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Environmental Risk Assessment for the Manufacture and Grow-out of EO-1α Salmon, Including the Sterile AquAdvantage® Salmon, at a Land-Based and Contained Facility near Rollo Bay, PEI

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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.

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ABSTRACT

On July 27, 2018, AquaBounty Canada Limited submitted a regulatory package to Environment and Climate Change Canada (ECCC) under the *New Substances Notification Regulations (Organisms)* of the *Canadian Environmental Protection Act,* for the manufacture of EO-1α Salmon (also known as the AquAdvantage® Salmon), a fast growing genetically engineered Atlantic Salmon (*Salmo salar*), at a land-based aquaculture facility near Rollo Bay, Prince Edward Island. Under a Memorandum of Understanding between the Department of Fisheries and Oceans (DFO), ECCC, and Health Canada, DFO conducted an environmental risk assessment of the notified organism and its proposed use.

The environmental risk assessment was conducted using the paradigm where risk is directly related to the exposure and hazard of the organism, and considers level of uncertainty for both exposure and hazard determinations. The exposure assessment considers the likelihood and magnitude of release into, and survival, reproduction, establishment, and spread of the organism, in the Canadian environment. The hazard assessment is focused on the potential for the organism to impact through hazard pathways, specifically: through environmental toxicity, horizontal gene transfer, trophic interactions, hybridization and as a vector of disease; and to impact environmental components, specifically: biogeochemical cycling, habitat, and biodiversity. The risk assessment included consideration of two scenarios, Scenario A where the company would include the production of non-transgenic fish for external parties at the same facility as EO-1α Salmon production, or Scenario B, where there is no production of non-transgenic fish for external parties.

EO-1α Salmon were determined to have the capacity to survive in Canadian environments, and hazard assessments to Canadian environments ranged from negligible (e.g. through environmental toxicity) to high (i.e. through trophic interactions or intraspecific hybridization). However, extensive and redundant physical containment of EO-1α Salmon at the proposed land-based facility, as well as >98% sterility (via triploidy) of the production form of EO-1α Salmon, resulted in a negligible environmental exposure ranking for the notified organism under Scenario B, and negligible to low risk. Under Scenario A, the potential for human error in shipping eggs increases potential exposure, resulting in low to moderate risk. Mitigation procedures were proposed to decrease exposure and hence environmental risk under use Scenario A, although it is not clear if they would decrease final risk conclusion to low. Notified containment measures are essential to minimizing risk of the EO-1α Salmon to the Canadian environment, and any changes to containment or expansion of the manufacture and production facilities could change the outcome of the environmental risk assessment.

LIST OF ACRONYMS

AAS: AquAdvantage® Salmon

ASCU: Atlantic Salmon Conservation Unit

bp: base pair

CEPA: *Canadian Environmental Protection Act, 1999*

CFIA: Canadian Food Inspection Agency

COSEWIC: Committee on the Status of Endangered Wildlife in Canada

DFO: Fisheries and Oceans Canada

DNA: Deoxyribonucleic acid

DU: Designatable Unit (COSEWIC)

ECCC: Environment and Climate Change Canada

FMA: Failure Modes Analysis

GE: Genetically Engineered

GH: Growth Hormone

GxE: Genotype by Environment interaction

HC: Health Canada

17α-MT: 17α-Methyltestosterone

IGF-1: Insulin-like growth factor

2n: Diploid

3n: Triploid

NSN-16528: New Substances Notification submission #16528. The original 2013 submission by AquaBounty Inc. for production of AAS eggs at Bay Fortune, PEI facility for grow-out in Panama Facility.

NSN-19702: New Substances Notification submission #19702. The current submission by AquaBounty Inc. for expansion of production of AAS eggs and grow-out to new facility near Rollo Bay, PEI.

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

PEI: Prince Edward Island

RAS: recirculating aquaculture system

rDNA: recombinant DNA

SOP: Standard operating procedure

USFDA: United States Food and Drug Agency

GLOSSARY

AAS-relatives: Atlantic Salmon containing the same opAFP-GHc2 construct as EO-1α Salmon but are from a different founding individual, i.e., a different transgenic line.

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): a triploid (99%) all-female Atlantic Salmon (*Salmo salar*), hemizygous for the *opAFP-GHc2* rDNA construct at the α-locus in the EO-1α lineage. The commercial form of EO-1α Salmon.

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.

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.

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

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

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

Diversity (ecological): 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.

EO-1α Salmon: The notified organism. An Atlantic Salmon (*Salmo salar*) bearing the *opAFP-GHc2* rDNA construct at the α-locus in the EO-1α lineage. Includes AquAdvantage® Salmon, EO-1α all-female homozygous broodstock, and EO-1α neomale homozygous broodstock

EO-1α Salmon descendant: offspring of EO-1α Salmon that are produced in the wild environment and carry the *opAFP-GHc2* rDNA construct at the α-locus.

Exposure: likelihood that the organism (EO-1α Salmon) will come into contact with susceptible species and/or environmental components in Canada.

Exposure pathway: the physical route by which EO-1α Salmon or EO-1α descendants move from a source to assessment endpoints.

Genotype: the genetic constitution of an individual organism.

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

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

Gynogenesis: Process by which an embryo contains only maternal chromosomes: generally produced by inactivation of male chromosomes prior to fertilization combined with doubling of maternal chromosomes (e.g., through pressure shock to retain the second polar body).

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.

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

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.

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.

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.

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

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).

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

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.

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

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

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

The sources used for the definitions in this glossary include Lincoln et al. 1988; CEPA 1999; Burgman 2005; Kapuscinski et al. 2007; Mair et al. 2007; Levin 2009; Moon et al. 2010;

EXECUTIVE SUMMARY

BACKGROUND

On July 27, 2018, AquaBounty Canada Limited (the notifier) submitted a regulatory package to Environment and Climate Change Canada (ECCC) under the *New Substances Notification Regulations (Organisms)* [NSNR(O)] of the *Canadian Environmental Protection Act* (CEPA) for the manufacture of an EO-1α Salmon, a fast growing genetically engineered (GE) Atlantic Salmon (*Salmo salar*), at a land-based aquaculture facility near Rollo Bay, Prince Edward Island (PEI) (NSN-19702).

In 2013, AquaBounty Canada submitted a similar notification (NSN-16528) to ECCC detailing its intent to commercially manufacture the AquAdvantage® Salmon (AAS), the commercial form of EO-1α Salmon, in a land-based contained facility near Bay Fortune, PEI. Fisheries and Oceans Canada (DFO) assisted in the regulatory process by conducting the environmental and indirect human health risk assessment, and by providing science advice to ECCC in support of its decisions regarding the management of risks. Under the defined containment conditions proposed by AquaBounty, DFO determined that the AAS poses low risk to the Canadian environment and indirect human health. However, DFO advised that this conclusion could change if activities in relation to the organism change from those proposed by AquaBounty in its earlier submission, and could potentially result in greater risk to the environment. ECCC and HC accepted this advice and decided to allow commercial production of AAS on the condition that it was contained in a land-based facility, with strict physical and biological containment measures in place as described in the 2013 notification (see Significant New Activity Notice No. 16528 published in Canadian Gazette in November 2013). In 2016, Health Canada (HC) and the Canadian Food Inspection Agency (CFIA) approved the AquAdvantage® Salmon for human food and animal feed use, respectively, on the basis that it is nutritionally the same as non-GE salmon.

It is within this context that DFO conducted an environmental risk assessment of the notified organism (EO-1α Salmon/AAS) and its proposed use at a land-based aquaculture facility near Rollo Bay, PEI. Here, risk is defined as a function of the potential for Canadian environments to be exposed to the notified organism, and the potential for the notified organism to pose hazards to the Canadian environment. Exposure and Hazard assessments are conducted separately and then integrated into an assessment of Risk. Uncertainty in Exposure and Hazard assessments are determined, and uncertainty associated with the final risk assessment discussed.

THE NOTIFIED ORGANISM

The EO-1α Salmon was developed by micro-injecting a gene construct (opAFP-GHc2) into the newly fertilized egg of a non-transgenic Atlantic Salmon (*Salmo salar*), followed by introgression of the transgene into the non-transgenic genetic background of the initial mosaic founder. 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 target phenotypic difference between EO-1α Salmon and non-transgenic Atlantic Salmon is a significant increase in growth rate. AAS are all-female, triploid (3n) EO-1α Salmon, and are the commercial form of EO-1α Salmon. The current notification includes the following forms of the notified organism: AquAdvantage® Salmon (AAS); EO-1α female diploid (2n) broodstock; and EO-1α neomale diploid (2n) broodstock. The company will also maintain a broodstock of non-transgenic St. John River domestic Atlantic Salmon for AAS manufacture.

Use Scenarios

In addition to the notified use of EO-1α Salmon above, the notifying company expressed its intensions to manufacture and sell diploid (2n) non-transgenic Atlantic Salmon eggs to external parties from the Rollo Bay facility. This raised the possibility of a containment failure resulting from human error, whereby transgenic eggs are accidentally shipped as non-transgenic, to customers who could inadvertently release the organism into the environment. Consequently, the risk assessment included consideration of two scenarios. Under Scenario A, company activities would include the production (manufacture) of non-transgenic fish, for external parties, occurring along-side transgenic fish production using existing and planned procedures for keeping eggs organized and separated and for keeping transgenic organisms contained. Under Scenario B, there is no production of non-transgenic fish for external parties, with all non-transgenic salmon housed at the facility used only for the production of AAS, as described in the regulatory package submitted by the company.

WAIVER REQUEST

Under paragraph 5(a) of Schedule 5 of the NSNR(O), the notifier is required to submit data from a test conducted to determine the invasiveness of the organism. However, it was concluded that the data provided was insufficient to conclude on invasiveness under paragraph 5(a) (DFO 2019). Therefore, in accordance with Section 106(8) of CEPA, the notifier has requested a waiver for the information required under paragraph 5(a). The waiver request is based on the notifier's assertion that the organism is manufactured at a location where the person requesting the waiver is able to contain the living organism so as to satisfactorily protect the environment and human health.

An evaluation of containment was conducted as part of the environmental exposure assessment to inform ECCC's decision regarding acceptance of the waiver request. Under Scenario B, redundant physical containment and strong operational oversight make the likelihood of exposure resulting from the accidental release of EO-1α Salmon from the Rollo Bay facility negligible. Under Scenario A, the potential for release increases due to the added possibility of human error leading to a mix up of transgenic and non-transgenic embryos/larvae, increasing exposure ranking to low. Uncertainty associated with this conclusion is low given the available information on facility design, containment structures, SOPs and internal compliance documentation. It is concluded that AAS will be manufactured at a location where AquaBounty is able to contain EO-1α Salmon so as to satisfactorily protect the environment and human health.

ENVIRONMENTAL RISK ASSESSMENT

The environmental risk assessment is conducted under AquaBounty Canada's proposed use; manufacture and grow-out of both sterile 3n (AAS) and fertile 2n EO-1α Salmon in a land-based, environmentally contained aquaculture facility near Rollo Bay, PEI. The assessment takes into account all information provided by AquaBounty Canada as part of its regulatory submission in 2013, new information regarding containment of the organism at the Rollo Bay facility, any new information or data elements the company submits as part of the current regulatory submission, relevant data submitted as part of a Public Engagement process, as well as relevant data from the scientific literature.

Exposure

The exposure assessment for living EO-1α Salmon addresses both its potential to enter the environment (release) and its fate once in the environment. All relevant information regarding

physical, chemical and biological containment strategies used at all life stages, and the potential for exposure resulting from the failure of containment were evaluated. All life-history stages and all pathways of entry into the environment for both sterile 3n AAS and fertile 2n EO-1α Salmon were considered. The proposed redundant physical containment and operational oversight make the likelihood of exposure resulting from the accidental release of EO-1α Salmon from the Rollo Bay facility negligible during proposed rearing and manufacture activities.

Though the conditions of triploidy, sex-reversal, domestication, and growth hormone transgenesis may have an effect on the overall fitness of EO-1α Salmon, they are not expected to prevent EO-1α Salmon from reaching the adult life stage given a favourable environment. There is also abundant evidence supporting the argument that escaped domesticated Atlantic Salmon can migrate to suitable habitat and successfully reproduce with wild Atlantic Salmon. Available evidence suggests that, despite the likelihood of diminished reproductive fitness, fertile forms of EO-1α Salmon still have the capacity to successfully reproduce with wild Atlantic Salmon. However, this potential for exposure is limited by the company's ability to contain EO-1α Salmon in a land-based facility. Under Scenario A, human error increases the likelihood of exposure to the Canadian environment. Consequently, the exposure assessment concludes with **low uncertainty** that the likelihood of EO-1α Salmon exposure to the Canadian environment is **low**. However, if non-transgenic eggs from the facility are not sold to external parties (Scenario B), the exposure to the Canadian environment would be reduced to **negligible.**

Hazard

The potential for EO-1α Salmon to cause a hazard to Canadian environments was examined in the context of environmental toxicity (i.e., potential to be poisonous), horizontal gene transfer, trophic interactions with other organisms, hybridization with wild populations, as a vector for disease, as well as impacts to biogeochemical cycling, habitat, and biodiversity. The potential level of hazard posed by EO-1α Salmon depends on the pathway to harm examined, and ranged from negligible (through environmental toxicity, horizontal gene transfer, as a vector of disease, and to biogeochemical cycling), low (to habitat), moderate (through interspecific hybridization and to biodiversity), with pathways to harm through trophic interactions and hybridization with wild Atlantic Salmon populations having high hazard ranking. Hazards ranked moderate to high are expected to be very context specific, where maximum hazards may only be present in specific circumstances (e.g., under environmental conditions that favour EO-1α Salmon in competition for resources or wild mates). Hazard rankings are likely to be affected by numerous factors including resilience of affected wild populations, community structure at site of interactions (e.g., structure of competitor, predator, and prey populations), and life stage of EO-1α Salmon escape. Uncertainty level is moderate to high for hazard assessments, due to limited data on EO-1α Salmon under a variety of relevant environmental conditions, limited understanding of genotype by environment interactions in surrogate models, and limited understanding of applicability of data from surrogate models to EO-1α Salmon. The current hazard assessment is in alignment with what was assessed in the 2013 notification. Some uncertainty levels have decreased, and two hazard assessments were concluded on in the current assessment but not the previous one, due to increased scientific information regarding relevant impacts and/or genotype by environment interactions in EO-1α Salmon and surrogate models. A single overall conclusion on hazards was not made, rather each hazard is considered separately for conclusions on risk.

CONCLUSIONS ON RISK

Based on a risk matrix (Risk ∝ Exposure × Hazard) integrating the negligible exposure and negligible to high hazards of EO-1α Salmon, manufacture and grow-out of the notified organism in a land-based facility near Rollo Bay, PEI poses **negligible to moderate risk** to Canadian environments depending on the specific use scenario. Under Scenario A, where non-transgenic eggs are sold from the Rollo Bay facility, the paradigm Risk ∝ Exposure × Hazard results in a final risk assessment of low to moderate (Low Exposure × Negligible to High Hazard). Consequently, EO-1α Salmon under the proposed use in Scenario A are expected to pose Low to Moderate Risk to Canadian environments. Under the use scenario where there is no production of non-transgenic eggs for third parties (Scenario B), the paradigm Risk ∝ Exposure × Hazard results in a final risk assessment of negligible to low (negligible to low Exposure × Negligible to High Hazard). Consequently, EO-1α Salmon under this use scenario are expected to pose Negligible to Low Risk to Canadian environments. Sources of uncertainty in the risk assessment are primarily due to uncertainty in hazard assessments as listed above. The level of proposed containment strategies (operational and physical) of EO-1α Salmon results in low uncertainty regarding the exposure of the environment to EO-1α Salmon, although uncertainty may increase under Scenario A. As containment is essential to minimizing risk of the EO-1α Salmon to the Canadian environment, any changes to containment or expansion of the manufacture and grow-out facilities could change the outcome of the environmental risk assessment and would require inspection to confirm that full containment will be maintained. To mitigate the potential for human error that may result in the mixing of transgenic and non-transgenic fish under Scenario A, measures were proposed to decrease the exposure level under this scenario. Mitigation measures included physical and temporal separation of transgenic and non-transgenic eyed-egg production, labelling throughout production, operating procedures and oversite, and verification of genotype prior to eyed-eggs leaving the facility.

PART 1: PROBLEM FORMULATION

1.1 PURPOSE OF PART 1

Part 1 of this document elaborates the problem formulation for the environmental risk assessment that will be conducted under the *Canadian Environmental Protection Act* (CEPA), with respect to EO-1α Salmon, including the commercial form AquAdvantage® Salmon (AAS), a genetically engineered (GE) Atlantic Salmon (*Salmo salar*) notified by AquaBounty Canada Inc. under the *New Substances Notification Regulations (Organisms)* [NSNR(O)] for manufacture and grow-out at a land-based, contained facility near Rollo Bay, PEI (NSN-19702). It identifies protection goals and assessment endpoints that are aligned with the legislative protection goals in CEPA and provides a characterisation of EO-1α Salmon, the comparator species, and the receiving environment in Canada.

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

1.2 BACKGROUND

In 2013, AquaBounty Canada submitted a notification (NSN-16528) to ECCC detailing its intent to commercially produce GE Atlantic Salmon in Canada in a land-based contained facility near Bay Fortune, PEI for grow-out in a land-based contained facility in Panama. Under CEPA, anyone proposing to import or manufacture a living animate product of biotechnology in Canada, including a GE fish, is required to provide ECCC 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 an assessment of indirect human health (risk to human health from environmental exposure to the living organism).

Fisheries and Oceans Canada (DFO) assisted in the regulatory process by conducting the environmental and indirect human health risk assessment for EO-1α Salmon (including AAS), and by providing science advice to ECCC and HC in support of decisions regarding the appropriate management of risks. Under the well-defined containment conditions proposed by AquaBounty in the 2013 notification, DFO determined that the AquAdvantage® Salmon poses low risk to the Canadian environment and indirect human health. However, DFO advised that this conclusion could change if activities in relation to the salmon change significantly from those proposed by AquaBounty in its submission, and could result in greater risk to the environment.

On July 27, 2018, AquaBounty Canada Limited submitted a regulatory package to ECCC under CEPA and the NSNR(O) for the manufacture and grow-out of AAS at a new land-based aquaculture facility near Rollo Bay, PEI, including housing and breeding of EO-1α Salmon broodstock (NSN-19702). This facility would produce eyed AAS eggs for grow-out at the PEI facility, as well as at AquaBounty facilities in Panama and the USA. For this notification, the New Substances program at ECCC provided a voluntary public engagement opportunity for the public to submit scientific information and test data to inform the risk assessments. Through this initiative, and with consultation with the notifying company, it became clear the notifying company also had plans to sell non-transgenic eyed eggs to third parties from the Rollo Bay facility. This raised the possibility of a containment failure resulting from human error whereby transgenic eggs are accidentally shipped as non-transgenic to customers who could inadvertently release the organism into the environment. Consequently, the following risk

assessment included consideration of two scenarios. Under Scenario A, company activities would include the production of non-transgenic fish, for external parties, occurring along-side transgenic fish production using existing and planned procedures for keeping eggs organized and separated and for keeping transgenic organisms contained. Under Scenario B, non-transgenic fish would not be produced for external parties, with all non-transgenic salmon housed at the facility used only for the production of AAS, as described in the regulatory package submitted by the company.

A waiver for one or more regulatory information requirements specified in Schedule 5 of the NSNR(O) may be requested by the notifier. As specified under paragraph 106(8) of CEPA, waivers may be granted if (a) in the opinion of the Minister of ECCC 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 a location where, in the opinion of the Ministers, the person requesting the waiver is able to contain the living organism so as to satisfactorily protect the environment and human health; or (c) it is not, in the opinion of the Ministers, practicable or feasible to obtain the test data necessary to generate the information. AquaBounty Canada Limited requested a waiver for paragraph 5(a) of Schedule 5 of the NSNR(O), *data from a test conducted to determine pathogenicity, toxicity or invasiveness* after it was concluded that insufficient information was available to conclude on invasiveness of the notified organism (DFO 2019).

The scope of a Schedule 5 assessment under the NSNR(O) must also take into account all likely “potential” use scenarios, not just the specific one(s) being proposed by the notifier. If there is not enough information to consider all potential applications, then a “case-by-case” approach is taken whereby the specific use scenario notified and elaborated by the notifier in the regulatory submission, including any containment or mitigation measures, sets the specific parameters around the risk assessments.

1.3 LEGAL CONTEXT, RISK ASSESSMENT FRAMEWORK, AND REGULATORY DECISION MAKING

A detailed overview of the legal context for the risk assessment, the risk assessment framework, and the regulatory decision making process is provided in Leggatt et al. 2018.

