty that consumption of AAS with potentially increased levels of GH would present a negligible hazard for predators. It also concludes with reasonable uncertainty that consumption of AAS would present a negligible hazard for predators. It is also concludes that there is a negligible hazard from processes used to produce AAS. Though it is possible that an increase in bioconcentration of waterborne contaminants could result in AAS, a conclusion about the magnitude of this hazard cannot be made.

4.2.1.2. Potential to Act as a Vector for Native or Introduced Pathogens

AAS may act as a vector for pathogens either by direct introduction of pathogens associated with escaped AAS from the PEI facility into the environment 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.

The relative disease susceptibility of AAS compared to wild Atlantic Salmon is not known. Nor is it known 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 (Kjøglum 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.

The significance of any altered pathogen susceptibility of AAS as an indicator of the ability of AAS to act as a vector for pathogens 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. 2007a). 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 Coho. 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.

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 gynogenic and sex-reversed fish has not been examined.

Given the uncertainty elaborated above, it cannot be concluded 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. This includes disease agents that may affect wild Atlantic Salmon, as well as predators, prey, and competitors of Atlantic Salmon.

The near-absence of disease outbreaks in fish, 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.

4.2.1.3. Potential Horizontal Gene Transfer

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 (ESFA 2013). Genetic analyses suggest 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 (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 HGT to occur, it would most likely be to prokaryotic organisms. 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 it can persist for weeks or longer (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, and so on (DFO 2006). 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 (NSN 16528), or other known factors that may increase the potential for DNA uptake/mobility to a new organism. Out of nine different classifications of transgenes, DFO (2006) listed the transgene type of EO-1α as being third least likely to have increased mobility. 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 (NSN 16528) 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 (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 gene 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.

4.2.1.4. Potential for AAS to Affect Wild Populations of Atlantic Salmon

The potential hazard of AAS to wild populations of Atlantic Salmon is strongly associated with the relative fitness of the two genotypes in nature (Devlin 2011). Relevant phenotypes include competitive, predatory, reproductive, and migratory behaviours of AAS, as well as its fecundity and potential to act as a vector for pathogens/parasites. The current status of an affected wild population (small or large; in decline or growing) will also play a role in the magnitude or effect of the hazard. Additional factors that must be taken into consideration are the effects of domestication, gynogenesis, and triploidy on AAS. Also, fitness traits in both AAS and wild conspecifics are affected by the rearing environment and experimental conditions (Sundström et al. 2007a; Devlin 2011), thus the importance of bearing in mind potential genotype-by-environment (G x E) effects. Finally, 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; Sundström and Devlin 2011).

4.2.1.4.1. Current status of wild Atlantic Salmon populations in Eastern Canada and PEI

Many Atlantic Salmon populations in Canada are currently in decline. Of the 16 designated units (DUs) of Atlantic Salmon in Canadian waters, 11 have COSEWIC status as extinct, endangered, threatened, or of special concern (COSEWIC 2010). At proximity to the PEI facility are the Gaspé-Southern Gulf of St. Lawrence DU (of special concern), the Anticosti Island and Eastern Cape Breton DUs (endangered), and the South Coast Newfoundland DU (threatened) (COSEWIC 2010). Threats to Atlantic Salmon populations include stream sedimentation, physical blockages (such as beaver dams, artificial impoundments, and poorly installed culverts), pesticides, and competition with non-native Rainbow Trout. Many of the populations in PEI are very small and face the likelihood of extirpation if current trends continue (Cairns et al. 2010).

Since the 1880s, the biological characteristics of the Atlantic Salmon populations on PEI have been modified by intensive stocking. Today, small rivers are dominated by fall runs of large (over 63 cm fork length) fish (much like the original PEI populations), while larger rivers, where stocking has been intense, are dominated by small (less than 63 cm), early-run salmon (Cairns

et al. 2010). Mean age at smoltification in the Gaspé-Southern Gulf of St. Lawrence DU (the closest to the PEI facility), is two to three years old. As with all rivers in Eastern Canada, large salmon are predominantly female whereas small salmon are predominantly male. Mean fork length range from 54 to 58 cm for small salmon and from 70 to 90 cm for large salmon (Chaput et al. 2006).

4.2.1.4.2. Potential for AAS to affect wild Atlantic Salmon populations when food is not limited

When food availability is high, AAS are expected to consume more prey, have higher feed conversion ratios and metabolic rates, and grow faster than non-transgenic Atlantic Salmon (Deitch et al. 2006; Levesque et al. 2008; Moreau and Fleming 2012a; NSN 16528). Higher survival is likely and would result in greater numbers of AAS reaching a reproductive stage in a relatively short period of time. Under these conditions, the potential for introgression of the transgene would be high.

Subsequent generations of “feral” AAS in the natural environment would likely differ from the founder generation due to phenotypic plasticity and the impact of early rearing conditions. Moreau (2011) and Moreau et al. (2011b) found no evidence to suggest the transgene would provide a fitness advantage at early life stages of AAS offspring; having no significant effect on time to hatching or territorial dominance following emergence. Experiments with Coho Salmon have demonstrated that transgenic fish are more similar in phenotype to non-transgenic fish when reared together, from early life stages, under naturalized conditions (Sundström et al. 2007a; Sundström et al. 2009; Sundström et al. 2010). Therefore, it is reasonable to expect that the phenotypes of subsequent generations containing the EO-1α gene will have phenotypes more similar to wild Atlantic Salmon than to AAS that have been raised in a hatchery or have been released from a hatchery.

Consequently, it can be concluded with reasonable uncertainty that, when food is abundant in the 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 could occur, resulting in a hazard that is moderate to high. It is difficult to determine if the transgene would persist or be purged from the wild population over a number of generations. The size of the wild population compared to that of the invading AAS population would influence the magnitude of this hazard, such that small, threatened, or endangered populations being more at-risk than large healthy populations.

4.2.1.4.3. Potential for AAS to affect wild Atlantic Salmon populations when food is limited

Information regarding the potential phenotypes of AAS under conditions of low food availability is limited and provides no evidence of increased fitness relative to non-transgenic Atlantic Salmon. Increased appetite of AAS and AAS-relatives under hatchery conditions (Abrahams and Sutterlin 1999; NSN 16528) could translate into more frequent foraging, and more competitive interactions in the natural environment, when food is limited (Devlin 2011). Growth hormone is known to stimulate appetite (Björnsson 1997; Lõhmus et al. 2008) and, under the control of the anti-freeze protein promoter, is expected to be expressed in AAS throughout the year, regardless of season (Fletcher et al. 1985; NSN 16528). Abrahams and Sutterlin (1999) have speculated that, under limited food availability, AAS would not have the resources to express accelerated growth rates and would likely suffer increased mortality due to a greater tolerance of predation risk while foraging. Moreau et al. (2011b) concluded that survival and growth of first feeding AAS in food limited naturalized streams was unaffected by transgenesis. However, these results are difficult to interpret given an overall loss of weight in all fish, including controls, during the experiment. Also, developmental data suggest that the phenotypic

response of the transgene in AAS is delayed and may not be expressed during the earliest life stages (Moreau 2011; NSN 16528).

In contrast, interactions of GH transgenic Coho Salmon and wild conspecifics fry in tanks resulted in a population collapse under low food conditions, whereas populations without transgenic fish did not collapse (Devlin et al. 2004). However, under naturalized conditions, and in the presence of natural predators, no collapse was observed. However, low survival rates suggested the potential for collapse given a longer period of time (Sundström and Devlin 2011).

Therefore, the assessment concludes with reasonable uncertainty that, under conditions of low food abundance, competitive interactions with AAS have the potential to significantly reduce the size of wild Atlantic Salmon populations, resulting in a hazard that is moderate to high.

4.2.1.4.4. Potential for introgression of AAS genes to affect wild populations of Atlantic Salmon

Observations of successful natural spawning events between AAS and wild conspecifics underline the potential for the introgression of the transgene from AAS into subsequent generations of wild populations (Moreau et al. 2011a). It has also been demonstrated that GH transgenic Coho Salmon can successfully reproduce with non-transgenic conspecifics under simulated natural environments (Bessey et al. 2004; Fitzpatrick et al. 2011). However, abnormal reproductive behaviour of GH transgenic salmonids also results in very low rate of reproductive success relative to non-transgenic or wild conspecifics (Fitzpatrick et al. 2011; Moreau et al. 2011a). Reduced sperm quality observed in GH transgenic Coho Salmon may also contribute to its inferior breeding performance relative to conspecifics (Fitzpatrick et al. 2011). The sperm quality of AAS relative to wild Atlantic Salmon has not been studied.

Greater size at maturation could give AAS an advantage over non-transgenic Atlantic Salmon during reproduction in the wild. The effect of the EO-1α transgene on the maximum size of AAS over successive reproductive periods is not known, nor is the role that environmental interactions in the wild may play determining the size of AAS at spawning. Consequently, AAS are expected to attain a size at spawning that is similar to non-transgenic Atlantic Salmon, but it is uncertain if they can exceed this size, or if the enhanced growth phenotype will have any effect on reproductive behaviour or success of AAS. Growth-enhanced transgenic Rainbow Trout are reported to mature at a much larger size than their wild counterparts (Devlin et al. 2001). When reared together in large marine aquariums at low density (to mimic the marine environment), GH transgenic Coho Salmon will also mature at a greater size than non-transgenic siblings (R. Devlin, personal communication, 2013).

Sexual maturation and reproduction of male Atlantic Salmon parr provide an alternative life-history strategy for the transfer of genes between generations (see section 2.3.4.4.). Individual parr can fertilize up to 44 per cent of the eggs in a redd (Hutchings and Myers 1988; Richard et al. 2013), thereby shortening the period between generations and increasing the probability of gene transfer (Hutchings and Myers 1994; Moreau and Fleming 2012a). However, Moreau et al. (2011a) observed a reduction in the occurrence of sexual maturation as parr in AAS, relative to non-transgenic Atlantic Salmon, under hatchery conditions. This difference may limit the chances of AAS parr contributing to the gene pool at this early life stage.

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 2012a; NSN 16528). Such phenotypes could, under nutrient rich conditions, also shorten the AAS life cycle, enabling it to reach the adult reproductive stage faster than wild conspecifics, providing a possible reproductive advantage.

Successful reproduction as anadromous adults would depend, in part, on the marine survival and migratory behaviour of AAS, neither of which has been investigated. Studies conducted on GH transgenic Coho Salmon by Sundström et al. (2010) concluded that early rearing conditions have a stronger effect on the migratory behaviour of Coho Salmon than the GH transgene, with an early onset of migration under hatchery conditions that does not occur when reared in an artificial stream.

Relative reproductive success of AAS would also depend on the survival of offspring. Moreau et al. (2011a) reported that genotype does not influence the offspring survival and growth at the onset of exogenous feeding when comparing AAS to non-transgenic Atlantic Salmon in artificial streams. However, the results of these experiments require cautious interpretation. Early survival of GH transgenic Coho Salmon fry was low relative to conspecifics when reared in tanks (Bessey et al. 2004) and lower or similar to non-transgenics under artificial stream conditions (Sundström et al. 2005, 2010).

It is difficult to predict the overall reproductive fitness of fertile AAS in the natural environment. In addition to the potential phenotypic changes listed above, complicating factors such as the effects of genetic background and rearing conditions on expression of the transgene phenotype, as well as knowledge gaps regarding the reproductive fitness of female AAS, make predictions even more difficult. However, observations of successful reproduction of escaped domesticated Atlantic Salmon in the wild (Jonsson 1997; see section 2.4.3.5.), implies the potential for successful reproduction of escaped AAS in the wild, despite its diminished reproductive capacity. Consequently, introgression of the AAS genotype and the GH transgene into wild populations of Atlantic Salmon, following entry of AAS into the environment, is indeed possible.

Though the transfer of domesticated genotypes to subsequent generations is expected to reduce the adaptive potential of wild populations (see Leggatt et al. 2010), the effects of GH transgene introgression on the fitness of wild populations remains unknown. Therefore, this assessment concludes 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.

4.2.1.4.5. Potential for AAS to affect wild populations of Atlantic Salmon via the Trojan gene effect

The Trojan gene effect is a simple model proposed by Muir and Howard (1999) to describe a possible outcome of transgene introgression into a wild population. The model predicts that when a transgene confers increased reproductive fitness as an adult, but diminished viability as a juvenile, there is a potential for the wild population to crash.

The extinction of a wild Atlantic Salmon population resulting from an AAS-mediated Trojan gene effect is unlikely given the observations of inferior reproductive behaviour and limited change in fitness at emergence and first feeding (Moreau 2011; Moreau et al. 2011b). In addition, models proposed by Ahrens and Devlin (2011) suggest that selection acting on background genetic variation would prevent a Trojan gene effect in most cases. However, there remain significant knowledge gaps regarding the reproductive capacity of female AAS in the natural environment and the reproductive behaviour of naturally reared AAS. Survival of GH transgenic Coho Salmon has been reported as similar or reduced relative to wild conspecifics, under naturalized stream conditions (Sundström et al. 2005, 2010). Consequently, the potential for AAS to affect wild populations of Atlantic Salmon via the Trojan gene effect is small, but cannot be completely discounted, and represents a hazard to wild populations that is high, but is also highly uncertain.

4.2.1.4.6. Potential for domestication to affect AAS hazards to wild populations of Atlantic Salmon

The effects of escaped domesticated Atlantic Salmon on wild populations are especially relevant since the processes of both domestication and GH transgenesis result in similar phenotypic outcomes (Devlin et al. 2009). However, additional hazards may be expected for transgenic fish as the number of genes and the magnitude of effects are magnified in transgenic animals (Devlin et al. 2009). For these reasons, it is important to consider both the overall and the incremental hazard potential of AAS compared to domesticated fish (DFO 2012b).

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. 2015). The magnitude of impact is dependent on several factors, including the scale and frequency of the escapes, the status of the native wild population, and the fitness of the escaped salmon relative to wild conspecifics in the natural environment (reviewed in Cote et al. 2015). Fitness depends on several factors such as the stage of release, extent of domestication, area and season of release, and the abundance of competitors and predators (reviewed in Cote et al. 2015). In general, there is consensus that domesticated Atlantic Salmon escapees have lower survival, inferior foraging abilities, irregular migration behaviour, and reduced reproductive capacity relative to wild conspecifics (Ferguson et al. 2007; Leggatt et al. 2010).

Despite reduced fitness, escaped domesticated salmon can have an effect on the growth of wild juveniles, can reduce the effective population size of wild populations, and can have direct genetic effects through the introgression of cultured genotypes into the genetic background of wild populations (Ferguson et al. 2007; Leggatt et al. 2010).

4.2.1.4.7. Potential for enhanced growth rate to affect AAS hazards to wild populations of Atlantic Salmon

In Atlantic Salmon, body size is the phenotype most related to overall fitness, as it is positively correlated with fresh water 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 AAS under hatchery conditions, there is also evidence that the magnitude of the growth-enhanced phenotype may change under different environmental conditions (Oakes et al. 2007; NSN 16528; Oke et al. 2013). A reduction, or absence, of enhanced growth rates in naturalized environments, relative to hatchery conditions, have also been reported in GH transgenic Coho Salmon (Devlin et al. 2004; Eales et al. 2004; Tymchuk et al. 2005; Sundström et al. 2004, 2005, 2007a, 2009; Sundström and Devlin 2011). Experiments in artificial streams have identified numerous environmental factors that may lead to growth rates that are equal to, or lower than, that of non-transgenic conspecifics including limited food availability (Sundström et al. 2004, 2005; Sundström and Devlin 2011), the presence of predators (Sundström et al. 2004), the early arrival of predators (Sundström et al. 2005), the presence of resident competitors, prior culture in the hatchery, and an increased complexity of habitat. Strong genotype by environmental interactions have also been noted where transgenic and non-transgenic fish differ in their response to environmental factors (e.g., Devlin et al. 2004; Tymchuk et al. 2005; Sundström et al. 2004, 2007a).

Although the natural environment is generally assumed to provide limited and stochastic food abundance (see Moreau 2011), it cannot be concluded that high food abundance will never be encountered. Proximity to hatchery outlets or to net pens could provide a constant food resource in the natural environment (Carss 1990). Consequently, the ability to predict growth rates of AAS in the wild is highly problematic. Depending on the circumstances and the

conditions of the receiving environment, the high growth rates observed for AAS juveniles under hatchery conditions will not necessarily be maintained in the wild.

4.2.1.4.8. Potential for gynogenesis and sex-reversal to affect AAS hazards to wild populations of Atlantic Salmon

Under most circumstances, gynogenesis and sex-reversal of AAS are expected to have little or no effect on genetic and competitive hazards to wild Atlantic Salmon populations. Quillet (1984) reported that gynogenic fish had decreased fecundity and delayed maturation, indicating a potential decrease of genetic hazard to wild populations. Models examining the release of sex-reversed fish in stocking programs suggest that the release of such fish could theoretically disrupt 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 fitness of sex-reversed fish has not been directly addressed, sex-reversed salmon tend to have abnormal gonad development (e.g., Johnstone and MacLachlan 1994, see Pandian and Koteeswaran 1998) that may limit genetic interactions in the wild. These techniques are not expected to alter the competitive hazards of AAS to wild populations.

