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**National Capital Region**

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# ENVIRONMENTAL AND INDIRECT HUMAN HEALTH RISK ASSESSMENTS FOR THE MANUFACTURE AND GROW-OUT OF EO-1α SALMON, INCLUDING THE AQUADVANTAGE® SALMON, AT A LAND-BASED AND CONTAINED FACILITY NEAR ROLLO BAY, PEI

*Figure 1. AquAdvantage® Atlantic Salmon (EO-1α Salmon) containing the opAFP-GHc2 transgenic construct (back) and non-transgenic Atlantic Salmon of equal age (front) (Photo courtesy AquaBounty Canada Inc.).*

## Context:

*The biotechnology provisions of the* Canadian Environmental Protection Act, 1999 *(CEPA) take a preventative approach to environmental protection by requiring all new living organism products of biotechnology, including genetically engineered fish, to be notified and assessed prior to their import into or manufacture in Canada, to determine whether they are "toxic" or capable of becoming "toxic", as defined in section 64 of CEPA.*

*Under a Memorandum of Understanding (MOU) between the Department of Fisheries and Oceans (DFO), Environment and Climate Change Canada (ECCC) and Health Canada (HC), DFO conducts environmental risk assessment, provides science advice to ECCC, and collaborates with HC to conduct an indirect human health risk assessment for any fish products of biotechnology notified under CEPA and the* New Substances Notification Regulations (Organisms) *[NSNR(O)]. The advice is provided to ECCC and HC in the form of a Canadian Science Advisory Secretariat (CSAS) Science Advisory Report that is used to inform the CEPA risk assessment.*

*On July 27, 2018, AquaBounty Canada Inc. submitted a regulatory package to ECCC under the NSNR(O) for the manufacture and grow-out of EO-1α Salmon, a fast growing, genetically engineered Atlantic Salmon (*Salmo salar*), at a new land-based aquaculture facility, near Rollo Bay, PEI.*

*The following Science Advisory Report summarizes the results of the December 11 to 13, 2018 "Environmental and Indirect Human Health Risk Assessments for the Manufacture and Grow-out of Sterile AquAdvantage® Salmon at a Land-Based Facility near Rollo Bay, PEI" CSAS peer-review meeting. In advance of the meeting, a CSAS Science Response process was held to establish whether information provided by the company in the regulatory package was sufficient to determine invasiveness (DFO 2019).*

*Additional publications from this meeting will be posted on the* Fisheries and Oceans Canada (DFO) Science Advisory Schedule *as they become available.*



Canada

# SUMMARY


- Pursuant to the *Canadian Environmental Protection Act* (CEPA), a notification under the *New Substances Notification Regulations (Organisms*) (NSNR(O)) was submitted by AquaBounty Canada Inc. to Environment and Climate Change Canada (ECCC) for the manufacture and grow-out of a genetically engineered Atlantic Salmon (i.e., the AquAdvantage® EO-1α Salmon) at a new site in PEI, near Rollo Bay.
- Environmental and indirect human health risk assessments were conducted that included an analysis of potential hazards, likelihoods of exposure, and associated uncertainties to reach conclusions on risk and to provide science advice to ECCC and Health Canada (HC) to inform their CEPA toxicity assessment. This risk assessment included consideration of two scenarios:
    - Scenario A: the production of non-transgenic fish, for external parties, occurring along-side transgenic fish production under existing and planned procedures; and
    - Scenario B: no production of non-transgenic fish, for external parties, at facilities producing transgenic fish.
    - In addition to the two scenarios, additional measures were identified that could further reduce exposure and risk of Scenario A (see below in Risk Mitigation section).

**Environmental Risk Assessment**

- The assessment concluded that environmental exposure attributed to the EO-1α Salmon will be negligible to low in the Canadian environment, recognizing that there are physical, biological and operational measures in place or planned at the Rollo Bay facility that could prevent unintentional environmental release.
    - For Scenario A, where the production of non-transgenic fish for external parties occurs with transgenic fish, the likelihood of exposure of EO-1α Salmon to the Canadian environment is ranked low.
    - For Scenario B, where there is no production of non-transgenic fish for external parties, the likelihood of exposure of EO-1α Salmon to the Canadian environment is ranked negligible.
- The uncertainty associated with this environmental exposure estimation is low, given the available information on physical, biological and operational containment of the organism. Production of non-transgenic fish under Scenario A could increase uncertainty in exposure.
- The environmental hazard assessment for EO-1α Salmon ranged from negligible to high depending on the type of interaction considered, such as through environmental toxicity, horizontal gene transfer, as a vector for disease, or effects to biodiversity, biogeochemical cycling, and habitat, with highest ratings through trophic interactions with other organisms and intraspecific hybridization.
- The uncertainty levels associated with the environmental hazard ratings range from moderate to high due to limited data on EO-1α Salmon under a variety of relevant environmental conditions, presence but limited understanding of genotype by environment (GxE) interactions in surrogate models (comparator species, non-transgenic siblings, or domestic strains), and limited understanding of how data from surrogate organisms can be extrapolated to the organism.

- There is negligible to moderate risk of adverse environmental effects at the exposure and hazard levels predicted for the Canadian environment from the use of EO-1α Salmon at the Rollo Bay facility:
    - under the proposed use scenario where non-transgenic fish are produced for external parties (Scenario A) the risk is low to moderate; and
    - if non-transgenic fish were not sold to external parties (Scenario B), there is negligible to low risk of adverse environmental effects.

**Indirect Human Health Risk Assessment**

- The indirect human health (IHH) exposure assessment concluded that human exposure potential of the EO-1α Salmon is low, as physical, biological and operational measures are in place or planned at the Rollo Bay facility to prevent unintentional environmental release, thus greatly limiting human exposure to the notified organism. This ranking is not expected to change taking into consideration Scenarios A and B above.
- Uncertainty associated with the IHH exposure assessment is low since adequate information is available regarding exposure scenarios in the Canadian environment given the existing or planned containment measures at the Rollo Bay facility. However, this uncertainty ranking could likely be higher in the event that non-transgenic and transgenic fish are produced alongside each other (Scenario A).
- The IHH hazard assessment concluded that the indirect human hazard potential of EO-1α Salmon is low as the source organisms for the inserted genetic materials are not pathogenic, there are no reported cases of zoonotic infections associated with the organism or Atlantic Salmon in general, and based on the sequence identity and the structure of the inserted transgenes, the production of allergens or toxins is not anticipated.
- Uncertainty associated with the IHH hazard assessment is low, under the recognition that information on human health effects are based on reports from suitable surrogate organisms.
- There is a low risk of adverse IHH effects at the exposure levels predicted for the Canadian population from the use of EO-1α Salmon.

**Conclusions**

- The overall assessment of the manufacture and grow-out of EO-1α Salmon at a land-based facility near Rollo Bay, PEI, is concluded to have:

    - a low to moderate risk of adverse environmental effects to the Canadian environment if non-transgenic fish for external parties are produced along-side transgenic fish (Scenario A);

    - a negligible to low risk of adverse environmental effects to the Canadian environment if no non-transgenic fish are produced for external parties at facilities producing transgenic fish (Scenario B); and

    - a low risk to the indirect human health of Canadians under both Scenario A and Scenario B.