1.4 CHARACTERISATION OF EO-1α SALMON

The notified organism (EO-1α Salmon, Figure 1.1) is an Atlantic Salmon containing a single insert of the opAFP-GHc2 transgene at the EO-1α locus (hereafter referred to as the EO-1α construct). The current notification includes the following forms of the notified organism:

- AquAdvantage® Salmon (AAS): triploid (3n, ≥98.5%), all-female transgenic fish that carry one copy (hemizygous) of the EO-1α construct. AAS are the fish that will be produced for commercial use.
- EO-1α diploid female broodstock: All-female transgenic fish that carry a double copy (homozygous) of the EO-1α construct. These will be used to maintain the EO-1α broodstock line.
- EO-1α diploid neomale broodstock. Genetically female transgenic fish that carry a double copy (homozygous) of the EO-1α construct, and were treated with 17α-methyltestosterone (17α-MT) to become functionally male. These will be used to maintain the EO-1α broodstock line and be crossed with St. John River Strain non-transgenic domesticated females to produce the AAS commercial form of EO-1α Salmon.

St. John River strain domestic Atlantic Salmon will also be reared at the Rollo Bay facility as broodstock for production of AAS. Detailed characterization of EO-1α Salmon (including AAS Salmon) is given in the 2014 document "*Environmental and Indirect Human Health Risk Assessment of the AquAdvantage® Salmon*" and summary and conclusions from DFO (2013) are given in modified version below. No modifications to the line, or outcrossing with other lines (other than to St. John River Strain to produce AAS), have been performed since the previous notification.

Though detailed information regarding the development of EO-1α Salmon, its phenotype, and the structure and function of the transgene integrant has been provided by the company for review, portions of this information are considered confidential and have been redacted from this report.

Figure 1.1: AquAdvantage® Atlantic Salmon (triploid all-female form of the EO-1α Salmon) containing the opAFP-GHc2 transgenic construct (back) and non-transgenic Atlantic Salmon of equal age (front). (Photo from AquaBounty Technologies Inc.)

1.4.1 Characterisation of the Transgene and Insert Construct

AquaBounty appropriately described the opAFP-GHc2 transgene construct in the 2013 notification. Briefly, the transgene construct was assembled through the use of standard molecular biology tools and techniques including plasmids, bacteriophage, restriction enzymes, linearization and ligation. No mobile genetic elements were used. The opAFP-GHc2 construct includes a 5'- antifreeze protein (AFP) promoter from the Ocean Pout, the complementary DNA sequence of growth hormone (GH) from the Chinook Salmon and a 3'-terminator from the Ocean Pout (Figure 1.2). AquaBounty provided *in vitro* and *in vivo* evidence of the functionality of the opAFP promoter to drive gene expression in salmonid species. Through complete sequencing of the integrant, AquaBounty provided evidence for:

1. The presence of expected regulatory elements in the promoter and terminator regions;
2. The presence of a full-length sequence encoding a mature hormone homologous to the endogenous GH-1 Chinook Salmon gene which is 95% homologous to the Atlantic Salmon GH; and
3. The absence of sequences for known toxic proteins.

Evidence demonstrates that the opAFP-GHc2 construct was randomly rearranged upon insertion into the host genome (Figure 1.3). The 4205 base pair (bp) EO-1α transgene integrant includes the last 613 bp of the Ocean Pout 5'-AFP promoter sequence, followed by the intact

Chinook Salmon GH cDNA, the complete Ocean Pout antifreeze 3' regulatory sequence, 25 bp from pUC9, 20 bp from pUC18 and the first 1678 bp of the Ocean Pout antifreeze 5' region. Excluding the above rearrangements, sequencing of the transgene integrant confirms complete identity with the transgene construct. The non-coding pUC sequences are not of concern. It was concluded in the previous assessment that the nature of the transgene construct and insert are not of concern (see DFO 2013).

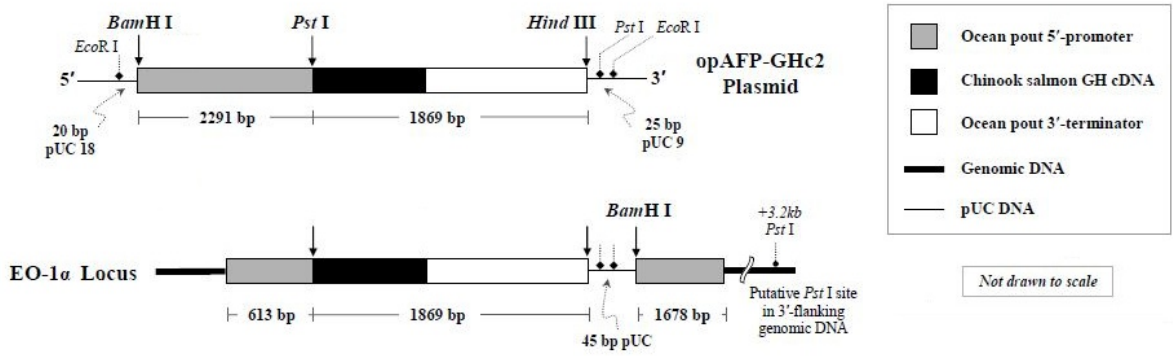

Figure 1.2: Physical structure of the microinjected plasmid construct (opAFPO-GHc2 Plasmid) and the integrated transgene (EO-1α locus) in the EO-1α Salmon genome (NSN-16528).

1.4.1.1 Strain History and Genealogy

EO-1α Salmon comprises the genetic background of several strains of Atlantic Salmon. Early generations of EO-1α Salmon were derived from and crossed with individuals from the Exploits, Colinet and Northeast Rivers in the Province of Newfoundland and Labrador. However, since 2000, subsequent generations used in the development of the EO-1α Salmon/AAS line intended for commercial application have been crossed predominantly with domesticated fish from the St. John River strain. EO-1α Salmon is, therefore, a domesticated transgenic Atlantic Salmon strain. Currently, the broodstock line is maintained by crossing EO-1α females with genetically female (XX) hormonally sex-reversed neomales to produce an all genetically female line.

1.4.1.2 Inheritance and Stability of the Transgene Integrant

In the previous assessment, Mendelian inheritance of the opAFP-GHc2 transgene inserted at the EO-1α locus was demonstrated over five generations through ratios of transgenic to non-transgenic progeny that were identified using PCR. It was concluded with high certainty that the opAFP-GHc2 transgene is stable at the EO-1α locus in EO-1α Salmon, although the notifier indicates that the exact location of the EO-1α locus within the native genome is not known. It was concluded with high certainty that the opAFP-GHc2 transgene inserted at the EO-1α locus is transmitted in accordance with Mendelian inheritance ratios.

Genotypic stability of the opAFP-GHc2 transgene inserted at the EO-1α locus was demonstrated over three generations, as there was consensus in nucleotide sequencing at the integrant and genomic flanking regions of individuals from the F2 and F4 generations (NSN-16528). Additional evidence was provided through PCR amplification of the 5' and 3' junctions of the EO-1α integrant in fish selected from the F2, F4 and F6 generations (NSN-16528), as well as PCR amplification of the GH transgene and either the 5' or 3' junction of the EO-1α integrant for the Bay Fortune facility 2015-2017 broodstock. The integration site of the 2015 broodstock was also confirmed via Southern blot. Therefore, it was concluded with high certainty that the opAFP-GHc2 transgene is stable at the EO-1α locus in EO-1α Salmon. However, it was noted that the insertion of the transgene in a simple sequence repeat region of the genome has the

potential to alter locus structure, with possible alterations in gene expression or protein structure and function, but only over evolutionary timeframes (Grechko 2011).

1.4.1.3 Other Manipulations

In addition to transgenesis, the production of genetically all-female broodstock and all female 3n AAS from the EO-1α line necessitates the application of gynogenesis (no longer used), sex-reversal and triploidy induction techniques. Gynogenesis was used in the early development of an all-female broodstock, but is no longer used for maintenance of EO-1α Salmon. It was concluded in the previous assessment that the generation of an all-female population through gynogenesis had been successful, though sampling to confirm the female status of the monosex broodstock population under rearing conditions at the Bay Fortune facility has been limited to phenotypic examination of the neomale gonads.

Sex-reversal of genetic females is achieved through 17α-MT treatment to produce phenotypic males. Neomales are confirmed by terminal gonad observations at maturity, where neomales are lacking a sperm duct. Since levels of exogenous 17α-MT are reported to be transient and decline to trace levels by 14 days post-treatment (Curtis et al. 1991), potential toxicological effects through the consumption of EO-1α neomales by predators would be over an extremely limited time frame. Consequently, it was concluded that sex-reversal treatment is not of concern.

To produce commercial AAS fish, EO-1α neomales are bred with St. John River domestic females, then triploidy is induced through pressure shock shortly after fertilization. This results in one chromosome set and the EO-1α gene from the EO-1α neomale, and 2 chromosome sets from the St. John River domestic female. Due to uneven chromosome segregation during meiosis, 3n fish are functionally sterile (Benfey 1999), providing a useful method of biological containment. However, the procedure is not always 100% effective, and the notifier reports success rates of 98.5% to 100% triploidy (from tests on 200 eggs per production batch, representing 0.5-1.3% of each batch). In GH transgenic Coho Salmon (*Oncorhynchus kisutch*), 3n failure rates in large scale trials ranged from 0.2% to 3.0% and included diploid (2n) offspring containing the transgene (Devlin et al. 2010), which were fertile and could pass the transgene to offspring (Devlin unpublished data). There were no noted toxicological concerns associated with induction of triploidy.

1.4.1.4 Methods to Detect Notified Organism

EO-1α Salmon can be distinguished from other Atlantic Salmon lines by detection of the opAFP-GHc2 transgene using PCR. The notifier provided one such test through amplification of three parts of the EO-1α integrant including the 5’ and 3’ ends integrated into the native genome. This procedure is suitable to distinguish transgenic EO-1α Salmon from non-transgenic Atlantic Salmon, but does not distinguish hemizygous from homozygous EO-1α Salmon.

1.4.2 Biological and Ecological Properties of EO-1α Salmon

Biological and ecological properties of EO-1α Salmon are summarized below. When relevant, the phenotypes of AAS-relatives (Atlantic Salmon microinjected with the same opAFC-GHc2 construct but from a different line than EO-1α Salmon) are also reported.

1.4.2.1 Body Size, Growth Rates and Hormone Levels

In Atlantic Salmon, body size is the phenotype most relevant to overall fitness, and is positively correlated with freshwater and marine survival, fecundity, egg size, reproductive success and offspring survival (Garcia de Leaniz et al. 2007). Since increased growth rate is the intended

phenotype of the genetic modification to EO-1α Salmon, size, growth rates and growth hormone expression profiles are important considerations.

The notifier provided *in vitro* and *in vivo* evidence of the ability of the truncated promoter of the transgene integrant to activate GH transgene expression. However, there is limited information available on functional GH protein levels in EO-1α Salmon from the transgene expression. NSN-16528 reported that GH levels (produced from both native and transgenic GH genes) in the muscle and skin of commercial size AAS were below the detection limit of 6.24 ng/g, as were those for unrelated domesticated salmon. The same study also detected no difference between the transgenic and domestic groups for insulin-like growth factor (IGF-1), the primary regulator of the effects of GH. Early studies by Du et al. (1992) in G0 (founding generation) AAS-relatives found no differences in plasma GH levels between transgenic and non-transgenic counterparts at the parr stage, and GH levels were not significantly related to growth rates, although sample sizes were small. The effect of GH transgenesis on GH protein levels in other life stages of EO-1α Salmon or AAS-relatives, or under different environmental conditions, have not been reported.

The primary phenotypic change of EO-1α Salmon is increased growth and size at equivalent age relative to non-transgenic siblings. This phenotype is consistently observed in standard hatchery practices by AquaBounty and in numerous published papers, and is also associated with improved feed conversion efficiency (Deitch et al. 2006; Levesque et al. 2008; Moreau and Fleming 2012; Oke et al. 2013; Tibbetts et al. 2013). However, increased growth rate is not observed in first feed EO-1α Salmon in simulated streams (Moreau et al. 2011a) and the notifier reports growth rate differences decrease with age, but data were not provided to support this assertion. The accelerated growth phenotype of salmon appears to be very plastic, and is strongly influenced by environmental conditions (e.g., Sundström et al. 2007b; Sundt-Hansen et al. 2012). Although accelerated growth may be limited in many circumstances including natural conditions, it cannot be concluded that salmon will never express growth rates that provide salmon a fitness advantage in the natural environment. Uncertainty remains around the maximum size of salmon.

1.4.2.2 Morphology, Metabolism and Physiology

Morphological irregularities reported in both 2n and 3n EO-1α Salmon are of low frequency and of a non-debilitating nature. At commercial size, and under commercial feed conditions, EO-1α Salmon have a body composition within the range of commercially grown Atlantic Salmon (NSN-16528). However, there is no information available regarding the body composition of EO-1α Salmon at other life stages, or for EO-1α Salmon fed natural prey.

Oxygen consumption in EO-1α Salmon is similar to non-transgenic fish during early life stages up to the beginning of exogenous feeding (Moreau et al. 2014), but is higher in adults (Deitch et al. 2006). Increased oxygen uptake and consumption rates have also been reported in juvenile AAS-relatives (Stevens and Sutterlin 1999; Cook et al. 2000a; Cook et al. 2000c). Other metabolic and physiological differences between EO-1α Salmon and non-transgenic counterparts include higher feed consumption rates, improved feed conversion ratios, reduced metabolic scope and reduced swimming performance in juveniles raised under hatchery conditions (Deitch et al. 2006; NSN-16528). Increased feed consumption rates have also been reported in AAS-relatives compared to non-transgenic counterparts (Abrahams and Sutterlin 1999; Cook et al. 2000b).

1.4.2.3 Health Status

There are limited data on EO-1α Salmon susceptibility to pathogens as compared to wild or non-transgenic Atlantic Salmon. Studies on 1 year old Atlantic Salmon found triploidy and EO-

1α transgenesis both decreased ability of fish to maintain homeostasis relative to wild type during starvation and stress (Cnaani et al. 2013), suggesting a diminished ability to respond to stressful conditions. Based on Fish Health Certificate data, it was concluded that fish disease risk at the AquaBounty facility in PEI is well managed.

1.4.2.4 Life History, Behaviour and Reproduction

Available information suggests that, although the GH transgene has a minimal effect on fitness-related traits during early stages of development (embryo to beginning of exogenous feeding juveniles, Moreau et al. 2014), it does appear to influence important life history traits as juveniles grow and mature. Specifically, EO-1α males have a reduced tendency to mature sexually as parr and appear to reach smolt status faster than non-transgenics under artificial conditions (Moreau et al. 2011b; Moreau and Fleming 2012). There is no information available on the maturation of female EO-1α Salmon relative to non-transgenic conspecifics. Data from the notifier indicate EO-1α Salmon appear to mature at a normal age and are larger in size than equal-aged non-transgenic salmon, but are smaller than one-year older mature non-transgenic salmon.

Limited information about the behaviour of EO-1α Salmon is available. AquaBounty reported normal avoidance, feeding and postural (positioning) behaviour of juvenile EO-1α Salmon in a hatchery environment (NSN-16528), presumably in comparison to domestic salmon. In a study with first-feeding EO-1α Salmon juveniles, territorial behaviour of transgenic and non-transgenic individuals was similar, suggesting no significant differences in competitive foraging at this critical life history stage (Moreau et al. 2011a). Abrahams and Sutterlin (1999) demonstrated that AAS-relatives are willing to incur greater risk of predation while foraging than non-transgenic comparators, a behaviour that has not been assessed for EO-1α Salmon. There is no information available about the predatory behaviour of EO-1α Salmon or AAS-relatives in the natural environment.

Triploid AAS females are expected to be functionally sterile; however, the process of generating 3n fish at a commercial scale is not always 100% effective. Commercial production of AAS by AquaBounty for grow-out in Panama has produced an average of 99.4% triploidy, with lowest level 98.5% (NSN-19702, 200 eggs tested per batch representing 0.5-1.3% of each batch). Diploid fertile EO-1α females will be present at the facility as broodstock. However, there is no information on the reproductive behaviour of female EO-1α Salmon (both 2n and 3n); a significant knowledge gap. Hatchery reared, 2n EO-1α males have reduced reproductive performance relative to wild (anadromous) or conspecific (mature parr) males for access to wild females, however, they can participate in natural spawning events, and are capable of producing offspring that will survive past the first feeding stage under food limited conditions (Moreau et al. 2011b). However, only neomales will be present at Rollo Bay facility. The notifier reports that neomales produce sperm but lack a sperm duct so could not spawn naturally. It is worth noting that Lee et al. (2004) reported neomale Atlantic Salmon having open sperm ducts in some cases (up to 92% depending on treatment), although whether this resulted in free-flowing milt at maturity was not reported. As well, whether EO-1α neomales display typical spawning behaviour and would attempt to spawn with females has not been determined.

1.4.2.5 Pleiotropic Effects of Growth Enhancement Transgenes in Other Fish Models

Numerous studies have investigated the phenotypic effects of growth-enhanced transgenesis in other fish models. Due to the participation of GH in many major physiological processes in the fish (see Section 1.6.4.9), GH transgenesis is reported to influence almost every phenotype and physiological system examined (see Devlin et al. 2015). The effect of GH transgenesis on fitness traits in fish models relative to their non-transgenic conspecifics indicate that the effect of growth hormone over-expression on the overall fitness of an organism is highly dependent on

both the background genetics, rearing environment and genotype by environment interactions (GxE, see Devlin et al. 2015 and below). Specific data from strains other than that being assessed should not be directly used in the assessment; however, general observations associated with factors affecting the biology of similar organisms and general principles affecting uncertainty associated with collection of data under certain conditions may provide valuable insight.

Increased growth rates with GH transgenesis have been reported in aquaculture-related species including various Pacific salmon and trout, Common Carp (*Cyprinus carpio*), Nile Tilapia (*Oreochromis niloticus*), Rohu (*Labeo rohita*), and Mud Loach (*Misgurnus mizolepis*) (see Devlin et al. 2015), as well as model research organisms such as Zebrafish (*Danio rerio*, Figueiredo et al. 2007) and Medaka (*Oryzias latipes*, Komine et al. 2016). While most studies report similar maximum size but earlier age at maturation, some fish models have greater obtainable size than non-transgenic (e.g., Rainbow Trout *Oncorhynchus mykiss*, Devlin et al. 2001; Mud Loach, Nam et al. 2001). Increased growth has been associated with altered appetite regulation and improved feed conversion efficiency in those models examined (e.g., Nam et al. 2001; Fu et al. 2007; Kobayashi et al. 2007; Zhong et al. 2013; Dalmolin et al. 2015; Kim et al. 2015). Commonly reported behavioural changes associated with increased appetite include increased foraging behaviour and competitiveness, as well as decreased predation avoidance (e.g., Devlin et al. 1999; Sundström et al. 2004b; Zhang et al. 2014; Crossin and Devlin 2017; Hollo et al. 2017). These traits are also observed in salmonids with growth hormone injections or implants (Johnsson and Björnsson 1994; Johnsson et al. 1996; Jönsson et al. 1998a; Jönsson et al. 1998b), although altered behaviour from exogenous GH does not necessarily impact outcome of contests (Johnsson et al. 1996; Neregard et al. 2008) or survival in natural conditions (Johnsson et al. 1999; Johnsson et al. 2000; Johnsson and Björnsson 2001; Sundt-Hansen et al. 2012).

Other commonly reported physiological effects of GH transgenesis in fish species include morphological or bone development abnormalities (Devlin et al. 2004a; Lu et al. 2013; Zhu et al. 2013) and reduced disease resistance or immune function (Jhingan et al. 2003; Kim et al. 2013; Batista et al. 2015). Impaired swimming ability has been noted in Common Carp and Coho Salmon (Farrell et al. 1997; Li et al. 2007; Leggatt et al. 2017a), but not Nile Tilapia or juvenile Rainbow Trout (McKenzie et al. 2003; Crossin et al. 2015). Time to reach specific life stages is generally reported to be compressed, e.g., in Coho Salmon include advanced time to egg hatching (Lõhmus et al. 2010), smoltification (Devlin et al. 1994; Devlin et al. 1995), and sexual maturation (Bessey et al. 2004). While some models have reported strong negative impacts to fertility (e.g., Zebrafish, Figueiredo et al. 2013; and Medaka, Komine et al. 2016), other species do not have reported negative effects on fecundity and limited or no effects on spawning success relative to equally-reared non-transgenic siblings (i.e., Coho Salmon and Common Carp, Bessey et al. 2004; Lian et al. 2013; Leggatt et al. 2014).

Research on growth-enhanced transgenic salmonids has also illustrated the importance of background genetics and the insertion event on the expression of the transgene. Devlin et al. (2001) observed that the insertion of a GH transgene into a wild Rainbow Trout resulted in a 17-fold increase in weight after 14 months, whereas introduction of the same construct into a highly domesticated strain of Rainbow Trout (previously selected for rapid growth) did not result in further growth enhancement. In contrast, when the same GH transgene was inserted into wild and domesticated strains of Coho Salmon, both strains expressed higher growth rates with the domesticated strain experiencing a much greater increase in growth rate than the wild strain (Devlin et al. 2001). It is difficult to determine the extent to which the observed phenotypes are influenced by background genetics and/or the point of insertion of the construct into the genome, which has also been demonstrated to affect the expression of the transgene in Coho

Salmon (Devlin et al. 2004a). Different strains of GH transgenic Coho Salmon that share one parent have differing success in both culture (Leggatt et al. 2012) and semi-natural streams (Leggatt et al. 2017b), suggesting transgene insertion site has some influence on success. Consequently, although there is clear evidence that background genetics and the insertion event can affect growth phenotype, it remains difficult to predict the direction of the resulting effect.