4.2.1.4.9. Potential for triploidy to affect AAS hazards to wild populations of Atlantic Salmon

Triploid fish are functionally sterile (Benfey 1999; see section 2.2.4.3.). Consequently, the potential hazard of genetic interactions between AAS triploid females and wild Atlantic Salmon populations is expected to be highly reduced or eliminated. Under most circumstances, triploidy is expected to decrease or have no effect on AAS hazards to wild populations of Atlantic Salmon that may result from competitive interactions. Though the potential effects of triploidy on the competitive ability in AAS have not been assessed, triploid AAS are known to grow at a slower rate than diploid AAS (NSN 16528), indicating a possible decrease in overall performance. Studies examining triploidy in other salmonid models demonstrate equal or lower competitive abilities relative to diploid counterparts. Fraser et al. (2012) found triploid fish to have lower or equal aggressive behaviour and food consumption relative to diploid fish. O'Keefe and Benfey (1997) found that one strain of triploid Brook Trout had lower competitive ability than diploid fish, whereas 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, an indication that triploid fish competed poorly for limiting resources.

Under some circumstances, triploid female fish do not experience the decreased growth rates and increased mortality associated with spawning diploids (Sumpter et al. 1991; Sheehan et al. 1999; Teuscher et al. 2003; Poontawee et al. 2007). It follows that triploid female AAS could theoretically obtain a larger size than their diploid counterparts, and could potentially become better competitors. However, there is only one anecdotal report of triploid female Rainbow Trout obtaining unusually large sizes in the wild . Consequently, the potential for triploid female AAS to reach a larger size than diploid female AAS or wild Atlantic Salmon is unknown. Therefore, the assessment concludes with reasonably uncertainty that, under some circumstances, triploid female adult AAS could pose increased competitive hazards to wild Atlantic Salmon. However, triploidy is also expected to decrease or have no effect on competitive hazards during other life stages and is expected, with reasonable certainty, to decrease or prevent genetic hazards to wild Atlantic Salmon.

4.2.1.4.10. Potential for AAS to carry more diseases than domesticated Atlantic Salmon

As reviewed under section 4.2.1.2, it is reasonably certain that AAS would not act as a vector for the introduction of new fish pathogens in the natural environment. The assessment

concludes with reasonable uncertainty that AAS will not carry more diseases than domesticated Atlantic Salmon.

4.2.1.4.11. Overall potential for AAS to affect wild populations of Atlantic Salmon

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. Most ecological studies regarding the potential effects of GH transgenic salmonids have been conducted on juvenile stages; this leaves a knowledge gap regarding the potential impacts of AAS at the adult life stage and during the marine phase of its life cycle. Highest hazards are expected from potential introgression of fertile broodstock with wild populations, or through competition under food-limiting environments. Therefore, the assessment concludes with reasonable uncertainty that the potential hazard of AAS to wild populations of Atlantic Salmon is high. However, induced triploidy is expected to reduce the overall hazards relative to fertile broodstock.

4.2.1.5. Potential for AAS to Affect the Prey of Wild Atlantic Salmon

The impact of AAS on the prey of wild Atlantic Salmon will depend in part on the feeding motivation of AAS in the natural environment and the ability of AAS to avoid predation while foraging. The magnitude of any impact will depend on the prey resources available in the environment, which may also influence the growth rate of AAS in the wild. Other relevant phenotypes include the maximum attainable size of AAS and its capacity to act as a vector for diseases in nature.

Information on the foraging behaviour of AAS in the presence or absence of predators and in a natural environment is not available. Observations in the hatchery indicate that AAS have increased feeding motivation and appetite (NSN 16528), which could lead to more active foraging behaviours in a natural environment (Devlin 2011). Abrahams and Sutterlin (1999) demonstrated that AAS-relatives had greatly increased feeding motivation relative to control salmon in both the presence and absence of predators. This difference could be attributed to the higher metabolic rate of AAS-relatives, even during periods of starvation (Cook et al. 2000c). In addition, as AAS-relatives maintain high metabolic rates over at least 24 hours of starvation, AAS could be expected, with reasonable certainty, to have increased feeding motivation. Sundström et al. (2004) reported that GH transgenic Coho Salmon attacked prey more often and more rapidly in aquarium conditions compared to non-transgenic controls. However, preliminary results suggest that both feeding and risk taking by GH transgenic Coho Salmon are more closely related to environmental food resources and the presence of predation than to genotype (R.H. Devlin, personal communication, 2013).

Growth hormone is known to stimulate appetite and, in Atlantic Salmon, its expression declines during the winter (Björnsson 1997; Lõhmus et al. 2008). However, under the control of a promoter for the anti-freeze protein, expression continues throughout the year and increases during the winter (Fletcher et al. 1985). AAS is therefore expected to have increased appetite, relative to conspecifics, year round, though a decrease in food intake by AAS associated with winter temperatures has been observed (D. Moreau, personal communication, 2013). Lõhmus et al. (2008) reported that, in contrast to wild conspecifics, GH transgenic Coho Salmon do not reduce their food intake during the winter. Also, when GH transgenic fish are satiated, they will return to active feeding more rapidly than non-transgenic fish, even when their digestive tract is still full (reviewed in Devlin 2011). Consequently, it is reasonable to expect that AAS in the natural environment would have similar or increased feeding motivation compared to wild conspecifics. However, an increased appetite in AAS will not necessarily result in greater pressure on the prey of Atlantic Salmon.

Contrasting results from several experiments that investigated the foraging behaviour of transgenic salmonids demonstrate the complexity of predicting this trait in AAS. Increased mortality from predation was observed in GH transgenic Coho fry when foraging in the absence of protective habitat (Sundström et al. 2004), but was not observed when fish had the option of hiding from predators in a refuge (Tymchuk et al. 2005). This difference was attributed in part to differences in the early rearing conditions of the two groups. Sundström et al. (2005) reported transgenic Coho 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. Also, Sundström and Devlin (2011) reported that newly emerged GH transgenic Coho Salmon had higher rates of survival in the presence of predators when fish had access to refuges in naturalized streams. Abrahams and Sutterlin (1999) predicted that increased tolerance of predators by AAS-relatives would lead to higher mortality while foraging. In addition, inconsistent results regarding the swimming speed of growth-enhanced transgenic salmonids (Farrell et al. 1997; Abrahams and Sutterlin 1999; Lee et al. 2003; Deitch et al. 2006) make it difficult to predict how effective AAS would be at catching prey or escaping predators.

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. Background genetics may also play a role in the foraging behaviour of AAS. Whereas the above experiments involving GH transgenic Coho aimed to minimize differences in the genetic background of the transgenic and control animals by crossing with wild fish at each generation (Sundström et al. 2005; Tymchuk et al. 2005; Sundström and Devlin 2011), AAS has been crossed with domesticated strains of Atlantic Salmon for over 12 generations (NSN 16528). Consequently, the effects of domestication would also contribute to divergent foraging behaviour in AAS relative to wild Atlantic Salmon (see sections 2.4.3.2 and 2.4.3.3.) and may obscure the effects that can be confidently attributed to the transgene. Consequently, the effect of the transgene on the foraging behaviour of AAS, or how any change relative to wild Atlantic Salmon may affect the prey of wild salmon, is difficult, if not impossible, to predict.

As far as the assessment was able to determine, there has been no investigation to assess prey selection of AAS under hatchery or naturalized conditions. Atlantic Salmon is known as an opportunistic feeder, with a broad diet that varies with life stage, size, resource availability, location, and season (reviewed in Johansen et al. 2011; Rikardsen and Dempson 2011). Under hatchery conditions, GH transgenic Coho Salmon, fed the same amount of food as satiated wild controls, attacked edible and non-edible prey items at the same frequency as control fish (Sundström et al. 2004). However, GH transgenic Coho Salmon fry have also demonstrated a tendency toward greater dispersal than non-transgenic conspecifics and are more likely to explore previously unused habitats (Sundström et al. 2007b) where different previously unexploited species may serve as prey. Should AAS reach a larger size than its wild conspecifics, they could potentially consume larger species not normally preyed upon by wild Atlantic Salmon. Though there is no information regarding the maximum size attained by AAS under natural conditions, there have been observations that GH transgenic Coho Salmon grow larger than non-transgenic fish when raised in mesocosms under high food abundance (R. Devlin, personal communication, 2013) and that GH transgenic Rainbow Trout mature at a much larger size than their wild counterparts (Devlin et al. 2001). Consequently, the assessment concludes with reasonable uncertainty that AAS may be able to feed on prey items outside of the normal range for wild Atlantic Salmon.

No information is available regarding the influence of triploidy, sex reversal, and gynogenesis on the predation behaviour of AAS. The lower growth rate of triploid AAS, and the equivalent or diminished feeding and competitive behaviour observed for other triploid salmonids, suggest that triploidy would decrease or have no effect on the predation hazard of AAS to the prey of

Atlantic Salmon under most circumstances. There is a theoretical chance that triploid female AAS could reach a larger size than diploid fish after maturation, thereby increasing the range of prey sizes or types that it could prey upon; however, there is no information available on this issue and uncertainty is high. There is also no information available regarding the influence of gynogenesis or sex-reversal on the predatory behaviour of fish, but these manipulations are not expected to increase or result in new predatory hazards of released fish.

Phenotypic plasticity combined with the wide range of environmental and genetic conditions make specific predictions about the impact of AAS on various Atlantic Salmon prey species inconclusive. The magnitude of the hazard associated with an overall increased pressure on prey in the presence of AAS will depend on factors such as early rearing conditions, the genetic background of AAS, and the availability of resources that will affect the growth rate and size of AAS over time. In a high-food environment, AAS is expected to fulfill its metabolic requirements and could have an enhanced growth phenotype, enabling it to consume more prey than wild conspecifics. In contrast, in food-limited environments, the enhanced growth rate phenotype could be suppressed (Sundström et al. 2007a; Oke et al. 2013) or the increased metabolic rate of AAS could lead to a depletion of muscle mass and energy reserves (Cook et al. 2000c; Sundström et al. 2010), which could result in higher mortality. In food-limited environments, any increase in predator abundance or consumption rates could have an impact on prey populations. Consequently, the assessment concludes that the overall potential for AAS to affect the prey of Atlantic Salmon is moderate with reasonable uncertainty.

4.2.1.6. Potential for AAS to Affect Predators of Wild Atlantic Salmon

As with domesticated Atlantic Salmon, AAS are expected to occupy the same habitat as wild Atlantic Salmon and be consumed 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 predator avoidance behaviour of AAS has not been examined, although an increased tolerance for risk of predation has been demonstrated for AAS-relatives under hatchery conditions and for GH transgenic salmonids under a variety of conditions. Studies assessing the mortality of GH transgenic salmonids due to predation provide inconsistent results and, given the effect of domestication, it is impossible to predict if AAS, relative to wild Atlantic Salmon, would be more or less prone to predation in the natural environment.

The consumption of AAS with potentially greater levels of plasma GH, IGF-1, and T_3 is not expected to be hazardous to predators (see review of potential environmental toxicity in section 4.2.1.1). Triploidy and gynogenesis are not expected to affect the hazard of AAS to predators; however, uncertainty remains around the consumption of diploid AAS with increased levels of steroid. Sex-reversal through 17α-methyltestosterone exposure is expected to increase the whole-body levels of methyltestosterone in treated fish, and could potentially impact predators if treated AAS was consumed in significant quantities. However, experiments in other fish models demonstrate that increase in 17α-methyltestosterone in treated fish is transient and that 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 would be restricted to a limited time frame and a very limited mass of fish (diploid AAS selected for the production of neomales are treated at the PEI facility with 17α-methyltestosterone for a period of 600 degree-days, or 60 days at 10^{o}C, commencing just after the start of first feeding).

Information on the allergenicity of AAS to wild predators of Atlantic Salmon is not available and experimental evidence regarding endogenous allergen production in AAS, relative to wild

Atlantic Salmon, is highly uncertain. It is therefore not possible to come to a conclusion on the allergenic impact of the AAS on potential predators.

The nutritional composition of Atlantic Salmon varies with life stage, size, and the quality and quantity of food that it consumes (Reinitz 1983; Shearer et al. 1994; Anderston et al. 1996). Protein content is believed to be endogenously controlled and closely related to fish size, whereas lipid levels are thought to be affected by both endogenous and exogenous factors and inversely related to moisture content (Shearer et al. 1994). ABC has reported that the fat content in muscle and skin of market-sized AAS is higher than in sponsor controls, but similar to farmed Atlantic Salmon (NSN 16528). Whether AAS differs from non-transgenic fish in body composition during other life stages, or under different environmental conditions or diets, is not known. However, Higgs et al. (2009) found that the body composition of GH transgenic Coho Salmon differed from controls in response to diets of low lipid or low protein content. Body composition of AAS in the environment would be expected to change with time, life stage, and diet. Effects on predators are expected to be minimal, if any, and of short duration.

It is reasonably certain that AAS would not act as a vector for the introduction of new fish pathogens in the natural environment. Given the available information, the assessment concludes with high uncertainty that the potential hazards of AAS to predators of wild Atlantic Salmon are low. Although the toxicological impact to predators through consumption of AAS is expected to be low, the absence of information regarding hormone concentrations, allergen levels, and the nutritional value of AAS throughout its life cycle make this assessment highly uncertain.

4.2.1.7. Potential for AAS to Affect the Competitors of Wild Atlantic Salmon

Atlantic Salmon are known to compete with Brook Trout (*Salvelinus fontinalis*), Rainbow Trout (*Oncorhynchus mykiss*), and Brown Trout (*Salmo trutta*) for habitats in fresh waters and may be limited by percids and cyprinids in slow moving freshwater habitats (Cairns 2006; DFO and MNRF 2008). The impact of AAS on the 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 the potential of AAS to transmit diseases to competitors.

The interspecies competitive behaviour of AAS has only been studied with respect to transgenic and non-transgenic hybrids of AAS and Brown Trout (Oke et al. 2013). Under hatchery conditions and in the absence of competition, both AAS and transgenic hybrids grew faster than their non-transgenic counterparts. However, in food-limited stream mesocosms and in the presence of either transgenic or non-transgenic hybrids, Atlantic Salmon and AAS had significantly reduced growth rates. These findings suggest a competitive dominance of hybrids, although complex interactions between hybridization and transgenesis are suggested since AAS had a greater reduction in growth rate than wild Atlantic Salmon in the presence of hybrids (either transgenic or non-transgenic). Parameters that may have contributed to the competitive advantage of hybrids over AAS and Atlantic Salmon include the increased foraging motivation of the transgenic individuals (Abrahams and Sutterlin 1999; Sundström et al. 2004), the potential competitive dominance of juvenile Brown Trout over juvenile Atlantic Salmon under experimental stream conditions (Van Zwol et al. 2012), or the hatchery-rearing conditions prior to experiments in artificial streams.

Experiments with GH transgenic Coho Salmon indicate that when fish are reared in artificial streams, competitive interactions between Coho, Chinook Salmon (*Oncorhynchus tshawytscha*), and Steelhead Trout (*Oncorhynchus mykiss*) are not affected by genotype. However, when fish are first raised in the hatchery, the impact of transgenic Coho on the survival and growth of competitors is greater than that of non-transgenic Coho (Sundström et al. 2014). 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. Consequently, the competitive interactions that involve AAS in the wild are likely to be affected by previous rearing conditions and are likely to be reduced under food-limiting conditions.

Although studies examining direct competitive interactions between GH transgenic salmonids and other species are limited to the ones described above, reports describing AAS phenotypes that are known to affect the relative fitness of competitors, such as growth rates, dominance, and swimming speed are also relevant. Moreau et al. (2011b) observed that dominance among first feeding AAS and non-transgenic Atlantic Salmon fry was determined by order of appearance in the nursery stream, rather than genotype. Predicting whether AAS will maintain a high-growth phenotype in the natural environment is problematic, although current studies suggest accelerated growth may be limited in many circumstances. Experiments in artificial streams with limited food conditions indicated that the competitive ability and performance of first feeding AAS and non-transgenic siblings were similar (Moreau et al. 2011b). However, in the same artificial streams, Oke et al. (2013) found that AAS fry have reduced growth rates relative to non-transgenic siblings when hybrid competitors are present. Under hatchery conditions, GH transgenic Coho Salmon have increased ability to compete for food (Devlin et al. 1999, 2004) and are better at seizing prey than non-transgenic conspecifics (Sundström et al. 2004), but are competitively equal to non-transgenic controls under the environmental conditions of an artificial stream (Tymchuk et al. 2005). This suggests that any competitive advantage of GH transgenic salmonids under hatchery conditions is lost in a naturalized environment. Results regarding the swimming performance of GH transgenic salmonids span a variety of models and life-history stages and are therefore not directly comparable (Farrell et al. 1997; Abrahams and Sutterlin 1999; Lee et al. 2003; Deitch et al. 2006). Deitch et al. (2006) reported a nine per cent reduction in the critical swimming speed (speed at which a fish will exhaust itself within a set time period) of post-smolt AAS, relative to non-transgenic Atlantic Salmon, that could be interpreted as a competitive disadvantage to AAS in the marine environment. In contrast, Abrahams and Sutterlin (1999) measured a threefold increase in the swimming speed of AAS-relatives at the parr stage, providing a potential advantage over competitors in fresh water.