- Additional measures were identified that could further reduce exposure and risk of Scenario A (see below in Risk Mitigation section). There was consensus that the

exposure rating could be reduced, but there was not consensus on whether the exposure can be reduced to negligible.

- Any changes to containment or expansion of the manufacture and grow-out facilities could change the outcome of the environmental risk and indirect human health assessments and could require additional information to be provided to ECCC.

# BACKGROUND

In 2013, AquaBounty Canada Inc. submitted a notification (NSN-16528) to ECCC detailing its intent to commercially manufacture AquAdvantage® Salmon (AAS) in a land-based contained facility near Bay Fortune, PEI. Under the containment conditions proposed by AquaBounty Canada Inc., DFO determined that the manufacture of AAS poses low risk to the Canadian environment and indirect human health, but advised that this conclusion could change if activities in relation to the organism change significantly from those proposed by AquaBounty Canada Inc. in its submission, and could result in greater risk to the environment (DFO 2013). Environment Canada and Health Canada accepted this advice and published a Significant New Activity Notice #16528 in the Canada Gazette in November 2013. In 2016, Health Canada (HC) and the Canadian Food Inspection Agency (CFIA) approved AAS for human food and animal feed use, respectively, on the basis that it is nutritionally the same as non-genetically engineered salmon. The current assessment examines an additional notification to expand manufacture and grow-out of AAS at a new land-based aquaculture facility near Rollo Bay, PEI.

## Characterization of the Notified Organism

The notified organism (EO-1α Salmon, Figure 1) is an Atlantic Salmon (*Salmo salar*) containing a single insert of the opAFP-GHc2 transgene at the EO-1α locus (hereafter referred to as the EO-1α construct). The EO-1α Salmon was developed by micro-injecting a gene construct (opAFP-GHc2) into the newly fertilized egg of a wild Atlantic Salmon, 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.

The current notification includes the following forms of the notified organism:

- AquAdvantage® Salmon (AAS): triploid sterilized (≥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 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 neomale broodstock: Genetically female transgenic fish that carry a double copy (homozygous) of the EO-1α construct, and were treated with 17α-methyltestosterone to render fish phenotypically male. These will be used to maintain the EO-1α broodstock line and be bred with St. John River Strain non-transgenic domesticated females to produce the AAS commercial form of EO-1α Salmon.

In addition to EO-1α Salmon, the company will maintain a broodstock of non-transgenic St. John River domestic strain that will be used as the source of non-transgenic Atlantic Salmon eggs

needed for the manufacture of AAS. The company also intends to sell fertilized non-transgenic Atlantic Salmon eggs to external parties (see Use Scenario below).

### Targeted Phenotypic Effect of the Modification

The primary phenotypic change of EO-1α Salmon is increased growth and size at equivalent age relative to non-transgenic siblings (Figure 1). This phenotype is consistently observed in standard hatchery practices by AquaBounty Canada Inc. and in numerous published papers (Levesque et al. 2008; Moreau and Fleming 2012; Oke et al. 2013; Tibbetts et al. 2013), and is also associated with increased feed conversion efficiency (Tibbetts et al. 2013).

### Non-targeted Phenotypic Effects of the Modification

Morphological irregularities reported in EO-1α Salmon are of low frequency and of a non-debilitating nature. 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) and juvenile AAS relatives (Stevens and Sutterlin 1999; Cook et al. 2000a; Cook et al. 2000c). Other metabolic and physiological attributes of EO-1α Salmon relative to non-transgenic counterparts include higher feed consumption rates, lower feed conversion ratios, reduced metabolic scope, and reduced swimming performance in juveniles raised under hatchery conditions (Deitch et al. 2006, and from 2013 notification 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).

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. 2011; Moreau and Fleming 2012). Abrahams and Sutterlin (1999) demonstrated that AAS relatives 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 on the reproductive behaviour of female EO-1α Salmon (both diploid and triploid) which is a significant knowledge gap.

### 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, GH transgenesis is reported to influence almost every phenotype and physiological system examined (see Devlin et al. 2015). The effect of GH 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, when environmental conditions influence the phenotype of different genotypes in dissimilar ways, 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 example, Sundström et al. (2007) demonstrated that growth-enhanced transgenic Coho Salmon grew three times longer than non-transgenic conspecifics under hatchery conditions, but grew only 20% longer under simulated stream conditions. 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.

## Comparator Conspecific

Atlantic Salmon is one of the most studied fish species in the world. 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. Comprehensive reviews of Atlantic Salmon ecology and genetics can be found in Aas et al. (2011) and Verspoor et al. (2007), respectively. The biology of the Atlantic Salmon has been recently summarized by the OECD (2017).

## Characterization of Potential Receiving Environment

The environment directly outside of the Rollo Bay facility, as well as those connected to the immediate environment, is expected to be highly conducive to 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 km 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 and is known to support a population of Brook Trout (*Salvelinus fontinalis*). The potential receiving environment is well within the natural range of Atlantic Salmon, and the physical and chemical components of the receiving habitat and connecting habitats (e.g., the Atlantic Ocean) would likely support all life-stages of Atlantic Salmon from embryo to adult.

## Use Scenarios

The primary activity under the notification is the commercial manufacture of triploid (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 diploid 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. 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, 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.

## Information Regarding Invasiveness

In addressing paragraph 5(a) of Schedule 5 of the NSNR(O), *data from a test conducted to determine pathogenicity, toxicity or invasiveness*, the notifier provided information and data from the scientific literature to support its claim that the organism is not pathogenic, toxic or invasive. For invasiveness, which was considered the most relevant endpoint under paragraph 5(a) for this environmental assessment, the notifier argued that EO-1α Salmon has lowered fitness relative to wild Atlantic Salmon and therefore would not be invasive. On November 2, 2018, a CSAS Science Response process was conducted to establish whether the information provided by the company in the regulatory package was adequate to fulfill paragraph 5(a). It was concluded that there were significant gaps in the data such that invasiveness potential of EO-1α Salmon could not be systematically assessed, and that the information provided by the notifier was not sufficient. The consensus recommendation was to not accept the information as complete for paragraph 5(a). In response, the notifier requested a waiver for this data element

on the basis that the organism will be suitably contained at the Rollo Bay facility and would not be released to the environment.

### Waiver Request

In accordance with Section 106(8) of CEPA, the notifier (AquaBounty Canada Inc.) has requested a waiver for the information required under information element 5(a) of Schedule 5 of the NSNR(O). This information element requires that data be submitted from a test conducted to determine pathogenicity, toxicity or invasiveness of the organism, where invasiveness is considered the most appropriate endpoint for this notification. 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, including a site visit by risk assessment staff, was conducted as part of the environmental exposure assessment and will be used 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, standard operating procedures (SOPs) and internal compliance documentation. It is concluded that AAS will be manufactured at a location where AquaBounty Canada Inc. is able to contain EO-1α Salmon so as to satisfactorily protect the environment and human health.