Strong consideration should also be given to potential interactions (i.e., GxE). In almost every trait examined, the effects of environment on phenotype have differed between GH transgenic Coho Salmon and non-transgenic cohorts (see Devlin et al. 2015). This can make predictions of GH transgenic phenotype in nature difficult to do with certainty when only laboratory or semi-natural studies are available. For instance, Devlin et al. (2004b) observed that growth and survival of transgenic and non-transgenic hatchery Coho Salmon fry can vary depending on food availability, ranging from enhanced growth for the transgenic fish when food is abundant, to a population crash when transgenic fish are present under conditions of low food abundance in simplified tanks (Devlin et al. 2004b). Sundström et al. (2007b) demonstrated that growth enhanced transgenic Coho Salmon grew three times longer than wild conspecifics under hatchery conditions, but grew only 20% longer under simulated stream conditions. Vandersteen et al. (2019) examined the effect of individual environmental components (habitat complexity, food availability and type, density, predation) on growth and survival of non-transgenic and GH transgenic Coho Salmon fry in simulated streams and found the effect of genotype on survival was influenced by all examined environmental factors, primarily through alterations in non-transgenic fish survival, such that no predictable trend in relative survival emerged. As well, transgenic fry were only able to gain an extreme size advantage over non-transgenic fish when fed artificial food regardless of food level (Vandersteen et al. 2019). Consequently, it is critical to consider GxE interactions in the risk assessment, with particular attention given to uncertainty where phenotype has not been examined under multiple relevant conditions, or where phenotype of notified and control organisms are unequally influenced by relevant environmental conditions.

1.4.3 History of Use

AquaBounty Technologies Inc. is an American biotechnology company with a land-based, contained research and development facility near Bay Fortune, Prince Edward Island (PEI). EO-1α Salmon were developed by micro-injecting the opAFP-GHc2 gene construct into the eggs of a non-transgenic Atlantic Salmon (Du et al. 1992), followed by introgression of the transgene in the initial mosaic founder genotype into a non-transgenic genetic background. Since 1996, male, female and neomale broodstock have been maintained at the Bay Fortune, PEI facility where EO-1α Salmon eggs and milt are produced, and where eggs are fertilized to generate both 2n broodstock and sterile 3n AAS production fish.

In 2013, AquaBounty Technologies submitted a notification (NSN-16528) to ECCC detailing its intent to commercially produce genetically-engineered (GE) Atlantic Salmon in Canada in a contained facility (not for food or feed approval). The proposed production scenario consisted of egg production and broodstock maintenance at the facility near Bay Fortune, PEI, and commercial grow-out at a contained facility in Panama as described in NSN-16528. Fisheries and Oceans Canada assisted in the regulatory process by conducting the environmental and indirect human health risk assessment for AquAdvantage® Salmon, and by providing science advice to ECCC in support of its decisions regarding the appropriate management of risks. Under the well-defined containment conditions proposed by AquaBounty, DFO determined that the AquAdvantage® Salmon poses low risk to the Canadian environment and indirect human health. However, DFO advised that this conclusion could change if activities in relation to the

salmon change significantly from those proposed by AquaBounty in its submission, and could result in greater risk to the environment (see DFO 2013). ECCC and HC accepted this advice and published Significant New Activity Notice #16528 in the Canada Gazette in November 2013.

In November 2015, the US Food and Drug Administration (FDA) approved AquaBounty's GE (AquAdvantage®) salmon for human consumption. However, the import of AquAdvantage® into the US was blocked at this time by a US Congress spending bill that called upon the FDA to prohibit the introduction of food that contains GE salmon into US interstate commerce until final labeling guidelines are published that inform consumers of such content.

In May 2016, Health Canada (HC) and the Canadian Food Inspection Agency (CFIA) approved the transgenic AquAdvantage® Atlantic Salmon for human food and animal feed use, respectively, making Canada the first market open for the sale of a transgenic animal. By 2017, reports of shipments from Panama to Canada were appearing in the news.

In April 2018, the US Food and Drug Administration approved an additional grow-out site for AAS in Albany, Indiana. In December 2018, the US government issued labelling guidelines for GE foods, and in March 2018 the FDA lifted the import restriction on AquAdvantage® Salmon (eyed eggs and processed meat) into the US.

1.5 CHARACTERISATION OF COMPARATOR SPECIES

The Atlantic Salmon (*Salmo salar*) is world renowned for both its spectacular life-history and its economic importance to recreational, commercial, and aboriginal subsistence and ceremonial 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. (2007a), respectively. A summary of the biology of Atlantic Salmon can be found in a consensus document on the biology on the Organization for Economic Co-operation and Development (OECD) web page (Safety Assessment of Transgenic Organisms in the Environment; Volume 7). Here, a brief overview of Atlantic Salmon biology is provided with an emphasis on domesticated Atlantic Salmon.

1.5.1 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).

1.5.2 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.

1.5.3 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.

1.5.4 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).

1.5.5 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 2009). 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, Figure 1.3). Since the COSEWIC assessment in 2010, significant work in population structure have identified population structure beyond these DUs in some areas (e.g., Moore et al. 2014; Jeffrey et al. 2018). As well, many regions have continued to decline since status designation in COSEWIC (2010) (e.g., DFO 2014b, 2018a).

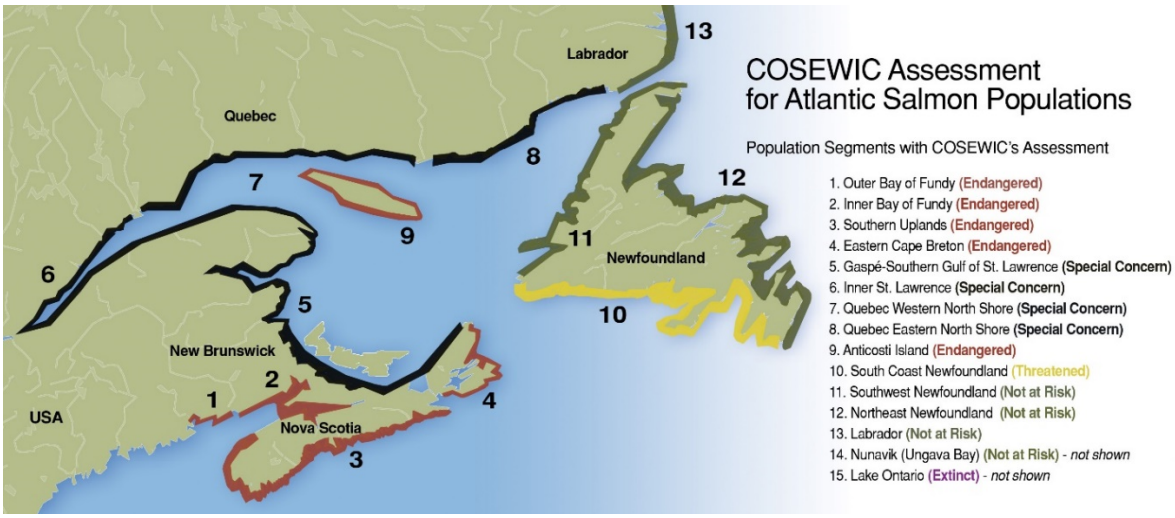

Figure 1.3: Designatable Units of Canadian Atlantic Salmon stocks assessed in 2010 (from COSEWIC 2010).

1.5.6 History of Invasiveness

With a few 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 their native range (Thorstad et al. 2011). Numerous attempts to establish self-sustaining populations of Atlantic Salmon outside of the native or historic range of the species in Canada have occurred in the western provinces of British Columbia and Alberta; however, no permanent populations were ever established (MacCrimmon and Gots 1979). However, escaped domesticated Atlantic Salmon have been well documented reproducing and hybridizing within wild Atlantic Salmon throughout the native range of Atlantic Salmon (see Glover et al. 2017, Section 1.6.7.4), and there is significant evidence of hybridization of stocked hatchery strains of Atlantic Salmon with local wild populations (e.g., Le Cam et al. 2015), demonstrating significant potential for invasiveness within the range of the species.

1.5.7 Biology of Domesticated Atlantic Salmon

The environment 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.

1.5.7.1 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).

1.5.7.2 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).

1.5.7.3 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.

1.5.7.3.1 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.

1.5.7.3.2 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; Fleming and Einum 1997; Jonsson and Jonsson 2006; Houde et al. 2009a).

1.5.7.3.3 Feeding

In the marine environment, Jacobsen and Hansen (2000) 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.

1.5.7.3.4 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).

1.5.7.3.5 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). However, hybridization between Atlantic Salmon domestic escapes and wild populations is most likely through female escapes (see Glover et al. 2017). Reproductive success of escaped domesticated Atlantic Salmon and hybridization with wild populations has been associated with landscape features, where domesticated fish success may be greater in areas with lower spawning migration effort required (Sylvester et al. 2018).

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. 2009b, a; Fraser et al. 2010a; Fraser et al. 2010b; Wringe et al. 2018; Sylvester et al. 2019, see Glover et al. 2017). 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).

1.5.7.4 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; Glover et al. 2017). 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 (Bolstad et al. 2017) and genetic integrity of wild Atlantic Salmon populations (Skaala et al. 2006; Bourret et al. 2011; Bolstad et al. 2017; Keyser et al. 2018; Wringe et al. 2018), decreased abundance of wild Atlantic Salmon (Sylvester et al. 2019), and altered maturity schedules and growth rates (Bolstad et al. 2017). Long-term consequences of invasiveness of domesticated Atlantic Salmon within the native range of the species include changes in life-history traits, expected reduced population productivity and expected decreased resilience to future challenges (see above and Glover et al. 2017). 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).

1.6 CHARACTERIZATION OF THE POTENTIAL RECEIVING ENVIRONMENT

The environment directly outside of the Rollo Bay facility, as well as those connected to the immediate environment, has potential to allow the survival of Atlantic Salmon. The facility drains into a small, sheltered stream that enters the local drainage system, which runs for about 2.0 kilometers before emptying into Rollo Bay and the Northumberland Strait. The stream will be fed year-round with water from the aquaculture facilities and natural sources of runoff. At the time of a site visit by DFO and ECCC officers in September 2018, the facility was not at full operation and much of the stream was dry. However, at full operation, and with 99.7% water recirculation, the volume of water discharged from the three buildings combined is expected to be equivalent to the amount of water discharged from the AquaBounty Canada facility in Bay Fortune, PEI. With these volumes of water in mind, the notifier has indicated that the stream will likely be hospitable to salmonids at all life stages (NSN-19702).

The receiving environment is located well within the natural range of Atlantic Salmon (Figure 1.3), and the physical and chemical components of the receiving habitat and connecting habitats (e.g., the Atlantic Ocean) have potential to support all life stages of Atlantic Salmon from embryo to adult. Should EO-1α smolts migrate into the Northumberland Strait, there are numerous salmon-bearing rivers within reported stray distance from the Rollo Bay Facility of a different domestic Atlantic Salmon strain (i.e., average stray distance of AquaGen strain was 178 km, Jonsson and Jonsson 2017), where EO-1α Salmon could potentially return to as adults, and survive and interact with wild salmon populations (Figure 1.3, 1.4). While many PEI watersheds have habitat impacts (e.g., sediment deposition, fragmentation, Cairns and MacFarlane 2015) that prevent or limit Atlantic Salmon population occupancy, currently 25 PEI rivers support populations of Atlantic Salmon (DFO 2018, Figure 1.4) and there are many salmon rivers across the Gulf in eastern New Brunswick and Nova Scotia.

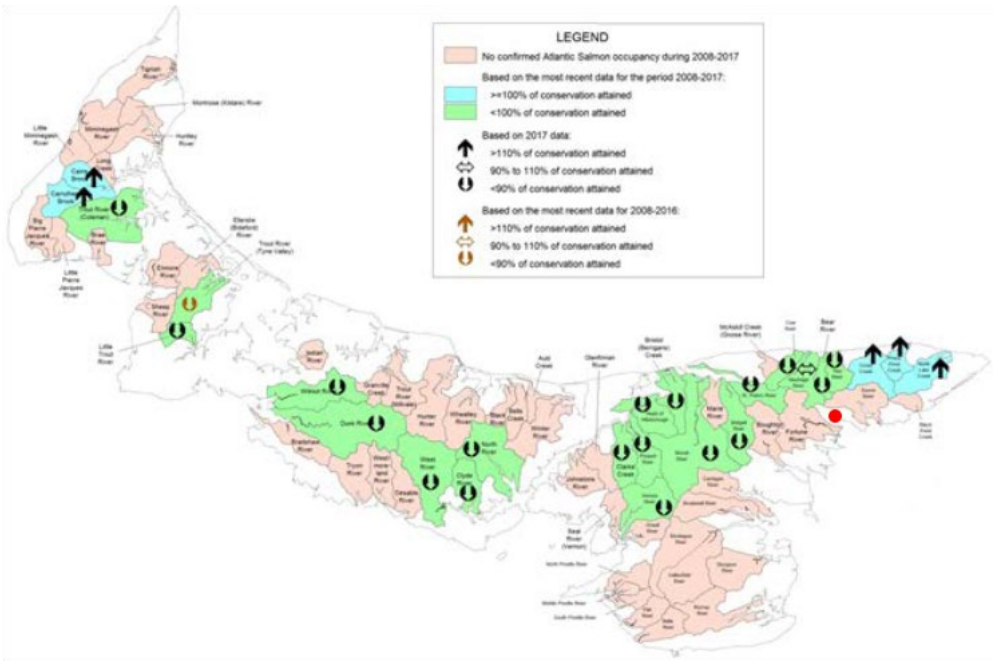

Figure 1.4: Watersheds of Prince Edward Island showing locations of historic and current Atlantic Salmon occupancy as of 2017. Blue shading indicates watersheds that have met or exceed conservation requirements, green shading indicates watersheds that are below conservation requirements, and pink shading indicates watersheds with historic populations, but no evidence of salmon presence since 2008. Down arrows indicate less than 90% of conservation requirements attained, sideways arrows indicates between 90-110%, and up arrow indicates greater than 110% of conservation requirements. (Taken from DFO 2018, red dot indicates approximate location of Rollo Bay facility).

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 (i.e., with potential to become endangered or threatened, COSEWIC 2010, Figure 1.4). 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 shifts in marine ecosystem resulting in extremely poor marine survival, salmonid aquaculture, depressed population phenomenon, freshwater habitat destruction and obstruction, illegal fishing, interbreeding with domestic salmon escapes, exposure to pesticides, disease and parasites, climate change and competition with invasive species (COSEWIC 2010; DFO 2014a). In PEI specifically, the Northeast Cluster of eastern PEI appear to represent an ancestral strain that is not documented elsewhere and are consequently particularly important to salmon biodiversity (Moore et al. 2014). The Northeast Cluster strain is vulnerable due to small stream size and resulting low population sizes (DFO 2018). This population represents the closest intact Atlantic Salmon population within migration distance of the Rollo Bay facility and may have highest potential to be exposed to EO-1α Salmon should they escape the facility. A loss or severe reduction of Atlantic Salmon in the Northeast Cluster, or an impairment of their genetic integrity, could lead to a significant loss of biodiversity in Atlantic Salmon. Other potential receiving waters of Rollo Bay escapees off PEI generally show salmon genotypes that have fairly broad geographic distributions (i.e., clustered with other Gulf of St. Lawrence populations with a strong influence of historic stocking with Miramichi River salmon, Moore et al. 2014), and local impacts in these areas may not have as wide-reaching effects on species genetic diversity. Other populations in PEI are very small and face the

likelihood of extirpation if current trends continue (Cairns and MacFarlane 2015). Recent work suggests small populations such as those in PEI are more prone to hybridization and negative effects of introgression (Sylvester et al. 2018; Wringe et al. 2018).

In addition to COSEWIC-listed populations of Atlantic Salmon (see Figure 1.4), the proposed Rollo Bay site is within migration distance of many other SARA- or COSEWIC-listed species including various species of whales, cetaceans, and fish.

1.7 SUMMARY

Within the legislative context of CEPA and the information requirements of the *New Substances Notification Regulations (Organisms)* Schedule 5, this document elaborates the Framework for the assessment of potential risks to the Canadian environment that may be associated with the manufacture or production of GE fish. The environmental risk assessment is conducted in accordance with the classical risk assessment paradigm where risk is directly related to the exposure and hazard of the organism. The exposure assessment is based on the likelihood and magnitude of release into the environment, and the likelihood and magnitude of survival, reproduction, establishment, and spread of the organism and potential descendants of the organism in the Canadian environment. The hazard assessment is focused on the potential for the organism to impact through hazard pathways, specifically: through environmental toxicity, horizontal gene transfer, trophic interactions, hybridization and as a vector of disease; and to impact the environmental components, specifically: biogeochemical cycling, habitat, and biodiversity. The level of uncertainty for both exposure and hazard determinations is evaluated and communicated in terms of impact to the final risk assessment. DFO makes recommendations to ECCC for regulatory decision-making under CEPA, based on risk to the environment and the uncertainty associated with the conclusion. This may include recommended measures to mitigate risk under the proposed notified use.

PART 2: ENVIRONMENTAL RISK ASSESSMENT

2.1 PURPOSE OF PART 2

Part 2 of this document comprises the environmental risk assessment conducted under the *Canadian Environmental Protection Act*, 1999 (CEPA) with respect to EO-1α Salmon, including the commercial form AquAdvantage® Atlantic Salmon (AAS), a GE Atlantic Salmon (*Salmo salar*) notified by AquaBounty Canada Inc. under the *New Substances Notification Regulations (Organisms)* [NSNR(O)].

2.2 EXPOSURE ASSESSMENT

The exposure assessment for living EO-1α Salmon addresses both their potential to enter the environment (release) and fate once in the environment. The likelihood and magnitude of environmental exposure is determined through an extensive cradle-to-grave assessment that details the potential for the release of EO-1α Salmon, as well as its survival, reproduction, and proliferation in the Canadian environment. All relevant information regarding physical, chemical and biological containment strategies used at all life stages is considered. The potential for unintentional release resulting from catastrophic events is also taken into consideration. Rankings for the likelihood of Exposure to the Canadian Environment are provided in Table 2.1.

Table 2.1: Rankings for exposure of EO-1α Salmon to the Canadian environment.

Exposure Ranking	Assessment
Negligible likelihood	No occurrence; Not observed in Canadian Environment
Low likelihood	Rare, isolated occurrence; Ephemeral presence
Moderate likelihood	Often occurs, but only at certain times of the year or in isolated areas
High likelihood	Often occurs at all times of the year and/or in diffuse areas

The exposure assessment requires two distinct approaches to assessing uncertainty; one for the physical containment (i.e., entry or release) and a second for the biological containment (i.e., fate of the organism). Since exposure related to physical containment relies on both the design and operational management of facilities, the evaluation of uncertainty relies upon the availability of accurate and detailed information that adequately demonstrates the efficacy and redundancy of mechanical barriers, and the efficacy of standard operating procedures (SOPs). This included diagrams of mechanical barriers and containment systems, incident reports, and training and compliance documentation (Table 2.2). Uncertainty associated with the exposure that may result from the survival, reproduction and proliferation of EO-1α Salmon in the Canadian environment depends on the availability and robustness of scientific information related to the biological and ecological parameters of EO-1α Salmon, valid surrogates, and the receiving environment (Table 2.3).

Table 2.2: Categorization of exposure uncertainty based on the assessment of physical containment (i.e., entry) of EO-1α Salmon in the Canadian environment.

Rank	Description
Negligible uncertainty	Detailed information on facility design, containment structures, water treatment equipment, SOPs, internal compliance documentation, facility incident reports and inspection reports are available.
Low uncertainty	Detailed information on facility design, containment structures, water treatment equipment, SOPs are available.
Moderate uncertainty	Information on facility design, containment structures, and water treatment equipment is available; however, SOPs are not available.
High uncertainty	Limited information on facility design, containment structures, and water treatment equipment is available.

Table 2.3: Categorization of exposure uncertainty based on the assessment of effectiveness of biological and environmental containment (i.e., fate) of EO-1α Salmon in the Canadian environment.

Rank	Description
Negligible uncertainty	High quality data on EO-1α Salmon (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.
Low uncertainty	High quality data on EO-1α relatives or valid surrogate. Data on environmental parameters of the receiving environment. Understanding of potential GxE effects across relevant environmental conditions. Some variability.
Moderate uncertainty	Limited data on EO-1α Salmon, 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.

To facilitate the assessment of physical containment, a Failure Modes Analysis (FMA) was conducted following guidance from Stamatis (2003) and McDermott et al. (2009). The FMA was intended to identify potential weaknesses along all potential pathways of entry into the environment, and provide a systematic method to examine and assess each and every element of physical containment. 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 are all taken into consideration. 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 if available), and the mitigation measures in place to prevent a potential failure (based on SOPs and oversight documentation). Severity (S), occurrence (O), and mitigation (M) are ranked as shown in Table 2.4, Table 2.5, and Table 2.6, respectively. 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 2.7).