Hybridization represents an indirect pathway through which AAS could have an impact on competitors of Atlantic Salmon. Atlantic Salmon are known to hybridize naturally with Brown Trout in both North America and Europe, although the causes behind the breakdown of pre-reproductive isolating mechanisms may vary (Verspoor 1988; McGowan and Davidson 1992; Youngson et al. 1993; Castillo et al. 2008). Oke et al. (2013) have recently demonstrated that the opAFP-GHc2 transgene is expressed in hybrids generated from a cross between AAS and Brown Trout. In artificial streams, the hybrids appeared to be at a competitive advantage regardless of transgenesis, suggesting that the Brown Trout genotype may have significant influence in the artificial stream environment, although competitive interactions involving pure Brown Trout were not included in the experiment. Consequently, it is difficult to conclude if transgenic hybrids that are produced in the wild would have any greater impact on Atlantic Salmon or its competitors than the non-transgenic hybrids that occur naturally. Through artificial fertilization, Arctic Charr (*Salvelinus alpinus*) and Brook Trout can also produce viable hybrids when crossed with Atlantic Salmon (Chevassus 1979). However, these crosses have never been observed in nature and do not produce fertile offspring. Introgression between Brown Trout and Atlantic Salmon appears to be effectively blocked (Galbreath and Thorgaard 1995b), though the possibility, however remote, cannot be completely ruled out (Castillo et al. 2008). Consequently, the indirect hazard to competitors of Atlantic Salmon through introgression of AAS genes into other species of fish is considered, with reasonable uncertainty, to be negligible.

It is reasonably certain that AAS would not act as a vector for the introduction of new fish pathogens in the natural environment. However, it cannot be concluded if, 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 competitors of Atlantic Salmon.

No information is available regarding the influence of triploidy, sex reversal, and gynogenesis on the competitive fitness of AAS. The lower growth rate of triploid AAS, and the equivalent or diminished feeding and competitive behaviour observed for other triploid salmonids, suggest that triploidy would decrease, or have no effect on the hazard of AAS to competitors of Atlantic Salmon under most circumstances. There is a theoretical chance that triploid female AAS could reach a larger size than diploid fish after maturation, possibly providing a competitive advantage; however, there is no information available on this issue and uncertainty is high. There is also no information available regarding the influence of gynogenesis or sex-reversal on the competitive abilities of fish; however, these manipulations are not expected to increase or result in new hazards if AAS enters the natural environment.

Overall, the limited potential for AAS to affect the competitors of Atlantic Salmon would result from ecological interactions with competitors rather than from introgression through interspecies hybridization. Interspecies competitive interactions involving AAS at juvenile stages is expected to be similar to that observed for non-transgenic Atlantic Salmon, whereas the impact of transgenic adults remains undetermined. Although the accelerated growth phenotype may be limited or repressed under many circumstances, it cannot be concluded with certainty that AAS would never grow faster than non-transgenic Atlantic Salmon in the wild; if AAS did grow faster, it would have a size advantage, relative to wild Atlantic Salmon, during competitive interactions with other species. Therefore, the assessment concludes that the hazard of AAS to potential competitors of Atlantic Salmon is moderate, but this conclusion is made with reasonable uncertainty due to a significant gap in knowledge and a limited understanding of genotype by environment effects.

4.2.1.8. Potential for AAS to Affect Habitat

4.2.1.8.1. Potential for 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). Salmonid behaviour during foraging, predator avoidance, and migration has not been associated with significant effects on habitat. 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). Salmonids can move large quantities of coarse sediments short distances downstream when constructing redds, which influences habitat attributes in a number of ways. Redd excavation affects substrate composition by disturbing and sorting the substrate, and it can remove various amounts of finer sediments through interaction with the water current (Moore 2006; Gottesfeld et al. 2008). Redd excavation can also increase concentration of suspended particulate matter (i.e., turbidity, Moore 2006), enhance the production of a stream by mobilizing nutrients, and change interstitial flows within the sediment that promotes survival of intermediate life stages (G. Chaput, personal communication, 2013).

Reported secondary effects of salmonid redd construction include a decrease in stream macrophyte, algae, and moss biomass, as well as alterations to insect communities (Field-Dodgson 1987; Minakawa and Gara 2003; Moore and Schindler 2008). Redd construction can also 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 such as temperature, dissolved oxygen, or pH.

4.2.1.8.2. Potential for 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. After behaviour, Moore (2006) identified body size and population density as the two most important factors 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 (the majority of the studies listed above examine Pacific salmon). The size and behaviour of spawning Atlantic Salmon indicate they have the potential to be ecosystem engineers and may play a role in forming aquatic habitats. The majority of mature, wild Atlantic Salmon females are expected to reach 55 to 75 cm in fork length (reviewed by Hutchings and Jones 1998). 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 35 cm deep in the gravel (De Vries 1997; Amiro 2006), which indicates a large potential to impact substrate composition within a stream. However, return estimates (Reddin and Veinot 2010; Reddin 2010; Jones et al. 2004; DFO 2012a), 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 the number of spawning Atlantic Salmon, or their importance as ecosystem engineers, can be expected to increase in the foreseeable future.

4.2.1.8.3. Potential for AAS to affect habitat

Reduced metabolic scope (Deitch et al. 2006) could affect its capacity to excavate redds or cause bioturbation of habitat. Experiments with growth-enhanced transgenic Coho Salmon indicate that GH transgenic females have a lower rate of spawning, redd digging, and redd covering than hatchery-reared non-transgenic controls (Bessey et al. 2004).

The potential for size to influence the effects of AAS on habitat during spawning is not clear. Increased spawning size could potentially influence substrate and stream position preference in salmon under some circumstances (Roni and Quinn 1995; cited in Beechie et al. 2008), altering the sections of stream habitat affected by spawning salmon.

Growth-enhanced transgenic Rainbow Trout are reported to mature at a much larger size than their wild counterparts (Devlin et al. 2001). When reared together in large marine aquariums at low density (to mimic the marine environment), GH transgenic Coho Salmon will also mature at a greater size than non-transgenic siblings (R. Devlin, personal communication, 2013).

Were AAS or descendants of AAS to follow a reproductive strategy more closely resembling that of the Pacific salmonids, which is characterized by large numbers of returning adults and a semelparous life-history strategy (death after spawning), greater bioturbation and nutrient

loading within the freshwater environment might be expected. However, assuming survival of AAS in the natural environment is not expected to be significantly greater than non-transgenic Atlantic Salmon, the likelihood of such a scenario is low. In addition, any conclusions regarding the potential effects of nutrient inputs above those (currently or historically) observed in rivers and streams supporting large runs of Atlantic Salmon would be highly speculative (see section 4.2.1.9 for a more detailed review of this issue). AAS are expected to have negligible effects on other aspects of habitat, such as stream flow rates, temperature, oxygen, and pH.

Due to the limited role of Atlantic Salmon in habitat alteration and the potential for a diminished ability to dig redds, AAS are expected to have a very limited effect on habitat. However, a lack of information on reproductive fitness, migration, spawning behaviour, longevity, and maximum size attained by AAS results in a high degree of uncertainty regarding these issues. High uncertainty is also attributed to a reliance on expert opinion for information.

4.2.1.9. Potential for AAS to Affect Biodiversity

Biodiversity is defined as the variability among all living organisms from all sources, including terrestrial, marine, and other aquatic ecosystems, and the ecological complexes of which they form a part. It includes the diversity within species and between species, as well as that of ecosystems (CEPA 1999). Potential pathways through which AAS could affect biodiversity include: genetic alteration through introgression and hybridization (assessed in previous sections); competitive exclusion or displacement of other fish species from available habitat; changes in species composition resulting from AAS feeding behaviours; transfer of diseases or parasites; and significant changes in nutrient cycles thereby potentially resulting in altered food-web dynamics and local community biodiversity.

Since the habitat preferences of AAS in natural environments are largely unknown, the effects that AAS may have on the distribution and habitat utilization of other fish species are difficult to predict. For example, in Eastern Canada, the pools in salmon rivers are often shared by Atlantic Salmon and non-salmonid species such as coregonids (whitefish) or cyprinids (minnows), but they divide the habitat according to the velocity of water currents (G. Chaput, personal communication, 2013). If, in the wild, AAS were to prefer a different current velocity than Atlantic Salmon or were restricted to slower moving waters, competitive interactions with fish that occupy a different niche may increase and could possibly result in the displacement of some species. However, information regarding the habitat preference of AAS relative to wild Atlantic Salmon is not available and any predictions regarding its preferred niche would be highly speculative.

Wild adult Atlantic Salmon do not typically feed while migrating upriver to spawn or during the spawning period, and kelts do not resume feeding until the end of winter as they migrate back to the marine environment (G. Chaput, personal communication, 2013). In the wild, if the enhanced and year-round motivation for AAS to feed continues to be expressed, AAS could potentially continue to feed as mature adults in fresh water. This departure from the normal feeding behaviour of wild Atlantic Salmon could lead to impacts on prey species at different trophic levels that are consumed by AAS, but not normally consumed by adult non-transgenic Atlantic Salmon. However, data on the foraging behaviour of adult AAS in the natural environment are not available; it is not known if AAS continue to feed after entering fresh water nor is there any knowledge of what types of prey they might consume. Consequently, it is not feasible to speculate if any impact to the biodiversity of potential prey species would be realized.

Nutrient availability is widely accepted as a principle factor limiting the primary production of ecosystems (DeAngelis et al. 1989). Salmon export nutrients from the freshwater environment to the marine environment during their migration as smolts, and return those nutrients, along with nutrients gathered from the marine environment, if they spawn and die in fresh water. The

role of Pacific salmon in river nutrient cycles is well established, and spawning mortality of Pacific salmon is reported to have inconsistent, but positive, effects on both aquatic and terrestrial ecosystems (see Jonsson and Jonsson 2003; Janetski et al. 2009). Large runs of semelparous Pacific salmon not only produce future generations of salmon, the nutrient release after spawned-out adults die contributes to the productivity of ecosystems, with impacts such as increased periphytoplankton biomass (Yoder et al. 2006), increased biofilms of bacteria and eukarya (Schuldt 1998), and increased output of juvenile salmon (Shaff and Compton 2009).

Atlantic Salmon may also have the potential to influence stream and river nutrient cycles though their contribution is unlikely to be as significant as that of the Pacific salmon. Unlike Pacific salmon that are semelparous and die soon after reproducing, Atlantic Salmon are iteroparous and have the capacity to return to the ocean after they spawn. Reported survival rates of Atlantic Salmon after spawning vary between systems and years, and range from 9 per cent to 74 per cent (Fleming 1996). As well, Atlantic Salmon spawning numbers in Canada are much lower than that of Pacific salmon.

Results from the few studies that have examined the influence of Atlantic Salmon on river nutrient cycling have been inconsistent, but they do indicate a potential for small, but positive, impacts. Williams et al. (2009) demonstrated that introduction of Atlantic Salmon carcasses to upland streams resulted in juvenile salmon biomass of up to twice that observed in reference streams. Using data collected over a period of 19 years on the River Imsa, Norway, Jonsson and Jonsson (2003) calculated that even small runs of spawning Atlantic Salmon (less than 200 fish) can contribute significant amounts of phosphorous (5 per cent) and nitrogen (0.2 per cent) to low-nutrient systems. The annual import of carbon, nitrogen, and phosphorous in seven English rivers was determined to account for only a fraction of the annual river export (0.09-0.24 per cent, Elliott et al. 1997), although Lyle and Elliott (1998) have postulated that the impact on localized areas in the upper reaches of the watersheds, where the salmon spawned and died, could be much greater. In contrast, Nislow et al. (2004) found that poor returns of adult Atlantic Salmon resulted in a net export of phosphorous from a Scottish river. In New Brunswick, Jardine et al. (2009) found no evidence of marine isotopes in resident sculpins (*Cottus bairdii*) and postulated that, in Canada, the input of carbon and nitrogen from marine sources may be insignificant due to a low number of returning Atlantic Salmon.

The potential for AAS to affect river nutrient cycles through migration and spawning mortality has not been investigated and there is no information available to determine if their tendency to survive or die after natural spawning is any different from wild Atlantic Salmon. Triploid all-female AAS are not expected to mature, and would likely have a diminished tendency to migrate upstream to spawn, as is observed in triploid Atlantic Salmon (Warrillow et al. 1997; Cotter et al. 2000; Wilkins et al. 2001). Consequently, the sponsored product form of AAS (triploid all-females) is not expected to import significant quantities of marine nutrients into river systems, although there is a potential to export river nutrients into the marine environment, but only if large numbers are reared in a wild, freshwater environment until they can migrate to sea as smolts. While the potential impact of diploid AAS, or descendants of AAS, on river nutrient cycling is not known, Atlantic Salmon appear to play a limited role in most Canadian river systems. 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.

In general, the effects that escaped farmed fish may have on overall community dynamics or ecosystem function are still unknown (Leggatt et al. 2010). Similarly, the potential hazards of AAS to biodiversity in Canada are also unknown. Although AAS are expected to have a negligible effect on nutrient cycles in rivers, its potential to displace non-salmonid fish species, or to affect the abundance of biota not typically consumed by wild Atlantic Salmon is impossible to predict given the lack of information regarding the behaviour and physiology of AAS in the

wild. In addition, limited understanding of the influence that wild or domesticated Atlantic Salmon have over ecosystem structure, function, and dynamics will increase the highly speculative nature of any prediction.

4.2.2. 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, that represent legislative protection goals and are selected based on potential and most relevant interactions of AAS with the ecosystem components, 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. 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 4.2.

Based on the thorough molecular characterization of the inserted construct in AAS and on supporting evidence from basic alignment sequence analyses, the assessment concludes with high certainty that the inserted construct at the EO-1α locus does not contain coding sequences for any known toxins, allergens, or proteins other than the intended growth hormone. The assessment also concludes with reasonable certainty that no other coding sequence was inserted into 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 at 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, 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, was concluded with reasonable uncertainty to be of negligible hazard to predators. Uncertainty remains around the hazard associated with the potential increase in other hormone levels. Finally, the assessment concludes with reasonable certainty that the bioconcentration factor of waterborne contaminants could be relatively higher in AAS compared to wild conspecifics, but the magnitude of any associated hazard cannot be predicted. Overall, the assessment concludes with reasonable uncertainty that the environmental hazard related to the potential toxicity of AAS is low.

Table 4.14: 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**	**Reasonable uncertainty**

Given the considerable knowledge gap related to pathogens, it cannot be determined if 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, the assessment concludes with reasonably certainty that AAS would not act as a vector for the introduction of new fish pathogens into the natural environment. Regardless, it cannot be concluded if there is an 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 its potential for HGT via exposure, uptake, stability, and selection. The assessment therefore concludes with reasonable uncertainty that the environmental hazard related to the potential for HGT is negligible.

Most ecological studies about the potential effects of GH transgenic salmonids have been conducted on juvenile stages. As a result, there is an existing gap in knowledge of the 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, the assessment concludes with reasonable uncertainty that AAS could pose high hazards to wild populations of Atlantic Salmon. The highest hazards are expected to be from potential introgression of fertile broodstock with wild populations, or through competition under food-limiting environments. Therefore, the assessment concludes with reasonable uncertainty that the overall environmental hazard of AAS to wild populations of Atlantic Salmon is high

In assessing the potential effects of AAS on potential prey of Atlantic populations, the predatory pressure and selection of AAS were considered. A conclusion cannot be made 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 the phenotype of AAS will be dependent on environmental conditions, especially food resources. However, the assessment concludes 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. Therefore, the assessment concludes with high uncertainty that the overall environmental hazard of AAS to prey of Atlantic Salmon is moderate.

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 nutritional value of AAS and its capacity to act as a vector for pathogens, the assessment concludes that any potential hazards to predators are expected to be minimal, and of short duration, as effects would require high and continuous consumption rates of AAS. Therefore, the assessment concludes with high uncertainty that the overall environmental hazard of AAS to predators of Atlantic Salmon is low.

Based on available studies, the assessment concludes that the limited potential for AAS to affect the competitors of Atlantic Salmon would result 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. An increased growth rate would provide AAS with a size advantage and theoretical increased interspecies competitiveness relative to wild conspecifics. Although accelerated growth may be limited in many circumstances, it cannot be concluded if AAS would never express increased growth rates in the environment. Hazard to Atlantic Salmon competitors through introgression and subsequent impacts on other competitors is considered, with reasonable uncertainty, to be negligible to low. Therefore, the assessment concludes with reasonable uncertainty that the overall environmental hazard of AAS to competitors of Atlantic Salmon is moderate.

