# Justification for the Mitigated Determination of Non-Significance (MDNS) for Washington Department of Fish and Wildlife SEPA 19-056 and for the Approval of Cooke Aquaculture Pacific's Marine Aquaculture Permit Application

Washington Department of Fish and Wildlife
(contact: Dr. Kenneth I. Warheit; kenneth.warheit@dfw.wa.gov)

January 21, 2020

## INTRODUCTION

In January 2019, Cooke Aquaculture Pacific (hereafter termed Cooke) submitted to the Washington Department of Fish and Wildlife (WDFW) two applications: (1) an application to renew an expiring 5-year Marine Aquaculture Permit to continue to culture Atlantic salmon (*Salmo salar*) at Cooke's marine net-pen facilities in Puget Sound; and (2) an application for a new 5-year Marine Aquaculture Permit to transition production from Atlantic salmon to all-female triploid (sterile) steelhead trout (*Oncorhynchus mykiss*) at Cooke's existing marine net-pen facilities in Puget Sound. Included with these applications were Fish Escape, Prevention, Response, and Reporting Plan; Regulated Finfish Pathogen Reporting Plan; Plan of Operation for All-female Triploid Rainbow Trout[1]; and Plan of Operation for Atlantic Salmon Rearing.

In March 2019, WDFW approved and issued to Cooke a renewal of their 5-year Marine Aquaculture Permit for Atlantic salmon, contingent on the requirement specified in EHB 2957 that farming of nonnative marine finfish in Puget Sound is valid only with a current lease of state-owned aquatic lands. At the same time, WDFW responded to Cooke's second application by informing them that before WDFW could decide on their permit application a SEPA process was required to determine the environmental effects of transitioning production from Atlantic salmon to all-female, triploid steelhead trout at their existing facilities. On July 25, 2019 Cooke submitted to WDFW a completed SEPA checklist and a set of supporting documents, including information that would add to or complement the 1990 PEIS for environmental impact of fish culture in floating net-pens located anywhere in Washington State marine waters.

On October 1, 2019 WDFW issued a Mitigated Determination of Nonsignificance (MDNS) for Cooke's proposed action described in their SEPA Checklist and supporting documents. We emphasize here and elsewhere that this determination is specific and limited to Cooke's proposed action: *to transition production from Atlantic salmon to all-female, triploid steelhead trout in existing Puget Sound net-pen facilities*. This SEPA determination is tied to WDFW's substantive decision on the 5-year Marine Aquaculture Permit application for steelhead trout. This SEPA determination anticipates and discusses Cooke's planned transfers of juvenile steelhead trout from its freshwater hatchery to marine net-pens

[1] Rainbow Trout is the standard commercial aquaculture terminology for the species *Oncorhynchus mykiss*, which also includes steelhead trout. In many of their documents, Cooke uses the commercial aquaculture terminology. Since these fish are being reared in salt water, in this document we will refer to the species as steelhead trout.

facilities as part of its regular operations. These transfers would require finfish transfer permits from WDFW. The current SEPA determination therefore is intended to double as the SEPA analysis for all anticipated transfer permits inherently connected to Cooke's operations approved under the 5-year Marine Aquaculture Permit. Each individual Finfish Transport Permit application would still require a fish health and net-pen facility evaluation.

On January 21, 2020, WDFW granted Cooke's application for a 5-year Marine Aquaculture Permit to transition production from Atlantic salmon to all-female, triploid steelhead trout at their existing Puget Sound net-pen facilities where they have valid aquatic lands leases from the Department of Natural Resources (DNR).

This document provides a description of WDFW's deliberative process associated with our SEPA determination and 5-year Marine Aquaculture Permit decision. In an upcoming document WDFW will provide a response summary to the comments we received during the 52-day Public Comment period associated with our October 1, 2019 SEPA determination. However, answers to most public comments can be found within this document.

# DELIBERATIVE PROCESS

## 1. Regulatory Authority

### 1.1. 2018 law sunsetting non-native finfish marine net-pen aquaculture

EHB 2957: "AN ACT Relating to reducing escape of nonnative finfish from marine finfish aquaculture facilities."