## ENVIRONMENTAL RISK ASSESSMENT

### Exposure

The exposure assessment for living EO-1α Salmon addresses both their potential to enter the environment (release) and fate (final outcome) once in the environment. All relevant information regarding physical, chemical and biological containment strategies used at all life stages and under both use scenarios is considered. The potential for unintentional release during catastrophic events or sensitive activities, such as the manufacture, incubation and transport of EO-1α Salmon, is also taken into consideration. Rankings for the likelihood of Exposure to the Canadian Environment are provided in Table 1.

The exposure assessment requires two distinct approaches to assessing uncertainty; one for the physical containment (i.e., entry or release; Table 2) and a second for the biological containment (i.e., fate; Table 3).

To facilitate the assessment of physical containment, a Failure Modes Analysis (FMA) was conducted following guidance from 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.

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

| Exposure Ranking | Assessment |
|---|---|
| Negligible likelihood | No occurrence[1]; 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 |

[1]extremely unlikely or unforeseeable

**Table 2. Categorization of exposure uncertainty based on the assessment of physical containment (i.e., entry) of EO-1α Salmon in the Canadian environment**

| Uncertainty Ranking | 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 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**

| Uncertainty Ranking | 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. Limited to low knowledge of GxE interactions. |
| High uncertainty | Significant knowledge gaps. Significant reliance on expert opinion. |

### Likelihood of Release

The AquaBounty Rollo Bay facility is located just north of the municipality of Rollo Bay, Prince Edward Island, on a parcel of land that is approximately 1.5 km from Rollo Bay and the Northumberland Straight. The facility is entirely land-based, with all fish maintained within the confines of three buildings (one newly renovated and two in the process of being constructed), each with a cement foundation, solid walls, and a roof. The assessment captures information on containment provisions for all three buildings, as well as for the transport of EO-1α Salmon and activities during the manufacturing process. Consideration is also given to the facility's security and susceptibility to natural disasters. When operational, all three buildings will be subject to routine inspection by ECCC, in accordance with CEPA Compliance and Enforcement Policy.

*1. The Hatchery*

The Hatchery is a recently renovated 8800 square foot building that will be used to house select lines of diploid EO-1α broodstock. Four potential pathways of entry into the environment were identified for EO-1α gametes, embryos, fry, juveniles and adults. Forty-four elements of containment (e.g., screens, filters, chlorine pucks, etc.) and 88 potential failure modes were examined using FMA. For viable EO-1α Salmon to reach the environment outside of the Hatchery there must be simultaneous failure of at least six independent containment measures along a single pathway of entry.

*2. The Grow-out Building*

The Grow-out Building is an in-progress construction (at time of assessment) of approximately 41,000 square feet, and will be used to raise all-female triploid AAS, from egg to market size (5 kg) adults, at a rate of approximately 250 metric tonnes (MT) per year. Four potential pathways of entry into the environment were identified for EO-1α embryos, fry, juveniles and adults. Thirty-five elements of containment and 77 potential failure modes were examined using FMA. For viable EO-1α Salmon to reach the environment outside of the Grow-out Building there must be simultaneous failure of at least five independent containment measures along a single pathway of entry.

*3. The Broodstock Building*

The Broodstock Building is an in-progress construction (at time of assessment) of similar size and design as the Grow-out Building. It will be used for the manufacture of the all-female triploid AAS eggs that will be used in commercial grow-out, and will house the diploid EO-1α homozygous females and the diploid EO-1α homozygous neomales required by the manufacturing process. The Broodstock building will also be used for incubation of the product, all-female triploid AAS eggs that will be shipped to grow out facilities in Panama and the United States prior to hatching. Four potential pathways of entry into the environment were identified for EO-1α gametes, embryos, fry, juveniles and adults. Thirty-four elements of containment and 72 potential failure modes were examined using FMA. For viable EO-1α Salmon to reach the environment outside of the Grow-out Building there must be simultaneous failure of at least five independent containment measures along a single pathway of entry.

*4. Containment during the manufacture of triploid AAS and EO-1α broodstock*

During the manufacturing process, eggs must be collected from fish, fertilized, then undergo pressure shocking to induce triploid sterilization (minimum 98.5% efficiency). As with the physical containment of EO-1α gametes and embryos, all three buildings will have multiple mechanical and chemical barriers in place to prevent the release of EO-1α Salmon at any point during the manufacturing process (fertilization and pressure shock). However, under Scenario A, non-transgenic eggs will be incubated alongside EO-1α eggs, and will be shipped to facilities

where containment provisions may be less stringent than those observed at the Rollo Bay facility. Though the company has SOPs and oversight documentation in place to prevent the mix-up of eggs during incubation, or prior to shipping, there remains a possibility of human error resulting in the release of transgenic eggs to customers who believe they have purchased non-transgenic eggs. Under Scenario B, this is no longer a possibility as all non-transgenic eggs will be kept within the facility, and any mistake will only result in the shipment of non-transgenic eggs to facilities that are internal to the company and have containment measures in place to prevent the escape of transgenic salmon. Consequently, the likelihood of exposure under Scenario A is low, whereas the likelihood of exposure under Scenario B is negligible.

*5. Containment during the transport of triploid AAS eggs*

When completely operational, the Rollo Bay facility will be used to manufacture triploid AAS for commercial grow-out and for export to approved commercial grow-out facilities in Panama and the United States. 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. During ground transport, the fertilized 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. 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 Committee.

*6. Natural events*

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

*7. Security*

AquaBounty Canada Inc. has put in place several security measures to protect both its property and personnel. These measures include backup generators and emergency SOPs, video surveillance, steel exterior doors with key control and entry logs, motion detectors, 24 hour surveillance by commercial security provider, staff that live on site, and exterior lighting throughout the premises at night.

**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 released into the Canadian environment. In the highly unlikely event of an unintentional release, 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. There are numerous salmon rivers in close proximity to the Rollo Bay Facility where EO-1α adults could survive and interact with wild Atlantic 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. Induction of successful triploidy in AAS will prevent reproduction of this form of EO-1α Salmon, but will not be used on broodstock. Though 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 (see Glover et al. 2017).

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 the interaction between genotype and environment (GxE interactions) indicate that organisms of different genetic backgrounds can respond to different environments in different ways. Consequently, it may be unfeasible 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 wild Atlantic Salmon.

### Exposure Assessment Conclusions

Under Scenario A, in which non-transgenic eggs are produced for external parties, the likelihood of exposure of EO-1α to the Canadian environment is ranked low. However, an alternate use scenario (B) where non-transgenic eggs are not sold to external parties resulted in the negligible likelihood of exposure to the Canadian environment. A high degree of certainty associated with the physical, biological and operational containment of EO-1α Salmon results from available information that adequately demonstrates the efficacy and redundancy of mechanical barriers, and the efficacy of SOPs and operational oversight. It includes detailed diagrams of facility design, mechanical barriers and containment systems, and training and compliance documentation. In contrast, uncertainty associated with the fate of EO-1α Salmon in the environment is derived largely from the limited availability of empirical data regarding the survival, fitness, and ability of EO-1α Salmon to reproduce in the natural environment. Regardless, the capacity of EO-1α Salmon to survive, reproduce and proliferate in the Canadian environment is dependent on the degree to which containment (both physical and biological) is achieved. Under Scenario A, human error increases the likelihood of exposure to the Canadian environment. Consequently, the exposure assessment concludes with **low uncertainty** (Table 3) that the likelihood of EO-1α Salmon exposure to the Canadian environment is **low** (Table 1). 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 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, such as biogeochemical cycling, habitat, and biodiversity. Table 4 categorizes the severity of the biological consequences based on the severity and reversibility of effects to the structure and function of the ecosystem.