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, the assessment concludes with high uncertainty that the overall environmental hazard of AAS to the habitat is low.

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, the assessment concludes that the potential for AAS to affect nutrient cycle in rivers is 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. Therefore, it cannot be concluded if AAS will pose an environmental hazard to biodiversity.

The assessment concludes with reasonable uncertainty that the overall potential hazards of AAS to the Canadian environment are high. If AAS was to enter the natural environment, it is expected 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. It cannot be concluded if there is a potential hazard to biodiversity.

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.

5. RISK

Both the indirect human health and the environmental risk assessments were conducted in accordance with the classical risk assessment paradigm in which risk is directly related to the exposure and hazard of the organism, or R = H x E (see section 1.3.5). Final indirect human health and environmental risk assessments are reported separately.

5.1. INDIRECT HUMAN HEALTH RISK ASSESSMENT

The exposure assessment examined the potential for AAS to enter the Canadian environment through four different pathways. The findings of the exposure assessment are summarized in Table 3.5, and conclude that, for the specific use scenario that has been notified, exposure of AAS to the Canadian environment is expected to be negligible with reasonable certainty.

Human contact with naturally occurring Atlantic Salmon during swimming is rare and catches in the recreational and aboriginal Atlantic Salmon fisheries provide limited opportunity for dermal exposure through handling of fish. For the purposes of the indirect human health risk assessment, the likelihood of any incidental human contact to AAS through activities such as recreational swimming or fishing is considered to be extremely remote, given that the likelihood of entry of AAS into the Canadian environment is negligible with reasonable certainty. Therefore, the assessment likewise concludes that the exposure of humans in Canada to AAS is negligible with reasonable certainty.

The indirect human health hazard assessment characterized and ranked the incremental human health hazards that could result from environmental exposure to AAS as compared to wild Atlantic Salmon. This comparison was based on the potential toxicity and allergenicity of AAS and the capacity of AAS to act as a vector for human pathogens. The assessment concludes that the final indirect human health hazard of AAS is low with reasonable certainty.

The outcome of the indirect human health risk assessment is summarized in Table 5.1 and concludes that the risk to human health resulting from environmental exposure to AAS is low with reasonable certainty.

Table 5.15: Indirect human health risk assessment under the proposed use scenario.

Assessment	Rank	Uncertainty
Exposure	Negligible	Reasonable certainty
Hazard	Low	Reasonable certainty
Risk	**Low**	**Reasonable certainty**

5.2. ENVIRONMENTAL RISK ASSESSMENT

The exposure assessment has examined the potential for AAS to enter the Canadian environment through four different pathways. The findings of the exposure assessment are summarized in Table 3.5, and conclude that, for the specific use scenario that has been notified, exposure of AAS to the Canadian environment is expected to be negligible with reasonable certainty.

The environmental hazard assessment characterized the nature and severity of potential harmful effects that AAS may cause to wild populations of Atlantic Salmon, prey of Atlantic Salmon, predators of Atlantic Salmon, competitors of Atlantic Salmon, habitat, and biodiversity. The assessment concludes that the final environmental hazard of AAS to the Canadian environment is high with reasonable uncertainty.

The outcome of the environmental risk assessment is summarized in Table 5.2 and concludes that the risks to the Canadian environment associated with the manufacture and production of AAS is low with reasonable certainty under the proposed use scenario specified in the notification by AquaBounty.

Table 5.16: Environmental risk assessment of AAS under the proposed use scenario.

Assessment	Rank	Uncertainty
Exposure	Negligible	Reasonable certainty
Hazard	High	Reasonable uncertainty
Risk	**Low**	**Reasonable certainty**

Changes 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 quantity, manner, or circumstances significantly different to the potential exposure of AAS assessed in the current risk assessment. Given the potential hazard of AAS to the Canadian environment and associated uncertainty, including potential invasiveness, any significant new activity may result in an altered exposure and consequently a different risk assessment conclusion than provided in this report.

6. CONCLUSIONS

The finding of negligible with reasonable certainty for the exposure assessment and low with reasonable certainty for the indirect human health hazard assessment resulted in a risk assessment outcome of low with reasonable certainty.

The finding of negligible with reasonable certainty for the exposure assessment and high with reasonable uncertainty for the environmental hazard assessment resulted in a risk assessment outcome of low with reasonable certainty.

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 proposed activity, as outlined above. The proposed parameters, which include physical, biological, and geographical containment provisions, have been deemed sufficient to result in a negligible likelihood of entry into the Canadian environment with reasonable certainty.

Changes 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 quantity, manner, or circumstances significantly different to the potential exposure of AAS assessed in the current risk assessment. Given the potential hazard of AAS to the environment and the uncertainty associated with that hazard, including potential invasiveness, any significant new activity may result in an altered exposure and consequently in a different risk assessment conclusion than provided in this report.

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 may be considered a significant new activity and could require a Significant New Activity Notice.

7. REFERENCES CITED

Aarestrup, K., Nielsen, C., and Koed, A. 2002. Net ground speed of downstream migrating radio-tagged Atlantic Salmon (*Salmo salar* L.) and Brown Trout (*Salmo trutta* L.) smolts in relation to environmental factors. Hydrobiol. 483:95-102.

Aas, Ø., Einum, S., Klemetsen, A., and Skurdal, J. 2011. Atlantic Salmon Ecology. Wiley-Blackwell, United Kingdom.

ABRAC (Agricultural Biotechnology Research Advisory Committee). 1995. Performance Standards for Safely Conducting Research with Genetically Modified Fish and Shellfish. U.S. Department of Agriculture, Office of Agricultural Biotechnology, Document No. 95-04, Washington, D.C.

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

Ágústsson T., Sundell, K., Sakamoto, T., Johansson, V., Ando, M., and Björnsson, B.T. 2001. Growth hormone endocrinology of Atlantic Salmon (*Salmo salar*): pituitary gene expression, hormone storage, secretion and plasma levels during parr–smolt transformation. J. Endocrinol. 170:227-234.

Ahrens, R.N.M., and Devlin, R.H. 2011. Standing genetic variation and compensatory evolution in transgenic organisms: a growth-enhanced salmon simulation. Trans. Res. 20:583-597.

Alabaster, S. 1967. The survival of salmon (*Salmo salar* L.) and sea trout (*S. trutta* L.) in fresh and saline water at high temperatures. Water Res. 1:717-730.

Amiro, P.G. 2006. A synthesis of freshwater habitat requirements for Atlantic Salmon (*Salmo salar*) in Canada. Can. Sci. Adv. Sec. Res. Doc. 2006/017. vi + 35 p.

Amundrud, T.L., and Murray, A.G. 2009. Modeling sea lice dispersion under varying environmental forcing in a Scottish sea loch. J. Fish Dis. 32:27-44.

Anonymous. 1996. Oxford Dictionary of Biology. Third edition. Oxford New York Press, New York.

Arismendi, I., Soto, D., Penaluna, B., Jara, C., Leal, C., and Leon-Munoz, J. 2009. Aquaculture, non-native salmonid invasions and associated declines of native fishes in Northern Patagonian lakes. Freshwater Biol. 54:1135-1147.

Arnesen, A.M., Toften, H., Agustsson, T., Stefansson, S.O., Handeland, S.O., and Björnsson, B.T. 2003. Osmoregulation, feed intake, growth and growth hormone levels in 0+ Atlantic Salmon (*Salmo salar* L.) transferred to seawater at different stages of smolt development. Aquaculture 222:167-187.

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

Atlantic Climate Adaptation Solutions Association. 2012. Climate Change Vulnerability Assessment – Souris and Souris West, Prince Edward Island. (July 2013).

Atterwill, C.K., Kennedy, S., Jones, C.A., Lee, D.M., Davies, S., and Poole, A. 1988. Comparison of the toxicity of orally-administered L-triiodothyronine (T3) in rat and cynomolgus monkey. Toxicol. 53:98-105.

Azuma, T., Takeda, K., Doi, T., Muto, K., Akutsu, M., Sawada, M., and Adachi, S. 2004. The influence of temperature on sex determination in sockeye salmon *Oncorhynchus nerka*. Aquaculture 234:461-473.

Barron, M.G. 1990. Bioconcentration. Will waterborne chemicals accumulate in aquatic animals? Environ. Sci. Technol. 24:1612-1618.

Beechie, T.J., Moir, H., and Pess, G. 2008. Hierarchical physical controls on salmonid spawning location and timing. Pages 83-102 *in* D.A. Sear and P. DeVries, editors. Salmonid Spawning Habitat in Rivers: Physical Controls, Biological Responses, and Approaches to Remediation. American Fisheries Society, Symposium 65, Bethesda, Maryland.

Behncken, S.N., Rowlinson, S.W., Rowland, J.E., Conway-Campbell, B.L., Monks, T.A., and Waters, J.M. 1997. Aspartate 171 is the major primate-specific determinant of human growth hormone. J. Biol. Chem. 272:27077-27083.

Benfey, T.J., Solar, I.I., deJong, G., and Donaldson, E.M. 1986. Flow-cytometric confirmation of aneuploidy in sperm from triploid Rainbow Trout. Trans. Am. Fish. Soc. 115:838-840.

Benfey, T.J., Dye, H.M., and Donaldson, E.M. 1989. Estrogen-induced vitellogenin production by triploid Coho Salmon (*Oncorhynchus kisutch*), and its effect on plasma and pituitary gonadotropin. Gen. Comp. Endocrinol. 75:83-87.

Benfey, T.J. 1999. The physiology and behavior of triploid fishes. Rev. Fish. Sci. 7:39-67.

Benfey, T.J. 2001. Use of sterile triploid Atlantic Salmon (*Salmo salar* L.) for aquaculture in New Brunswick, Canada. ICES J. Mar. Sci. 58:525-529.

Bennett, S.N., Olson, J.R., Kershner, J.L., and Corbett, P. 2010. Propagule pressure and stream characteristics influence introgression: cutthroat and rainbow trout in British Columbia. Ecol. Appl. 20:263-277.

Benz, H.M., Tarr, A.C., Hayes, G.P., Villaseñor, A., Furlong, K.P., Dart, R.L., and Susan, R. 2011. Seismicity of the Earth 1900–2010 Caribbean plate and vicinity. U.S. Geological Survey Open-File Report 2010–1083-A.

Berrill, I.K., MacIntyre, C.M., Noble, C., Kankainen, M., and Turnbull, J.F. 2012. Bio-economic costs and benefits of using triploid Rainbow Trout in aquaculture: reduced mortality. Aquacult. Econ. Manage. 16:365-383.

Bessey, C., Devlin, R.H., Liley, N.R., and Biagi, C.A. 2004. Reproductive performance of growth-enhanced transgenic Coho Salmon. Trans. Am. Fish. Soc. 133:1205-1220.

Biro, P.A., Abrahams, M.V., Post, J.R., and Parkinson, E.A. 2004. Predators select against high growth rates and risk-taking behaviour in domestic trout populations. Proc. R. Soc. London B 271:2233-2237.

Björnsson, B.T, Taranger, G.L., Hansen, T., Stefansson, S.O., and Haux, C. 1994. The interrelation between photoperiod, growth-hormone, and sexual-maturation of adult Atlantic Salmon (*Salmo salar*). Gen. Comp. Endocrinol. 93:70-81.

Björnsson, B.T. 1997. The biology of salmon growth hormone: from daylight to dominance. Fish Physiol. Biochem. 17:9-24.

Björnsson, B.T., and Bradley, T.M. 2007. Epilogue: past successes, present misconceptions and future milestones in salmon smoltification research. Aquaculture 273:384-391.

Björnsson, B.T., Stefansson, S.O., and McCormick, S.D. 2011. Environmental endocrinology of salmon smoltification. Gen. Comp. Endocrinol. 170:290-298.

Boeuf, G., Le Bail, P.Y., and Prunet, P. 1989. Growth hormone and thyroid hormones during Atlantic Salmon, *Salmo salar* L., smolting, and after transfer to seawater. Aquaculture 82:257-268.

Bourret, V., O'Reilly, P.T., Carr, J.W., Berg, P.R., and Bernatchez, L. 2011. Temporal change in genetic integrity suggests loss of local adaptation in a wild Atlantic Salmon (*Salmo salar*) population following introgression by farmed escapees. Heredity 106:500-510.

Boylan, S. 2011. Zoonoses associated with fish. Vet. Clin. North Am. Exot. Anim. Pract. 14:427-438.

Burgman, M. 2005. Risk and Decisions for Conservation and Environmental Managers. Cambridge University Press, Cambridge.

Butler, T.M., and Fletcher, G.M. 2009. Promoter analysis of a growth hormone transgene in Atlantic Salmon. Theriogenol. 72:62-71.

Cairns, D.K. 2006. A review of predator-prey and competitive inter-specific interactions in Atlantic Salmon (*Salmo salar*). Can. Sci. Advis. Sec. Res. Doc. 2006/019. v + 17 p.

Cairns D.K., Guignion, D.L., Dupuis, T., and MacFarlane, R.E. 2010. Stocking history, biological characteristics, and status of Atlantic Salmon (*Salmo salar*) on Prince Edward Island. Can. Sci. Advis. Sec. Res. Doc. 2010/104. iv + 50 p.

Caissie, D. 2000. Hydrological conditions for Atlantic Salmon rivers in 1999. Can. Sci. Advis. Sec. Res. Doc. 2000/011. 29 p.

Carr, J.W., and Whoriskey, F.G. 2006. The escape of juvenile farmed Atlantic Salmon from hatcheries into freshwater streams in New Brunswick, Canada. ICES J. Mar. Sci. 63:1263-1268.

Carss, D.N. 1990. Concentration of wild and escaped fishes immediately adjacent to fish farm cages. Aquaculture 90:29-40.

Castillo, A.G.F., Ayllon, F., Moran, P., Izquierdo, J.I., Martinez, J.L., Beall, E., and Garcia-Vazquez, E. 2008. Interspecific hybridization and introgression are associated with stock transfers in salmonids. Aquaculture 278:31-36.

CEPA (Canadian Environmental Protection Act). 1999. Canadian Environmental Protection Act S.C. 1999, c. 33. Published by the Minister of Justice. Government of Canada.

Chaput, G., Dempson, J.B., Caron, F., Jones, R., and Gibson, J. 2006. A synthesis of life history characteristics and stock grouping of Atlantic Salmon (*Salmo salar* L.) in eastern Canada. Can. Sci. Advis. Sec. Res. Doc. 2006/15.

Chevassus, B. 1979. Hybridization in salmonids: results and perspectives. Aquaculture 17:113-128.

Chittenden, C.M., Rikardsen, A.H., Skilbrei, O.T., Davidsen, J.G., Halttunen, E., Skarodhamar, J., and McKinley, R.S. 2011. An effective method for the recapture of escaped farmed salmon. Aquacult. Environ. Interact. 1:215-224.

Colautti, R.I., Grigorovich, I.A., and MacIsaac, H.J. 2006. Propagule pressure: a null model for biological invasions. Biol. Invas. 8:1023-1037.

Cook, J.T., McNiven, M.A., Richardson, G.F., and Sutterlin, A.M. 2000a. Growth rate, body composition and feed digestibility/conversion of growth-enhanced transgenic Atlantic Salmon (*Salmo salar*). Aquaculture 188:15-32.

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

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

COSEWIC (Committee on the Status of Endangered Wildlife in Canada). 2010. COSEWIC assessment and status report on the Atlantic Salmon *Salmo salar* (Nunavik population, Labrador population, Northeast Newfoundland population, South Newfoundland population, Southwest Newfoundland population, Northwest Newfoundland population, Quebec Eastern North Shore population, Quebec Western North Shore population, Anticosti Island population, Inner St. Lawrence population, Lake Ontario population, Gaspé-Southern Gulf of St. Lawrence population, Eastern Cape Breton population, Nova Scotia Southern Upland population, Inner Bay of Fundy population, Outer Bay of Fundy population) in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa.

Cote, D., Fleming, I.A., Carr, J.W., and McCarthy, J. 2015. Ecological impact assessment of the use of European origin Atlantic Salmon in Newfoundland aquaculture facilities. Can. Sci. Advis. Sec. Res. Doc. 2015/073.

Cotter, D., O'Donovan, V., O'Maoiléidigh, N., Rogan, G., Roche, N., and Wilkins, N.P. 2000. An evaluation of the use of triploid Atlantic Salmon (*Salmo salar* L.) in minimising the impact of escaped farmed salmon on wild populations. Aquaculture 186:61-75.

Cotter, D., O'Donovan, V., Drumm, A., Roche, N., Ling, E.N., and Wilkins, N.P. 2002. Comparison of fresh water and marine performances of all-female diploid and triploid Atlantic Salmon (*Salmo salar* L.). Aquacult. Res. 33:43-53.

Craig, J.K., Foote, C.J., and Wood, C.C. 1996. Evidence for temperature-dependent sex determination in sockeye salmon (*Oncorhynchus nerka*). Can. J. Fish. Aquat. Sci. 53:141-147.