EHB 2957 became 2018 session law June 7, 2018, after passing the Washington Legislative House on February 14, 2018 and Senate on March 2, 2018, and signed by Governor Inslee on March 22, 2018. In signing the bill, Governor Inslee issued a partial veto, deleting Section 1 of the bill from the enacted law. The Governor stated that "[s]ection 1 is unnecessary to implement the bill and [he does] not agree with all the assertions made in this section." Despite the Acts title, the law's intent is three-fold: (1) the elimination of commercial nonnative finfish marine aquaculture; (2) the elimination of escapes of finfish from commercial marine net-pens; and (3) the completion of a guidance document for the planning and permitting of commercial finfish marine net-pen aquaculture. With Governor Inslee's veto of Section 1, the new law does not characterize commercial marine net-pen aquaculture as posing unacceptable risks to native salmon or the marine environment.

The new law, with bipartisan support, and the clear and explicit backing from many tribes and environmental NGOs *unambiguously allows for the continued operation of commercial net-pen aquaculture in Puget Sound, including in areas where current operations currently exist*. The new law imposes only a few constraints related to the continued operations of commercial net-pen aquaculture: (1) Washington Department of Natural Resources (DNR) may not allow the commercial culturing of nonnative finfish as an authorized use under any new state-owned

aquatic lands lease, and DNR cannot renew or extend current leases for nonnative finfish aquaculture beyond their current termination date; (2) Washington departments of Ecology (Ecology) and Fish and Wildlife (WDFW) may authorize or permit the commercial culturing of nonnative finfish, or related activities only if these activities are performed under a valid lease of state-owned aquatic lands; (3) approximately every two-years, when net-pens are fallow, each facility must be inspected by an independent marine engineering firm, approved by WDFW, and to receive fish the facility must be considered in good working order; and (4) WDFW is authorized to require the immediate removal of fish from a net-pen, or deny a transport permit if the facility is in "imminent danger of collapse or release of finfish."

The commercial culturing of native finfish (e.g., all-female triploid steelhead trout) in marine net-pens in Puget Sound is not constrained by this new law. If the commercial aquatic farmer has an aquatic farm registration, valid DNR lease, and appropriate permits from WDFW and Ecology, based on state law, **that farmer (e.g., Cooke) can legally operate native finfish net-pen aquaculture in the marine waters of Washington State**.

### 1.2. Washington State Regulations

WDFW received from Cooke in January 2019 an application for a new Marine Finfish Aquaculture Permit to raise all-female triploid steelhead trout in existing net-pen facilities in Puget Sound. Along with the permit application itself, Cooke also submitted a "Fish Escape Prevention, Response and Reporting Plan," "Regulated Finfish Pathogen Reporting Plan," and a "Plan of Operation for All-female Triploid Rainbow Trout (*Oncorhynchus mykiss*)." In March 2019 WDFW notified Cooke that the Marine Finfish Aquaculture Permit application would require analysis under SEPA (RCW Chapter 43.21C and WAC Chapter 197-11).

#### 1.2.1. WDFW Aquaculture Rules and Regulations

WDFW's aquaculture rules and regulations are described in RCW Chapters 77.115 and 77.125, and WAC Chapter 220-370. WAC 220-370 was last updated June 6, 2017, roughly two months prior to the collapse of Cooke's Cypress #2 net-pen facility. WDFW currently is updating this WAC Chapter to be reflect changes in the new law (see Section 1.1 above), as detailed in RCW 77.125.

##### 1.2.1.1. Limitations to WDFW Authority

With respect to commercial marine net-pen finfish aquaculture, WDFW's authority is constrained by RCWs 77.115 and 77.125, and WAC 220-370. In general, WDFW's authority is limited to (1) assessing and controlling the transmission of disease; (2) assessing genetic and ecological risk of net-pen operations to native species and their habitat; (3) preventing, reporting, and recapturing finfish released from commercial net-pen facilities; and (4) determining if the structural integrity of net-pen facilities is sufficiently adequate to receive or continue to hold the aquacultural product (e.g., Atlantic salmon or steelhead trout). In administering a disease control program, the Director of WDFW "shall not place constraints on or take enforcement actions in respect to the aquaculture industry that are more rigorous than those placed on the department or other fish-rearing entities" (RCW 77.115.010(6)).