Table 2.4: 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 present
2	Medium; No entry possible; 1 downstream barrier present
3	High; entry possible; no downstream barrier present

Table 2.5: 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
3	High; O ≥ 5 recorded incidents per year or no records available

Table 2.6: 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 2.7: 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 EO-1α Salmon 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.

2.2.1 Use Scenarios

The primary activity under the notification is the commercial manufacture of 3n (sterile) AquAdvantage® eyed eggs for grow-out to market size at the Rollo Bay facility, or for transport to approved grow-out facilities in Panama or the United States. The company also expressed its intensions to manufacture and sell 2n non-transgenic Atlantic Salmon eggs to external parties. This raised the possibility of a containment failure resulting from human error, whereby transgenic eggs are accidentally shipped as non-transgenic, to customers who could inadvertently release the organism into the environment. Consequently, the risk assessment included consideration of two scenarios as follows:

- Scenario A: company activities would include the production of non-transgenic fish, for external parties, occurring along-side transgenic fish production using existing and planned procedures for keeping eggs organized and separated and for keeping transgenic organisms contained.
- Scenario B: no production of non-transgenic fish for external parties takes place, with all non-transgenic salmon housed at the facility used only for the production of AAS, as described in the regulatory package submitted by the company.

2.2.2 Likelihood of Release

The Rollo Bay facility is located just north of the municipality of Rollo Bay, Prince Edward Island, on a parcel of land that is approximately 1.15 km from Rollo Bay and the Northumberland Strait. The facility is entirely land-based, with all EO-1α Salmon to be maintained within the confines of three buildings (one newly renovated and two newly constructed), each will have a cement foundation, solid walls, and a roof. Each building will have its own recirculating aquaculture systems (RAS), operating at 99.7% recycled water (as claimed by the notifier), with small amounts of fresh water supplied by any of several wells located on the property. In each building, a variety of mechanical and chemical barriers, such as tank and floor drain screens and covers, jump nets, and redundant screens and filters in pipes and plumbing system, designed to prevent the accidental release of EO-1α Salmon into the environment are in place. These are supported by SOPs and internal compliance documentation for the proper employment and maintenance of all containment provisions. Most of the 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, have been adapted from those in use at the approved AquaBounty facility in Bay Fortune PEI, for use at the Rollo Bay facility. When operational, all three buildings will be subject to routine inspection by ECCC, in accordance with CEPA Compliance and Enforcement Policy.

Here, each of the three buildings are considered in turn, using detailed information provided by the notifier regarding floor plans, drainage systems, operational procedures, and redundant barriers that are designed to contain EO-1α Salmon in all of its forms. 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 aquatic organism.

Information gathered by DFO and ECCC officials during a planned site visit to the Rollo Bay facility in September 2018 is included in the assessment. Containment of 2n EO-1α Salmon used in the manufacture of 3n AAS (i.e., pressure shocking eggs to induce triploidy) is

examined along with the likelihood of containment failure during the transport of AAS. Finally, the potential for catastrophic natural events or security violations to result in the release of EO-1α Salmon is taken into consideration.

Though details regarding the physical containment measures, drainage systems, and operational oversight at the facility have been provided by the company for review, they are considered confidential business information and are not included in this report. The FMA tables generated using this information have also been redacted.

2.2.2.1 The Hatchery

The Hatchery is a recently renovated 8800 square foot building that will be used to house select lines of 2n EO-1α broodstock. At the time of the site visit in September 2018, the Hatchery was fully operational, though parts were not in use. All aspects of physical containment in the Hatchery are governed with written SOPs and compliance documentation in the form of daily checklists.

2.2.2.1.1 Physical containment of EO-1α gametes in the Hatchery

EO-1α gametes will be present in the Hatchery during spawning and fertilization activities. Atlantic Salmon gametes have limited viability once they enter an aqueous environment, and are generally inactivated within 10 minutes (Vladiĉ and Järvi 1997; Lahnsteiner 2002), although viability is possible if sperm and eggs were released simultaneously in a way that allowed fertilization. The FMA for this stage of development identified six components of containment designed to prevent the release of EO-1α gametes into the environment, all of which are subject to daily inspection as dictated by written SOPs and checklists. For unfertilized ova to reach the environment outside of the Hatchery there must be simultaneous failure of all six containment measures. For milt to reach the environment there must be simultaneous failure of at least three containment measures. Risk Priority Numbers (RPNs) generated for 10 failure modes varied from three to nine (low to medium concern).

2.2.2.1.2 Physical containment of EO-1α embryos in the Hatchery

EO-1α embryos (fertilized eggs and sac-fry) will be housed in stacks of Heath Tray incubators. The FMA for this stage of development identified 18 containment measures designed to prevent the release of EO-1α embryos into the environment, all of which are subject to daily inspection as dictated by written SOPs and checklists. For an embryo to reach the environment outside of the Hatchery there must be simultaneous failure of at least six containment measures along a single pathway of entry. RPNs generated for 37 potential failure modes varied from three to nine (low to medium concern). Internal compliance documentation collected for an incubation unit of similar design at the company's facility in Bay Fortune, PEI indicate zero incidents of full failure (i.e., escape from facilities) over a period of 12 years (NSN-16528).

2.2.2.1.3 Physical containment of EO-1α fry in the Hatchery

When ready, EO-1α Salmon will be transferred from the Heath stack incubators to early rearing tanks. The FMA for this stage of development identified 13 containment measures designed to prevent the release of EO-1α fry into the environment, all of which are subject to daily inspection as dictated by written SOPs and checklists. For fry to reach the environment outside of the Hatchery there must be simultaneous failure of at least eight containment measures along a single pathway of entry. RPNs generated for 25 failure modes varied from three to nine (low to medium concern).

2.2.2.1.4 Physical containment of EO-1α juveniles and adults in the Hatchery

When fry are large enough, they are transferred to a different area of the Hatchery, where EO-1α Salmon are housed as juvenile and adult broodstock. The FMA for this stage of development

identified seven containment measures designed to prevent the release of EO-1α juveniles and adults into the environment, all of which are subject to daily inspection as dictated by written SOPs and checklists. For juvenile and adult EO-1α Salmon to reach the environment outside of the Hatchery there must be simultaneous failure of at least four containment measures. RPNs generated for 16 potential failure modes varied from three to nine (low to medium concern).

2.2.2.2 The Grow-out Building

The Grow-out Building is a new construction of approximately 41,000 square feet, and will be used to raise 3n AAS, from egg to market size (5 kg) adults, at a rate of approximately 250 metric tons per year. Though it is built with this purpose in mind, it may also be used to house 2n EO 1α Salmon depending on the circumstance or needs of the company. At the time of the site visit, in September 2018, construction of the facility was still underway. Though much of the drainage system and many tanks were in place, the assessment relies heavily on the documentation and schematic designs provided in the notification. The building has been built to code with a concrete foundation and reinforced steel structure, and has been designed to withstand local weather extremes such as high winds or heavy snow fall.

2.2.2.2.1 Physical containment of EO-1α embryos in the Grow-out Building

Triploid AAS embryos (fertilized eggs and sac-fry) will be housed in stacks of Heath Tray incubators. The FMA for this stage of development identified ten containment measures designed to prevent the release of EO-1α embryos into the environment, nine of which are subject to daily inspection as dictated by written SOPs and checklists. For an embryo to reach the environment outside of the Grow-out Building there must be simultaneous failure of at least six containment measures along a single pathway. RPNs generated for 21 failure modes varied from three to nine (low to medium concern). Internal compliance documentation collected for an incubation unit of similar design at the company's facility in Fortune, PEI indicate zero incidents of full failure (i.e., escape from facilities) over a period of 12 years.

2.2.2.2.2 Physical containment of EO-1α fry and young juveniles in the Grow-out Building

When ready, EO-1α fry will be transferred from the Heath stack incubators to early rearing tanks. The FMA for this stage of development identified seven containment measures designed to prevent the release of EO-1α fry into the environment, all of which are subject to daily inspection as dictated by written SOPs and checklists. For fry or young juveniles to reach the environment outside of the Grow-out Building, there must be simultaneous failure of at least five containment measures along a single pathway. Risk Priority Numbers generated for 16 failure modes varied from three to nine (low to medium concern).

2.2.2.2.3 Physical containment of EO-1α older juveniles and adults in the Grow-out Building

When EO-1α Salmon are large enough, they are transferred to a different area of the Grow-out Building and grown to a market weight of approximately 5 kg. If EO-1α Salmon were to enter the drainage system, there are five additional mechanical barriers (25 mm screens) in place to prevent fish from leaving the building and entering the environment. The FMA for this stage of development identified nine containment measures used to prevent the release of EO-1α juveniles and adults into the environment, all of which are subject to daily inspection as dictated by written SOPs and checklists. For juvenile and adult EO-1α Salmon to reach the environment outside of the Grow-out Building there must be simultaneous failure of at least seven barriers along a single pathway. RPNs generated for 20 failure modes varied from three to nine (low to medium concern).

2.2.2.2.4 Physical containment of market size EO-1α adults in the Grow-out Building

When EO-1α Salmon are large enough, they are transferred to a different area of the Grow-out Building and grown to a market weight of approximately 5 kg. If EO-1α Salmon were to enter the drainage system, there are five additional mechanical barriers (25 mm screens) in place to prevent fish from leaving the building and entering the environment. The FMA for this stage of development identified nine containment measures used to prevent the release of EO-1α juveniles and adults into the environment, all of which are subject to daily inspection as dictated by written SOPs and checklists. For juvenile and adult EO-1α Salmon to reach the environment outside of the Grow-out Building there must be simultaneous failure of at least seven barriers along a single pathway. Risk Priority Numbers generated for 20 failure modes varied from three to nine (low to medium concern).

2.2.2.3 The Broodstock Building

The Broodstock Building is a new construction of similar size and design as the Grow-out Building. It will be used for the manufacture of the all-female 3n AAS eggs that will be used in commercial grow-out, and will house the 2n EO-1α homozygous females and the 2n EO-1α homozygous neomales required by the manufacturing process. The Broodstock Building will also be used for incubation of the product, all-female 3n AAS eggs, that will be shipped to grow out facilities in Panama and the United States prior to hatching. At the time of the site visit, in September 2018, construction of the facility was still underway with no tanks or drainage system in place. Consequently, the assessment of containment must rely entirely on the documentation, descriptions and schematic designs provided in the notification. The building has been built to code with a concrete foundation and reinforced steel structure, and has been designed to withstand local weather extremes such as high winds or heavy snow fall.

2.2.2.3.1 Physical containment of EO-1α gametes in the Broodstock Building

EO-1α gametes will be present in the Broodstock Building during spawning and fertilization activities. The FMA for this stage of development identified six components of containment designed to prevent the release of EO-1α gametes into the environment, all of which are subject to daily inspection as dictated by written SOPs and checklists. For unfertilized ova to reach the environment outside of the Broodstock Building, there must be simultaneous failure of five containment measures along a single pathway of entry. For milt to reach the environment there must be simultaneous failure of at least three containment measures along a single pathway. RPNs generated for 11 failure modes varied from three to nine (low to medium concern).

2.2.2.3.2 Physical containment of EO-1α embryos in the Broodstock Building

Diploid and 3n AAS embryos will be housed in two different locations of the Broodstock Building. The FMA for this stage of development identified 12 containment measures designed to prevent the release of EO-1α embryos into the environment, nine of which are subject to daily inspection as dictated by written SOPs and checklists. For an embryo to reach the environment outside of the Broodstock Building there must be simultaneous failure of at least six containment measures. RPNs generated for 25 failure modes varied from three to nine (low to medium concern). Internal compliance documentation collected for incubation units of similar design at the company's facility in Fortune, PEI indicate zero incidents of full failure (i.e., escape from facilities) over a period of 12 years.

2.2.2.3.3 Physical containment of fry and young juveniles in the Broodstock Building

When ready, EO-1α fry will be transferred from the Heath stack incubators to early rearing tanks. Written SOPs are used to direct staff on procedures for the husbandry of fish, the disposal of mortalities, and for ensuring that fish do not enter the floor drains. The FMA for this

stage of development identified seven containment measures designed to prevent the release of EO-1α fry or young juveniles into the environment, all of which are subject to daily inspection as dictated by written SOPs and checklists. For fry or young juveniles to reach the environment outside of the Broodstock Building, there must be simultaneous failure of at least five containment measures. RPNs generated for 16 failure modes varied from three to nine (low to medium concern).

2.2.2.3.4 Physical containment of older juveniles and adults in the Broodstock Building

When EO-1α Salmon are large enough, they are transferred to a different part of the Broodstock Building, where they will be grown to sexual maturity. The FMA for this stage of development identified nine containment measures used to prevent the release of EO-1α older juveniles and adults into the environment, all of which are subject to daily inspection as dictated by written SOPs and checklists. For juvenile and adult EO-1α to reach the environment outside of the Broodstock Building there must be simultaneous failure of at least seven containment measures along a single pathway. Risk Priority Numbers generated for 20 failure modes varied from three to nine (low to medium concern).

2.2.2.4 Containment During the Manufacture of Triploid AAS and Diploid EO-1α Broodstock

AAS eggs that are exported from the Rollo Bay facility to a commercial grow-out facility must first be collected from fish, fertilized, then undergo pressure shocking to induce triploidy sterilization. During these activities, which could occur in any of the three buildings, there is potential for both 2n and 3n EO-1α embryos to spill onto the floor and enter the floor drains. As with the physical containment of EO-1α gametes or embryos (see sections 2.2.1.1.1 and 2.2.1.1.2) all three buildings will have multiple mechanical and chemical barriers in place to prevent the release of EO-1α at any point during the manufacturing process (fertilization and pressure shock). If EO-1α embryos were to enter the floor drains, additional mechanical and chemical barriers are in place to prevent a release from any of the three buildings.

Physical containment and operational oversight make the likelihood of exposure resulting from the accidental release of EO-1α embryos during the manufacturing process negligible. Uncertainty associated with this conclusion is low given the available information on facility design, containment structures, SOPs and internal compliance documentation.

2.2.2.5 Containment During the Transport of Triploid AAS Eggs to Other Facilities and EO-1α Salmon Between Buildings at Rollo Bay

When completely operational, the Rollo Bay facility will be used to manufacture 3n AAS for commercial grow-out and for export to additional approved commercial grow-out facilities in Panama and the United States. All transport of EO-1α and non-transgenic Salmon, including transport between buildings at the Rollo Bay facility will require approval and licensing through the DFO Introductions and Transfers Committees. During transport between any of the facilities, or between buildings within the Rollo Bay facility, fertilized eggs will be contained in a sturdy plastic cooler with a secured lid. The cooler is placed inside a cardboard shipping crate that is sealed and labelled according to the packaging requirements imposed by the United States Food and Drug Agency (USFDA) as part of their approval for the sale of AAS in the United States.

Written SOPs are used to direct staff on procedures for the storage, shipping, and handling of EO-1α eggs when in transit. During ground transport, the eggs will be in the possession of AquaBounty staff. Air transport will be facilitated by a commercial freight-forward company to maintain chain-of custody.

Transportation of fish between facilities will be via lockable transport tanks, follow a facility SOP and be performed by trained AquaBounty staff. However, it has been noted that the current transport SOP for fish could be strengthened to minimize potential for release during transport between buildings on the Rollo Bay site (see Section 2.4.2).

Physical containment and operational oversight makes the likelihood of exposure resulting from the accidental release of EO-1α embryos during transport between facilities negligible. Uncertainty associated with this conclusion is low given available information on the packaging of eggs, SOPs and internal compliance documentation.

2.2.2.6 Natural Events

An acute release of EO-1α Salmon resulting from natural disasters, such as earthquakes, tsunamis, tornados, hurricanes, tidal surges, flooding or fires, is highly unlikely. The most likely natural disaster to challenge the facility's infrastructure and physical containment of EO-1α Salmon 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.

In response to these natural threats, the buildings are built to local building codes by professional contractors with a steel and concrete infrastructure. In addition, the physical facility complied with the Province of Prince Edward Island's Environmental Impact Assessment requirements. The facility is sited approximately 19 meters above sea level and it is highly unlikely that a storm or tidal surge would ever cause damage to infrastructure. Employees are trained on emergency procedures and follow SOPs designed to limit the effects of catastrophic events or a loss of operational capacity.

2.2.2.7 Security

Like natural events, security violations are difficult to predict, but have the potential to result in large scale releases of EO-1α Salmon. AquaBounty has put in place several security measures to protect both its property and personnel. These measures include two backup electricity generators, emergency SOPs adopted from the facility in Bay Fortune, video surveillance, steel exterior doors with key control and entry logs, steel screen window covers, motion detectors, 24 hour surveillance by commercial security provider, and exterior lighting throughout the premises at night. There have been no reports of security violations.

2.2.2.8 Sale of Non-transgenic Eggs to Third Parties

Under Scenario A, where non-transgenic eyed eggs are produced and sold to third parties, there is an additional potential pathway of release, where transgenic eggs could be accidentally shipped as non-transgenic eggs to third parties who could inadvertently release the organism into the environment (e.g., rearing in sea-pens with direct contact with marine environment and higher likelihood of escape). This is expected to increase the likelihood of release relative to Scenario B (no non-transgenic eggs are sold), as well as increase uncertainty associated with likelihood of release as the potential fate of any EO-1α Salmon accidentally sold to third parties is not known and outside the control of the notifying company.

2.2.2.9 Conclusion Regarding the Physical Containment of EO-1α Salmon

The likelihood and potential magnitude of EO-1α Salmon exposure to the Canadian aquatic environment resulting from a failure of physical containment at the Rollo Bay facility was assessed. All life-history stages and all currently conceivable pathways of entry into the environment for both sterile 3n and fertile 2n EO-1α Salmon were considered. A Failure Modes Analysis (FMA) was used to identify potential weaknesses along all potential pathways, and to provide a systematic method for examining each and every element of physical containment.

Twelve different potential pathways of entry into the environment were identified and over 113 elements of physical and chemical containment examined. At the AquaBounty Rollo Bay facility, all pathways to entry have a minimum of four independent barriers in place to prevent the release of viable EO-1α Salmon at all life stages. In all cases, suitable operational measures and oversight are in place to avert or to mitigate potential failures and avert living EO-1α Salmon from entering the Canadian environment. The facility is sited in a location and constructed to standards that effectively prevent the unintentional release of EO-1α Salmon that may result from a catastrophic event and extensive security measures are in place to prevent unlawful entry that may result in damage to property.

The greatest source of uncertainty regarding the physical containment of EO-1α at the Rollo Bay facility is the absence of data on the occurrence of single-point containment failures. This is due to the fact that the facility is new, and there has been no time to accumulate these data, which is normally collected by the company through the use of containment incident reports. At its facility in Bay Fortune PEI, data accumulated over a twelve-year period indicate that incidence of single-point containment failure are rare, and that the severity of consequences small due to redundant containment measures (NSN-16528). Indeed, records from Bay Fortune indicate a facility that is well managed and well designed to prevent accidental release.

Redundant physical containment and strong operational oversight make the likelihood of exposure resulting from the accidental release of EO-1α Salmon from the Rollo Bay facility negligible under Scenario B. Uncertainty associated with this conclusion is low given the available information on facility design, containment structures, SOPs and internal compliance documentation. However, the potential for accidental sale of EO-1α Salmon to third parties raises the likelihood of exposure resulting from accidental release to low under Scenario A. Uncertainty associated with this rating would also increase under this scenario, but it was concluded it would not increase beyond low.

2.2.3 Likelihood of Survival, Reproduction, and Proliferation

The capacity of EO-1α Salmon to survive, reproduce and proliferate in the Canadian environment is precluded by the fact that live EO-1α Salmon is contained, and will not be entering the Canadian environment (see section 2.2.1 Likelihood of Release).

In the highly unlikely event of a release (intentional or unintentional), the principle factors limiting the survival of EO-1α Salmon will be the chemical and physical elements of the receiving environment. As discussed in the Problem Formulation (see 1.7 Characterization of the Potential Receiving Environment), the receiving environment is located well within the natural range of Atlantic Salmon and the physical and chemical components of the release habitat and connecting habitats would likely support all life stages of Atlantic Salmon (or EO-1α Salmon) from embryo to adult, with numerous salmon rivers in close proximity of the Rollo Bay Facility where EO-1α adults could survive and interact with wild salmon populations. Though the conditions of triploidy, sex-reversal, domestication, and growth hormone transgenesis may have an effect on the overall fitness of EO-1α Salmon, they do not prevent EO-1α Salmon from reaching the adult life stage.

Principle factors limiting the reproduction and proliferation of EO-1α Salmon will be its reproductive fitness, the availability of suitable spawning habitat, and the availability of suitable mates. Though 3n fish are functionally sterile (Benfey 1999), and sex-reversal is expected to greatly decrease or remove the ability of EO-1α neomales to reproduce naturally (Johnstone and MacLachlan 1994; see Pandian and Koteeswaran 1998), a portion of broodstock held at the PEI facility will be 2n (for example homozygous EO-1α females) and physiologically capable of reproducing if released into the environment. Also, the process of generating 3n populations at

a commercial scale is not absolute (e.g., < 99%, Devlin et al. 2010) and may leave some individuals fertile. While domestication may diminish the reproductive fitness of EO-1α Salmon, it does not prevent the organism from reaching sexual maturity or ascending rivers to mate with appropriate 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; Karlsson et al. 2016; Glover et al. 2017; Wringe et al. 2018).