Cross, T.F., McGinnity, P., Coughlan, J., Dillane, E., Ferguson, A., Coljonen, M.L., Milner, N., O'reilly, P., and Vasemagi, A. 2007. Stocking and ranching. Pages 325-256 *in* E. Verspoor, L. Stradmeyer, and J.L. Nielsen. The Atlantic Salmon: Genetics, Conservation, and Management. Blackwell Publishing. Oxford. UK.

Cunjak, R.A. 1992. Comparative feeding, growth and movements of Atlantic Salmon (*Salmo salar*) parr from riverine and estuarine environments. Ecol. Freshwater Fish 1:26-34.

Curtis, M.A., Rau, M.E., Tanner, C.E., Prichard, R.K., Faubert, G.M., Olpinski, S., and Trudeau, C. 1988. Parasitic zoonoses in relation to fish and wildlife harvesting by Inuit communities in northern Quebec, Canada. Arct. Med. Res. 47 (Supp. 1):693-696.

Curtis, L.R., Diren, F.T., Hurley, M.D., Seim, W.K., and Tubb, R.A. 1991. Disposition and elimination of 17μ-methyltestosterone in Nile Tilaplia (*Oreochromis niloticus*). Aquaculture 9:193-201.

Dabrowski, K., and Glogowski, J. 1977. Studies on the role of exogenous proteolytic enzymes in digestion processes in fish. Hydrobiologica 54:129-134.

Daley, E.L., Andrews, C.W., and Green, J.M. 1983. Precocious male Atlantic Salmon parr (*Salmo salar*) in insular Newfoundland. Can. J. Fish. Aquat. Sci. 40:647-652.

Danie, D.S., Trial, J.G., and Stanley, J.C. 1984. Species profiles: life histories and environmental requirements of coastal fish and invertebrates (north Atlantic) -- Atlantic Salmon. U.S. Fisheries and Wildlife Service. FWS/OBS-82/11.22. U.S. Army Corps of Engineers, TR EL-82-4.

DeAngelis, D.L., Mulholland, P.J., Palumbo, A.V., Steinman, A.D., Huston, M.A., and Elwood, J.W. 1989. Nutrient dynamics and food-web stability. Ann. Rev. Ecol. Syst. 20:71-95.

Debes, P.V., Fraser, D.J., McBride, M.V., and Hutchings, J.A. 2013. Multigenerational hybridization and its consequences for maternal effects in Atlantic Salmon. Heredity 111:238-247.

Deitch, E.J., Fletcher, G.L., Petersen, L.H., Costa, I.A., Shears, M.A., Driedzic, W.R., and Gamperl, A.K. 2006. Cardiorespiratory modifications and limitations in post-smolt growth hormone transgenic Atlantic Salmon. J. Exp. Biol. 209:1310-1325.

Devlin, R.H., Yesaki, T.Y., Biagi, C.A., Donaldson, E.M., Swanson, P., and Chan, W.K. 1994. Extraordinary salmon growth. Nature 371:209-210.

Devlin, R.H., Yesaki, T.Y., Donaldson, E.M., Du, S.J., and Hew, C.L. 1995. Production of germline transgenic Pacific salmonids with dramatically increased growth performance. Can. J. Fish. Aquat. Sci. 52:1376-1384.

Devlin, R.H., Johnsson, J.I., Smailus, D.E., Biagi, C.A., Jonsson, E., and Björnsson, B. 1999. Increased ability to compete for food by growth hormone-transgenic Coho Salmon *Oncorhynchus kisutch* (Walbaum). Aquacult. Res. 30:479-82.

Devlin, R.H., Swanson, P., Clarke, W.C., Plisetskaya, E., Dickhoff, W., Moriyama, S., Yesaki, T.Y., and Hew, C.L. 2000. Seawater adaptability and hormone levels in growth-enhanced transgenic Coho Salmon, *Oncorhynchus kisutch*. Aquaculture 191:367-385.

Devlin, R.H., Biagi, C.A., Yesaki, T.Y., Smailus, D.E., and Byatt, J.C. 2001. Growth of domesticated transgenic fish. Nature 409:781-782.

Devlin, R.H., D'Andrade, M., Uh, M., and Biagi, C.A. 2004. Population effects of growth hormone transgenic Coho Salmon depend on food availability and genotype by environment interactions. PNAS 101:9303-9308.

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

Devlin, R.H., Sakhrani, D., Tymchuk, W.E., Rise, M.L., and Goh, B. 2009. Domestication and growth hormone transgenesis cause similar changes in gene expression profiles in salmon. PNAS 106:3047-3052.

Devlin, R.H., Sakhrani, D., Biagi, C.A. and Eom, K. 2010. Occurrence of incomplete paternal-chromosome retention in GH-transgenic Coho Salmon being assessed for reproductive containment by pressure-shock-induced triploidy. Aquaculture 304:66-78.

Devlin, R.H. 2011. Growth hormone overexpression in transgenic fish. Pages 2016-2024 *in* A.P Farrell, E.D. Stevens, J.J. Cech Jr., and J.G. Richards, editors, Encyclopedia of Fish Physiology: From Genome to Environment, Volume 3. Academic Press, Waltham, MA.

DFO. 2006. Proceedings of the expert panel meeting on the potential risks associated with horizontal gene transfer from novel aquatic organisms. DFO Can. Sci. Advis. Sec. Proceed. Ser. 2006/036.

DFO. 2012a. Stock status of Atlantic Salmon (*Salmo salar*) in DFO Gulf Region (Salmon Fishing Areas 15 to 18). DFO Can. Sci. Advis. Sec. Sci. Advis. Rep. 2012/040.

DFO. 2012b. Temperature threshold to define management strategies for Atlantic Salmon (*Salmo salar*) fisheries under environmentally stressful conditions. DFO Can. Sci. Advis. Sec. Sci. Advis. Rep. 2012/019.

DFO and MRNF. 2008. Conservation Status Report, Atlantic Salmon in Atlantic Canada and Québec: Part I – Species Information. Can. MS Rep. Fish. Aquat. Sci. No. 2861.

Drinkwater, K., and Gilbert, D. 2004. Hydrographic variability in the waters of the Gulf of St. Lawrence, the Scotian Shelf and the Eastern Gulf of Maine (NAFO Subarea 4) during 1991–2000. J. Northwest Atlantic Fish. Sci. 34:85-101.

Du, S.J., Gong, Z., Fletcher, G.L., Shears, M.A., King, J.M., Idler, D.R., and Hew, C.L. 1992a. Growth enhancement in transgenic Atlantic Salmon by the use of an "all-fish" chimeric growth hormone gene construct. Nat. Biotechnol. 10:176-181.

Du, S.J., Gong, Z., Fletcher, G.L., Shears, M.A., and Hew, C.L. 1992b. Chapter 10 – Growth hormone gene transfer in Atlantic Salmon: use of antifreeze/growth hormone chimeric gene construct. Pages 176-189 *in* C.L. Hew and G. Fletcher, editors. Transgenic Fish. World Scientific Publishing. Signapore.

Duan, C., and Hirano. T. 1991. Plasma kinetics of growth hormone in the Japanese eel, *Anguilla japonica*. Aquaculture 95:179-188.

Dunham, R.A. 2011. Aquaculture and Fisheries Biotechnology: Genetic Approaches (2nd edition). CAB International, Oxfordshire, UK.

Eales, J.G., Devlin, R.H., Higgs, D.A., McLeese, J.M., Oakes, J.D., and Plohman, J. 2004. Thyroid function in growth-hormone-transgenic Coho Salmon (*Oncorhynchus kisutch*). Can. J. Zool. 82:1225-1229.

Ebbesson, L.O.E., Björnsson, B.T., Ekström, P., and Stefansson, S.O. 2008. Daily endocrine profiles in parr and smolt Atlantic Salmon. Comp. Biochem. Physiol. A 151:698-704.

EFSA (European Food Safety Authority). 2013. Guidance on the environmental risk assessment of genetically modified animals. GMO Panel (EFSA Panel on Genetically Modified Organisms). EFSA Journal 11(5):3200.

Einum, S., and Fleming, I.A. 1997. Genetic divergence and interactions in the wild among native, farmed and hybrid Atlantic Salmon. J. Fish Biol. 50:634-651.

Eisbrenner, W.D., Botwright, N., Cook, M., Davidson, E.A., Dominik, S., Elliott, N.G., Henshall, J., Jones, S.L., Kube, P.D., Lubieniecki, K.P., Peng, S., and Davidson, W.S. 2013. Evidence for multiple sex-determining loci in Tasmanian Atlantic Salmon (*Salmo salar*). Heredity 113:86-92.

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

Elliott, J.M., Lyle, A.A., and Campbell, R.N.B. 1997. A preliminary evaluation of migratory salmonids as vectos of organic carbon between marine and freshwater environments. Sci. Total Environ. 194/195:219-223.

Elliott, J.M., and Elliott, J.A. 2010. Temperature requirements of Atlantic Salmon *Salmo salar*, Brown Trout *Salmo trutta* and Arctic charr *Salvelinus alpinus*; predicting the effects of climate change. J. Fish Biol. 77:1793-1817.

Enders, E., Boisclair, D., and Roy, A.G. 2004. The cost of habitat utilization of wild, farmed and domesticated juvenile Atlantic Salmon (*Salmo salar* L.). Can. J. Fish. Aquat. Sci. 61:2302-2313.

Environment Canada. 2010. Research and Development Exemption for Organisms other than Micro-organisms. Guidance concerning the application of subsection 2(4) of the *New Substances Notification Regulations (Organisms)*. New Substances Notification Advisory Note 2010-02.

Fagerlund, U.H.M., and Dye, H.M. 1979. Depletion of radioactivity from yearling Coho Salmon (*Oncorhynchus kisutch*) after extended ingestion of anabolically effective doses of 17µ-methyltestosterone-1, 2-3 H. Aquaculture 18:303-315.

Farrell, A.P., Bennett, W., and Devlin, R.H. 1997. Growth-enhanced transgenic salmon can be inferior swimmers. Can. J. Zool. 75:335-337.

Ferguson, A., Fleming, I.A., Hindar, K., Skaala, O., McGinnity, P., Cross, T., and Prodohl, P. 2007. Farm escapes. Pages 367- 409 *in* E. Verspoor, L. Stradmeyer, and J.L. Nielsen editors. Atlantic Salmon: Genetics, Conservation and Management. Blackwell Publishing. Oxford, UK.

Field-Dodgson, M.S. 1987. The effects of salmon redd excavation on stream substrate and benthic community of two salmon spawning streams in Canterbury, New Zealand. Hydrobiol. 154:3-11.

Fine, M., Sakal, E., Vashdi, D., Daniel, V., Levanon, A., Lipshitz, O., and Gertler, A. 1993. Recombinant carp (*Cyprinus carpio*) growth hormone: expression, purification anf determination of biological activity *in vitro* and *in vivo*. Gen. Comp. Endocrinol. 89:51-61.

Finstad, B.F., Økland, F., Thorstad, E.B., Bjørn, P.A., and McKinley, R.S. 2005. Migration of hatchery-reared Atlantic Salmon and wild, anadromous Brown Trout post-smolts in a Norwegian fjord system. J. Fish Biol. 66:86-96.

Fiske, P., Lund, R.A., and Hansen, L.P. 2006. Relationships between the frequency of farmed Atlantic Salmon, *Salmo salar* L., in wild salmon populations and fish farming activity in Norway, 1989-2004. ICES J. Mar. Sci. 63:1182-1189.

Fitzpatrick, J.L., Akbarashandiz, H., Sakhrani, D., Biagi, C.A., Pitcher, T.E., and Devlin, R.H. 2011. Cultured growth hormone transgenic salmon are reproductively out-competed by wild-reared salmon in semi-natural mating arenas. Aquaculture 312:185-191.

Fleming, I.A., Jonsson, B., and Gross, M.R. 1994. Phenotypic divergence of sea-ranched, farmed and wild salmon. Can. J. Fish. Aquat. Sci. 51:2808-2824.

Fleming, I.A. 1996. Reproductive strategies of Atlantic Salmon: ecology and evolution. Rev. Fish Biol. Fish. 6:379-416.

Fleming, I.A., Jonsson, B., Gross, M.R., and Lamberg, A. 1996. An experimental study of the reproductive behaviour and success of farmed and wild salmon (*Salmo salar*). J. Appl. Ecol. 33:893-899.

Fleming, I.A., Hindar, K., Mjølnerød, I.B., Jonsson, B., Balstad, T., and Lamberg, A. 2000. Lifetime success and interactions of farm salmon invading a native population. Proc. R. Soc. London B 267:1517-1523.

Fleming, I.A., Augustsson, T., Finstad, B., Johnsson, J.I., and Björnsson, B.T. 2002. Effects of domestication on growth physiology and endocrinology of Atlantic Salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. 59:1323-1330.

Fleming I. A., and Einum, S. 2011. Reproductive ecology: A tale of two sexes. Pages 33-65 *in* Ø. Aas, S. Einum, A. Klemetsen, and J. Skurdal, editors. Atlantic Salmon Ecology. Wiley-Blackwell, Chichester, United Kingdom.

Fletcher, G.L., Hew, C.L., Li, X., Haya, K., and Kao, M.H. 1985. Year-round presence of high levels of plasma antifreeze peptides in a temperate fish, ocean pout (*Macrozoarces americanus*). Can. J. Zool. 63:488-493.

Fraser, D.L., Minto, C., Calvert, A.M., Eddington, J.D., and Hutchings, J.A. 2010a. Potential for domestic-wild interbreeding to induce maladaptive phenology across multiple populations of wild Atlantic Salmon. Can. J. Fish. Aquat. Sci. 67:1768-1775.

Fraser, D.L., Houde, A.L.S., Debes, P.V., O'Reilly, P.T., Eddington, J.D., and Hutchings, J.A. 2010b. Consequences of farmed-wild hybridization across divergent wild populations and multiple traits in salmon. Ecol. Appl. 20:935-953.

Fraser, T.W.K., Fjelldal, P.G., Hansen, T., and Mayer, I. 2012. Welfare considerations of triploid fish. Rev. Fish. Sci. 20:192-211.

Galbreath, P.F., and Thorgaard, G.H. 1995a. Saltwater performance of all-female triploid Atlantic Salmon. Aquaculture 138:77-85.

Galbreath, P.F., and Thorgaard, G.H. 1995b. Sexual maturation and fertility of diploid and triploid Atlantic Salmon x Brown Trout hybrids. Aquaculture 137:299-311.

Garcia de Leaniz, C., Fleming, I.A., Einum, S., Verspoor, E., Jordan, W.C., Consuegra, S., Aubin-Horth, N., Lajus, D., Letcher, B.H., Youngson, A.F., Webb, J.H., Vøllestad, L.A., Villanueva, B., Ferguson, A., and Quinn, T.P. 2007. A critical review of adaptive genetic variation in Atlantic Salmon: implications for conservation. Biol. Rev. Cambridge Philos. Soc. 82:173-211.

Garcia, H.E., Locarnini, R.A., Boyer, T.P., Antonov, J.I., Baranova, O.K., Zweng, M.M., and Johnson, D.R. 2010. World Ocean Atlas 2009, Volume 3: Dissolved oxygen, apparent oxygen utilization, and oxygen saturation. *In* S. Levitus, editor. NOAA Atlas NESDIS 68. US Government Printing Office, Washington, D.C.

Garside, E.T. 1973. Ultimate upper lethal temperature of Atlantic Salmon *Salmo salar* L. Can. J. Zool. 51:898-900.

Gaudemar, B., Schroder, S.L., and Beal, E.P. 2000. Nest placement and egg distribution in Atlantic Salmon redds. Environ. Biol. Fishes 57:37-47.

German, D.P., Horn, M.H., and Gawlicka, A. 2004. Digestive enzyme activities in herbivorous and carnivorous prickleback fishes (Teleostei:Stichaeidae): ontogenetic, dietary, and phylogenetic effects. Physiol. Biochem. Zool. 77:789-804.

Gong, Z., Fletcher, G.L., and Hew, C.L. 1992. Tissue distribution of fish antifreeze protein mRNAs. Can. J. Zool. 70:810-814.

Gong, Z., Maclean, N., Devlin, R.H., Martinez, R., Omitogun, O., and Estrada, M.P. 2007. Gene construct and expression: Information relevant for risk assessment and management. Pages 95-111 *in* A.R. Kapuscinski, K.R. Hayes, S. Li, and G. Dana. Environmental Risk Assessment of Genetically Modified Organisms, Volume 3: Methodologies for Transgenic Fish. CABI Publishing, Oxfordshire, UK.

Gottesfeld, A.S., Hassan, M.A., Tunnicliffe, J.F., and Poirier, R.W. 2004. Sediment dispersion in salmon spawning streams: The influence of floods and salmon redd construction. J. Am. Water Res. Assoc. 40:1071-1086.