#### 1.2.1.2. Marine Finfish Aquaculture Permit (WAC 220-370-100)

The Marine Finfish Aquaculture Permit is described wholly in WAC 220-370-100. This Section requires that the Marine Finfish Aquaculture Permit applications be accompanied by escape prevention and escape reporting and recapture plans. The Director of WDFW can either approve or deny a permit application, and the reasons for denying an application are explicitly stated as "significant genetic, ecological or fish health risks of the proposed fish rearing program on naturally occurring fish and wildlife, their habitat or other existing fish rearing programs" (WAC 220-370-100(1)). WDFW's aquaculture regulations do not allow the Director to deny a Marine Finfish Aquaculture Permit application based on economics, social, political, or other concerns, nor is the decision subject to a vote of the people. The Director's concerns here are limited to *significant* genetic, ecological, and fish health risks.

A provision in this rule stipulates that "transgenic" fish are prohibited from being used in marine finfish aquaculture. The rule defines transgenic as the "actual transfer of genetic material from *one species to another*" (WAC 220-370-100(1)).

#### 1.2.1.3. Finfish Transport Permit (WAC 220-370-190)

It is unlawful for any person to import into or transport within the state of Washington finfish aquaculture products (e.g., live fish, embryos (fertilized eggs), or gametes) without a Finfish Transport Permit (FTP). An FTP application is complete when all required information is submitted, including laboratory results from disease testing. No FTP application will be approved unless the aquaculture products being transported are free of regulated pathogens (see WAC 220-370-050(20)). In addition, the Director of WDFW can condition an FTP (1) "to ensure the protection of aquaculture products and native finfish from disease when the director concludes that there is a reasonable risk of disease transmission associated with the finfish aquaculture products" (WAC 220-370-190(2)); (2) to ensure the structural integrity of the net-pen facility; (3) and to prevent the captive finfish from escaping (see above Section 1.1).

#### 1.2.1.4. Aquaculture Disease Control (WAC 220-370 -080, -180, -190, -240)

All aquatic farms, including marine net-pens, are subject to inspection by WDFW "for the prevention and suppression of aquaculture diseases, including, but not limited to, taking samples for detection of regulated finfish pathogens and other diseases" (WAC 220-370-080). Aquatic farmers are required to report by the end of the following day the detection of regulated pathogens regardless of whether fish are showing symptoms of disease or appear healthy (WAC 220-370-190(2)). If an outbreak occurs at any aquaculture facility, the aquatic farmer is required to report the outbreak immediately to WDFW (WAC 220-370-180). WDFW has great latitude to order emergency actions if the Director determines that such actions are necessary to protect native stocks from disease that will cause severe mortality. These actions include denial of a transport permit, quarantining the aquaculture products, confiscating or ordering the destruction of the aquaculture products, or requiring that the products be removed from state waters (WACs 220-370-190 and 220-370-240).

**When Cooke submitted their completed Marine Finfish Aquaculture Permit application in January 2019, they complied with the requirements of WAC 220-370-100 by submitting with their application an Escape Prevention, Response and Reporting Plan, which includes all elements required by the Escape Prevention plan (WAC 220-370-110) and Escape reporting and recapture plan (WAC 220-370-120). In their Marine Finfish Aquaculture Permit application Cooke proposed a legal activity. WDFW's regulatory authority is limited and applications can be denied only if there are significant genetic, ecological, and fish health risks, or if the net-pen infrastructure is impaired enough to risk the escapement of the aquacultural product. WDFW's regulatory oversight allows for the inspection of the infrastructure, evaluation of the facilities biosecurity, and the testing of finfish for pathogens of concerns.**

### 1.2.2. Washington State SEPA Rules (WAC 197-11)

After receiving an environmental checklist and all supporting documents, the lead agency undertakes a deliberative process and makes a threshold determination (WAC 197-11-797). In making a threshold determination, the lead agency must: (1) review the environmental checklist and all supporting documents; (2) determine if the proposed action is "likely to have a probable significant adverse environmental impact"; (3) consider procedures that may mitigate or minimize environmental impacts (WAC 197-11-768); and (4) determine if the proposed action had been analyzed in a previous environmental document (e.g., a previously prepared EIS), which can be adopted or incorporated by reference; among other elements (see WAC 197-11-330).