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. Moreau et al. (2011b) conducted a series of experiments comparing the reproductive success of EO-1α 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 EO-1α Salmon, both as adults and parr. However, these experiments also demonstrated that EO-1α males are capable of reproduction in the wild and did not address effects of culture on anadromous male success. The reproductive success of EO-1α females has not been reported. Fitzpatrick et al. (2011) found the reproductive fitness of growth enhanced transgenic Coho Salmon to be less than that of hatchery Coho Salmon when reared in small-scale culture conditions, which was in turn, inferior to that of hatchery salmon reared in nature from smolt. Leggatt et al. (2014) found that hatchery and transgenic salmon reared in seawater mesocosms had similar spawning success in most circumstances, though transgenic males were less able to compete for nature-reared females. Though growth-enhanced transgenesis could potentially have a negative effect on the reproductive fitness of EO-1α Salmon, it does not preclude reproduction in the wild.

If reproduction of EO-1α Salmon were to occur in the wild, the potential fate or reproductive fitness of the resulting offspring is highly uncertain. Studies on GxE indicate that organisms of different genetic backgrounds will respond to different environments in different ways. With growth enhanced transgenic Coho Salmon, GxE interactions have been noted in the phenotype of almost all characteristics examined; including life stage timing, growth, behaviour, and reproductive success (see Devlin 2015). Consequently, it may be impossible to predict how the wild offspring of EO-1α Salmon carrying the EO-1α locus will perform in the wild, or how their reproductive fitness in the wild will compare to that of their wild cousins.

The 2013 assessment of AAS concluded with high certainty that should EO-1α Salmon be released from the Bay Fortune rearing facility, there would be high likelihood for them to survive, migrate, reproduce and establish in Canadian waters. The current assessment agrees with high likelihood of occurrence of the EO-1α Salmon in the Canadian environment, should they be released, due to the location of the facility within the native range of wild populations and lack of data demonstrating complete constraints on any life stage of EO-1α Salmon (other than sterility of the 3n AAS form of EO-1α Salmon). However, the current assessment designates a moderate uncertainty level to this rating, due to limited data on EO-1α Salmon survival, dispersal and reproduction across relevant environments, and incomplete understanding of the role of the EO-1α transgene, domestication, culture, and failed triploidy on the potential of EO-1α Salmon to establish in Canadian environments should they be released.

2.2.4 Overall Conclusions on Exposure

Based on what is known of the life history characteristics of EO-1α Salmon and the suitability of the potential receiving environment, should EO-1α Salmon be released from the Rollo Bay facility, they would be expected to be capable of survival, reproduction (fertile forms only), and spread in the Canadian environment. Consequently, exposure would be limited only by the

capacity to contain the organism, both physically and biologically. Under Scenario B, the assessment concludes with low uncertainty that the likelihood of EO-1α Salmon exposure to the Canadian environment is negligible due to negligible potential for release from the contained facility. The high degree of certainty associated with the physical containment of EO-1α Salmon 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, and training and compliance documentation. Under Scenario A, the detailed physical containment at the Rollo Bay facility would remain in effect, but a potential novel pathway for release is present where EO-1α eyed eggs are accidentally released to third parties. This would raise the likelihood of release to low, and could increase uncertainty in exposure. Consequently, the exposure assessment concludes that the likelihood of EO-1α Salmon exposure to the Canadian environment is **negligible** to **low**, with **low to moderate uncertainty,** depending on the use scenario.

2.3 HAZARD ASSESSMENT

The hazard assessment examines potential impacts that could result from environmental exposure to EO-1α Salmon in the environment. The hazard identification process considers the potential hazards through environmental toxicity (i.e., potential to be poisonous), through gene transfer (horizontal gene transfer, hybridization), through trophic interactions, as a vector of disease, and to environmental components, biogeochemical cycling, habitat, and biodiversity. Table 2.8 categorizes the severity of the biological consequences based on the severity and reversibility of effects to the structure and function of the ecosystem. The rank (negligible, low, moderate, high) of the potential impacts to the assessment endpoints is evaluated in the risk assessment as well as the uncertainties; taking into consideration the appropriateness of control experiments and data, rearing conditions, interaction effects, phenotypic plasticity, and genetic background, in order to minimize uncertainty regarding assessment of potential ecological consequences of EO-1α Salmon. Any difference in measurement endpoint is evaluated relative to ‘normal’ variation, based on published studies and expert opinion.

Given the lack of empirical data around the behaviour and fitness of EO-1α Salmon in the natural environment, significant attention to uncertainty considerations in the hazard assessment is required. Uncertainty around the hazard assessment may be significant due to clear knowledge gaps and lack of empirical data regarding the behaviour and effects of EO-1α Salmon in the natural environment.

Table 2.8: Ranking of hazard to the environment resulting from exposure to the organism.

Hazard Ranking	Assessment
Negligible	No effects[1]
Low	No harmful effects[2]
Moderate	Reversible harmful effects
High	Irreversible harmful effects

[1]No biological response expected beyond natural fluctuations (NOTE: this has been clarified from the original 2013 description of "No effects: when no biological responses are expected"). [2]Harmful effect: an immediate or long-term detrimental impact on the structure or function of the ecosystem including biological diversity beyond natural fluctuations.

Fisheries and Oceans Canada's (DFO) Centre of Expertise for Aquatic Biotechnology Regulatory Research has conducted a significant amount of laboratory research on the fitness and behaviour of GE fish to aid in estimating the fitness of GE fish in the natural environment through use and comparison of results of studies conducted in tanks, semi-natural streams, and mesocosms, and many sources of uncertainty have been identified (Devlin et al. 2015). Though research may not be conducted on the organism per se, it has highlighted several broad principles that may be applicable to the organism and that represent potential sources of uncertainty about the extent to which laboratory data can be depended upon as a reliable indicator of how GE fish would behave in the natural environment. These findings are described below:

- The environment in which fish are reared can significantly affect the phenotypic expression of the transgene (e.g., Sundström et al. 2007b). The influence of rearing environment limits our ability to extrapolate laboratory data as a reliable indicator of how a GE fish may behave (e.g., compete, survive) in the natural environment unless it can be demonstrated that non-transgenic controls reared in the laboratory environment behave the same way as non-transgenic fish in the natural environment, and it is demonstrated that there are no GxE interactions between non-transgenic and GE fish, or GxE interactions are well defined. In the absence of such control data, there is uncertainty around the extent to which we can rely upon laboratory data as an accurate indicator of behaviour in the natural environment;
- The gene expression and phenotypic effects of the transgene can vary significantly with the genetic background of the parent (e.g., wild vs. hatchery vs. domesticated species, Devlin et al. 2009; Devlin et al. 2013). For example, the performance of a wild or hatchery 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 (e.g., Leggatt et al. 2017b); and
- A single transgene may result in several or many phenotypic traits, termed pleiotropic effects. For example, some empirical data demonstrates that increased growth due to transgenesis in some fish species may also affect disease resistance (e.g., Jhingan et al. 2003). Thus, unless the investigator has specifically directed attention towards an unintended effect, it may go undetected. It should also be noted that different types of modified genes may have vastly different pleiotropic effects. For example, modification of a

central fitness trait, such as size or growth rate, is expected to have broad pleiotropic and fitness implications for all traits related to growth, whereas the pleotropic implication for more benign traits, such as eye colour or flesh pigmentation, may be limited.

Criteria for the assessment of uncertainty address potential effects to the environment, which may rely heavily on information and data found in published and peer-reviewed scientific literature. A description of rankings for uncertainty regarding the potential hazards of the organism in the environment is provided in Table 2.9. Here, the quality of data refers to the data or information available for each parameter being examined, the integration of this information and breadth of experimental conditions examined, sample size, appropriateness of controls, statistical analysis, as well as the 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 a GE fish in the receiving environment.

Table 2.9: Ranking of uncertainty associated with the environmental hazard.

Uncertainty Ranking[1]	Available Information
Negligible	High quality data on EO-1α Salmon. Demonstration of absence of GxE effects or complete understanding of GxE effects across relevant environmental conditions. Evidence of low variability.
Low	High quality data on relatives of EO-1α Salmon or valid surrogate. Understanding of GxE effects across relevant environmental conditions. Some variability.
Moderate	Limited data on EO-1α Salmon, EO-1α relatives or valid surrogate. Limited understanding of GxE effects across relevant environmental conditions. Knowledge gaps. Reliance on expert opinion.
High	Significant knowledge gaps. Significant reliance on expert opinion.

[1]Uncertainty rating for assessment of NSN-16528 were named High certainty, Reasonable certainty, Reasonable uncertainty, and High uncertainty, but ranking and descriptions were equal.

2.3.1 Potential Hazards Through Environmental Toxicity

For NSN-16528, it was concluded with moderate uncertainty that EO-1α Salmon pose negligible hazards through environmental toxicity. There have been no known scientific studies published since 2013 that alter this conclusion. In brief: EO-1α Salmon could be considered environmentally toxic or “poisonous” if they contained or excreted GH or other molecules in sufficient concentration to cause harm to predators or other organisms that come in contact with EO-1α Salmon. Information about growth hormone (GH) concentration has not been reported throughout its life cycle, with only one study reporting that GH levels remain below a detection limit of 6.24 ng/g in the muscle of commercial size EO-1α Salmon (NSN-16528). Average plasma GH levels in juvenile G0 AAS-relatives were reported to be 39.9 ± 14.8 ng/mL which were not significantly different from non-transgenic siblings (20.5 ± 7.8 to 28.2 ± 8.8 ng/mL depending on size, Du et al. 1992). Plasma GH concentrations in other GH transgenic salmonids can range from 0 to 40-fold higher than non-transgenic counterparts (Du et al. 1992; Devlin et al. 1994; Raven et al. 2008; Higgs et al. 2009; Leggatt et al. 2012). Average levels over 60 ng/mL were detected in an F1 generation of Coho Salmon bearing a growth hormone construct, compared to less than 5 ng/mL in non-transgenic fish (Devlin et al. 2000). In contrast,

only one of seven F3 transgenic Coho Salmon strains examined had significantly higher plasma GH levels than life stage matched non-transgenic fish (average of 13.0 ng/mL compared to 3.2 ng/mL in non-transgenic fish and 3.6 ng/mL in other transgenic strains, Leggatt et al. 2012).

Circulating GH concentration can vary in response to internal and external stimuli and can vary between life stages (Björnsson 1997; Ebbesson et al. 2008). The current characterization of GH levels in EO-1α Salmon is insufficient to determine if GH levels increase above the normal range throughout their lifespan and it remains unknown if predators consuming EO-1α Salmon in the environment would be exposed to higher levels of GH than if they consumed non-transgenic Atlantic Salmon. Among vertebrates, the ability of GH to bind to the growth hormone receptor and induce somatotropic effects is not universal among GH source and recipient treatment organisms (USFDA 2010). 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 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. 2011). While high doses of orally administered GH can elicit a biological response in fish (Duan and Hirano 1991; Moriyama et al. 1993; Moriyama 1995; Xu et al. 2001; Liu et al. 2011), the maximum potential concentration of GH in EO-1α Salmon is unlikely to reach concentrations that are high enough to elicit a biological effect. Consequently, GH levels in EO-1α Salmon represent a negligible hazard to predators or scavengers.

No differences were reported for other measured hormones: insulin-like growth factor 1 (IGF-1), 3,5,3'-triiodothyronine (T3), thyroxine (T4), estradiol, testosterone, and 11-keto-testosterone, in the muscle-skin samples from commercial sized AAS compared to sponsor controls (NSN-16528), although levels of these hormones in other life stages of EO-1α Salmon have not been reported. In other GH transgenic salmonids, several studies have reported up to 4-fold increases in IGF-1 levels (Raven et al. 2008; Devlin et al. 2009; Higgs et al. 2009; Leggatt et al. 2012). IGF-1 has been reported to be more resistant 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.

For thyroid hormones, juvenile G0 AAS-relatives had lower plasma T3 concentrations than non-transgenic siblings, but did not differ from non-related control fish (Du et al. 1992). In contrast, GH transgenic Coho Salmon had 3-fold increase in plasma T3 levels and no difference on T4 levels (Devlin et al. 2000; Eales et al. 2004), although such levels were not enough to elicit an effect in rats and primates (Atterwill et al. 1988). Current evidence does not indicate EO-1α Salmon could harm other species through elevated thyroid hormones.

No studies have examined the relative potential for EO-1α Salmon to bioaccumulate toxicants compared to domesticated or wild conspecifics. There is a positive correlation between waterborne toxicant uptake and oxygen consumption in fish (Rodgers and Beamish 1981; Yang et al. 2000). Oxygen consumption rates in EO-1α Salmon are similar to non-transgenic wild siblings during early life stages (Moreau et al. 2014), and up to 25 per cent higher in adult fish (Deitch et al. 2006). Larger differences in oxygen consumption have been reported in AAS-relative fry, reaching 1.70-fold increase while feeding, and 2.30-fold increase after 24 hours starvation (Cook et al. 2000c). The potential increased oxygen consumption in EO-1α Salmon at some life stages could lead to an increased uptake and subsequently to higher concentration factors of waterborne contaminants compared to wild conspecifics. In a study that directly compared contaminant levels in wild and farmed Atlantic Salmon (in Norway, Lundebye et al. 2017), domesticated salmon from aquaculture sites had lower contaminant levels than wild salmon in nature, and escaped domesticated Atlantic Salmon generally had high (e.g., similar to

wild) or intermediate levels of contaminants, although all levels were well below maximum allowable levels. This suggests EO-1α domesticated salmon may not have higher contaminant levels, but the effect of GH over-expression and interactions with genotype and environment have not been examined. Whether EO-1α Salmon could bioaccumulate greater than non-transgenic fish to a level of harm to other species could be context specific, i.e., influenced by the status of the predator population as well as on the mode of action, effect, and concentration of the contaminants in the natural environment. However, current evidence does not demonstrate a situation where harm from bioaccumulation in EO-1α Salmon would be greater than non-transgenic fish.

No toxicological concerns are associated with the triploidy induction processes used in the production of EO-1α Salmon. Sex-reversal through 17α-MT exposure increases whole body levels of methyltestosterone in treated fish, which could potentially impact predator fish if consumed in significant quantities. Experiments in other fish models demonstrate that increase in 17α-MT in treated fish is transient and exogenous methyltestosterone is removed by 10 days post-treatment (Fagerlund and Dye 1979; Johnstone et al. 1983; Cravedi et al. 1989; Curtis et al. 1991). As such, any potential hazards to predators of escaped treated fish would be over an extremely limited time frame to a limited number of fish.

Overall, EO-1α Salmon is expected to pose **negligible** hazards through environmental toxicity. The limited data on full life cycle assessments (e.g., GH and other hormone levels), and reliance on indirect data (e.g., bioaccumulation) results in a **moderate** uncertainty level associated with this rating.

2.3.2 Potential Hazards Through Horizontal Gene Transfer

For NSN-16528, it was concluded with moderate uncertainty that EO-1α Salmon pose negligible hazards through Horizontal Gene Transfer (HGT). There have been no known relevant scientific studies published since the previous assessment and, consequently, the rating remains low with moderate uncertainty. In brief: 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 (EFSA 2013). Gene transfers in eukaryotes primarily involve transposable elements (i.e., DNA that is able to move from one locus in the genome to another, see Peccoud et al. 2017). In order for HGT of a specified transgene to take place on a biologically relevant scale and result in hazardous effects, 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, neutral or positive selection of the novel organism expressing the transferred gene (DFO 2006), and finally harmful effects to the environment as a result of expression of the transferred gene in the novel organism. 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 focuses on harm from HGT of the EO-1α transgene to prokaryotes.

Pathways of exposure of free transgenic DNA to novel organisms include exposure within the EO-1α Salmon's gut, or through feces, mucus, and other waste sloughed off by the fish into the water. Bacteria could be exposed to free DNA sloughed from escaped fish, but also from DNA released in wastewater, in solid waste used as fertilizer (NSN-19702), and from carcasses (including for those in the commercial market) disposed of in landfill. Due to the nature of free DNA to rapidly degrade, particularly in water (e.g., Turner et al. 2015), exposure of Canadian environments to free DNA from EO-1α Salmon is expected to be low. As well, all pathways of exposure are not expected to differ from that of native Atlantic Salmon genes, and marine exposure is expected to be less than domestic Atlantic Salmon reared in net-pens. In terms of

uptake, the EO-1α construct 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 the EO-1α construct as being third least likely to have increased mobility. As such, the EO-1α construct is not expected to have increased uptake relative to native Atlantic Salmon genes. The EO-1α construct 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. In order for the transgene to be expressed resulting in phenotypic change, it requires co-transfer of regulatory elements. The close proximity of the Ocean Pout antifreeze promoter to the GH gene could increase the likelihood of them being co-transferred and expressed. However, vertebrate promoters commonly used in transgenesis have low activity in prokaryotic hosts (DFO 2006), although this has not been directly addressed for the AFP promoter. Expression of piscine GH could potentially occur if coding regions of the transgene were inserted next to bacterial promoters (e.g., Burgess et al. 1993), although this is not expected to have higher probability than native Atlantic Salmon genes. Should all of the above steps occur, neutral or positive selection for the organisms with the novel phenotype would then have to occur for the mobile transgene to persist in the population. The potential for this to occur has not been explored, nor has the potential hazards that could occur from HGT and expression of the transgene, but would not be expected to be different than other native piscine growth hormone genes. While the introduction of the EO-1α construct to novel organisms in Canadian environments through HGT cannot be excluded, the potential for such an introduction and consequent hazards are not expected to be different than for growth hormone genes from native fish species in Canada.

Consequently, the potential for hazards through HGT is **negligible**. While the transgene is well defined, this hazard has a **moderate** uncertainty rating due to limited knowledge of the transgene location within the salmon genome, and lack of studies examining HGT of the transgene and resulting consequences.

2.3.3 Potential Hazards Through Trophic Interactions with Other Organisms

The assessment for NSN-16528 was structured to focus on potential to affect wild populations of Atlantic Salmon (through hybridization and competition, and affecting populations of wild Atlantic Salmon prey, predators, and competitors). The current assessment is structured to assess impacts through specific pathways (i.e., Section 2.3.3. trophic – competition, predation, as prey, and Section 2.3.4 hybridization), and while these sections focus on wild Atlantic Salmon populations they also consider harm to other wild populations. In addition, there have been new relevant studies on EO-1α Salmon, as well as comparator species (other GH transgenic fish species, domesticated Atlantic Salmon) that have resulted in some minor shifts in hazard and uncertainty ratings of Sections 2.3.3 and 2.3.4 from the NSN-16528 assessment.

2.3.3.1 Through Competition

Escaped EO-1α Salmon could impact native species through competition with any organism occupying similar niches, most notably wild Atlantic Salmon populations. The potential hazard of EO-1α Salmon to wild populations of Atlantic Salmon (or other competitors) is strongly associated with the relative fitness of the two genotypes in nature (see Devlin 2011). Relevant phenotypes include competitive, predatory, reproductive (see Section 2.3.4), and migratory behaviours of EO-1α Salmon, as well as its potential to act as a vector for pathogens/parasites (see Section 2.3.5). 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. Many Atlantic Salmon populations in Canada are currently in decline, and the Rollo Bay facility is within migration

distance (i.e., unobstructed routes within migration distance variation of domestic fish) of many COSEWIC-listed populations of Atlantic Salmon (see Section 1.7). Additional factors that must be taken into consideration are the effects of domestication, sex reversal, and triploidy of EO-1α groups. Also, fitness traits in both EO-1α Salmon and wild conspecifics are expected to be affected by the rearing environment and experimental conditions (see Devlin et al. 2015), and potential GxE effects should be considered. 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 EO-1α Salmon, several of their conclusions do. Research on other GH transgenic salmonids provides evidence that resource levels, background genetics, rearing conditions, life stages, and predation levels have critical effects on the ecological consequences of transgenic fish in the environment (e.g., Devlin et al. 2004b; Sundström et al. 2009; Sundström and Devlin 2011; Leggatt et al. 2014; Vandersteen et al. 2019, see Devlin et al. 2015).

2.3.3.1.1 Through competition in freshwater

The freshwater system EO-1α Salmon could potentially escape to (Rollo Bay brook) does not contain wild populations of Atlantic Salmon. Therefore, in order for EO-1α escapes to impact wild populations of Atlantic Salmon through freshwater competition, EO-1α escapes would need to survive freshwater and marine environments, migrate to river systems containing wild Atlantic Salmon populations, and either reproduce successfully in these systems (see Sections 2.2.2 and 2.3.4.1), and/or compete as fish returned to freshwater. Reproduction would be restricted to 2n female EO-1α Salmon (EO-1α homozygous broodstock or failed 3n heterozygous AAS Salmon) in the first generation, which could potentially reproduce with wild males or any St. John River broodstock that co-escaped and migrated with female EO-1α Salmon. While there is much uncertainty regarding the level of success that EO-1α Salmon may have in natural systems, there is no evidence to date that demonstrates they would not be capable of survival, migration, and reproduction in the wild.