Gottesfeld, A.S., Hassan, M.A., and Tunnincliffe, J.F. 2008. Salmon bioturbation and stream process. Pages 175-193 *in* D.A. Sear and P. De Vries, editors. Salmonid Spawning Habitat in Rivers: Physical Controls, Biological Responses, and Approaches to Remediation. American Fisheries Society, Symposium 65, Bethesda, Maryland.

Government of Canada. 2003. Government of Canada Framework for the Application of Precaution in Science-based Decision Making about Risk.

Grant, C.G.J., and Lee, E.M. 2004. Life history characteristics of freshwater fishes occurring in Newfoundland and Labrador, with major emphasis on riverine habitat requirements. Can. MS Rpt. Fish. Aquat. Sci. 2672.

Grechko, V.V. 2011. Repeated DNA sequences as an engine of biological diversification. Mol. Biol. 45:704-727.

Gross, M.R. 1998. One species with two biologies: Atlantic Salmon (*Salmo salar*) in the wild and in aquaculture. Can. J. Fish. Aquat. Sci. (Suppl. 1) 55:131–144.

Guignion, D.L. 2009. A Conservation Strategy for Atlantic Salmon in Prince Edward Island. Council of the Atlantic Salmon Federation. Charlottetown, Prince Edward Island.

Habibi, H.R., Ewing, E., Bajwa, R., and Walker, R.L. 2004. Gastric uptake of recombinant growth hormone in Rainbow Trout. Fish Physiol. Biochem. 28:463-467.

Handeland, S.O., Björnsson, B.T., and Arnesen, A.M. 2003. Seawater adaptation and growth of post-smolt Atlantic Salmon (*Salmo salar*) of wild and farmed strains. Aquaculture 220:367-384.

Hansen, L.P., Døving, K.B., and Jonsson, B. 1987. Migration of farmed, adult Atlantic Salmon with and without olfactory sense, released on the Norwegian coast. J. Fish Biol. 30:713-721.

Hansen, L.P., and Quinn, T.R. 1998. The marine phase of the Atlantic Salmon (*Salmo salar*) life cycle, with comparison to Pacific salmon. Can. J. Fish. Aquat. Sci. (Suppl. 1) 55:104-118.

Hansen, L.P. 2006. Migration and survival of farmed Atlantic Salmon (*Salmo salar* L.) released from two Norwegian fish farms. ICES J. Mar. Sci. 63:1211-1217.

Hansen, L.P., and Youngson, A.F. 2010. Dispersal of large farmed Atlantic Salmon, *Salmo salar*, from simulated escapes at fish farms in Norway and Scotland. Fish. Manage. Ecol. 17:28-32.

Hassan, M.A., Gottesfeld, A.S., Montgomery, D.R., Tunnicliffe, J.F., Clarke, G.K.C., Wynn, G., Jones-Cox, H., Poirier, R., MacIsaac, E., Herunter, H., and MacDonald, S.J. 2008. Salmon-driven bed load transport and bed morphology in mountain streams. Geophys. Res. Lett. 35:L04405.

Hayes, K.R. 2002. Identifying hazards in complex ecological systems. Part 2: infection modes and effects analysis for biological invasions. Biol. Invas. 4:251-261.

Heinimaa, S., and Heinimaa, P. 2004. Effect of female size on egg quality and fecundity of the wild Atlantic Salmon in the sub-arctic River Teno. Boreal Environ. Res. 9:55-62.

Hendry, K., and Cragg-Hine, D. 2003. Ecology of the Atlantic Salmon. Conserving Natura 2000 Rivers Ecology Series No. 7. English Nature, Peterborough, UK.

Hidalgo, M.C., Urea, E., and Sanz, A. 1999. Comparative study of digestive enzymes in fish with different nutritional habits. Proteolytic and amylase activities. Aquaculture 170:267-283.

Higgs, D.A, Sutton, J., Kim, H., Oakes, J.D., Smith, J., Biagi, C., Rowshandeli, M., and Devlin, R.H. 2009. Influence of dietary concentrations of protein, lipid and carbohydrate on growth, protein and energy utilization, body composition, and plasma titres of growth hormone and insulin-like growth factor-1 in non-transgenic and growth hormone transgenic Coho Salmon, *Oncorhynchus kisutch* (Walbaum). Aquaculture 286:127-137.

Hindar, K., Fleming, I.A., McGinnity, P., and Diserud, O. 2006. Genetic and ecological effects of salmon farming on wild salmon: modelling from experimental results. ICES J. Mar. Sci. 63:1234-1247.

Hoar, W.S. 1988. The physiology of smolting salmonids. Pages 275-343 *in* W.S. Hoar and D.J. Randall editors, Fish Physiology: The Physiology of Developing Fish, Viviparity and Posthatching Juveniles, vol. XIB. Academic Press, NewYork.

Hobbs, R.S., and Fletcher, G.L. 2008. Tissue specific expression of antifreeze protein and growth hormone transgenes driven by the ocean pout (*Macrozoarces americanus*) antifreeze protein OP5a gene promoter in Atlantic Salmon (*Salmo salar*). Trans. Res. 17:33-45.

Hori, T.S., Xu, Q., Plouffe, D.A., Buchanan, J.T., and Rise, M.L. 2013. Functional genomic analysis of immune-relevant transcript expression response to triploidization in growth hormone (GH) transgenic Atlantic Salmon. Presentation at Aquaculture 2013. World Aquaculture Society, Nashville, USA.

Houde, A.L.S., Fraser, D.J., and Hutchings, J.A. 2010a. Fitness-related consequences of competitive ability between farmed and wild Atlantic Salmon at different proportional representations of wild-farmed hybrids. ICES J. Mar. Sci. 67:657-667.

Houde, A.L.S., Fraser, D.J., and Hutchings, J.A. 2010b. Reduced anti-predator responses in multi-generational hybrids of farmed and wild Atlantic Salmon (*Salmo salar* L.). Conserv. Genet. 11:785-794.

Hutchings, J.A. 1986. Lakeward migrations by juvenile Atlantic Salmon, *Salmo salar*. Can. J. Fish. Aquat. Sci. 43:732-741.

Hutchings, J.A., and Myers, R.A. 1988. Mating success of alternative maturation phenotypes in male Atlantic Salmon, *Salmo salar*. Oecologia 75:169-174.

Hutchings, J.A., and Myers, R.A. 1994. The evolution of alternative mating strategies in variable environments. Evol. Ecol. 8:256-268.

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

Jacobsen, J.A., and Hansen, L.P. 2001. Feeding habits of wild and escaped farmed Atlantic Salmon, *Salmo salar* L., in the Northeast Atlantic. ICES J. Mar. Sci. 58:916-933.

Janetski, D.J., Chaloner, D.T., Tiegs, S.D., and Lamberti, G.A. 2009. Pacific salmon effects on stream ecosystems: a quantitative synthesis. Oecologia 159:583-595.

Jardine, T.D., Roussel, J.M., and Mitchell, S.C. 2009. Detecting marine nutrient and organic matter inputs into multiple trophic levels in streams of Atlantic Canada and France. Am. Fish. Soc. Symp. 69:427-445.

Jensen, A.J., Karlsson, S., Fiske, P., Hansen, L.P., Hindar, K., and Østborg, G.M. 2013. Escaped farmed Atlantic Salmon grow, migrate and disperse throughout the Arctic Ocean like wild salmon. Aquacult. Environ. Interact. 3:223-229.

Jhingan, E., Devlin, R.H., and Iwama, G.K. 2003. Disease resistance, stress response and effects of triploidy in growth hormone transgenic Coho Salmon. J. Fish Biol. 63:806-823.

Jobling, M. 1981. Temperature tolerance and the final preferendum; rapid methods for the assessment of optimum growth temperatures. J. Fish Biol. 19:439-455.

Johansen, M., Erkinaro, J., and Amundsen, P.A. 2011. The when, what and where of freshwater feeding. Pages 89-114 *in Ø*. Aas, S. Einum, A. Klemetsen, and J. Skurdal. Atlantic Salmon Ecology. Blackwell Publishing. Oxford, UK.

Johnson, O.W., Dickhoff, W.W., and Utter, F.M. 1986. Comparative growth and development of diploid and triploid Coho Salmon, *Oncorhynchus kisutch*. Aquaculture 57:329-336.

Johnsson, J.I., Hojesjo, J., and Fleming, I.A. 2001. Behavioural and heart rate responses to predation risk in wild and domesticated Atlantic Salmon. Can. J. Fish. Aquat. Sci. 58:788-794.

Johnstone, R., Macintosh, D.J., and Wright, R.S. 1983. Elimination of orally administered 17μ-methyltestosterone by *Oreochromis mossambicus* (Tilaplia) and *Salmo gairdineri* (Rainbow Trout) juveniles. Aquaculture 35:249-257.

Johnstone, R., and MacLachlan, P.M. 1994. Further observations on the sex inversion of Atlantic Salmon, *Salmo salar* L., using 17α methyl testosterone. Aquacult. Fish. Manage. 25:855-859.

Johnstone, R., and Stet, R.J.M. 1995. The production of gynogenetic Atlantic Salmon, *Salmo salar* L. Theor. Appl. Genet. 90:819-826.

Jones, C.G., Lawton, J.H., and Shachak, M. 1994. Organisms as ecosystem engineers. Oikos 69:373-386.

Jones, R.A., Anderson, L., and Goff, T. 2004. Assessments of Atlantic Salmon stocks in southwest New Brunswick, an update to 2003. DFO Can. Sci. Advis. Sec. Res. Doc. 2004/019.

Jonsson, B., Jonsson, N., and Hansen, L.P. 1991. Differences in life history and migratory behavior between wild and hatchery-reared Atlantic Salmon in nature. Aquaculture 98:69-78.

Jonsson, N., Hansen, L.P., and Jonsson, B. 1993. Migratory behaviour and growth of hatchery-reared, post-smolt Atlantic Salmon *Salmo salar* L. J. Fish Biol. 42:435-443.

Jonsson, B. 1997. A review of ecological and behavioural interactions between cultured and wild Atlantic Salmon. ICES J. Mar. Sci. 54:1031-1039.

Jonsson, B., and Jonsson, N. 2003. Migratory Atlantic Salmon as vectors for the transfer of energy and nutrients between freshwater and marine environments. Freshwater Biol. 48:21-27.

Jonsson, N., Jonsson, B., and Hansen, L.P. 2003. The marine survival and groth of wild and hatchery-reared Atlantic Salmon. J. Appl. Ecol. 40:900-911.

Jonsson, B., and Jonsson, N. 2004. Factors affecting marine production of Atlantic Salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. 61:2369-2383.

Jonsson, B., and Jonsson, N. 2006. Cultured Atlantic Salmon in nature: a review of their ecology and interaction with wild fish. ICES J. Mar. Sci. 63:1162-1181.

Juskevich, J., and Guyer, G. 1990. Bovine growth hormone: human food safety evaluation. Science 249:875-884.

Kanaiwa M., and Harada, Y. 2002. Genetic risk involved in stock enhancement of fish having environmental sex determination. Popul. Ecol. 44:7-15.

Kanaiwa, M., and Harada, Y. 2008. Collapse of one-locus two-allele sex determining system by releasing sex-reversed hatchery fish. Rev. Fish. Sci. 16:296-302.

Kapuscinski, A.R., Hayes, K.R., Li, S., and Dana, G. 2007. Environmental Risk Assessment of Genetically Modified Organisms, Volume 3: Methodologies for Transgenic Fish. CABI Publishing, Oxfordshire, UK.

Khalil, W.K.B., Hasheesh, W.S., Marie, M.A.S., Abbas, H.H., and Zahran, E.A. 2011. Assessment the impact of 17α-methyltestosterone hormone on growth, hormone concentration, molecular and histopathological changes in muscles and testis of Nile Tilaplia; *Oreochromis niloticus*. Life Sci. J. 8:329-343.

Kim, D.H., Kim, Y.T., Cho, J.J., Bae, J.H., Hur, S.B., Hwang, I., and Choi, T.J. 2002. Stable integration and functional expression of flounder growth hormone gene in transformed microalga, *Chlorella ellipsoidea*. Mar. Biotechnol. 4:63-73.

Kim, J.H, Balfry, S., and Devlin, R.H. 2013. Disease resistance and health parameters of growth-hormone transgenic and wild-type Coho Salmon, *Oncorhynchus kisutch*. Fish Shellfish Immunol. 34:1553-1559.

Kimura, T., Murakawa, Y., Ohno, M., Ohtani, S., and Higaki, K. 1997. Gastrointestinal absorption of recombinant human insulin-like growth factor-I in rats. J. Pharmacol. Exp. Therap. 283:611-618.

King, T.L., Verspoor, E., Spidle, A.P., Gross, R., Phillips, R.B., Koljonen, M.L. Sanchez, J.A., and Morrison, C.L. 2007. Biodiversity and population structure. Pages 118-166 *in* E. Verspoor, L. Stradmeyer, and J.L. Nielsen. The Atlantic Salmon, Genetics, Conservation, and Management. Blackwell Publishing. Oxford, UK.

King, H., Ruff, N., Evans, B., and Elliott, N. 2012. Evidence that sex differentiation in Atlantic Salmon (*Salmo salar*) is thermolabile. 1st International Conference on Integrative Salmonid Biology, Oslo, Norway, 17-20 June 2012.

Kjøglum, S., Henryon, M., Aasmundstad, T., and Korsgaard, I. 2008. Selective breeding can increase resistance of Atlantic Salmon to furunculosis, infectious salmon anaemia and infectious pancreatic necrosis. Aquacult. Res. 39:498-505.

Koenig, M.K., Kozfkay, J.R., Meyer, K.A., and Schill, D.J. 2011. Performance of diploid and triploid Rainbow Trout stocked in Idaho alpine lakes. North Am. J. Fish. Manage. 31:124-133.

Komen, H., and Thorgaard, G.H. 2007. Androgenesis, gynogenesis and the production of clones in fishes: a review. Aquaculture 269:150-173

Kozfkay, J.R., Dillon, J.C., and Schill, D.J. 2006. Routine use of sterile fish in salmonid sport fisheries: Are we there yet? Fisheries 31:392-399.

Kuraku, S., Qiu, H., and Meyer, A. 2012. Horizontal transfers of Tc1 elements between teleost fishes and their vertebrate parasites, lampreys. Genome Biol. Evol. 4:929-936.

Lahnsteiner, F. 2002. The influence of ovarian fluid on the gamete physiology in the Salmonidae. Fish Physiol. Biochem. 27:49-59.

Lecomte, F., Beall, E., Chat, J., Davaine, P., and Gaudin, P. 2013. The complete history of salmonid introductions in the Kerguelen Islands. South. Ocean Polar Biol. 36:457-475.

Lee, C.G., Devlin, R.H., and Farrell, A.P. 2003. Swimming performance, oxygen consumption and excess post-exercise oxygen consumption in adult transgenic and ocean-ranched Coho Salmon. J. Fish Biol. 62:753-766.

Leggatt, R.A., O'Reilly, P.T., Blanchfield, P.J., McKindsey, C.W., and Devlin, R.H. 2010. Pathway of effects of escaped aquaculture organisms or their reproductive material on natural ecosystems in Canada. DFO Can. Sci. Advis Sec. Res. Doc. 2010/019.

Leggatt, R.A., Biagi, C.A., Smith, J.L., and Devlin, R.H. 2012. Growth of growth hormone transgenic Coho Salmon *Oncorhynchus kisutch* is influenced by construct promoter type and family line. Aquaculture 356-357:193-199.

Lehane, L., and Rawlin, G.T. 2000. Topically acquired bacterial zoonoses from fish: a review. Med. J. Aust. 173:256-259.

Lema, S.C., Hodges, M.J., Marchetti, M.P., and Nevitt, G.A. 2005. Proliferation zones in the salmon telencephalon and evidence for environmental influence on proliferation rate. Comp. Biochem. Physiol. A 141:327-335.

Levesque, H.M., Shears, M.A., Fletcher, G.L., and Moon, T.W. 2008. Myogenesis and muscle metabolism in juvenile Atlantic Salmon (*Salmo salar*) made transgenic for growth hormone. J. Exp. Biol. 211:128-137.

Levin, S.A. 2009. Princeton Guide to Ecology. Princeton University Press, Princeton NJ.

Lima dos Santos, C.A.M., and Howgate, P. 2011. Fishborne zoonotic parasites and aquaculture: a review. Aquaculture 318:253-261.

Lincoln, R., Boxshall, G., and Clark, P. 1998. A dictionary of ecology, evolution and systematics. Cambridge University Press, Cambridge, UK.

Liu B., Zang, X.N., Liu, X.F., Zhang, X.C., and Lei, J.L. 2012. Stable cell-surface expression of Japanese flounder growth hormone in yeast *Saccharomyces cerevisiae* and growth-promoting effect on juvenile fish by oral administration. Fish Sci. 78:99-107.

Locarnini, R.A., Mishonov, A.V., Antonov, J.I., Boyer, T.P., Garcia, H.E., Baranova, O.K., Zweng, M.M., and Johnson, D.R. 2010. Temperature. Volume 1 *in* S. Levitus, editor. NOAA Atlas NESDIS 68, World Ocean Atlas 2009. US Government Printing Office, Washington.

