ASSESSMENT OF THE ACCEPTANCE OF DATA SUBMITTED UNDER *THE CANADIAN ENVIRONMENTAL PROTECTION ACT NEW SUBSTANCES NOTIFICATION REGULATIONS (ORGANISMS)* TO DETERMINE INVASIVENESS OF THE AQUADVANTAGE® SALMON

Context

The *Canadian Environmental Protection Act*, 1999 (CEPA), administered by Environment and Climate Change Canada (ECCC) and Health Canada (HC), is the key authority for the Government of Canada to ensure that all new substances, including living organisms, are assessed for their potential harm to the environment and human health. The *New Substances Notification Regulations (Organisms)* [NSNR(O)] under CEPA, prescribe the information that must be provided to ECCC prior to the import or manufacture in Canada of new living organisms that are animate products of biotechnology, including fish products of biotechnology. Fisheries and Oceans Canada (DFO), ECCC and HC have signed a Memorandum of Understanding respecting the implementation of the NSNR(O) for new living fish products of biotechnology, whereby DFO provides science advice to ECCC and HC in support of their CEPA risk assessment and decision making process.

On July 27, 2018, AquaBounty Canada Limited submitted a regulatory package to ECCC for the manufacture and production (grow-out) of AquAdvantage® Salmon (EO-1α salmon), a fast-growing, genetically-engineered Atlantic Salmon (*Salmo salar*), at a land-based aquaculture facility near Rollo Bay, PEI. 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. This information was assessed by DFO officials to determine if it meets the information requirements of the NSNR(O).

This Science Response Report results from the Science Response Process of November 2, 2018 on the Assessment of the acceptance of data submitted under the Canadian Environmental Protection Act New Substances Notification Regulations (Organisms) to determine invasiveness of the AquAdvantage® salmon.

Background

The following document elaborates the DFO analysis of information submitted under paragraph 5(a) of Schedule 5 of the NSNR(O)*The data from a test conducted to determine the pathogenicity, toxicity or invasiveness* for Notification NSN-19702 AquAdvantage® Salmon (EO-1α salmon), and advice to ECCC and HC on whether to accept the submitted data as fulfilling requirements. The document was peer-reviewed by representatives from DFO, ECCC, HC as well as external reviewers at a CSAS Special Response meeting on November 2nd, 2018.The document represents an overall consensus of the reviewers. To meet requirements under paragraph 5(a), the notifier provided peer-reviewed data from the scientific literature to address pathogenicity, toxicity and invasiveness of the notified organism; however, invasiveness is discussed here as the most relevant pathway in respect of ecological effects of the organism.

Information requirements for a test of invasiveness for organisms other than microorganisms can be found in Section 4.3.5.1. of *Guidelines for the Notification and Testing of New Substances: Organisms*. Specific tests are not described, rather: "A broad range of types of organisms can be notified under this schedule of the Regulations, and therefore, a broad range of types of tests can be conducted". However, it is stipulated that: "For cases of suspected invasiveness, the duration of the test should permit time for colonization and manifestation of effects in the test system."

Analysis and Response

In the case of EO-1α salmon, the complex life history of the organism negates the possibility for a single test to determine the organism's potential to establish in, and cause harm to, Canadian ecosystems (i.e., be invasive). The notifier provided a summary of available scientific literature relevant to fitness and potential effects of the organism, its relatives (i.e., different Atlantic Salmon lines containing the same transgene construct), and some non-Atlantic Salmon growth hormone (GH) transgenic fish models, to comply with data requirements for the test of invasiveness under paragraph 5(a) of Schedule 5 of the NSNR(O). However, there were significant gaps in the data such that invasiveness potential of EO-1α salmon under NSN-19702 cannot be systematically assessed. The assessment of EO-1α salmon invasiveness potential should be based on three key points: 1) potential for EO-1α salmon to survive in natural environments across all life stages; 2) the potential for EO-1α salmon to reproduce/spawn in natural environments or hybridize with native populations; and 3) potential for naturalized EO-1α salmon to cause harm to Canadian ecosystems. The information provided by the notifier is not sufficient to assess the invasive potential of the EO-1α salmon. In detail:

1. Survival

Data on survival in simulated natural conditions are only provided for EO-1α salmon at the first feeding stage, and under these conditions EO-1α fry had similar survival as non-transgenic fry (Moreau et al. 2011a). Other survival tests found EO-1α salmon had similar survival as non-transgenic fish in culture conditions over 6 months fed an experimental diet (Tibbetts et al. 2013), and when injected with Infectious Salmon Anaemia Virus (NSN-19702). The data provided by the notifier does not allow for a conclusion on potential survival of EO-1α in natural conditions over a full life cycle and in the various environments EO-1α salmon may inhabit.