The competitive success of EO-1α juveniles relative to wild type is not extensively studied. In a study of first-feeding fry, EO-1α fish were equally likely to be as dominant as non-transgenic fish in paired competition, and prior residency was more important to determine success in competition (Moreau et al. 2011a). As well, transgenic and non-transgenic fry did not differ in survival or growth at different densities in stream microcosms (Moreau et al. 2011a). Consequently, EO-1α fry are not expected to impact wild populations through competition at this early life stage unless resources are limiting (see below), particularly as EO-1α life history timing at this life stage (Moreau et al. 2014) does not indicate they would start feeding and set up territories much earlier than non-transgenic fish, unless they were from an earlier spawning event. Fitness differences between genotypes at this life stage are also minimal and less important than family differences (Moreau et al. 2014).

At later life stages, phenotypic effects, both targeted (fast growth) and off-target (e.g., behavioural changes), may be more evident with increased potential for EO-1α juveniles to impact wild populations through competition. Competition at life stages later than first-feeding has not been determined in EO-1α Salmon or relatives although AAS-relatives had greater foraging activity than non-transgenic Atlantic Salmon juveniles (Abrahams and Sutterlin 1999). In other models, GH transgenic fish have greater competitive ability in culture conditions (Devlin et al. 1999; Duan et al. 2009; Duan et al. 2011), and as with AAS-relatives have diminished predator avoidance behaviour (Abrahams and Sutterlin 1999; Sundström et al. 2004b; Zhang et al. 2014). However, in Coho Salmon diminished predator avoidance of transgenic fish was not observed when compared with hatchery fish that had been reared in simulated streams from first feed (Sundström et al. 2016), indicating the importance of culture effects of both transgenic and non-transgenic fish on behavioural differences. GH treated salmonids also have decreased

antipredator response (Johnsson et al. 1996; Jönsson et al. 1998b) and aggression (Neregard et al. 2008), but this does not necessarily translate to altered success in competition (Neregard et al. 2008) or altered survival in natural systems with predation (Johnsson et al. 1999), suggesting, at least in these models, altered behaviour does not have large impacts on success in nature. GH treatment has also been reported to increase growth rate at the expense of maintenance (i.e., lipid and energy reserves) in natural streams (Johnsson et al. 1999; Johnsson et al. 2000), suggesting GH enhanced fish may have lower success in times of limited resources.

The reported influence of GH transgenesis on potential to impact wild populations through competition in other models at the juvenile stage varies widely, including population crashes and cannibalization of wild juveniles by transgenic fish in simple environments with limited resources (Devlin et al. 2004b), to non-transgenic juveniles having several fold greater survival in semi-natural streams with varying levels of natural food items in the presence of predators (e.g., Sundström et al. 2004b; Sundström et al. 2005; Crossin et al. 2015). However, determining relative juvenile success of transgenic versus wild juveniles in semi-natural conditions does not necessarily follow predictable patterns in other models, where transgenic survival can be greater under predation under some circumstances (e.g., Sundström and Devlin 2011; Leggatt et al. 2016; Leggatt et al. 2017b), and relative survival and growth can be greater than, equal to, or lesser than non-transgenic fish, and is influenced by level and type of food, type and timing of predator entry, timing of simulated escape, habitat complexity, density, and presence of additional competitors (see Vandersteen et al. 2019, Crossin et al. 2015). Based on data from other GH-enhanced fish, there may be situations where EO-1α juveniles could significantly outcompete wild fish, resulting in decreased survival and/or growth and consequently decreased productivity of local wild fish populations. In situations where EO-1α juveniles may compete equally with wild fish and resources are limiting, the additional competition from EO-1α Salmon also has potential to decrease productivity of local wild fish populations (as observed in domestic salmonids, e.g., Berg and Jorgensen 1991; McGinnity et al. 1997; Fleming et al. 2000). The increased intrinsic growth rate of EO-1α Salmon may allow EO-1α juveniles to gain a size and possible competitive advantage over wild juveniles under some conditions, such as in areas where resources are not limiting, and/or if EO-1α smolts migrate at a larger size but similar time as wild fish (as observed in GH transgenic Coho Salmon, Sundström et al. 2010). If EO-1α juveniles are able to obtain larger size relative to wild fish, this may give them a competitive advantage (e.g., Johnsson and Björnsson 2001; Blann and Healey 2006) and result in decreased wild juvenile productivity, although size does not always influence competition outcomes (see Jonsson and Jonsson 2006). Should EO-1α Salmon become established in these areas, there is potential for continuing harm to local wild fish productivity. Where local populations are already endangered (see COSEWIC 2010), decreased productivity may result in those populations being non-viable (i.e., extirpated). However, in situations where transgenic fish are inferior competitors, or similar competitors but resources are not limiting, impacts to wild fish populations through competition under these specific conditions may be minimal.

Rollo Bay stream, which is connected to Rollo Bay brook contains a population of Brook Trout (*Salvelinus fontinalis*) that could potentially be directly impacted through trophic interactions with escapes of EO-1α Salmon. As well, other species of fish that may occupy similar niches as EO-1α Salmon may be impacted by established populations from EO-1α Salmon escape outside of Rollo Bay brook. Effects of EO-1α Salmon through interspecific competition in freshwater are expected to depend on habitat use and competition level relative to non-Atlantic Salmon species. In Eastern Canada, the pools in salmon rivers are often shared by juvenile Atlantic Salmon, other salmonids (e.g., Brook Trout) and non-salmonid species such as Catostomidae (White Suckers) or cyprinids (minnows). If, in the wild, EO-1α Salmon were to prefer or use a different current velocity than wild Atlantic Salmon, or were more active foragers in later life

stages than wild Atlantic Salmon (see Section 2.3.3.2), this may result in EO-1α Salmon competing with other species for resources and habitat that don't normally interact with wild Atlantic Salmon. In another model, GH transgenic Coho Salmon impacted other species of salmonids greater than non-transgenic siblings in simulated stream tanks only if the transgenic salmon were allowed to grow larger under hatchery conditions before release (Sundström et al. 2014), suggesting that impacts through interspecific competition may occur only where EO-1α Salmon are able to realize increased growth or have larger size than competing wild fish. However, information regarding the habitat preference and interspecific competition of EO-1α Salmon relative to wild Atlantic Salmon is not available and any predictions regarding its preferred niche would be highly speculative.

2.3.3.1.2 Through competition in marine environments

The potential for EO-1α Salmon to impact wild fish populations through competition in the marine environment has not been assessed. Behaviour of Atlantic Salmon in the marine environment is pelagic and generally migratory, which contrasts to freshwater where juveniles often set up and defend territories (see COSEWIC 2010). Consequently, competition success in the marine environment is more likely dictated by locomotory constraints or advantages (see Domenici et al. 2007). Culture-reared marine-stage EO-1α Salmon have increased heart size and cardiac output, but decreased maximum swim speed, metabolic scope, and maximum obtainable oxygen uptake compared to size-matched non-transgenic domestic fish (Deitch et al. 2006). This indicates that the presence of the EO-1α transgene would impair the ability of escaped EO-1α Salmon to compete for prey in situations that required maximum swimming and metabolic capacity. This concurs with lower maximum swimming speed and/or increased cost of transport in other models (Coho Salmon, Common Carp, AAS-relatives, Farrell et al. 1997; Stevens et al. 1998; Lee et al. 2003; Li et al. 2007; Leggatt et al. 2017a), suggesting impaired swimming performance is a common feature associated with GH transgenesis, although this was not observed in a tilapia model (McKenzie et al. 2003). GH transgenic Coho Salmon maintain overall faster swim speeds during rearing than hatchery non-transgenic fish while reared in seawater mesocosms, and spend more time actively foraging for food (Hollo et al. 2017). If EO-1α Salmon also have higher foraging rates in the marine environment this may improve their competitive success, although this has not been examined. In a different fast-growing Atlantic Salmon model, domestic fish released into rivers in Norway had slightly greater growth than wild fish over first sea-winter but lower recapture rates (Jonsson and Jonsson 2017), suggesting increased competitive advantage from fast growth may be counteracted by poor survival.

Marine survival of Canadian Atlantic Salmon populations is very low in specific areas of their distribution, the mechanisms of which are not known but are suspected to be related to climate change-driven shifts in marine ecosystems. Additional stress from competition with escaped or naturalized EO-1α Salmon at this life stage may further threaten wild populations. However, the current evidence indicates poor swim and metabolic capacity may limit EO-1α Salmon competitive ability in the marine environment, and there is no evidence for density-dependent marine survival of wild Atlantic Salmon (see Chaput 2012). Consequently, competition in the marine environment is not expected to be as important a pathway to harm as freshwater competition.

2.3.3.1.3 Conclusion: Potential hazards through trophic competition

Given the potential for competition in variable habitats (i.e., there are 25 river systems that support wild Atlantic Salmon in PEI alone, that differ in flow rate, drainage size, habitat restoration level, invasive species, etc., Cairns and MacFarlane 2015; DFO 2018, Figure 1.5), and the many wild Atlantic Salmon populations in nearby rivers of New Brunswick and Nova

Scotia, isolated conditions may exists where EO-1α Salmon can gain an advantage, be neutral, or be disadvantaged relative to wild Atlantic Salmon or other competitors. The potential for high impacts should transgenic fish establish and outcompete at-risk populations of Atlantic Salmon results in a **high hazard rating to wild fish populations from competition with EO-1α Salmon**. It is important to note that hazards of EO-1α Salmon through this pathway are expected to be very context specific, and may be negligible under one set of conditions and high under a different set of conditions. **There is moderate uncertainty with this rating**, due to limited data specific to EO-1α juveniles, on competition of marine-stage EO-1α Salmon and factors influencing marine survival of wild Atlantic Salmon populations, and limited ability to define GxE in surrogate organisms, although the presence of significant GxE is well defined in other models including non-transgenic Atlantic Salmon. This concurs with high hazard with moderate uncertainty to wild Atlantic Salmon populations from juvenile competition with EO-1α Salmon assessed in NSN-16528.

2.3.3.2 Through Predation on Other Species

In the natural environment, EO-1α Salmon are expected to have similar or increased feeding motivation relative to wild Atlantic Salmon (see Section 2.3.3.1). In the absence of predators this may result in increased foraging pressure on prey populations, although as discussed in Section 2.3.3.1, this may be very context specific. The predation ability of EO-1α Salmon has not been specifically examined, but as with competition ability is expected to be influenced by abundance of prey items, presence of predators and competitors, timing and number of escapes, swimming ability, etc.

Atlantic Salmon are known as opportunistic feeders, 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). If EO-1α Salmon prey on similar species as wild Atlantic Salmon, this would include aquatic invertebrates in freshwater and early marine stages, and small fish species in later marine stages including COSEWIC-listed Gadids, and important fisheries stocks with declining populations (e.g., herring and capelin, COSEWIC 2010).

Non-transgenic Atlantic Salmon are expected to decrease GH expression and consequent feeding motivation in the winter (Björnsson 1997; Lõhmus et al. 2008), while EO-1α Salmon are expected to maintain year-round steady expression of GH (Fletcher et al. 1985). This could result in increased feeding motivation particularly in the winter (as observed in GH Coho Salmon, Lõhmus et al. 2008), and increased prey consumption relative to wild Atlantic Salmon. As well, this may result in adult EO-1α Salmon continuing to feed while migrating upriver to spawn, or as kelts returning to the ocean, behaviour that is rarely observed in wild Atlantic Salmon (G. Chaput, personal communication). This could result in EO-1α anadromous adults consuming larger and different prey species than wild Atlantic Salmon in freshwater. Conversely, decreased swimming efficiency and maximum sustainable swim speed (see Section 2.3.3.1.2) may decrease the ability of EO-1α Salmon to capture prey, particularly in marine environments.

In GH transgenic Coho Salmon, transgenic fish consumed several fold more prey than non-transgenic hatchery salmon but only if they had been ration-restricted prior to release to semi-natural environments (Sundström et al. 2009). As well, prey were less visible in the presence of transgenic versus non-transgenic hatchery predators (Sundström et al. 2009) suggesting presence of transgenic fish may modify behaviour of prey. GH transgenic Coho Salmon under limiting food and simple habitat structure have also been observed cannibalizing conspecifics (Devlin et al. 2004b), suggesting EO-1α juveniles may also prey on wild Atlantic Salmon juveniles should similar behaviour be present in EO-1α Salmon, although this may not be present in more complex environments (see Devlin et al. 2015). GH transgenic Coho Salmon

attack edible and inedible prey more quickly and were more likely to repeat attack prey items than wild type (Sundström et al. 2004a) suggesting potential for increased predation pressure on typical prey species as well as novel prey species. GH transgenic Coho Salmon fry have demonstrated a tendency toward greater dispersal than non-transgenic conspecifics, are more likely to explore previously unused habitats (Sundström et al. 2007a), and undergo smolt migration at a similar time but larger size than non-transgenic siblings (Sundström et al. 2010). Differences in dispersal and migration patterns, if present in EO-1α Salmon, could expand prey species to those in different habitats or larger sizes than normally consumed by wild Atlantic Salmon. In Common Carp, predation by GH transgenic or non-transgenic carp had similar effects on overall prey numbers, but different prey preferences resulted in different effects on prey community composition (Zhu et al. 2017). These studies suggest EO-1α Salmon may exert predation pressures on prey populations differently than non-transgenic Atlantic Salmon, potentially resulting in altered prey population community dynamics and ecosystem processes. However, whether altered preferences for prey are present in EO-1α Salmon, and whether this would translate to alterations in prey communities in natural ecosystems is not known.

Considering all of the above, the assessment concludes that the **overall potential for EO-1α Salmon to affect the prey species is moderate with moderate uncertainty**. The level of uncertainty is due to limited studies on foraging of EO-1α Salmon and AAS-relatives, limited studies across relevant environments in other models, and limited understanding of relevance of other models to EO-1α Salmon. The level of uncertainty has dropped from high in assessment for NSN-16528 due to increased scientific studies of trophic interactions in surrogate models (i.e., other GH transgenic fish).

2.3.3.3 As Prey to Other Species

EO-1α Salmon are expected to occupy the same habitat as wild Atlantic Salmon and be consumed by the same predators. The impact of EO-1α Salmon on these predators will depend on the predator avoidance behaviour of EO-1α Salmon, the toxicity, allergenicity, and nutrition value of EO-1α Salmon, and its capacity to act as a vector for pathogens and parasites in nature.

The predator avoidance behaviour of EO-1α Salmon 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 and GH treated salmonids under many conditions (see Section 2.3.3.1.1). Studies assessing the mortality of GH transgenic salmonids relative to non-transgenic fish due to predation provide inconsistent results (see Vandersteen et al. 2019), and, as with competition success, relative potential of EO-1α Salmon to be prey could be either greater or lesser than non-transgenic fish depending on environmental conditions.

The consumption of EO-1α Salmon with potentially greater levels of plasma GH, IGF-1, and T3, as well as transient 17α-MT levels in sex-reversed fish, is not expected to be hazardous to predators (see Section 2.3.1). The nutritional composition of Atlantic Salmon varies with life stage, size, and the quality and quantity of food that it consumes (Reinitz 1983; Shearer 1994; Anderson et al. 1996). The company 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). The higher fat content of AAS-relatives to control fish was more pronounced in the spring when control fish had depleted reserves (NSN-16528). Whether EO-1α Salmon including AAS differ from non-transgenic fish in body composition during other life stages, or under different environmental conditions or diets, is not known. Higgs et al. (2009) found that the body composition of GH transgenic Coho Salmon differed from non-transgenic hatchery controls in response to diets of low lipid or low protein content, but in most cases differences between genotypes were less than 10%. As well, the CFIA under the Feeds Act, has

determined that the EO-1α Salmon is safe to be consumed by livestock animals when mixed as a feed ingredient. Consequently effects on individual predators from consumption of EO-1α Salmon are not expected to be significantly different than for consumption of wild fish or escaped domestic fish. Consequently, there is a **negligible hazard to wild fish through predation on EO-1α Salmon. There is a moderate level of uncertainty** to this due to limited information regarding hormone concentrations, toxicity, and the nutritional value of EO-1α Salmon throughout its life cycle. The assessment of NSN-16528 stated a high level of uncertainty for low hazard through this pathway. This has changed in part due to clarification of the "Negligible" classification (i.e., addition of "beyond natural fluctuations" to the description "no biological response"), the presence of limited data on EO-1α Salmon and surrogates, and also to align with assessment of hazards through environmental toxicity as this is expected to be the main pathway to harm for predators.

2.3.3.4 Impact of Domestication, Triploidy, and Sex Reversal on Potential Hazards Through Trophic Interactions

For studies on EO-1α Salmon and other GH transgenic fish listed in Sections 2.3.3.1 through 2.3.3.3, GH transgenic fish are generally compared to sibling or relative fish without the transgene. An important difference to note between EO-1α Salmon and some other GH transgenic fish models discussed above is that EO-1α Salmon have a domestic background while GH transgenic Coho Salmon and Rainbow Trout models are in a hatchery or wild background. Domestication can have similar effects as GH transgenesis; for example increased competition and aggression in domestic Atlantic Salmon parr has been reported in some cases although, as with EO-1α Salmon, prior residence has a strong effect on competition success (see Jonsson and Jonsson 2006). Consequently, the effects of domestication may also contribute to divergent trophic behaviour in EO-1α Salmon relative to wild Atlantic Salmon and may obscure the effects that can be confidently attributed to the transgene. There are few studies directly comparing effects of GH transgenesis versus domestication. GH transgenesis and domestication have similar effects on overall gene expression in salmonids, suggesting they act through similar, but not identical, pathways (Devlin et al. 2009; Devlin et al. 2013). In GH transgenic Coho Salmon, transgenic fry are much more similar in phenotype to hatchery-strain fish when reared in simulated stream tanks compared to rearing in culture tanks (Sundström et al. 2007b), while domestic-strain Coho Salmon were better able to maintain high growth and had higher survival in stream tanks than GH transgenic and hatchery-strain fry in the presence or absence of predators (Leggatt et al. 2016). In Rainbow Trout, hybridization of GH transgenic with a domestic line did not alter juvenile survival or growth in stream mesocosms with or without predators (Crossin et al. 2015). The conflicting results could be due to species or environmental differences, level of domestication, and differing interactions with the transgene and genetic background. While domestication and GH transgenesis can bring about similar off-target phenotypes, the differences between EO-1α Salmon and domestic non-transgenic siblings reported in the literature and two notifications do indicate that the presence of the transgene causes effects above those of domestication.

Triploidy of the AAS-form of EO-1α Salmon is expected to decrease or have no effect on hazards to wild populations through trophic interactions. Though the potential effects of triploidy on the competitive ability in AAS have not been assessed, 3n AAS grow at a slower rate than 2n EO-1α Salmon (Tibbetts et al. 2013), and both triploidy and EO-1α transgenesis diminish the ability of Atlantic Salmon to maintain homeostasis during stress (Cnaani et al. 2013), indicating a possible decrease in overall performance from triploidy in AAS. Triploid fish in other salmonid models demonstrate equal or lower competitive ability and aggression relative to 2n counterparts (O'Keefe and Benfey 1997; Fraser et al. 2012). Preston et al. (2014) reported that while 2n Brown Trout were more aggressive than 3n trout in experimental streams, 3n fish

adopted a sneak feeding strategy and spent less time defending a territory, and therefore decreased aggression may not equate to decreased impact. Kozfkay et al. (2006) found stocked 3n trout had decreased survival in systems with low productivity, while 3n Atlantic Salmon had higher feeding rates at low temperatures, but equal at moderate to high temperatures (Sambraus et al. 2018). These latter studies indicate the effect of triploidy on trophic interactions may be context specific and depend on factors such as temperature and level of competition. Triploid Atlantic Salmon are less likely to migrate to freshwater (Glover et al. 2016) and would consequently decrease potential for trophic interactions in this environment post-smolt.

As the proposed location of manufacture and growth of AAS requires successful survival and reproduction of escaped fish to impact through juvenile competition in freshwater (see Section 2.3.3.1.1), the sterile nature of the AAS form of EO-1α Salmon would prevent the potential for harm through this trophic interaction.

Under some circumstances, 3n female fish do not experience the decreased growth rates and increased mortality associated with spawning 2n fish (Sumpter et al. 1991; Sheehan et al. 1999; Teuscher et al. 2003; Poontawee et al. 2007). It follows that 3n female AAS could theoretically obtain a larger size than their 2n counterparts, and could potentially become better competitors. However, there is only one report (accessed February 2020) of escaped aquaculture 3n female Rainbow Trout obtaining unusually large sizes in natural environments, and there are no known reports of unusually large 3n salmonids from extensive stocking programs for recreational fisheries. Consequently, the potential for 3n female AAS reared in nature to reach a size larger than the maximal obtained by wild Atlantic Salmon is unknown but may be unlikely under many conditions.