Lockwood, J.L., Cassey, P., and Blackburn, T. 2005. The role of propagule pressure in explaining species invasions. Trends Ecol. Evol. 20:223-228.

Lõhmus, M., Raven, P.A., Sundström, L.F., and Devlin, R.H. 2008. Disruption of seasonality in growth hormone-transgenic Coho Salmon (*Oncorhynchus kisutch*) and the role of cholecystokinin in seasonal feeding behavior. Horm. Behav. 54:506-513.

Lowry, T., and Smith, S.A. 2007. Aquatic zoonoses associated with food, bait, ornamental, and tropical fish. Vet Med Today: Zoonoses update. J. Am. Vet. Med. Assoc. 231:876-880.

Lyle, A.A., and Elliott, J.M. 1998. Migratory salmonids as vectors of carbon, nitrogen and phosphorous between marine and freshwater environments in north-east England. Sci. Total Environ. 210/211:457-468.

MacCrimmon, H.R., and Gots, B.L. 1979. World distribution of Atlantic Salmon, *Salmo salar*. J. Fish. Res. Brd. Can. 36:422-457.

MacDonald, R., and Ekker, M. 2012. Spatial and temporal regulation of transgene expression in fish. Chapter 15, Pages 235-251 *in* G.L. Fletcher and M.L. Rise, editors, Aquaculture Biotechnology, First Edition. . John Wiley & Sons, Oxford, UK.

Magnuson, J.J., Crowder, L.B., and Medvick, P.A. 1979. Temperature as an ecological resource. Am. Zool. 19:331-343.

Mair, G.C., Nam, Y.K, and Solar, I.I. 2007. Risk management: reducing risk through confinement of transgenic fish. Pages 209-238 *in* A.R. Kapuscinski, K.R. Hayes, S. Li, and G. Dana, editors, Environmental Risk Assessment of Genetically Modified Organisms, Volume 3: Methodologies for Transgenic Fish. CABI Publishing, Oxfordshire, UK.

McCarthy, I.D., Carter, C.G., Houlihan, D.F., Johnstone, R., and Mitchell, A.I. 1996. The performance of all-female diploid and triploid Atlantic Salmon smolts on transfer together to sea water. J. Fish Biol. 48:545-548.

McCormick, S.D., Hansen, L.P., Quinn, T.P., and Saunders, R.L. 1998. Movement, migration, and smolting of Atlantic Salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. 55:77-92.

McDermott, R.E., Mikulak, R.J., and Beauregard, M.R. 2009. The Basics of FMEA. Second Edition. CRC Press. Taylor & Francis Group, New York.

McGinnity, P., Stone, C., Taggart, J.B., Cook, D., Cotter, D., Hynes, R., McCamley, C., Cross, T., and Fergusson, A. 1997. Genetic impact of escaped farmed Atlantic Salmon (*Salmo salar* L) on native populations: use of DNA profiling to assess freshwater performance of wild, farmed, and hybrid progeny in a natural river environment. ICES J. Mar. Sci. 54:998-1008.

McGinnity, P., Prodöhl, P., Ferguson, A., Hynes, R., Maoiléidigh, N.O., Baker, N., Cotter, D., O'Hea, B., Cooke, D., Rogan, G., Taggart, J., and Cross, T. 2003. Fitness reduction and potential extinction of wild populations of Atlantic Salmon, *Salmo salar*, as a result of interactions with escaped farm salmon. Proc. R. Soc. London B 270:2443-2450.

McGowan, C., and Davidson, W.S. 1992. Unidirectional natural hybridization between Brown Trout (*Salmo trutta*) and Atlantic Salmon (*S. salar*) in Newfoundland. Can. J. Fish. Aquat. Sci. 49:1953-1958.

McNair, A. Sr., Lim, J.N., and Nakagawa, S. 2012. The fitness consequences of environmental sex reversal in fish: a quantitative review. Biol. Rev. 87:900-911.

Meysman, F.J.R., Middelburg, J.J., and Heip, C.H.R. 2006. Bioturbation: a fresh look at Darwin's last idea. Trends Ecol. Evol. 21:687-695.

Minakawa, N., and Gara, R.I. 2003. Effects of chum salmon redd excavation on benthic communities in a stream in the Pacific Northwest. Trans. Am. Fish. Soc. 132:598-604.

Monafo, V., Marseglia, G.L., Maghnie, M., Dyne, K.M., and Cetta, G. 2000. Transient beneficial effect of GH replacement therapy and topical GH application on skin ulcers in a boy with prodiase deficiency. Pediatr. Dermatol. 17:227-230.

Moore, J.W. 2006. Animal ecosystem engineers in streams. BioScience 56:237-246.

Moore, J.W., and Schindler, D.E. 2008. Biotic disturbance and benthic community dynamics in salmon-bearing streams. J. Anim. Ecol. 77:275-284.

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

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

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

Moreau, D.T.R., and Fleming, I.A. 2012a. Enhanced growth reduces precocial male maturation in Atlantic Salmon. Funct. Ecol. 26:399-405.

Moreau, D.T.R., and Fleming, I.A. 2012b. The potential ecological and genetic impacts of aquaculture biotechnologies: Eco-evolutionary considerations for managing the blue revolution. Pages 319-342 *in* G.L. Fletcher and M.L. Rise editors. Aquaculture Biotechnology, first edition, Wiley-Blackwell, Oxford, UK.

Moriyama, S., Yamamoto, H., Sugimoto, S., Abe, T., Hirano, T., and Kawauchi, H. 1993. Oral administration of recombinant salmon growth hormone to Rainbow Trout, *Oncorhynchus mykiss*. Aquaculture 112:99-106.

Moriyama, S. 1995. Increased plasma insulin –like growth factor-I (IGF-I) following oral and intraperitoneal administration of growth hormone to Rainbow Trout, *Oncorhynchus mykiss*. Growth Regul. 5:164-167.

Morris, M.R.J., Fraser, D.J., Heggelin, A.J., Whoriskey, F.G., Carr, J.W., O'Neil, S.F., and Hutchings, J.A. 2008. Prevalence and recurrence of escaped farmed Atlantic Salmon (*Salmo salar*) in eastern North American rivers. Can. J. Fish. Aquat. Sci. 65:2807-2826.

Mortensen, A., and Damsgård, B. 1998. The effect of salinity on desmoltification in Atlantic Salmon. Aquaculture 168:407-411.

Muir, W.M., and Howard, R.D. 1999. Possible ecological risks of transgenic organism release when transgenes affect mating success: sexual selection and the Trojan gene hypothesis. PNAS 96:13853-13856.

Murota, T. 2003. The marine nutrient shadow: a global comparison of anadromous salmon fishery and guano occurrence. Pages 17-32 *in* J. G. Stockner, editor. Nutrients in Salmon Ecosystems: Sustaining Production and Biodiversity. American Fisheries Society, Symposium 34, Bethesda, Maryland, USA.

Nam, Y.K., Maclean, N., Fu, C., Pandian, T.J., and Eguia, M.R.R. 2007. Development of transgenic fish: Scientific background. Pages 61-94 *in* A.R. Kapuscinski, K.R. Hayes, S. Li, and G. Dana. Environmental Risk Assessment of Genetically Modified Organisms, Vol. 3: Methodologies for Transgenic Fish, CABI Publishing, UK.

Naylor, R., Hindar, K., Feming, I.A., Goldburg, R., Williams, S., and Volpe, J. 2005. Fugitive salmon: assessing the risks of escaped fish from net-pen aquaculture. BioScience 55:427-437.

New Brunswick. 2013. New Brunswick Rainbow Trout Aquaculture Policy. Department of Agriculture and Aquaculture.(July 2013).

Nislow, K.H., Armstrong, J.D., and McKelvey, S. 2004. Phosphorus flux due to Atlantic Salmon (*Salmo salar*) in an oligotrophic upland stream: effects of management and demography. Can. J. Fish. Aquat. Sci. 61:2401-2410.

O'Connell, M.F., Dempson, J.B., and Chaput, G. 2006. Aspects of the life history, biology, and population dynamics of Atlantic Salmon (*Salmo salar* L.) in Eastern Canada. DFO Can. Sci. Advis. Sec. Res. Doc. 2006/014.

Oakes, J.D., Higgs, D.A., Eales, J.G., and Devlin, R.H. 2007. Influence of ration level on the growth performance and body composition of non-transgenic and growth-hormone-transgenic Coho Salmon (*Oncorhynchus kisutch*). Aquaculture 265:309-324.

OECD (Organisation for Economic Co-operation and Development). 2010. Consensus document on molecular characterisation of plants derived from modern biotechnology. Series on Harmonisation of Regulatory Oversight in Biotechnology No. 51.

O'Flynn, F.M., McGeachy, S.A., Friars, G.W., Benfey, T.J., and Bailey, J.K. 1997. Comparisons of cultured triploid and diploid Atlantic Salmon (*Salmo salar* L.). ICES J. Mar. Sci. 54:1160-1165.

Ohigashi, I., Yamasaki, Y., Hirashima, T., and Takahama, Y. 2010. Identification of the transgenic integration site in immunodeficient tgε26 human CD3ε transgenic mice. PLoS ONE 5(12): e14391.

Ojolick, E.J., Cusack, R., Benfey, T.J., and Kerr, S.R. 1995. Survival and growth of all-female diploid and triploid Rainbow Trout (*Oncorhynchus mykiss*) reared at chronic high temperature. Aquaculture 13:177-187.

Oke, K.B., Westley, P.A., Moreau, D.T.R., and Fleming, I.A. 2013. Hybridization between genetically modified Atlantic Salmon and wild Brown Trout reveals novel ecological interaction. Proc. R. Soc. B 280:20131047.

O'Keefe, R.A., and Benfey, T.J. 1997. The feeding response of diploid and triploid Atlantic Salmon and Brook Trout. J. Fish Biol. 51:989-997.

Ozerov, M.Y., Lumme, J., Päkk, P., Rintamäki, P., Zietara, M.S., Barskaya, Y., Lebedeva, D., Saadre, E., Gross, R., Primmer, C.R., and Vasemägi, A. 2010. High *Gyrodactylus salaris* infection rate in triploid Atlantic Salmon *Salmo salar*. Dis. Aquat. Org. 9:129-136.

Pandian, T.J., and Koteeswaran, R. 1998. Ploidy induction and sex control in fish. Hydrobiologia 384:167-243.

Parry, G. 1960. The development of salinity tolerance in the salmon, *Salmo salar* (L.) and some related species. J. Exp. Biol. 37:425-434.

Parsons, J.E., Busch, R.A., Thorgaard, G.H., and Scheerer, P.D. 1986. Increased resistance of triploid Rainbow Trout x Coho Salmon hybrids to infectious hematopoietic necrosis virus. Aquaculture 57:337-343.

Pepper, V.A., Nicholls, T., and Collier, C. 2004. Reproductive technologies applied to Newfoundland salmonid aquaculture to enhance commercial production. Can. Tech. Rep. Fish. Aquat. Sci. 2541: v + 50 p.

Peter, R.E., and Marchant, T.A. 1995. The endocrinology of growth in carp and related species. Aquaculture 129:299-321.

Petrie, B., Drinkwater, K., Sandstrom, A., Pettipas, R., Gregory, D., Gilbert, D., and Sekhon, P. 1996. Temperature, salinity and sigma-t atlas for the Gulf of St. Lawrence. Can. Tech. Rep. Hydrogr. Ocean Sci. 178: v + 256 p.

Phillips, R.B., and Devlin, R.H. 2009. Integration of growth hormone gene constructs in transgenic strains of Coho Salmon (*Oncorhynchus kisutch*) at centromeric or telomeric sites. Genome 53:79-82.

Piccolo, J.J., and Orlikowska, E.H. 2012. A biological risk assessment for an Atlantic Salmon (*Salmo salar*) invasion in Alaskan waters. Aquat. Invas. 7:259-270.

Piferrer, F. 2001. Endocrine sex control strategies for the feminization of teleost fish. Aquaculture 197:229-281.

Poole, W.R., Nolan, D.T., Wevers, T., Dillane, M., Cotter, D., and Tully, O. 2003. An ecophysiological comparison of wild and hatchery-raised Atlantic Salmon (*Salmo salar* L.) smolts from the Burrishoole system, western Ireland. Aquaculture 222:301-314.

Poontawee, K., Werner, C., Muller-Belecke, A., Horstgen-Schwark, G., and Wicke, M. 2007. Flesh qualities and muscle fibre characteristics in triploid and diploid Rainbow Trout. J. Appl. Ichthyol. 23:273-275.

Poppe, T.T., Johansen, R., Gunnes, G., and Torud, B. 2003. Heart morphology in wild and farmed Atlantic Salmon *Salmo salar* and Rainbow Trout *Oncorhynchus mykiss*. Dis. Aquat. Org. 57:103-108.

Potter, E.C.E., Maoileidigh, N.O., and Chaput, G. 2003. Marine mortality of Atlantic Salmon, *Salmo salar* L: methods and measures. DFO Can. Sci. Advis. Sec. Res. Doc. 2003/101.

Prunet, P., Bœuf, G., Bolton, J.P., and Young, G. 1989. Smoltification and seawater adaptation in Atlantic Salmon (*Salmo salar*) olasma orolactin, growth hormone, and thyroid hormones. Gen. Comp. Endocrinol. 74:355-364.

Quillet, E. 1984. Survival, growth and reproductive traits of mitotic gynogenetic Rainbow Trout females. Aquaculture 123:223-236.

Quillet, E., and Gaignon, J.L. 1990. Thermal induction of gynogenesis and triploidy in Atlantic Salmon (*Salmo salar*) and their potential interest for aquaculture. Aquaculture 89:351-364.

Quillet, E., Aubard, G., and Quéau, I. 2002. Mutation in a sex-determining gene in Rainbow Trout: detection and genetic analysis. J. Heredity 93:91-99.

Randall, D.J., and Wright, P.A. 1995. Circulation and gas transfer. Pages 439-458 *in* C. Groot, L. Margolis and W.G. Clarke, editors. Physiological Ecology of Pacific salmon. UBC Press Vancouver.

Raven, P.A., Uha, M., Sakhrani, D., Beckman, B.R., Cooper, K., Pinter, J., Leder, E.H., Silverstein, J., and Devlin, R.H. 2008. Endocrine effects of growth hormone overexpression in transgenic Coho Salmon. Gen. Comp. Endocrinol. 159:26-37.

Reddin, D.G. 2010. Atlantic Salmon return and spawner estimates for Labrador. DFO Can. Sci. Advis. Sec. Res. Doc. 2009/045.

Reddin, D.G., and Veinott, G.I. 2010. Atlantic Salmon return and spawner estimates for insular Newfoundland. DFO Can. Sci. Advis. Sec. Res. Doc. 2009/044.

Reid, J.E., and Chaput, G. 2012. Spawning history influence on fecundity, egg size, and egg survival of Atlantic Salmon (*Salmo salar*) from the Miramichi River, New Brunswick, Canada. ICES J. Mar. Sci. 69:1678-1685.

Reinecke, M., Björnsson, B.T., Dickhoff, W.W., McCormick, S.D., Navarro, I., Power, D.M., and Gutiérrez, J. 2005. Growth hormone and insulin-like growth factors in fish: Where we are and where to go. Gen. Comp. Endocrinol. 142:20-24.

Reinitz, G. 1983. Relative effect of age, diet, and feeding rate on the body composition of young Rainbow Trout (*Salmo gairdneri*). Aquaculture 35:19-27.

Rhodes, J.S., and Quinn, T.P. 1998. Factors affecting the outcome of territorial contests between hatchery and naturally reared Coho Salmon parr in the laboratory. J. Fish Biol. 53:1220-1230.

Richard, A., Dionne, M., Jinliang, W., and Bernatchez, L. 2013. Does catch and release affect the mating system and individual reproductive success of wild Atlantic Salmon (*Salmo salar* L.)? Mol. Ecol. 22:187-200.

Rikardsen, A.H., and Dempson, J.B. 2011. Dietary life-support: the food and feeding of Atlantic Salmon at sea. Pages 115-143 *in* Ø. Aas, S. Einum, A. Klemetsen, and J. Skurdal editors. Atlantic Salmon Ecology. Wiley-Blackwell, Oxford, UK.

Riley, S.C., Tatara, C.P., and Scheurer, J.A. 2005. Aggression and feeding of hatchery-reared and naturally reared steelhead (*Oncorhynchus mykiss*) fry in a laboratory flume and a comparison with observations in natural streams. Can. J. Fish. Aquat. Sci. 62:1400-1409.

Roberts, H.E., Palmeiro, B., and Weber III, E.S. 2009. Bacterial and Parasitic Diseases of Pet Fish. Veterinary Clinics of North America: Exot. Anim. Pract. 12:609-638.