A "determination of significance" (DS) is made when the lead agency concludes that the proposed action would have a *probable significant* adverse environmental impact, and a DS would then require an EIS (WAC 197-11-736). It is important to note that an EIS is required *after* a determination of significance is made. SEPA defines "significant" as "a reasonable likelihood of more than a moderate impact on environmental quality" (WAC 197-11-794). SEPA does not define reasonable, likelihood, or moderate, but WDFW considered an action to have significant adverse environmental impact if a review of the scientific literature, including any existing regulatory documents, including prior EISs, supplemented by data analysis and consultation with experts, suggest that the proposed action under consideration will produce a more than moderate adverse effect. A "determination of nonsignificance" (DNS) is the opposite of a DS; that is, the proposed action will not have a significant adverse environmental impact or that the impact is something less than moderate (WAC 197-11-340). A proposed action can lead to a mitigated DNS, if the implementation of the mitigating provisions minimizes environmental impacts that otherwise may have resulted in a DS.

**SEPA anticipates an evaluation of an application prior to making a threshold determination, and if based on that evaluation, a DNS or a mitigated DNS is made, an EIS is not required. <u>Since Cooke's proposed action is limited to switching production from Atlantic salmon to all-female triploid (sterile) steelhead trout</u>, WDFW's evaluation of their**

**application was limited to the genetic and biological risks associated with that action, and to the structural integrity of the net-pen infrastructure, as required by EHB 2957.**

### 1.2.3. Fish Culture in Floating Net-Pens Final Programmatic EIS (January 1990)

At the direction of the Washington State Legislature, the Washington Department of Fisheries (WDF) in 1990 prepared a non-project or programmatic EIS (PEIS), in consultation with the departments of Ecology, Natural Resources, and Agriculture. The purpose of the EIS was two-fold: to assess the adequacy of the existing regulations that affect commercial marine net-pen aquaculture; and to present a Preferred Alternative that identifies governmental actions aimed at reducing or eliminating significant adverse environmental impacts. For each of nine elements of the Natural Environment[2], and the nine elements of the Built Environment[3] the PEIS describes the affected environmental element, impacts from commercial net-pen aquaculture on the affected elements, mitigation measures, and unavoidable significant adverse impacts. The PEIS also considered the cumulative impacts of the number and geographic distribution of fish farms (commercial net-pen facilities) in Puget Sound. This PEIS is foundational to any serious evaluation of the commercial finfish net-pen aquaculture in Washington State. Not only does the PEIS lay out 18 wide-ranging environmental elements that may be impacted by net-pen aquaculture, it provides actions that would mitigate for adverse impacts, discusses unavoidable significant adverse impacts (based on 1990 technology), and includes 22 pages of references. **WDFW is required by SEPA to ascertain if previous environmental documents are relevant to or have already addressed marine net-pen aquaculture in Washington State, and therefore, we incorporated by reference the PEIS in our SEPA determination (see above 1.2.2, first paragraph, #4). The incorporation of the 1990 PEIS in our SEPA determination does not indicate that the document was the only or main source used for our determination.**

## 2. Tribal Consultation

The Centennial Accord between federally recognized Indian tribes in Washington State and the State of Washington, dated Aug 4, 1989, provides a framework to implement government to government relationships. WDFW recognizes the sovereignty of each federally recognized Indian tribe in Washington and strives to implement government to government coordination to improve communication around Department decisions that may impact treaty resources. As such, WDFW and the Swinomish Indian Tribal Community (SITC) held a government to government consultation on December 17, 2019 to discuss impacts to treaty resources presented by Cooke's proposal to raise all-female triploid steelhead trout. The SITC emphasized that Cooke's Hope Island net-pen was of paramount concern as it lies within the boundaries of their ancestral homelands. In addition, the SITC presented testimony that the presence of the net-pens impedes their fishing treaty rights. The SITC raised additional concerns about disease transmission, genetic introgression, and ecosystem quality, which we address below in Section 4.