Given the lack of empirical data around the behaviour and fitness of EO-1α Salmon in the natural environment, 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. A description of rankings for uncertainty regarding the potential hazards of the organism in the environment is provided in Table 5.

**Table 4. 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.

[2]Harmful effect: an immediate or long-term detrimental impact on the structure or function of the ecosystem, including biological diversity, beyond natural fluctuations.

**Table 5. Ranking of uncertainty associated with the environmental hazard**

| Uncertainty Ranking | 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. |

**Potential Hazards through Environmental Toxicity**

Information about GH concentration has not been reported throughout the life cycle of EO-1α Salmon, with only one study reporting that GH levels remain below a detection limit of 6.24 ng/mL in the muscle of commercial size EO-1α Salmon (NSN-16528). Average plasma GH levels in juvenile G0 (founder individuals) AAS-relatives were not significantly different from non-transgenic siblings (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). Though 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 in organisms that consume or come into contact with EO-1α Salmon. Consequently, GH levels in EO-1α Salmon represent a negligible hazard to predators or scavengers.

No differences were reported for other measured hormones in the muscle-skin samples from commercial sized AAS compared to sponsor controls (NSN-16528). As well, data from domestic Atlantic Salmon suggest EO-1α Salmon will not bioaccumulate environmental toxins at a greater rate than wild fish (Lundebye et al. 2017). 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 **moderate uncertainty**.

### Potential Hazards through Horizontal Gene Transfer

Horizontal gene transfer (HGT) is the non-sexual exchange of genetic material between organisms of the same or different species (DFO 2006). Pathways of exposure of free transgenic DNA to novel organisms (most likely prokaryotes) include exposure within the EO-1α Salmon's gut, or through feces, mucus, and other waste sloughed off by the fish into the water, and do not necessarily require the EO-1α Salmon to escape containment for exposure. 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. 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, although vertebrate promoters generally have poor activity in prokaryotes. The lack of expected differences in likelihood of HGT, or its effects, between the EO-1α transgene and native Atlantic Salmon genes results in **negligible** hazard through HGT. While the transgene is well defined, the limited knowledge of the location of the transgene within the salmon genome, and lack of studies examining HGT of the transgene and resulting consequences, results in a **moderate uncertainty** for this hazard rating.

### Potential Hazards through Trophic Interactions with other Organisms

*Through competition*

Escaped EO-1α Salmon could compete with any organism occupying similar niches, most notably wild Atlantic Salmon. 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). 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 (see Devlin et al. 2015; Vandersteen et al. 2019).

Given the potential for competition in variable habitats (i.e., there are 26 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), isolated conditions may exist where EO-1α Salmon can gain an advantage, be neutral, or have a disadvantage 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**, though 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 interactions in surrogate organisms.

*Through predation on other species*

The predation ability of escaped EO-1α Salmon has not been specifically examined, but, as with competition, is expected to be influenced by abundance of prey items, presence of predators and competitors, timing and number of escapes, swimming ability, etc. Wild 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 high 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 not typical in wild Atlantic Salmon. 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 may decrease the ability of EO-1α Salmon to capture prey, particularly in marine environments. Consequently, the potential for EO-1α Salmon to affect the prey species is **high** with **moderate uncertainty**. The level of uncertainty is due to limited studies on foraging of EO-1α Salmon and their relatives, limited studies across relevant environments in other models, and limited understanding of relevance of other models to EO-1α Salmon. As with competition, the potential level of hazard through predation is expected to be context specific, and may vary under different environmental circumstances.

*As prey to other species*

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 most conditions. Studies assessing the mortality of GH transgenic salmonids relative to non-transgenic hatchery 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 wild fish depending on environmental conditions. The 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. The Canadian Food Inspection Agency 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, there is a **negligible hazard** to wild fish through predation on EO-1α Salmon. There is a **moderate** level of **uncertainty** due to limited information regarding hormone concentrations, toxicity, and the nutritional value of EO-1α Salmon throughout its life cycle.

*Trophic hazard conclusions*

Trophic interactions with EO-1α Salmon, should they escape containment, have highest potential to harm wild native populations through freshwater competition and predation (high hazard rating), although this would be context specific and the level of harm may vary under different conditions. Potential for harm as prey had a negligible rating, 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 but presence of GxE in other models, and limited understanding of relevance of other models to EO-1α Salmon.

**Potential Hazards through Hybridization**

*With Atlantic Salmon*

The potential for harm to wild populations from hybridization with EO-1α Salmon has not been examined. In a relatively well studied model with accelerated growth (escaped domestic Atlantic Salmon), impacts to wild populations from hybridization with escaped fish are well understood, and include decreased productivity of wild populations from lower fitness or increased straying of hybrid offspring (Bolstad et al. 2017; Glover et al. 2017; Jonsson and Jonsson 2017), with

long term consequences from introgression including alterations in life-history traits, decreased population productivity, and decreased resilience to environmental changes (see McGinnity et al. 2003; Glover et al. 2017). 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.

There are no known wild Atlantic Salmon spawning sites adjacent to the Rollo Bay facilities. Consequently, for escaped female EO-1α diploid Salmon to impact wild Atlantic Salmon populations through introgression they would need to survive in the drainage brook, migrate to and survive in marine ecosystems, migrate to spawning grounds of wild populations at the same time as wild fish, then successfully reproduce. The closest stream with wild Atlantic Salmon populations is within 50 km of the Rollo Bay facility, which is within the potential stray distance of domestic Atlantic Salmon (Glover et al. 2017). The potential for survival and reproduction of EO-1α Salmon (see Exposure) and long-term impacts from introgression results in a **high hazard** to wild Atlantic Salmon populations through hybridization 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 and limited to no data on potential effects/success over multiple generations in nature.

*With other species*

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. The study suggests that both types of offspring of EO-1α Salmon and Brown Trout hybridization could negatively impact wild Atlantic Salmon in the same niches, although since competitive differences of non-transgenic versus transgenic fish were not examined, it cannot be determined if EO-1α hybrids could pose greater harm than domestic Atlantic Salmon through this pathway. Whether EO-1α Salmon x Brown Trout hybrids could further introgress with wild Atlantic Salmon populations, and subsequent impacts of this, are not known. 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.

**Potential to act as a Vector of Disease Agents**

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. Potential hazards as a vector of disease could also occur from contained fish that shed pathogens from rearing facilities to natural ecosystems through waste water. The relative disease susceptibility of EO-1α Salmon has not been formally examined, although preliminary data provided in previous and current notifications does not indicate consistent alterations in disease susceptibility.