The notifier suggested that EO-1α salmon would have diminished fitness and/or survival relative to wild type, as EO-1α salmon (Deitch et al. 2006; Polymeropoulos et al. 2014) or related GH transgenic Atlantic Salmon lines (Stevens et al. 1998; Abrahams and Sutterlin 1999; Cook et al. 2000a, 2000b) have altered metabolic and swimming capacity, altered stress response (EO-1α salmon, Cnaani et al. 2013), and decreased predator avoidance (related GH transgenic Atlantic Salmon, Abrahams and Sutterlin 1999) under culture conditions. However, whether these physiological alterations would be present when reared in diverse natural environments, or would impart a survival disadvantage under natural conditions has not been examined.

The notifier also provided data that hatchery/culture rearing can greatly diminish fitness in salmonids in nature (e.g., Muir 2004; Hansen 2006; Olsen and Skilbrei 2010; Milot et al. 2013).

While culture rearing, domestication and laboratory culture does decrease fitness in Atlantic Salmon, it does not prevent establishment or introgression with wild populations, the implications of which are not fully understood (see Glover et al. 2017 for the most recent review). As well, the potential survival and productivity of EO-1α salmon born in nature should they become naturalized (i.e., in the absence of culture effects) has not been assessed.

Finally, the notifier indicated that any escaped pre-smolt EO-1α salmon would not survive the change from fresh water to sea water. However, they did not provide evidence that pre-smolt life stages could not persist in local drainage or estuaries until ready to migrate. As such, it cannot be presumed that pre-smolt EO-1α salmon survival is not possible should they escape the facility.

2. Reproduction

The majority of EO-1α salmon at the proposed facility will be the sterile triploid all-female AquAdvantage® form. However, fertile female EO-1α salmon broodstock will be present in smaller numbers. In addition, as triploidy is not 100% effective in sterilization of salmon, there can be diploid fertile fish in the AquAdvantage® population (expected to be ≤1.5% of the AquAdvantage® population based on reported average triploidy induction success, NSN-19702). Fertile St. John River domestic broodstock will also be present at the proposed site. Consequently, should diploid female EO-1α salmon escape the facility they have potential to reproduce with St. John domestic fish should they co-escape, or with wild populations they may encounter. However, there are no data provided on the reproductive success and spawning capacity of female EO-1α salmon from culture, or that have come from nature or nature-like environments.

In addition to female EO-1α broodstock, there will be EO-1α neomale (genetic female, hormone treated to be phenotypically male) broodstock. Though these fish are expected to lack sperm-ducts and be functionally sterile, whether they will mature and participate in spawning competition is not known. This information is required for item 3 below (potential to cause harm), as neomales that successfully spawn with wild females would leave unfertilized and non-viable eggs, potentially decreasing wild population productivity. The notifier did provide data demonstrating culture-reared anadromous male EO-1α salmon have lower spawning success than nature-reared wild males (Moreau et al. 2011b). However, the effect of culture was not examined and whether the poor success of the EO-1α males is due to the presence of the transgene, or rearing history (as has been observed in other GH transgenic salmonids, Bessey et al. 2004; Leggatt et al. 2014), is not known. In other fast-growing salmonid models (GH transgenic Coho Salmon, Leggatt et al. 2014; domestic Atlantic Salmon, Glover et al. 2017), introgression into a wild population is much more likely through female than male fish. Without data demonstrating the reproductive potential of EO-1α female salmon, the potential for EO-1α salmon to establish in natural systems, or reproduce with wild populations, cannot be definitively assessed.

3. Potential to cause harm

The notifier stated in NSN-19702 “The data and comparisons provided in NSN-16528 clearly documented that, aside from the presence of the EO-1α transgene and the rapid-growth phenotype that results from the genetic modification, no significant differences existed between transgenic and non-transgenic Atlantic Salmon with respect to factors that might produce adverse ecological effects”.