[2] Bottom sediments and benthos, water quality, phytoplankton, chemicals, food fish and shellfish, importation of new fish species, genetic issues, disease, and marine mammals and birds.

[3] visual quality, navigations, commercial fishing, human health, recreation, noise, odors, upland and shoreline use, and local services

WDFW appreciates the concerns raised by the SITC regarding the presence of the net-pens and the possible impacts to their fishing rights. As summarized above, *Cooke's proposed action is limited to switching production from Atlantic salmon to all-female triploid steelhead trout*. WDFW's evaluation of Cooke's application is limited by our regulatory authority over the genetic and biological risks associated with Cooke's proposed action, and to the structural integrity of net-pen infrastructure, as required by EHB 2957. WDFW's SEPA review and determination is limited in scope to Cooke's proposed action, which does not include siting issues related to already existing net-pen infrastructure. Therefore, for this SEPA action, WDFW will not review the impact of Cooke's Hope Island net-pen on SITC's fishing treaty rights.

## 3. Cooke's SEPA Checklist and Supporting Documents

As part of the SEPA process, Cooke submitted the required SEPA Environmental Checklist, with the following supporting documents: (1) Troutlodge Triploid Testing Results (Attachment A); (2) Additional Information: Response to WDFW Questions (Attachment B); (3) Annotated Bibliography, Prepared by Walton Dickoff, Ph.D. and Don Weitkamp, Ph.D (Attachment C); (4) Threatened and Endangered Species: 1990 Programmatic EIS Update (Attachment D); and (5) Curriculum Vitae: Don Weitkamp, Ph.D, and Walton Dickoff, Ph.D. (Attachment E). Also included with the SEPA package was a transmittal letter from Cooke and a map of the net-pen facility locations. Cooke's SEPA submission was filed by WDFW as SEPA #19056 and the entire package can be downloaded from WDFW's SEPA website: https://wdfw.wa.gov/licenses/environmental/sepa/open-comments, while the file remains open, or https://wdfw.wa.gov/licenses/environmental/sepa/closed-final, after the SEPA process closes.

## 4. WDFW's Environmental Review

### 4.1. Summary of types of data included

WDFW first began our analysis of Cooke's proposal using the information provided by Cooke in the SEPA Checklist and supporting documents (Section 3). Initially, we considered the 1990 PEIS, and these summary publications: Nash 2001, Waples et al. 2012, Price and Morris 2013, Rust et al. 2014, Hawkins et al. 2019. However, our primary evaluation was based on over 300 publications, including publications as recent as 2020. This document cites nearly 150 documents. In addition to the literature, we consulted with experts within and outside of WDFW, used unpublished data or analyses when required, and considered public comment.

### 4.2. Disease, Pathogen, and Parasite Control

#### 4.2.1. Introduction

Among the often-stated concerns associated with open net-pen aquaculture, voiced in public comment and in some scientific publications, is that marine aquaculture promotes (1) the introduction of non-native pathogens, (2) amplifies rate of infection and therefore amplifies pathogen abundance, (3) promotes the increase in virulence of existing pathogens or is the nexus for the emergence of new pathogens, and (4) promotes disease in wild finfish. All these elements are thought to add risk to the viability of listed populations.

Disease in an organism is a function of the interaction between the environment (e.g., stress resulting from too high or low temperatures, high densities, lack of food; pollution), the infectious (e.g., pathogen) or non-infectious (e.g., toxin) agent, and the organism itself (e.g., genetics, immune system) (Reno 1998). In aquaculture there is an attempt to manage all three components to control pathogens and parasites, and to prevent disease (McVicar 1997).