In other models, altered resistance to pathogens and impaired immune response is reported in GH transgenic Coho Salmon (Jhingan et al. 2003; Kim et al. 2013) and Zebrafish (Batista et al. 2014), although whether this would decrease or increase vector capabilities is not known. 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. 2007).

The available information from the current PEI facility indicates fish health is well managed. As well, the proposed recirculating system with UV and ozone treated water would decrease potential for pathogen leak from the facility relative to a traditional net-pen aquaculture site. However, the presence of altered disease susceptibility in surrogate organisms results in **low hazard** rating for EO-1α Salmon to cause harm as a vector of disease above that of domestic Atlantic Salmon. 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** with this rating.

**Potential to Impact Biogeochemical Cycling**

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), although studies in semi-natural conditions suggest spawning salmon may contribute significant marine-derived nutrients to river systems where populations are self-sustaining (Samways and Cunjak 2015). 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.

**Potential to Affect Habitat**

Reproductive behaviour of salmonids, including Atlantic Salmon, has been shown to influence habitat through ecosystem engineering and bioturbation (Grant and Lee 2004; Gottesfeld et al. 2008). Redd construction and excavation in streams by spawning female salmonids, when spawning at high densities, can significantly disturb the streambed (Gottesfeld et al. 2004; Hassan et al. 2008). While digging behaviour in EO-1α females has not been examined, domestic Atlantic Salmon and GH transgenic Coho Salmon have lower digging frequency than wild or hatchery fish (e.g., Fleming et al. 1996; Leggatt et al. 2014). Due to the potential for a diminished ability to dig redds in diploid EO-1α Salmon, and the lack of effects expected from sterile triploid AAS (not including up to 1.5% diploid from failed triploidy), 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 the 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.

**Potential to Affect 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, should they escape containment, EO-1α Salmon could impact biodiversity through alterations in competitive and predation ability and preferences. 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). 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** to this rating, as there are 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 (see Leggatt et al. 2010).

### 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, and to biogeochemical cycling), low (as a vector of disease and 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 6). It is important to note that those hazards ranked above negligible 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 at escape.

As discussed in DFO (2018), it is important to be able to articulate to regulators the rating and uncertainty associated with hazard and exposure assessments. 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 and vector of disease may not require an escape event and could occur when EO-1α Salmon are contained), 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.

The hazard assessment concurs for the most part with what was assessed for the 2013 notification (see Table 6). The primary difference between the two assessments is where conclusions were drawn in the current assessment but not the previous assessment. One uncertainty level decreased (to habitat), to reflect the available information on surrogate organisms.

**Table 6. Environmental hazard ranking of EO-1α Salmon through various pathways for the current assessment, as well as what was assessed in 2013 (bold text in final column indicates where the two assessments differ)**

| Hazard | Rank | Uncertainty | 2013 Assessment 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 |
| 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** |

### Uncertainty Associated with Hazard Assessments

Uncertainty level is moderate to high for all 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.

## 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 and severity of the harmful effect, the likelihood that the harmful effect is realized, and the uncertainty associated with each conclusion.

Environmental exposure assessment of EO-1α Salmon under the proposed manufacture and grow-out at the Rollo Bay facility concluded with low uncertainty of negligible to low exposure of EO-1α Salmon to the Canadian environment depending on the use scenario. There is suitable physical containment of the EO-1α Salmon during proposed manufacture, transport and grow-out of AAS (i.e., negligible potential for escape). However, the possibility of an exposure pathway due to the unintentional release (sale) of transgenic eggs as non-transgenic eggs to external parties resulted in a low likelihood of exposure (Scenario A). If non-transgenic eggs are not sold to external parties, the likelihood of exposure of EO-1α Salmon to the Canadian environment would be ranked negligible (Scenario B). If non-transgenic eggs would be produced at the Rollo Bay facilities, but under conditions outlined in Risk Management below, the potential for escape and exposure would decrease relative to Scenario A. If EO-1α Salmon were present in the Canadian environment, the potential for their survival, dispersal, reproduction and establishment cannot be discounted, but is limited by low potential for release. It should be noted that exposure to free DNA released from the EO-1α Salmon, and pathogens potentially shed from the EO-1α Salmon is possible through release of waste water etc. from the Rollo Bay facility, and exposure through these pathways may not require an escape event. As such, exposure pathways for these hazards are expected to be low in any of the scenarios listed above.

Environmental hazards of EO-1α Salmon through different pathways or to different environmental components were assessed separately, and ranged from negligible to high, with highest hazards through trophic interactions and intraspecific hybridization (see Table 6). In this assessment, where a range of potential hazard levels was identified for a single pathway to harm (e.g., through competition, predation or hybridization), the highest potential hazard rating was used. Consequently, current hazard assessment ratings represent the highest hazard levels expected. Factors that have been identified as potentially influencing hazard level of EO-1α Salmon include health and resilience of affected population, community structure at site of interactions, and life-stage and number of EO-1α Salmon escape. Level of uncertainty of hazard ratings ranges from moderate to high.

Under Scenario A, where non-transgenic eggs are sold from the Rollo Bay facility, the paradigm Risk $\propto$ Exposure $\times$ Hazard results in a final risk assessment of low to moderate (Low Exposure $\times$ Negligible to High Hazard, see Figure 2). Consequently, EO-1α Salmon under the proposed use in NSN-19702 Scenario A are expected to pose **Low to Moderate** Risk to Canadian environments.

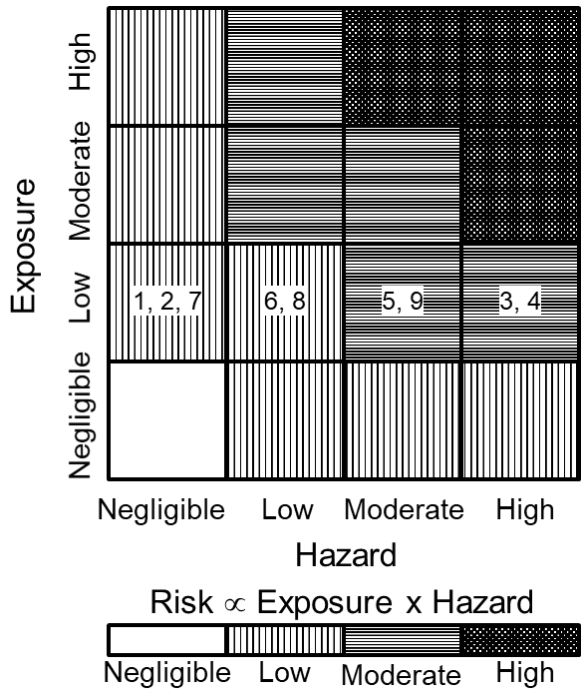


**Figure 2.** *Risk matrix and colour scale to illustrate how exposure and hazard are integrated to establish a level of risk in the environmental risk assessment for proposed use Scenario A. Risk assessments associated with assessed hazard components at the assessed exposure are identified by number: 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.*

Under the use scenario where non-transgenic eggs are not sold (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, see Figure 3). Consequently, EO-1α Salmon under this use scenario are expected to pose **Negligible to Low Risk** to Canadian environments.