Overall, the 3n state of AAS form of EO-1α Salmon is expected to prevent (i.e., through juvenile competition), diminish, or have no effect on the potential hazards of EO-1α Salmon through trophic interactions. However, there may be some circumstances (e.g., in cooler water temperatures, or if 3n fish are able to gain a size advantage) where triploidy could potentially increase harm through trophic interactions. The sterilization of 3n fish will prevent establishment of the AAS form of escaped fish, and will decrease overall potential for long-term harm caused by discrete release of AAS, even if circumstances temporarily favour 3n fish.

There is also no information available regarding the influence of sex-reversal on potential trophic interactions of fish. However, based on reports from the company, neomales are expected to be functionally sterile and would therefore diminish or prevent potential harm through trophic interactions dependent on reproduction (e.g., freshwater juvenile competition, see Section 2.3.3.1.1), or long-term harm dependent on establishment of EO-1α Salmon in nature. However, Lee et al. (2004) have suggested that functional sterility is not an absolute state for neomale Atlantic Salmon.

2.3.3.5 Conclusions on Hazards of EO-1α Salmon Through Trophic Interactions

Trophic interactions with EO-1α Salmon have highest potential to harm wild native populations through freshwater competition (high hazard rating), although this would be context specific and primarily from 2n EO-1α Salmon that had escaped and naturally reproduced in Canadian waters. Potential for harm through other trophic interactions range from moderate (through predation) to negligible (as prey), and all hazard ratings through trophic interactions have moderate uncertainty. As hazards through different trophic interactions are expected to be mainly independent from one another, the highest rating is used. Consequently, **the potential for EO-1α Salmon to impact wild populations through trophic interactions is ranked high, with moderate uncertainty.** The moderate uncertainty level is due to limited direct studies on trophic impacts of EO-1α Salmon and their relatives, limited studies across relevant

environments in other models, limited understanding of identified GxE in other models, and limited understanding of relevance of other models to EO-1α Salmon.

2.3.4 Potential Hazards Through Hybridization

2.3.4.1 Potential Hazards Through Hybridization with Atlantic Salmon

Of the three forms of EO-1α Salmon, only 2n EO-1α females (either broodstock or failed 3n AAS) could have potential to successfully reproduce with wild Atlantic Salmon, resulting in introgression of the transgene and domesticated background into the wild population. Triploid AAS are sterile, and while studies in domestic Atlantic Salmon suggests a small percentage may return to freshwater (Cotter et al. 2000; Wilkins et al. 2001), female 3n Atlantic Salmon do not return to spawning grounds (Glover et al. 2016) and are, therefore, not expected to interfere with wild spawning events. Neomale EO-1α broodstock are reported to have incomplete sperm ducts (NSN-19702) and would not be expected to successfully reproduce with wild stocks. Should neomales be present during wild spawning events, they could interfere with wild spawning if they successfully compete for wild female mates but fail to fertilize eggs during spawning. This could potentially decrease wild population productivity. However, whether neomales would have the drive to compete for mates in a natural setting is not known. As well, EO-1α anadromous males reared in culture have greatly diminished spawning success in competition with wild anadromous males for wild females (Moreau et al. 2011b), and the neomale state is expected to further diminish spawning success. Moreau et al. (2011b) did not determine whether diminished spawning in EO-1α males was due to the genetic structure of the fish, culture rearing, or a combination of both. Whether neomales that escaped early in their life stage, thereby decreasing culture effect, would be at a disadvantage in spawning competition is not known, although in other fast growing models (domestic Atlantic Salmon, GH transgenic Coho Salmon), male fish are much less successful than female fish in spawning with wild populations (Fleming et al. 1996; Fleming et al. 2000; Leggatt et al. 2014; Glover et al. 2017), and interference from EO-1α neomales is expected to be a less important pathway to harm than from EO-1α fertile females. It is worth noting up to 92% of neomales with complete sperm duct formation has been noted in other Atlantic Salmon (Lee et al. 2004), suggesting functional sterility is not an absolute state for neomale Atlantic Salmon, although whether sperm duct development may be influenced by other conditions later in life (i.e., natural rearing after escape of neomales) has not been examined.

There are no wild Atlantic Salmon spawning sites adjacent to the Rollo Bay facilities. Consequently, for escaped female EO-1α 2n Salmon to impact wild Atlantic Salmon populations through introgression they would need to survive in the drainage brook, migrate to the Atlantic Ocean, survive the marine life stage, migrate to spawning grounds of wild populations at the same time as wild fish, then successfully reproduce with wild male mates. Survival and migration potential are discussed under Exposure Section 2.2.2, where potential for survival and spawning were ranked high.

In general, salmonids home to the river where they originated. However, straying to other rivers has been reported in Atlantic Salmon populations (e.g., Cauwelier et al. 2018; Ulvan et al. 2018), and in domestic Atlantic Salmon in Norway straying was extremely high (88% stray rate, Jonsson and Jonsson 2017), although this may be an extreme case and homing has been observed in the domestic Atlantic Salmon (e.g., Fleming et al. 2000; McGinnity et al. 2004). Consequently, potential straying of EO-1α females to areas where wild populations spawn must be considered. The potential effect of the GH transgene on straying and migration to freshwater has not been examined in EO-1α Salmon or any other model. The mean stray distance of domestic Atlantic Salmon in Norway was >150 km, and was greater than for wild Atlantic Salmon (Jonsson and Jonsson 2017), and stray rates of much farther have been recorded in

Atlantic Salmon (e.g., over 1000 km, Leunda et al. 2013). Should EO-1α females follow a similar pattern, there are several populations of Atlantic Salmon in PEI and Nova Scotia that are less than 150 km from Rollo Bay brook, including populations of special concern (COSEWIC 2010; Cairns and MacFarlane 2015, NSN-19702), and straying of EO-1α Salmon further than 150 km could put them in freshwater contact with populations from NB or QC. While the potential for EO-1α female salmon to migrate to wild spawning grounds is not known, current evidence does not indicate the potential can be excluded.

The potential reproductive behaviour and success of EO-1α female salmon has not been reported. In GH transgenic Coho Salmon, female transgenic fish had diminished spawning success compared to non-transgenic fish when both were reared in small-scale culture facilities (Bessey et al. 2004; Fitzpatrick et al. 2011), but equal reproductive success as non-transgenic fish when both were reared in seawater mesocosms designed to minimize culture effects, though both groups had lower success than wild-reared non-transgenic fish (Leggatt et al. 2014). As well, transgenic females had similar reproductive behaviour as non-transgenic fish reared in equal conditions, but performed fewer digs five minutes before and immediately after spawning (Leggatt et al. 2014), which may impart a survival disadvantage to offspring from transgenic females. In non-salmonid fish models, GH transgenic Common Carp have equal reproductive success as non-transgenic carp (Lian et al. 2013), while GH transgenic Medaka (Komine et al. 2016) and Zebrafish (Figueiredo et al. 2013) both have greatly diminished reproductive capabilities, demonstrating altered reproductive success is not necessarily a function of GH transgenesis but may be strongly affected by species or line. In domestic Atlantic Salmon, introgression with wild populations is primarily through escaped female rather than male fish (see Glover et al. 2017), although there is some evidence that introgression may be driven by precocial maturation of male hybrids (see Sylvester et al. 2019). While lingering effects of culture may diminish escaped EO-1α female reproductive success, other models indicate some successful reproduction with wild male Atlantic Salmon should be expected.

If EO-1α female salmon successfully reproduce in nature, either with wild males or St. John River domestic males that co-escape, mixed sex offspring will be all transgenic if from EO-1α broodstock, or half transgenic if from failed 3n AAS females containing the EO-1α transgene. Non-transgenic offspring would be expected to have similar success and potential for harm as naturalized or hybrid domestic salmon (see Glover et al. 2017 for review), while transgenic offspring would have additional short- and long-term impacts on success and harm from the GH transgene. EO-1α Salmon are reported to have diminished proportion of mature male parr when reared under hatchery conditions, and these parr may have decreased reproductive success relative to equally reared non-transgenic fish (Moreau et al. 2011b; Moreau and Fleming 2012). Individual parr that mature at least a year earlier than anadromous fish 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 2012). Consequently, diminished proportion and spawning success of EO-1α mature male parr may decrease further introgression of the EO-1α transgene into wild populations, although whether the effects on EO-1α mature male parr would be present under natural conditions and food supply is not known. Further introgression via anadromous EO-1α Salmon would be expected to be greater than the released generation, as the effects of culture would not be present. The reproductive success of naturalized EO-1α Salmon with wild salmon over multiple generations has not been assessed, but there is no evidence to date indicating it may not be possible. As well, EO-1α Salmon and other GH transgenic salmonids have reported accelerated smoltification and adult maturation under culture conditions (Devlin et al. 1995; Devlin et al. 2000; Devlin et al. 2004a; Moreau et al. 2011b; Moreau and Fleming 2012; NSN-16528). Such phenotypes could potentially shorten the EO-1α Salmon life cycle should the phenotypes be present in nature, enabling it to reach the adult reproductive stage

faster than wild conspecifics, and increase frequency of reproductive events with wild populations. Whether conditions would be present in the natural environment to allow for accelerated growth and life history of EO-1α Salmon is not known.

The potential for harm to wild populations from introgression with EO-1α Salmon has not been examined. Even in a relatively well studied model (escaped domestic Atlantic Salmon), impacts to wild populations from introgression with escaped fish are not well understood (see Glover et al. 2017), but include potential decreased productively of wild populations from lower fitness and/or competitive interactions, or increased straying of hybrid offspring (Glover et al. 2017; Jonsson and Jonsson 2017; Sylvester et al. 2019), with long-term consequences including alterations in life-history traits (Bolstad et al. 2017), decreased population productivity, and decreased resilience to environmental changes (see Glover et al. 2017). The closest populations to the Rollo Bay site are the Northeast PEI populations that represent a unique ancestral strain not located elsewhere (Moore et al. 2014) and many wild populations of Atlantic Salmon in New Brunswick and Nova Scotia. Introgression of EO-1α and domestic genotypes into these populations would impact genetic biodiversity of the species as a whole. Domestication and GH transgenesis generally have a similar goal of fast growth, and in other species (Rainbow Trout and Coho Salmon) have resulted in similar down-stream effects on expression of multiple genes (Devlin et al. 2009; Devlin et al. 2013), but not overall muscle proteomics (Causey et al. 2019), or juvenile success in semi-natural streams (Leggatt et al. 2016). While the genetic effects of domestication decrease with each generation (Tymchuk et al. 2006), the EO-1α transgene and associated phenotype will be passed down in an “all-or-nothing” manner, and resulting phenotypic changes could remain stable in individuals containing the transgene over multiple generations. Consequently, introgression with EO-1α Salmon may pose unique challenges to wild populations from the EO-1α transgene beyond those from the domestic background.

The potential effects of introgression of a growth hormone transgene into a wild population have been examined in other fish models. Muir and Howard (1999) proposed a “Trojan gene effect” of the GH transgene in a Medaka line. In this line, the transgene conferred an increased reproductive fitness as an adult, but diminished viability as a juvenile, and computer modelling predicted these phenotypes could lead to a crash in wild populations. While potential reproductive success and early survival of EO-1α Salmon in natural conditions is not well predicted, current data from culture and microcosms does not directly demonstrate increased reproductive success or decreased juvenile survival in EO-1α Salmon (see above and Sections 2.2.2 and 2.3.3). In addition, computer models proposed by Ahrens and Devlin (2011) suggest that selection acting on background genetic variation would prevent a Trojan gene effect in most cases.

In the Coho Salmon model, computer modelling and quantitative trait loci (QTL) analysis suggest presence of a GH transgene can result in evolutionary changes in wild populations. Ahrens and Devlin (2011) modeled selection of a transgene in the presence of single or multiple modifier loci (loci that can modify fitness parameter values in both transgenic and non-transgenic genotypes). In this exercise, genetic background had a strong influence on transgene effects, and was found to shift in both non-transgenic and transgenic individuals, theoretically shifting phenotype away from naturally selected optima (Ahrens and Devlin 2011). While this model may not encompass expected phenotypes of EO-1α Salmon, it demonstrates that transgene effects in a wild population may evolve over time, making long-term predictions of potential harm from introgression problematic, but important. Kodama et al. (2018) performed QTL mapping of a GH transgene introgressed through one generation into a hatchery population of Coho Salmon to identify how presence of a transgene might influence selection. This study found that the presence of the transgene altered the genetic basis for growth-related

traits, and the authors postulated the transgene may alter evolutionary rates and/or directions, with resulting ecological consequences, in response to selection in a wild population (Kodama et al. 2018).

Computer modelling of GH transgene impacts in a wild population using relevant phenotypes from EO-1α Salmon has not been performed, nor has the impact of the EO-1α transgene on genotype and phenotype of wild salmon over one or multiple generations. The above studies examine the effect of a transgene in isolation in a Coho hatchery population, while introgression with EO-1α Salmon would also contribute domestic genes from the St. John River background. Introgression of the St. John River domestic background has been predicted to decrease wild populations abundances in Atlantic Salmon in some scenarios (Sylvester et al. 2019). How the GH transgene, domestic genetic background, and wild genetic background may interact through introgression of EO-1α Salmon into a wild population is poorly understood. In Rainbow Trout, GH transgenic fish had similar growth and transcriptome-level gene expression when from a domestic x wild background or a pure wild background (Devlin et al. 2013), suggesting the domestic background of EO-1α Salmon may not have a large impact above that of the GH transgene. The above studies do suggest the presence of a GH transgene can have long-term evolutionary-scale impacts on wild populations. Genetic structure of Atlantic Salmon populations in Canada are demonstrated to be strongly influenced by freshwater habitat, climactic forces and local adaptation (Bradbury et al. 2014; Jeffrey et al. 2018; Sylvester et al. 2019). Introduction of the EO-1α transgene could potentially alter adaptation in discrete populations away from the locally-adapted optima, resulting in decreased productivity and/or resiliency of the wild population over time. Many Atlantic Salmon populations are listed under COSEWIC as special concern, threatened or endangered, and face multiple stressors including one or a combination of changes in marine ecosystems, habitat destruction, illegal fishing, interbreeding with domestic salmon, and invasive species (COSEWIC 2010). These populations are expected to be more sensitive to potential negative effects from introgression (see ICES 2016).

Overall there is a **high** hazard to wild Atlantic Salmon populations through introgression with EO-1α Salmon. There is a **moderate** level of uncertainty regarding this rating due to limited data on reproductive success of EO-1α Salmon, limited data on effects of introgression in comparator species, and limited to no data on potential effects/success over multiple generations in nature. This concurs with the NSN-16528 assessment.

2.3.4.2 Potential Hazards Through Hybridization with Other Species

EO-1α Salmon may impact species related to Atlantic Salmon through interspecific hybridization. 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). Domestication appears to increase incidence of hybridization between Brown Trout and Atlantic Salmon, at least in males, and introgression between the two species has been observed in nature (Castillo et al. 2008). Oke et al. (2013) demonstrated that the opAFP-GHc2 transgene is expressed in hybrids generated from EO-1α Salmon and Brown Trout crosses. In artificial streams, the hybrids (transgenic and non-transgenic combined) appeared to be at a competitive advantage and greatly decreased growth of both non-transgenic and transgenic Atlantic Salmon, although competitive interactions involving pure Brown Trout were not included in the experiment. This study suggests non-transgenic and transgenic offspring of EO-1α Salmon and Brown Trout hybridization could negatively impact wild Atlantic Salmon in the same niches, although competitive differences of non-transgenic versus transgenic hybrids were not examined. Whether transgenic hybrids could pose greater harm than non-transgenic hybrids produced from escaped domestic Atlantic Salmon is not known. Whether EO-1α Salmon and Brown Trout hybrids could further introgress with wild

Atlantic Salmon populations, and subsequent impacts of this, are not known. 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. Overall, the hazard through introgression of EO-1α Salmon genes into other species of fish is considered, with **moderate** uncertainty, to be **moderate**. The moderate level of uncertainty is due to inability to separate potential impacts of EO-1α transgenic versus non-transgenic hybrids, and limited data regarding hazards from interspecific hybridization across relevant environmental conditions. In the assessment for NSN-16528, it was concluded that EO-1α Salmon posed negligible hazard to competitors of Atlantic Salmon, including through hybridization. However, the moderate hazard rating here is due to downstream potential for harm to Atlantic Salmon populations from competition with EO-1α – Brown Trout hybrids, not for harm to Atlantic Salmon competitors from hybridization, and is consequently measuring a different endpoint than the NSN-16528 assessment.

2.3.5 Potential to Act as a Vector of Disease Agents

The previous assessment of NSN-16528 determined there was insufficient data to conclude on the hazard of the notified organism through its ability to act as a vector. However, further information from the notifier, data from other models, and detail information on containment and effluent treatment in the proposed use scenario allow for conclusions in the current assessment.

EO-1α Salmon may act as a vector for pathogens to wild populations should diseased EO-1α Salmon escape from the facility. However, the proposed land-based facility, with 99.7% recirculation and UV and ozone treatment, has significantly lower potential to be a source of pathogens from cultured fish to natural populations that the typical aquaculture rearing in sea pens. Disease risk at the current PEI facility is generally well managed, and updated infrastructure suggests disease risk at the proposed facility will be improved above the current facility. It is therefore unlikely that EO-1α Salmon would introduce new pathogens into the surrounding area in the event that they were to escape from the proposed facility. There does remain the potential for impacts from opportunistic agents initiating an outbreak at an Aqua Bounty facility and the agent then entering the environment via the effluent, where UV treatment is likely far less efficacious on effluent than influent. However, impacts through this pathway are still expected to be lower compared to net-pen operations.

EO-1α Salmon could impact wild populations should escaped fish act as a reservoir in the environment for diseases of significance to wildlife including other fishes. The relative disease susceptibility of EO-1α Salmon has not been formally examined. It is not known to what extent disease resistance has been selected for in the St. John River domestic stock to which EO-1α Salmon neomales are crossed. There is strong evidence that selectively breeding Atlantic Salmon for disease resistance can be highly successful (Kjøglum et al. 2008). Consequently, the disease susceptibility of EO-1α Salmon may not remain constant with subsequent generations, as EO-1α Salmon continue to be selected and crossed with the St. John River domestic strain, which is itself subject to selective breeding. In other models altered resistance to pathogens and impaired immune response is known to occur in GH transgenic Coho Salmon (Jhingan et al. 2003; Kim et al. 2013) and Zebrafish (Batista et al. 2014). Decreased disease resistance coupled with enhanced fitness may heighten the capacity of transgenic salmon to act as a reservoir for the transmission of disease agents to other organisms (Jhingan et al. 2003). However, if EO-1α Salmon were to have increased disease susceptibility and succumb to the disease quickly, then EO-1α Salmon 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 significance of any altered pathogen susceptibility of EO-1α Salmon as an indicator of its ability 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 (e.g., Jhingan et al. 2003; Sundström et al. 2007b). Kim et al. (2013) observed higher susceptibility in two year classes of growth hormone transgenic Coho Salmon challenged with *Aeromonas salmonicida* as compared to non-transgenic hatchery Coho Salmon. Similarly, Jhingan et al. (2003) reported that growth hormone transgenic 2n Coho Salmon smolts displayed higher cumulative mortality when exposed to *Vibrio anguillarum* than did non-transgenic smolts. However, 2n transgenic and non-transgenic Coho fry had approximately equal susceptibility to high doses of *V. anguillarum*, though transgenic 3n fish were more susceptible than non-transgenic 3n fish. In contrast, at a lower pathogen dose, transgenic 2n and 3n 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 3n 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, 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 sex-reversed fish has not been examined.

Given the expected health profiles of any escaped fish and water treatment at the proposed facility, but presence of altered disease susceptibility in surrogate organisms, there is **low hazard** rating for EO-1α Salmon to cause harm as a vector of disease above that of domestic Atlantic Salmon. However, due to the lack of studies directly examining vector capabilities of EO-1α Salmon, the limited understanding of applicability of lower disease resistance in other models, and limited understanding of significance of altered resistance to vector capabilities, there is **high uncertainty** associated with this rating.

2.3.6 Potential to Impact Biogeochemical Cycling

The current assessment corresponds to the NSN-16528 negligible hazard assessment of EO-1α Salmon through impacts to biogeochemical cycling. In brief: 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. Anadromous Atlantic Salmon can influence stream and river nutrient cycles through excretion and gamete production (e.g., Samways and Cunjak 2015), and through spawning mortality (Jonsson and Jonsson 2003). Anadromous Atlantic Salmon are reported to contribute significantly to river ecosystem nutrients at all tropic levels (Jonsson and Jonsson 2003; Williams et al. 2009; Samways et al. 2017; Samways et al. 2018), and poor returns of adult Atlantic Salmon can result in a net export of nutrients from freshwater systems (Nislow et al. 2004). As well, juvenile Atlantic Salmon in streams without parental carcasses have much larger selection intensities acting on them, with consequent increased phenotypic divergence, than those with parental carcass nutrient pulses (Auer et al. 2018).