Rodgers, D.W., and Beamish, F.W.H. 1981. Uptake of waterborne methylmercury by Rainbow Trout (*Salmo gairdneri*) in relation to oxygen consumption and methylmercury concentration. Can. J. Fish. Aquat. Sci. 38:1309-1315.

Roni, P., and Quinn, T.P. 1995. Geographic variation in size and age of North American Chinook salmon (*Oncorhynchus tshawytscha*). North Am. J. Fish. Manage. 15:325-345.

Ruggles, C.P. 1980. A review of the downstream migration of Atlantic Salmon. Can. Tech. Rep. Fish. Aquat. Sci. No. 952.

Saegrov, H., Hindar, K., Kalas, S., and Lura, H. 1997. Escaped farmed Atlantic Salmon replace the original salmon stock in the River Vosso, western Norway. ICES J. Mar. Sci. 54:1166-1172.

Saloniemi, I., Jokikokko, E., Kallio-Nyberg, I., Jutila, E., and Pasanen, P. 2004. Survival of reared and wild Atlantic Salmon smolts: size matters more in bad years. ICES J. Mar. Sci. 61:782-787.

Santigosa, E., Sanchez, J., Médale, F., Kaushik, S., Pérez-Sanchez, J., and Gallardo, M.A. 2008. Modifications of digestive enzymes in trout (*Oncorhynchus mykiss*) and sea bream (*Sparus aurata*) in response to dietary fish meal replacement by plant protein sources. Aquaculture 282:68-74.

Saunders, R.L., and Henderson, E.B. 1969a. Survival and growth of Atlantic Salmon parr in relation to salinity. Fish. Res. Brd. Can. Tech. Rep. No. 147.

Saunders, R.L., and Henderson, E.B. 1969b. Survival and growth of Atlantic Salmon fry in relation to salinity and diet. Fish. Res. Brd. Can. Tech. Rep. No. 148.

Saunders, R.L., and Henderson, E.B. 1970. Influence of photoperiod on smolt development and growth of Atlantic Salmon (*Salmo salar*). J. Fish. Res. Brd. Can. 27:1295-1311.

Saunders, R.L., Fletcher, G.L., and Hew, C.L. 1998. Smolt development in growth hormone transgenic Atlantic Salmon. Aquaculture 168:177-193.

Schoonmaker, P.K., Gresh, J., Lichatowich, J., and Radtke, H.D. 2003. Past and present Pacific Salmon abundance: bioregional estimates for key life history stages. Pages 33-40 in J.G. Stockner, editor. Nutrients in Salmon Ecosystems: Sustaining Production and Biodiversity. American Fisheries Society, Symposium 34, Bethesda, Maryland.

Schuldt, J.A. 1998. Impact of Chinook salmon spawning migrations on stream ecosystems. Dissertation Abstracts International Part B: Science and Engineering, page 59.

Scott, W.B., and Crossman, E.J. 1973. Freshwater Fishes of Canada. Fisheries Research Board of Canada. Bulletin 184. Ottawa, Canada.

Shaff, C.D., and Compton, J.E. 2009. Differential incorporation of natural spawners vs. artificially planted salmon carcasses in a stream food web: evidence from δ15N of juvenile Coho Salmon. Fisheries 34:62-72.

Shahsavarani, A., Delgaty, K.L., Ali, K., and Beardall, J. 2008. The *Canadian Environmental Protection Act, 1999* and the regulation of animal products of biotechnology: Organisms other than microorganisms. Poster presented at the 8th International Meeting on Zebrafish Development & Genetics, Madison, Wisconsin.

Shearer, K.D., Asgard, T., Andorsdottir, G., and Aas, G.H. 1994. Whole body elemental and proximate composition of Atlantic Salmon (*Salmo salar*) during the life cycle. J. Fish Biol. 44:785-797.

Sheehan, R.J., Shasteen, S.P., Suresh, A.V., Kapuscinski, A.R., and Seeb, J.E. 1999. Better growth in all-female diploid and triploid Rainbow Trout. Trans. Am. Fish. Soc. 128:491-498.

Sheehan, T.F., Reddin, D.G., Chaput, G., and Renkawitz, M.D. 2012. SALSEA North America: a pelagic ecosystem survey targeting Atlantic Salmon in the Northwest Atlantic. ICES J. Mar. Sci. 69:1580-1588.

Sherrod, D.R., Vallance, J.W., Tapia-Espinosa, A., and McGeehin, J.P. 2008. Volcan Baru; eruptive history and volcano-hazards assessment. US Geological Survey Open-File Report 2007-1401.

Skaala, O., Wennevik, V., and Glover, K.A. 2006. Evidence of temporal genetic change in wild Atlantic Salmon, *Salmo salar* L., populations affected by farm escapees. ICES J. Mar. Sci. 63:1224-1233.

Skilbrei, O.T., Holst, J.C., Asplin, L., and Holm, M. 2009. Vertical movements of “escaped” farmed Atlantic Salmon (*Salmo salar* L.); a simulation study in a western Norwegian fjord. ICES J. Mar. Sci. 66:278-288.

Skilbrei, O.T. 2010. Reduced migratory performance of farmed Atlantic Salmon post-smolts from a simulated escape during autumn. Aquacult. Environ. Interact. 1:117-125.

Skilbrei, O.T., and Jørgensen, T. 2010. Recapture of cultured salmon following a large-scale escape experiment. Aquacult. Environ. Interact. 1:107-115.

Souza, S.C., Frick, G.P., Wang, X., Kopchick, J.J., Lobo, R.B., and Goodman, H.M. 1995. A single arginine residue determines species specificity of the human growth hormone receptor. PNAS 92:959-963.

Stamatis, D.H. 2003. Failure Mode Effect Analysis: FMEA from Theory to Execution. Second Edition. ASQ Quality Press, Milwaukee, Wisconsin.

Stefansson, S.O., Berge, A.I., and Gunnarsson, G.S. 1998. Changes in seawater tolerance and gill Na+, K+-ATPase activity during desmoltification in Atlantic Salmon kept in fresh water at different temperatures. Aquaculture 168:271-277.

Stevens, E.D., and Sutterlin, A. 1999. Gill morphometry in growth hormone transgenic Atlantic Salmon. Environ. Biol. Fishes 54:405-411.

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

Stevens, E.D., Wagner, G.N., and Sutterlin, A. 1999. Gut morphology in growth hormone transgenic Atlantic Salmon. J. Fish Biol. 55:517-26.

Sumpter, J.P., Lincoln, R.F., Bye, V.J., Carragher, J.F., and Lebail, P.Y. 1991. Plasma growth hormone levels during sexual maturation in diploid and triploid Rainbow Trout (*Oncorhynchus mykiss*). Gen. Comp. Endocrinol. 83:103-110.

Sundström, L.F., Devlin, R.H., Johnsson, J.I., and Biagi, C.A. 2003 Vertical position reflects increased feeding motivation in growth-transgenic Coho Salmon (*Oncorhynchus kisutch*). Ethology 109:701-712.

Sundström, L.F., Lõhmus, M., Johnsson, J.I., and Devlin, R.H. 2004. Growth hormone transgenic salmon pay for growth potential with increased predation mortality. Proc. R. Soc. London B 271:350-352.

Sundström, L.F., Lõhmus, M., and Devlin, R.H. 2005. Selection on increased intrinsic growth rates in Coho Salmon, *Oncorhynchus kisutch*. Evolution 59:1560-1569.

Sundström, F., Lõhmus, M., Tymchuk, W.E., and Devlin, R.H. 2007a. Gene-environment interactions influence ecological consequences of transgenic animals. PNAS 104:3889-94.

Sundström, L.F., Lõhmus, M., Johnsson, J.I., and Devlin, R.H. 2007b. Dispersal potential is affected by growth-hormone transgenesis in Coho Salmon (*Oncorhynchus kisutch*). Ethology 113:403-410.

Sundström, L.F., Tymchuk, W.E., Lõhmus, M., and Devlin, R.H. 2009. Sustained predation effects of hatchery-reared transgenic Coho Salmon *Oncorhynchus kisutch* in semi-natural environments. J. Appl. Ecol. 46:762-769.

Sundström, L.F., Lõhmus, M., and Devlin, R.H. 2010. Migration and growth potential of Coho Salmon smolts: implications for ecological impacts from growth-enhanced fish. Ecol. Appl. 20:1372-1383.

Sundström, L.F., and Devlin, R.H. 2011. Increased intrinsic growth rate is advantageous even under ecologically stressful conditions in Coho Salmon (*Oncorhynchus kisutch*). Evol. Ecol. 25:447-460.

Sundström, L.F., Vandersteen, W.E., Lõhmus, M., and Devlin, R.H. 2014. Growth-enhanced Coho Salmon invading other salmon species populations: effects on early survival and growth. J. Appl. Ecol. 51:82-89

Sundt-Hansen L., Neregard, L., Einum, S., Hojesjo, J., Bjornsson, B.T., Hindar, K., Okland, F., and Johnsson, J.I. 2009. Growth enhanced Brown Trout show increased movement activity in the wild. Funct. Ecol. 23:551-558.

Taylor, J.F., Needham, M.P., North, B.P., Morgan, A., Thompson, K., and Migaud, H. 2007. The influence of ploidy on saltwater adaptation, acute stress response and immune function following seawater transfer in non-smolting Rainbow Trout. Gen. Comp. Endocrinol. 152:314-325.

Teuscher, D.M., Schill, D.J., Megargle, D.J., and Dillon, J.C. 2003. Relative survival and growth of triploid and diploid Rainbow Trout in two Idaho reservoirs. North Am. J. Fish. Manage. 23:983-988.

Thomas, J., Schaack, S., and Pritham, E.J. 2010. Pervasive horizontal transfer of rolling-circle transposons among animals. Genome Biol. Evol. 2:656-664.

Thorpe, J.E., Metcalfe, N.B., and Fraser, N.H.C. 1994. Temperature dependence of the switch between nocturnal and diurnal smolt migration in Atlantic Salmon. Pages 83-86 *in* D.D. MacKinlay, editor. High-Performance Fish. Fish Physiology Association, Vancouver, Canada.

Thorstad, E.B., Fleming, I.A., McGinnity, P., Soto, D., Wennevik, V., and Whoriskey, F. 2008. Incidence and Impacts of Escaped Farmed Atlantic Salmon (*Salmo salar*) in Nature. NINA Special Report 36.

Thorstad, E.B., Whoriskey, F., Rikardsen, A.H., and Aarestrup, K. 2011. Aquatic nomads: The life and migrations of the Atlantic Salmon. Pages 1-32 *in* O. Aas, S. Einum, A. Klemetsen, J. Skurdal, editors. Atlantic Salmon Ecology. Wiley-Blackwell, Oxford, UK.

Tymchuk, W.E.V., Abrahams, M.V., and Devlin, R.H. 2005. Competitive ability and mortality of growth-enhanced transgenic Coho Salmon fry and parr when foraging for food. Trans. Am. Fish. Soc. 134:381-389.

Uh, M., Khattra, J., and Devlin, R.H. 2006. Transgene constructs in Coho Salmon (*Oncorhynchus kisutch*) are repeated in a head-to-tail fashion and can be integrated adjacent to horizontally-transmitted parasite DNA. Trans. Res. 15:711-727.

USFDA (U.S. Food and Drug Administration). 2010. Briefing Packet for AquAdvantage® Salmon Veterinary Medicine Advisory Committee. Center for Veterinary Medicine, US Food & Drug Administration. (September 2010)

Valiente, A.G., Ayllon, F., Nunez, P., Juanes, F., and Garcia-Vazquez, E. 2010. Not all lineages are equally invasive: genetic origin and life history in Atlantic Salmon and Brown Trout acclimated to the Southern Hemisphere. Biol. Invas. 12:3485-3495.

Van Zwol, J., Neff, B.D., and Wilson, C.C. 2012. The effect of competition among three salmonids on dominance and growth during the juvenile life stage. Ecol. Freshwater Fish 21:533-540.

Vasseur, L., and Catto, N. 2008. Atlantic Canada. Pages 119-170 *in* D.S. Lemmen, F.J. Warren, J. Lacroix, and E. Bush, editors. From Impacts to Adaptation: Canada in a Changing Climate 2007. Government of Canada, Ottawa.

Verspoor, E. 1988. Widespread hybridization between native Atlantic Salmon, *Salmo salar*, and introduced Brown Trout, *S. trutta*, in eastern Newfoundland. J. Fish Biol. 32:321-334.

Verspoor, E., García de Leániz, C., and McGinnity, P. 2007. Genetics and habitat management. Pages 339-424 *in* E. Verspoor, L. Stradmeyer and J. L. Nielsen, editors. The Atlantic Salmon : Genetics, Conservation, and Management. Blackwell Publishing, Oxford, UK.

Vladic, T., and Jarvi, T. 1997. Sperm motility and fertilization time span in Atlantic Salmon and Brown Trout; the effect of water temperature. J. Fish Biol. 50:1099-1093.

Volpe, J.P., Taylor, E.B., Rimmer, D.W., and Glickman, B.W. 2000. Evidence of natural reproduction of aquaculture-escaped Atlantic Salmon in coastal British Columbia river. Conserv. Biol. 14:899-903.

Volpe, J., Anholt, B.R., and Glickman, B. 2001. Competition among juvenile Atlantic Salmon (*Salmo salar*) and steelhead (*Oncorhynchus mykiss*): relevance to invasion potential in British Columbia. Can. J. Fish. Aquat. Sci. 58:197-207.

Waago, H. 1987. Local treatment of ulcers in diabetic foot with human growth hormone. Lancet 1(8548):1485.

Warrillow, J.A., Josephson, D.C., Youngs, W.D., and Krueger, C.C. 1997. Differences in sexual maturity and fall emigration between diploid and triploid Brook Trout (*Salvelinus fontinalis*) in an Adirondack lake. Can. J. Fish. Aquat. Sci. 54:1808-1812.

Webb, J., Verspoor, E., Aubin-Horth, N., Romakkaniemi, A., and Amiro, P. 2007. The Atlantic Salmon. Pages 17-56 *in* E. Verspoor, L. Stradmeyer and J.L. Nielsen, editors. The Atlantic Salmon : Genetics, Conservation, and Management. Blackwell Publishing, Oxford, UK.

Weir, L.K., Hutchings, J.A., Fleming, I.A., and Einum, S. 2004. Dominance relationships and behavioural correlates of spawning success in farmed and wild male Atlantic Salmon, *Salmo salar*. J. Anim. Ecol. 73:1069-1079.

Weir, L.K., Hutchings, J.A., Fleming, I.A., and Einum, S. 2005. Spawning behaviour and success of mature male Atlantic Salmon (*Salmo salar*) parr of farmed and wild origin. Can. J. Fish. Aquat. Sci. 62:1153-1160.

Welcomme, R.L. 1988. International Introductions of Inland Aquatic Species. FAO Fisheries Technical Paper 294, Food and Agriculture Organization of the United Nations. Rome, Italy.

Whoriskey, F.G., Brooking, P., Doucette, G., Tinker, S., and Carr, J.W. 2006. Movements and survival of sonically tagged farmed Atlantic Salmon released in Cobscook Bay, Maine, USA. ICES J. Mar. Sci. 63:1218-1223.

Wilkins, N.P., Cotter, D., and O'Maoiléidigh, N. 2001. Ocean migration and recaptures of tagged, triploid, mixed-sex and all-female Atlantic Salmon (*Salmo salar* L.) released from rivers in Ireland. Genetica 111:197-212.

Williams, F.R., Jung, G.H., and Renard, R.J. 1989. Forecasters Handbook for Central America and Adjacent Waters. Naval Environmental Prediction Research Facility, Monterey, California.

Williams, K.L., Griffiths, S.W., Nislow, K.H., and McKelvey, S. 2009. Response of juvenile Atlantic Salmon, *Salmo salar*, to the introduction of salmon carcasses in upland streams. Fish. Manag. Ecol. 16:290-297.

Withler, R.E., Beacham, T.D., Solar, I.I., and Donaldson, E.M. 1995. Freshwater growth, smelting, and marine survival and growth of diploid and triploid Coho Salmon (*Oncorhynchus kisutch*). Aquaculture 136:91-107.

Xu, B., Mai, K., Xu, Y.L., Miao, H.Z., Liu, Z.H., Dong, Y., Lan, S., Wang, R., and Zhang, P.J. 2001. Growth promotion of red sea bream, *Pagrosomus major*, by oral administration of recombinant eel and salmon growth hormone. Chin. J. Oceanol. Limnol. 19:141-146.

Yamamoto, A., and Iida, T. 1994. Oxygen consumption and hypoxic tolerance of triploid rainbow-trout. Fish Pathol. 29:245-251.

Yamamoto, A., and Iida, T. 1995. Susceptibility of triploid rainbow-trout to IHN, furunculosis and vibriosis. Fish Pathol. 30:69-70.

Yang, R., Brauner, C., Thurston, V., Neuman, J., and Randall, D.J. 2000. Relationship between toxicant transfer kinetic processes and fish oxygen consumption. Aquat. Toxicol. 48:95-108.