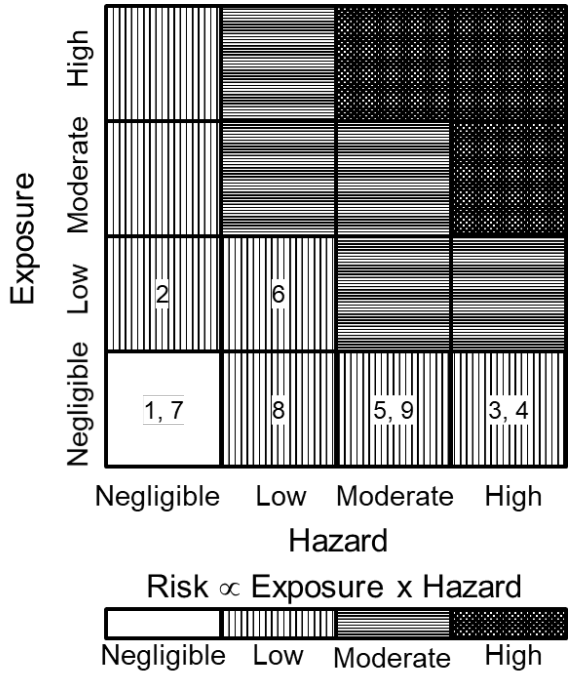


**Figure 3.** *Risk matrix and colour scale to illustrate how exposure and hazard are integrated to establish a level of risk in the environmental risk assessment for the use scenario of no sale of non- transgenic fish (Scenario B). Risk assessments associated with assessed hazard components at the assessed 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.*

# INDIRECT HUMAN HEALTH RISK ASSESSMENT

A determination of whether one or more criteria of section 64 of CEPA are met is based on an assessment of potential risks to the environment and/or to human health associated with exposure in the general environment. For humans, this includes, but is not limited to, exposure from air, water and the use of products containing the substances. Risks from workplace exposure to the notified strain are not considered in this assessment , which are covered Hazardous Products Regulations for products intended for workplace use. As well, risks via exposure to the commercial AAS product for human consumption was assessed and approved by Health Canada under the Food and Drug Regulations.

## Exposure

The main source of human exposure to the notified organism is expected to be from the manufacture of AAS eggs and the production of different age-classes of EO-1α Salmon including fry, smolts, juveniles, adult broodstock and the 400 g to 5 kg market weight grow-out fish. The physical state of the manufactured product will be largely eggs and live or killed fish but may include milt to be used to fertilize the eggs.

### Manufacture in Canada

The notifier intends to expand manufacturing capabilities to produce AAS using contained land-based facilities at Rollo Bay, Prince Edward Island. The site is in eastern PEI (Kings County) and is approximately 1.5 km north of the closest coastal waters. The site is about 7 km northwest of Souris, PEI (estimated population: 1,232), which is approximately 78 km northeast of the provincial capital of Charlottetown (estimated population: 38,174).

Operationally, human exposure to the notified organism could arise from the:

- manufacture of AAS eyed-eggs and EO-1α broodstock eggs;
- grow-out of AAS from first feeder fry to juvenile fish of approximately 400 g until about 5 kg market weight;
- purging, harvesting and slaughter of market weight AAS fish for delivery to off-site processing facilities;
- propagation of the two lines of fish used to produce the AAS, the homozygous EO-1α neomales and the non-transgenic females derived from the St. John River strain that was the original source of the EO-1α line of salmon; and
- activities involving third parties that may arise under Scenario A, if production of non-transgenic fish for sale is to occur along-side that of transgenic fish at the Rollo Bay facility.

### Introduction of the Organism

The only intended current use of the notified organism is commercial aquaculture production in land-based contained facilities. The Rollo Bay facility will be used for the purpose of keeping EO-1α Salmon broodstock to be used to manufacture AAS eggs, and to grow AAS for commercial sale as food for human consumption. At harvest, AAS will be sold into existing seafood-distribution channels for processing, export, and retail sale.

### Environmental Fate

While sex-reversal, domestication and growth hormone transgenesis may impact fitness, they have not been demonstrated to prevent survival and reproduction, although successful triploid induction in the AquAdvantage® Salmon will prevent reproduction in this form of EO-1α Salmon.

Consequently, EO-1α Salmon have potential to survive and reproduce in the wild, should they escape confinement.

### Other Potential Uses

The notifier states that the only intended use of AAS is commercial aquaculture production in land-based contained facilities in Canada, United States (proposed) and Panama. To date, manufacture and grow-out of AAS has been confined to the notifier's land-based freshwater facilities in Prince Edward Island and Panama. In the United States, no life stage of AAS may be raised in ocean net pens under the New Animal Drug Application approval granted by the USFDA in 2015. Similarly, in Canada, physical containment is required to manufacture AAS. Use in research and development is possible, such as the testing of DNA-based vaccinations in fish (US Patent 5780448A). There may also be other unknown potential uses under Scenario A but these are difficult to identify in this assessment due to lack of information on activities involving third parties.

### Exposure Characterization

The ranking system used to determine potential human exposure through release to the environment is given in Table 7. Taking into account both Scenarios A and B, the human exposure potential of the EO-1α Salmon is assessed to be low because:

1) The main source of human exposure to the notified organism is expected to be from the manufacture of AAS eyed-eggs and up to 250 MT of AAS fish per year in contained land-based facilities at Rollo Bay and Bay Fortune in Prince Edward Island, each of which have multiple and redundant means of containment designed to prevent the release of EO-1α Salmon into the Canadian environment;

2) There is no intentional release of EO-1α Salmon into the environment and the physical state of the manufactured products (eggs, different age-classes of AAS including fry, smolts, juveniles, adult broodstock, killed fish and milt to be used to fertilize the eggs) is not expected to lead to increased human exposure;

3) Human exposure is expected to be further reduced through the use of operational controls, including procedures for operating the redundant layers of containment, documentation, reporting of containment breaches, staff training, and other site-specific SOPs, which have been developed based on experiences in current operations at Bay Fortune (broodstock and hatchery) and in Panama (grow-out);

4) Since there is no intentional environmental release of EO-1α Salmon from the facility and the fact that only market weight fish will be harvested and killed before leaving the facility, the likelihood of human exposure to live EO-1α Salmon will be greatly minimized;

5) While there are uncertainties associated with the expected fitness of EO-1α Salmon in natural environments, conditions may be favourable for survival and dispersal of EO-1α Salmon if released into the freshwater brook that runs through the Rollo Bay facility and potentially result in human exposure;

6) Physical and operational measures in place at Rollo Bay and Bay Fortune facilities including multiple containment barriers, wastewater treatment, and the processing of solid waste are expected to decrease the likelihood of human exposure to the notified EO-1α Salmon; and

7) Apart from the use in research, there are no other foreseeable potential uses outside of containment if the Rollo Bay facility is used to produce fish only for internal uses. However, there may be unknown potential uses if production of non-transgenic fish for sale to third parties is to occur along-side that of transgenic fish at the Rollo Bay facility (Scenario A).