In other GH transgenic fish models, strong alteration of appetite control genes have been reported, (Carp, Coho Salmon, Zebrafish, Zhong et al. 2013; Dalmolin et al. 2015; Kim et al. 2015), as well as alterations in behaviour (increased feed motivation, decreased predator avoidance). GH transgenic Coho Salmon fry reared in semi-natural streams have been demonstrated, in some circumstances, to have a growth or survival advantage over cohabiting wild-type fry (e.g., Sundström and Devlin 2011; Sundström et al. 2014; Leggatt et al. 2016; Leggatt et al. 2017). Further, the interaction between genotype and environment has been shown to affect phenotypes (body size and behaviour) of GH transgenic and wild-type fish in non-parallel ways, and can result in different consequences to experimental populations (i.e., biomass, numbers of individuals) between genotypes (Devlin et al. 2004; Sundstrom et al. 2007; Vandersteen et al. in press). As well, computer modelling and quantitative trait loci analysis in GH transgenic Coho Salmon found introgression of the transgene could potentially shift phenotypes in wild populations away from naturally selected optima (Ahrens and Devlin 2011), and have potential to influence evolutionary changes in wild populations (Kodama et al. 2018). Current data from other models (see above) does suggest the presence of a GH transgene and/or its phenotypic effects have potential to cause harm to ecosystem components (e.g., wild populations) under some conditions. However, genotype-by-environment interactions (see Devlin et al. 2015; Vandersteen et al. in press), different effects in different lines of transgenic fish in the same strain and species (Leggatt et al. 2017), and different effects of transgenesis in different strains and species (Devlin et al. 2001) have been demonstrated. Consequently, studies from other models should not be used as direct evidence for EO-1α salmon, but rather can be used to make general conclusions, identify areas of uncertainty, or identify areas for study on potential invasiveness of GH transgenic fish. The notifier provided data demonstrating EO-1α first-feeding fry did not have a competitive advantage or disadvantage relative to wild type, and had similar growth and survival under low and high density in stream microcosms (Moreau et al. 2011a). As well, hybrids of EO-1α salmon and Brown Trout suppressed the growth of populations of both EO-1α and wild-type fry in simulated streams (Oke et al. 2013), although whether this was due to hybridization, the transgene, or a combination was not examined. Potential effects of EO-1α salmon at other life stages and/or under other naturalized conditions have not been reported, nor have potential effects of introgression of the EO-1α transgene into wild Atlantic Salmon populations or potential for neomales to decrease wild salmon productivity through reproductive interference. Consequently, the potential for EO-1α salmon to cause harm, as part of invasiveness, cannot be definitively assessed.

Discussion, Conclusions and Recommendations

The limited or missing data on full lifecycle survival and female reproductive potential of EO-1α salmon under a range of relevant natural conditions, and limited data on the potential for EO-1α salmon to cause harm to natural ecosystems, prevent a systematic assessment of the invasiveness of EO-1α salmon in this context. It was highlighted during the discussion that there's additional information available in the literature on similar organisms that the company did not address. Though some data for each section (survival, reproduction, potential to cause harm) was provided, when taken together in the context of invasiveness, it was considered insufficient to draw meaningful conclusions. Consensus was that considering the missing information on EO-1α salmon (i.e., information from public domain and GoC's research), and the difficulty in extrapolating information from other organisms, the data provided were not robust enough to support any conclusions on invasiveness. Additional issues to address include the potential downstream effects of culture, multigenerational (including epigenetic) effects of

EO-1α salmon in naturalized conditions, and responses of EO-1α salmon to a range of different relevant environmental conditions in the context of invasiveness. The consensus of the November 2nd , 2018 meeting was that the information and data provided in NSN-19702 to address requirements for paragraph 5(a) of Schedule 5 of the NSNR(O) on invasiveness were not sufficient to make a systematic assessment of invasiveness of EO-1α salmon. The consensus recommendation was to not accept the information as complete for paragraph 5(a). As a consequence, a waiver request was submitted for this requirement with a rationale indicating that the containment measures to be put in place at the Rollo Bay facility will effectively prevent release of the organism into the environment and thus mitigate any potential risks to the environment from invasiveness. This was considered in the environmental risk assessment.

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