The potential for EO-1α Salmon to affect river nutrient cycles through migration and spawning has not been investigated. Triploid all-female AAS are not expected to mature, and would likely have a diminished tendency to return to freshwater, as is observed in 3n Atlantic Salmon

(Warrillow et al. 1997; Cotter et al. 2000; Wilkins et al. 2001; Glover et al. 2016). Consequently, the commercial product form of AAS is not expected to import significant quantities of marine nutrients into river systems. There is a potential for escaped EO-1α Salmon to export freshwater nutrients into the marine environment if they remain in the drainage brook for significant time after release. Should EO-1α Salmon significantly impact the density of anadromous wild Atlantic Salmon populations, this could potentially alter the contribution of these populations to river nutrient cycles. As well, should EO-1α anadromous salmon have higher or lower propensity for spawning mortality, this could impact their role in river nutrient cycling, though spawning mortality has not been examined in EO-1α Salmon. However, the role of wild Atlantic Salmon populations on river nutrient cycles in Canada is postulated to be limited due to low numbers of returning Atlantic Salmon (Jardine et al. 2009), and as many post-spawners leave the river before they die. As such, the impact of EO-1α Salmon on river nutrient cycling in Canada is expected to be **negligible**. There is a **moderate** uncertainty associated with this rating due to limited understanding of the role of Atlantic Salmon in nutrient cycling in Canada, and of potential effects of EO-1α Salmon on wild population densities.

2.3.7 Potential to Affect Habitat

The current assessment corresponds to the NSN-16528 low hazard rating with high uncertainty of impacts of EO-1α Salmon to habitat. In brief: 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). 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. 2007b; Gottesfeld et al. 2008). Redd construction and excavation in stream gravel beds by spawning female salmonids, when spawning at high densities, can significantly disturb the streambed (Gottesfeld et al. 2004; Hassan et al. 2008). Redd excavation affects substrate composition (Moore 2006; Gottesfeld et al. 2008), can increase concentration of suspended particulate matter (i.e., turbidity, Moore 2006), increase the interstitial flow within the site (DeVries 2008) and modify pool-riffle characteristics (Field-Dodgson 1987). Reported secondary effects of salmonid redd construction include a decrease in stream macrophyte, algae, and moss biomass, as well as alterations to insect communities (for Pacific salmon, Field-Dodgson 1987; Minakawa and Gara 2003; Moore and Schindler 2008).

The scale of streambed bioturbation during redd construction depends on the species, behaviour, female size, number and density of spawning salmon, and the spatial extent of the spawning beds in the stream (Moore 2006). 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 as those performed by large-sized, high-density Pacific salmon 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.

There is no information on redd building and spawning behaviour of female EO-1α Salmon. Triploid female AAS do not mature and are not expected to contribute to habitat through redd building. Experiments with growth-enhanced transgenic Coho Salmon indicate that GH transgenic females can have a lower rate of redd digging and redd covering than non-transgenic controls in some circumstances (Bessey et al. 2004; Fitzpatrick et al. 2011; Leggatt et al. 2014), suggesting decreased potential for EO-1α Salmon to influence stream habitat structure relative to wild Atlantic Salmon.

Should EO-1α Salmon establish a reproducing population that spawn at a significant density relative to the spawn area, or should escape of EO-1α Salmon result in decreased wild population that significantly contribute to habitat structure, EO-1α Salmon could potentially alter habitat structure on a local level. However, it is unclear whether current population levels of Atlantic Salmon are sufficient to impact local habitat structure, and if they are, whether EO-1α Salmon could establish populations large enough to impact habitat on a local level.

Due to the limited role of Atlantic Salmon in habitat alteration, the potential for a diminished ability to dig redds in 2n EO-1α Salmon, and the lack of redd building in 3n EO-1α Salmon, the assessment concludes with **moderate** uncertainty that the potential hazards of EO-1α Salmon to habitat are **low**. The moderate degree of uncertainty is attributable to limited information on migration and spawning behaviours of adult EO-1α Salmon spawners or surrogates, propensity for spawning, and overall longevity of repeat EO-1α Salmon spawners.

2.3.8 Potential 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 EO-1α Salmon could affect biodiversity include: genetic alteration through introgression and hybridization (moderate to high hazard, Section 2.3.4); competitive exclusion or displacement of other fish species from available habitat; changes in species composition resulting from EO-1α Salmon feeding behaviours (moderate to high hazard, Section 2.3.3); transfer of diseases or parasites (negligible hazard, Section 2.3.5); and significant changes in nutrient cycles (negligible hazard, Section 2.3.6) thereby potentially resulting in altered food-web dynamics and local community biodiversity.

The negligible to low hazard ratings through transfer of disease, nutrient cycles, habitat alterations, and environmental toxicity indicate there is limited to negligible potential for EO-1α Salmon to impact biodiversity through these pathways. However, EO-1α Salmon could impact biodiversity through alterations in competitive and predation ability and preferences. In Section 2.3.3, it was concluded that alterations in appetite, behaviour, potential food preferences, and life history could result in moderate to high hazard to prey species and competitors (primarily Atlantic Salmon) of EO-1α Salmon. Potential endpoints included alterations to individual species productivity, as well as community structure. However, these hazards are expected to be very context specific, and hazard level expected to be influenced by numerous factors including health of affected populations, community structure (i.e., density, presence of predators, availability of prey), life stage of EO-1α escape, etc. EO-1α Salmon also have high hazard to wild Atlantic Salmon populations through intraspecific hybridization (see Section 2.3.4). Computer modelling of the effects of GH transgenic Coho Salmon escapes in the Strait of Georgia, BC demonstrated escaped fish could theoretically impact biomass of different groups when large numbers were released in repeat escape events, and effects depended on predicted diet of escaped fish (Li et al. 2015). Again, the potential for this hazard is expected to be context specific, and the downstream consequences, if any, on biodiversity are not known.

Overall, the potential for EO-1α Salmon to impact prey and competitor community dynamics through altered appetite, behaviour, and possible habitat use at different life stages results in **moderate** hazard of EO-1α Salmon to biodiversity. There is a **high** degree of uncertainty for this rating, as limited indirect data on effects of GH transgenic fish on community dynamics, and, even in well studied domestic Atlantic Salmon, the effects that escaped farmed fish may have on overall community dynamics or ecosystem function are not known (Leggatt et al. 2010). In the previous assessment of NSN-16528, this hazard was not concluded on due to lack of data.

2.3.9 Environmental Hazard Conclusions

The potential level of hazard posed by EO-1α Salmon depends on the pathway to harm examined, and ranges from negligible (through environmental toxicity, horizontal gene transfer, as a vector of disease, and to biogeochemical cycling), low (to habitat), moderate (through interspecific hybridization and to biodiversity), with pathways to harm through trophic interactions and interspecific hybridization having highest potential for hazards (high ranking, see Table 2.10). It is important to note that those hazards ranked moderate to high are expected to be very context specific, where maximum hazards may only be present in specific circumstances. These hazard rankings are likely to be affected by numerous factors including resilience of affected wild populations, community structure at site of interactions (e.g., structure of competitor, predator, and prey populations), and life stage of EO-1α Salmon escape. Uncertainty level is moderate to high for hazard assessments, due to limited data on EO-1α Salmon under a variety of relevant environmental conditions, presence but limited understanding of genotype by environment interactions in surrogate models, and limited understanding of applicability of data from surrogate models to EO-1α Salmon.

The current assessment is well aligned with what was assessed of EO-1α Salmon under NSN-16528. Uncertainty levels have decreased in some hazard assessments due to improved understanding of impacts and/or genotype by environment interactions in EO-1α Salmon and surrogate models. As well, two hazard assessments were concluded on in the current assessment but not the previous one due to new scientific information relevant to the hazards. The assessment of NSN-16528 had a final overall conclusion of High Hazard of EO-1α Salmon to the Canadian environment. However, as discussed in DFO (2018c), it is important to be able to articulate to regulators the rating and uncertainty associated with hazard and exposure assessments, and individual hazard ratings may be considered transient, as they can be context-specific and as uncertainty levels are high enough to warrant future studies in these areas. As well, exposure routes may be different in certain hazards (i.e., HGT could occur through exposure to EO-1α carcasses or waste, while all other hazards require exposure to living EO-1α Salmon), and consequent synthesis of exposure and hazard to conclude on risk will be different for different hazard pathways. Therefore, an overall conclusion on hazard is not made, but rather each hazard will be considered separately for conclusions on risk.

Table 2.10: Environmental hazard ranking of EO-1α Salmon through various pathways for the current assessment, as well as what was assessed for NSN-16528. Bold text in final column indicates where the two assessments differ.

Hazard	Rank	Uncertainty	NSN-16528 Rank/Uncertainty
Through environmental toxicity	Negligible	Moderate	Negligible/Moderate
Through horizontal gene transfer	Negligible	Moderate	Negligible/Moderate
Through trophic interactions	High	Moderate	High/Moderate
Through intraspecific hybridization	High	Moderate	High/Moderate
Through interspecific hybridization	Moderate	Moderate	Moderate/Moderate

Hazard	Rank	Uncertainty	NSN-16528 Rank/Uncertainty
As a vector of disease	Low	High	**Not concluded on**
To biogeochemical cycling	Negligible	Moderate	Negligible/**not concluded on**
To habitat	Low	Moderate	Low/**High**
To biodiversity	Moderate	High	**Not concluded on**

2.4 ASSESSMENT OF RISK

Risk is the likelihood that a harmful effect is realized as a result of exposure to a hazard. The risk assessment incorporates the nature, severity, and reversibility of the harmful effect, the likelihood that the harmful effect is realized, and the uncertainty associated with each conclusion. DFO's science advice to ECCC and HC for a regulatory decision is based on the overall risk of the organism, carried out in the context of the notifier's proposed use scenario, and all other potential use scenarios.

An overall conclusion on Risk is based on the classic paradigm where risk is proportional to Hazard and Exposure:

Risk $\propto$ Exposure $\times$ Hazard

For each endpoint, hazard and exposure are ranked as: negligible, low, moderate, or high, and include an analysis of uncertainty for both. Overall Risk is estimated by plotting Hazard against Exposure, using a matrix or heat map (see Figure 2.1 and 2.2). The matrix cannot be used as a tool for establishing a discreet conclusion or decision on risk, but can be used as a device to facilitate communication and discussion. The uncertainty associated with overall Risk rating is not estimated, rather uncertainty in the hazard and exposure assessments are discussed in the context of a final conclusion on risk.

2.4.1 Environmental Risk Assessment of EO-1α Salmon

Environmental exposure assessment of EO-1α Salmon under the proposed manufacture and grow-out at the Rollo Bay facility concluded with low to moderate uncertainty of negligible to low exposure of EO-1α Salmon to the Canadian environment depending on the use scenario. This assessment is due to the suitable containment of the EO-1α Salmon (i.e., negligible potential for escape, Scenario B), but production of non-transgenic eggs for third parties would increase potential exposure to low (Scenario A). If EO-1α were present in the Canadian environment, the potential for their survival, dispersal, reproduction and establishment cannot be discounted, but is prevented by negligible potential for release. It should be noted that exposure to free DNA from the EO-1α Salmon is possible through release of waste and/or carcasses from the Rollo Bay facility, during processing and marketing, and through use by Canadian consumers. However, due to the nature of free DNA to rapidly degrade, particularly in water (e.g., Turner et al. 2015) exposure of Canadian environments to free DNA from EO-1α Salmon is expected to be low.

Environmental hazards of EO-1α Salmon through different pathways or to different environmental components are assessed separately, and ranged from negligible to high, with highest hazards through trophic interactions and intraspecific hybridization (see Table 2.10). However, it is important to note that hazards with moderate to high ratings are considered very context specific, where EO-1α Salmon may pose high hazards under one set of environmental

conditions or lower to negligible hazards under a different set of environmental conditions. In this assessment, where a range of potential hazard levels was identified for a single pathway to harm, the highest potential hazard rating was used. Consequently, current hazard assessment ratings represent the most extreme hazard levels expected. Continued research into this area may change uncertainty of specific hazards, and alter the final hazard ratings in the future. Factors that have been identified as potentially influencing hazard level of EO-1α Salmon include health and resilience of exposed populations (e.g., wild Atlantic Salmon), community structure at site of interactions, and life stage and number of EO-1α Salmon escape. Level of uncertainty of hazard ratings ranges from low to high.

Under the paradigm Risk ∝ Exposure × Hazard, and given conditions under **Scenario A** (i.e., non-transgenic eggs are sold to third parties), the final risk assessment is calculated as **Low** to **Moderate** Risk to Canadian environments (Low Exposure × Negligible to High Hazard, see Figure 2.1). Risk to the Canadian Environment under this scenario may be lessened by following proposed mitigation procedures to decrease the potential for accidental release of transgenic eggs to external partners (see Section 2.4.2 below). Under **Scenario B** (i.e., non-transgenic eggs are not produced for third parties), the paradigm Risk ∝ Exposure × Hazard results in a final environmental risk assessment of **Negligible** to **Low** Risk to Canadian environments (Negligible Exposure × Negligible to High Hazard, see Figure 2.2). It is important to note that the hazards with moderate risk under Scenario A are classified as having potential to cause reversible (through interspecific hybridization or to biodiversity) or irreversible harm (through trophic interactions or intraspecific hybridization) to Canadian environments.

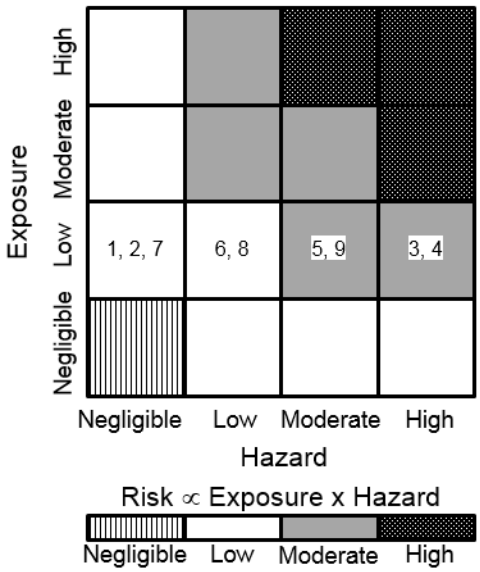

Figure 2.1: Risk matrix and pattern scale to illustrate how exposure and hazard are integrated to establish a level of risk in the environmental risk assessment for the proposed use under Scenario A. Risk assessments associated with assessed hazard components at the assessed exposure are identified by number: 1) through environmental toxicity; 2) through horizontal gene transfer; 3) through trophic interactions with other organisms; 4) through intraspecific hybridization; 5) through interspecific hybridization; 6) as a vector of disease; 7) to biogeochemical cycling; 8) to habitat; 9) to biodiversity.

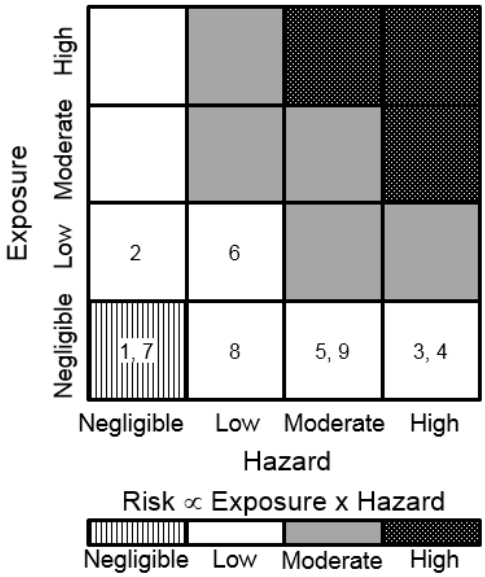

Figure 2.2: Risk matrix and pattern scale to illustrate how exposure and hazard are integrated to establish a level of risk in the environmental risk assessment for the proposed use under Scenario B. Risk assessments associated with assessed hazard components at the assessed exposure are identified by number: 1) through environmental toxicity; 2) through horizontal gene transfer; 3) through trophic interactions with other organisms; 4) through intraspecific hybridization; 5) through interspecific hybridization; 6) as a vector of disease; 7) to biogeochemical cycling; 8) to habitat; 9) to biodiversity.

Sources of uncertainty in the risk assessment are primarily due to uncertainty in hazard assessments. Sources of uncertainty in hazards include limited data directly examining hazards of EO-1α Salmon under a variety of relevant environmental conditions, presence but limited understanding of GxE in surrogate models, and limited understanding of applicability of data from surrogate models to EO-1α Salmon. The given level of detail of containment of EO-1α Salmon at the proposed facility results in low uncertainty regarding the exposure of the environment to EO-1α Salmon. There is some uncertainty in some hazard ratings themselves, notably for hazards through trophic interactions or hybridization, where the hazard rating may be context specific – i.e., high under one set of conditions but lower under another set of conditions. Continued research into this area may decrease uncertainty of specific hazards, and alter the final hazard ratings in the future, but any alterations would be expected to be downgraded, not upgraded, as the current assessment designated the highest conceivable hazard rating where ratings were considered context specific. Consequently, overall risk ratings of EO-1α Salmon under the current context would not be expected to increase should further research into the potential hazards of EO-1α Salmon be provided.

2.4.2 Proposed Mitigation Procedures

To mitigate the potential for human error that may result in the mixing of transgenic and non-transgenic fish under Scenario A, the production of non-transgenic fish for use by external parties should be conducted under all of the following conditions:

a) be undertaken in a different building from transgenic eggs, or a physically separate area within a building, with a separate and secured entrance, and in locations where there is no production of transgenic fish, through the production cycle, from egg fertilization to the end of the egg shipping process;

b) be undertaken where there is no overlap in time between transgenic and non- transgenic spawning events, and between egg shipping events;

c) be undertaken with staff trained on all applicable SOPs;

d) require a statistically appropriate sampling methodology for validation of a non-transgenic genotype, as close to the time of shipping as possible, and for all shipments; and

e) require labelling inside and outside of shipping boxes to indicate contents, and shipping of eggs as soon as possible following validation (e.g., eggs are selected, sampled for genotyping, genotyped, packaged, and shipped prior to a new batch of eggs being selected for shipping).

2.4.3 Other Considerations

The above assessment was conducted under current climate conditions. However, ongoing climate change could potentially lead to more extreme weather events that could have an impact on physical containment. Additional mitigation measures to address these highly unlikely events could include developing procedures (SOPs) for discreet catastrophic events, such as tornados or hurricanes, with elements for the capacity of trained staff to capture escapes in the nearby settling pond or brook, or erect a temporary barrier in the nearby brook to mitigate the risk of an escape event.

Minimizing potential for release is paramount in minimizing risk of EO-1α Salmon under the proposed notified use. The potential for accidental release of EO-1α Salmon while transporting between buildings at the Rollo Bay facility could be further decreased by development or strengthening of SOPs regarding the transport of transgenic and non-transgenic fish at all life stages (e.g., mitigation of spills, locked transport boxes, labelling, do not transport during hazardous weather, etc.). Third party auditing of SOPs, as well as third party monitoring of the near-by brook by electrofishing, would help minimize release potential and confirm negligible release from the site.

It should be recognized that the notified use of EO-1α Salmon will have other provincial and federal requirements (e.g., Code for Introductions and Transfers, Fish Health programs, Environmental Impact Assessments), as well as regulatory requirements for programs in other jurisdictions (e.g., USFDA transport requirements).

2.5 SUMMARY AND CONCLUSIONS

AquaBounty Canada Limited has indicated its intent to expand current operations for the manufacture and grow-out of AquAdvantage® Salmon (AAS) to a land-based, contained facility near Rollo Bay, PEI. This facility would house transgenic EO-1α Salmon and non-transgenic St. John River domesticated broodstock used in the production of AAS eggs. Grow-out of AAS for sale as food into approved markets will occur at the Rollo Bay Facility, and approved facilities in Panama and the United States. The company also intends to sell non-transgenic eyed eggs from the Rollo Bay facility to third parties.

Extensive and redundant physical containment of EO-1α Salmon at the proposed land-based facility result in a negligible potential for the notified organism to enter the Canadian environment under Scenario B (non-transgenic eggs are not sold to third parties). In addition, a large percentage of EO-1α Salmon at the proposed Rollo Bay facility, and all fertilized eggs leaving the proposed facility for grow-out will be treated to induce triploidy, providing an additional level of biological containment to minimize exposure. Though EO-1α Salmon have the capacity to survive, reproduce (fertile female broodstock and failed 3n AAS only), and cause harm in Canadian environments, the confirmed high level of containment at the proposed facility

results in Negligible to Low Risk of EO-1α Salmon to Canadian Environments under Scenario B. Under Scenario A, where non-transgenic fertilized eggs will be produced for external parties, the potential for human error in shipping eggs increases potential exposure. Consequently, the likelihood of exposure of EO-1α Salmon to the Canadian environment is ranked low, and therefore, results in low to moderate risk of EO-1α Salmon to Canadian environments under Scenario A.

Mitigation procedures have been proposed to decrease exposure and hence environmental risk under use Scenario A. While these procedures may decrease exposure, it is not clear if they would decrease final risk conclusion to Low. As containment is essential to minimizing risk of the EO-1α Salmon to the Canadian environment, it is imperative that the use scenario proposed by AquaBounty Canada Ltd be maintained, including all physical, biological, and operational containment measures. Any changes to containment or expansion of the manufacture and production facilities could change the outcome of the environmental risk assessment and would require additional information to be provided to ECCC.

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