**Table 7. Exposure considerations (humans)**

| EXPOSURE | CONSIDERATIONS |
|---|---|
| **High** | • The release quantity, duration and/or frequency are high.<br>• The organism is likely to survive, persist, disperse proliferate and become established in the environment.<br>• Dispersal or transport to other environmental compartments is likely.<br>• The nature of release makes it likely that susceptible humans or ecosystems will be exposed and/or that releases will extend beyond a region or single ecosystem.<br>• In relation to exposed humans, routes of exposure are permissive of toxic, zoonotic or other adverse effects in susceptible humans. |
| **Medium** | • It is released into the environment, but quantity, duration and/or frequency of release is moderate.<br>• It may persist in the environment, but in low numbers.<br>• The potential for dispersal/transport is limited.<br>• The nature of release is such that some susceptible humans may be exposed and/or exposure will be of intermittent frequency and/or short duration.<br>• In relation to exposed humans, routes of exposure are not expected to favour toxic, zoonotic or other adverse effects. |
| **Low** | • **It is used in containment (no intentional release).**<br>• **The nature of release and/or the biology of the organism are expected to contain the organism such that susceptible populations or ecosystems are not exposed.**<br>• **Low quantity, duration and frequency of release of organisms that are not expected to survive, persist, disperse or proliferate in the environment where released.** |

### Uncertainty Related to Indirect Human Health Exposure Assessment

The ranking of uncertainty associated with the indirect human health exposure assessment is presented in Table 8. Although adequate information was provided by the notifier on the sources of exposure and measures in place at the two land-based facilities, conditions may be favourable for survival and dispersal of EO-1α Salmon if released from the Rollo Bay facility into the fresh water brook that leads to the Northumberland Strait. Since the notified organism is not intended for environmental release, uncertainty on potential human exposure may only arise as a result of accidental or unintended releases of EO-1α Salmon. Available information in the scientific literature indicates a potential for survival of these fish in the Canadian environment. Therefore, based on the available information on exposure scenarios in the Canadian environment, the human exposure to the notified organisms is considered **low** with **low** uncertainty. However, the uncertainty level for this exposure ranking is not expected to remain the same when taking into account Scenario A where non-transgenic and transgenic fish are produced alongside each other. Under this scenario, the uncertainty could likely be higher due to lack of information on operational procedures and potential use scenarios for activities involving external parties. Consequently, the uncertainty level for human exposure in such a scenario would increase as many of the factors involved (e.g., frequency, number of organisms involved, etc.) would be difficult to predict at any given time.

**Table 8. Uncertainty ranking associated with the indirect human health exposure**

| Available Information | Uncertainty Ranking |
|---|---|
| High quality data on the organism, the sources of human exposure and the factors influencing human exposure to the organism. Evidence of low variability. | Negligible |
| **High quality data on relatives of the organism or valid surrogate, the sources of human exposure and the factors influencing human exposure to the organism or valid surrogate. Evidence of variability.** | **Low** |
| Limited data on the organism, relatives of the organism or valid surrogate, the sources of human exposure and the factors influencing human exposure to the organism. | Moderate |
| Significant knowledge gaps. Significant reliance on expert opinion. | High |

## Hazard

### Zoonotic Potential

Fish-borne zoonoses are rare and tend to be restricted to a small number of opportunistic bacterial pathogens (Boylan 2011). Bacterial species that have been isolated from wounds and systemic infections following aquatic injuries and exposures include *Aeromonas hydrophyla, Chromobacterium violaceum, Edwardsiella tarda, Erysipelothrix rhusiopathiae, Mycobacterium marinum, Shewanella* species, *Streptococcus iniae* and *Vibrio vulnificus* (Diaz and Lopez 2015; Savini et al. 2017). There are no reported cases in the scientific literature of infections from these bacterial species resulting from Atlantic Salmon exposure. The land-based, closed containment structure of the Rollo Bay facility will help to mitigate zoonotic infections of cultured salmon.

Bacterial pathogens that have caused serious epidemics in farmed salmonids include *Renibacterium salmoninarum*, the causative agent in bacterial kidney disease, and *Aeromonas salmonicida* which causes furunculosis (OECD 2017). While there are no reported cases in the scientific literature of human infections by *R. salmoninarum*, there are recently reported cases from *A. salmonicida* (Acosta-García and Aguilar-García 2014; Tewari et al. 2014; Moore et al. 2017; Varshney et al. 2017). However, these infections resulted from other types of environmental exposure and were not zoonoses.

In addition to bacterial infections, humans suffer from numerous parasitic fishborne zoonoses (e.g., opisthorchiasis, intestinal trematodiasis, anisakiasis or diphyllobothriasis) many of which are caused by helminths (Chai et al. 2005). However, in most of these cases involving waterborne parasites, infections in humans are acquired through the consumption of raw or improperly cooked or processed fish (Boylan 2011) and not environmental exposure.

No pathogens of human health significance have ever been reported at the Bay Fortune facility, and no apparent adverse human health effects attributable to EO-1α Salmon exposure have been reported by the notifier's staff during two decades of operation. The notifier provided standard operating procedures (SOPs) for the Rollo Bay facility outlining the pathogen barrier procedure for staff and visitors as well as for handling mortalities and moribund salmonids. If EO-1α Salmon were to have an increased capacity to act as a reservoir for human pathogens, the nature and severity of adverse effects is expected to be relatively mild based on what is

known of topically acquired fish zoonoses reported in the scientific literature (Lehane and Rawlin 2000; Boylan 2011).

**Allergenicity/Toxigenicity**

Prevalence of fish allergy in the general population ranges from 0.2 to 2.29% and up to 8% in fish processing workers, with salmon among the major species of fish reported to cause allergic reactions (Sharp and Lopata 2014). Sensitization through aerosol and dermal exposure to fish protein allergens has been reported in occupational settings (Onesimo et al. 2012; Lopata and Jeebhay 2013) including a salmon processing plant (Dahlman-Höglund et al. 2012). Dermatitis and bronchial hyperreactivity have been reported in fish processing workers following exposure to fish infected with *Anisakis* sp. (Nieuwenhuizen et al. 2006). No apparent allergic reactions have been reported after more than 20 years of occupational exposure by staff of the notifier. However, susceptible individuals that are already allergic to fish proteins may also be highly likely to have an allergic response if exposed to EO-1α Salmon.

In 2014, the notifier requested an independent evaluation of the extant allergenicity data provided with NSN 16528 by the co-directors of the Food Allergy Research and Resource Program at the University of Nebraska - Lincoln. Their review concluded that both the diploid and triploid genetically modified Atlantic Salmon did not present any greater risk than non-modified Atlantic Salmon to those with fish allergies. Rehbein and Devlin (2009) found no indication of an increase in expression of parvalbumin (an animal food allergen common in fish) at either the mRNA or protein level in transgenic rapid-growing Coho Salmon (*Oncorhynchus kisutch*) compared to non-transgenics. Similarly, Nakamura et al. (2009) reported no difference in endogenous allergen expression in genetically modified Amago Salmon (*Oncorhynchus masou ishikawae*).

**Hazard Characterization**

Human Health Hazard considerations are presented in Table 9. The human hazard potential of the EO-1α Salmon is assessed to be low because:

1) The notified organism is a transgenic Atlantic Salmon containing a single copy of the opAFP-GHc2 integrant at a single locus that was confirmed to be stably integrated by PCR and Southern blots;

2) The methods used to produce the notified living organism do not raise any indirect human health concerns. Neither of the source organisms from which the inserted genetic material was derived (the Chinook Salmon and Ocean Pout) are known to produce toxins, nor are the inserted genetic material or expressed growth hormone associated with any toxicity or pathogenicity in humans;

3) While there are reported cases of zoonotic infections associated with fish, particularly for immunocompromised individuals, there are no reported cases attributed to either the notified organism or the wild-type Atlantic Salmon;

4) Data from allergenicity testing submitted previously did not indicate any increases in allergenic potential compared to non-transgenic counterparts, and sequence identities of the inserted transgene or any potentially expressed proteins from the constructs do not match any known allergens or toxins; and

5) The notifier states that there have been no apparent adverse indirect human health effects reported by staff of the Bay Fortune facility during 20 years of operation.

**Table 9. Considerations for hazard severity (human health)**

| HAZARD | CONSIDERATIONS |
|---|---|
| **High** | • Effects in healthy humans are severe, of longer duration and/or sequelae in healthy individuals or may be lethal.<br>• Prophylactic treatments are not available or are of limited benefit.<br>• High potential for community level effects. |
| **Medium** | • Effects on human health are expected to be moderate but rapidly self-resolving in healthy individuals and/or effective prophylactic treatments are available.<br>• Some potential for community level effects. |
| **Low** | • **No effects on human health or effects are expected to be mild, asymptomatic, or benign in healthy individuals.**<br>• **Effective prophylactic treatments are available.**<br>• **No potential for community level effects.** |

### Uncertainty Related to Indirect Human Health Hazard Assessment

The ranking of uncertainty associated with the indirect human health hazard assessment is presented in Table 10. Sequence analyses of the inserted genetic material did not match any known toxins or allergens and no reports in the scientific literature were found for adverse effects attributed to the inserted material in humans. Cases of zoonotic infections from fish are rare and most often associated with immunocompromised individuals. Consequently, the indirect human health hazard of EO-1α Salmon is considered to be **low,** with **low uncertainty** since much of the information on human health effects are based on reports from suitable surrogate organisms.

**Table 10. Categorization of uncertainty related indirect human health hazard.**

| Description | Uncertainty Ranking |
|---|---|
| There are many reports of human health effects related to the hazard, and the nature and severity of the reported effects are consistent (i.e., low variability); OR<br>The potential for human health effects in individuals exposed to the organism has been monitored and there are no reports of effects. | Negligible |
| **There are some reports of human health effects related to the hazard, and the nature and severity of the effects are fairly consistent; OR**<br>**There are no reports of human health effects and there are no effects related to the hazard reported for other mammals.** | **Low** |
| There are some reports of human health effects that may be related to the hazard, but the nature and severity of the effects are inconsistent; OR<br>There are reports of effects related to the hazard in other mammals but not in humans. | Moderate |
| Significant knowledge gaps (e.g., there have been a few reports of effects in individuals exposed to the organism but the effects have not been attributed to the organism). | High |

## Risk Characterization

In this assessment, risk is characterized according to a paradigm embedded in section 64 of CEPA that a hazard and exposure to that hazard are both required for there to be a risk. The

risk assessment conclusion is based on the hazard, and on what can be predicted about exposure from the notified use as well as any other potential uses.

Owing to the low potential hazard and the low potential exposure, and the effective containment procedures implemented at the land-based facilities, the human health risk associated with the use of the AquAdvantage® Salmon for commercial aquaculture production in land-based, contained facilities in Rollo Bay, PEI is assessed to be **low**.

## CONCLUSIONS AND ADVICE

For the environmental risk assessment, extensive and highly redundant physical containment during manufacture, transport and grow-out of EO-1α Salmon at the proposed land-based facility limits the potential for the notified organism to enter the Canadian environment. In addition, a large percentage of EO-1α Salmon at the proposed Rollo Bay facility, and all fertilized transgenic eggs leaving the proposed facility, will be treated to induce triploidy with high (>98.5%) efficiency, providing an additional level of biological containment to minimize exposure.

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. An alternate use scenario (Scenario B) where non-transgenic eggs are not sold to external parties, resulted in the likelihood of exposure of EO-1α Salmon to the Canadian environment to be ranked negligible and would result in negligible to low risk of EO-1α Salmon to Canadian environments.

There is no evidence to suggest a risk of adverse human health effects at the exposure levels predicted for the general Canadian population from commercial aquaculture production of EO-1α Salmon in land-based, contained facilities in Rollo Bay, PEI. This risk to human health associated with the EO-1α Salmon is not suspected to meet criteria in paragraph 64(c) of CEPA 1999.

## SOURCES OF UNCERTAINTY

Sources of uncertainty in the environmental 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 interactions in surrogate models, and limited understanding of applicability of data from surrogate models to EO-1α Salmon. The high level of detail of multiple and redundant forms 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 non-negligible 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). Where hazard ranking was expected to be context-specific, the highest conceivable ranking was used. Continued research into this area may change uncertainty of specific hazards, and alter the final hazard ratings in the future.

Sources of uncertainty in the exposure assessment for IHH included limited information on exposure scenarios in the Canadian environment, and lack of information on operational procedures and potential use scenarios for activities involving external parties. Sources of

uncertainty in the hazard assessment for IHH include reliance on reports from surrogate organisms.

## RISK MANAGEMENT

As containment is essential to minimizing risk of the EO-1α Salmon to the Canadian environment, it is imperative that all physical, biological, and operational containment measures proposed by AquaBounty Canada Inc. be maintained. Any changes to containment or expansion of the manufacture and grow-out facilities beyond what is being currently proposed could change the outcome of the environmental risk assessment and would require additional information to be provided to ECCC.

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, 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).

## OTHER CONSIDERATIONS

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.

The company might develop or strengthen SOPs regarding the transport of transgenic and non-transgenic fish at all life stages (e.g., mitigation of spills, locked transport boxes, labelling, don't transport during hazardous weather, etc.), and consider third party monitoring of the near-by brook by electrofishing. They might also consider third party auditing of SOPs.

It should be recognized that there are 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).

## LIST OF MEETING PARTICIPANTS

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## SOURCES OF INFORMATION

This Science Advisory Report is from the December 11-13, 2018 peer-review meeting, Environmental and Indirect Human Health Risk Assessments for the Manufacture and Grow-out of Sterile AquAdvantage® Salmon at a Land-Based Facility near Rollo Bay, PEI.

Additional publications from this meeting will be posted on the Fisheries and Oceans Canada (DFO) Science Advisory Schedule as they become available.

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