Disease management in marine aquaculture of salmonids begins with the source material – the origin and health status of the broodstock, of the embryos, and of juvenile fish reared in freshwater hatcheries. By preventing the introduction of pathogens, especially non-native pathogens, into the cultured environment, the health status of the populations may be maintained. WDFW's regulatory authority is designed to prevent the introduction of specific pathogens by testing fish, gametes, and embryos at their source and preventing their transport if they test positive for these specific pathogens. Best management practices while the fish are cultured in marine waters can reduce stress thereby reducing risk of infection, and disease amplification and transmission. Vaccinations prepare the individual organisms' immune system to combat pathogens, and to reduce the risks of infection, pathogen amplification and transmission, and disease.

### 4.2.2. Importation of non-native pathogens

There are both Federal (50 CFR 16) and Washington State (see Section 1.2 above) regulations that govern the importation of salmonid gametes, embryos, and live fish into Washington State. Federal rules apply only to international importation, while Washington State rules apply to any gametes, embryos, or live fish that are transported into or through Washington State, regardless of their origin. Both Federal and State rules require that the live fish or the broodstock that produced the gametes or embryos be free of the viruses causing viral hemorrhagic septicemia (VHS), infectious hematopoietic necrosis (IHN), infectious salmon anemia (ISA; WDFW only), and infectious pancreatic necrosis (IPN), and *Oncorhynchus masou* virus, (all five viruses collectively referred to as "regulated viruses"). Rules also apply to the disinfection of the surface of embryos. In addition, since early 2018, WDFW requires that the live fish or the broodstock that produced the gametes or embryos be tested for both piscine orthoreovirus-1 (PRV-1) and PRV-3. Transport permits will be denied if the fish or broodstock test positive for North Atlantic Ocean variants of PRV-1, any variant of PRV-3, or any of the regulated viruses listed above. Lastly, WDFW requires a second round of tests after hatching when the fry's yolk sack is absorbed. If at this time the lot of fish tested positive for regulated pathogens or North Atlantic PRV-1, WDFW would require either destruction of the lot or deny any transport permit application to move live fish out of the freshwater hatchery.

Since the WDFW PRV testing requirements went into effect in 2018, WDFW denied two separate transport permit applications from Cooke because their Atlantic salmon that originated from Iceland tested positive for North Atlantic variants of PRV-1.

In their Marine Aquaculture Permit application Cooke proposes to culture only all-female triploid steelhead trout from Troutlodge, a Washington State company based out of Bonney

Lake. The broodline Cooke will use in their operation was locally derived from Puyallup River (Puget Sound) steelhead trout around 1960. WDFW will verify genetically that each lot is from this locally derived, native population of steelhead trout. **Therefore, by switching from culturing Atlantic salmon, originating from the North Atlantic, to native, locally derived steelhead trout, Cooke will dramatically reduce the risk of importing non-native pathogens.**

### 4.2.3. Disease Prevention

#### 4.2.3.1. Biosecurity

We define biosecurity as precautions taken to minimize the risk of introducing, establishing, and spreading an infectious disease in an aquatic animal population. This includes, but is not limited to, disinfection of equipment, use of foot baths, limiting personnel movement, fish health monitoring, and general cleaning practices. Biosecurity also includes management activities that are designed to reduce or eliminate stress to the cultured fish. Stress can negatively affect the immune system, which can increase the fish's vulnerability to disease.

"To promote good health in farm stocks, it is in the self-interest of fish farmers to maintain good environmental conditions in their farms and in the surrounding areas" (McVicar 1997:1095). To accomplish this, and as required by WDFW, each year Cooke provides an updated "Regulated Finfish Pathogen Reporting Plan" that is reviewed and requires approval by WDFW. Within this Plan is a biosecurity section entitled "Disease Prevention and Control Measures." This section includes descriptions of specific management activities that "are designed to reduce the risk of disease occurrence at each farming location and help prevent transmission of pathogens" (p. 2 of Plan). The biosecurity activities start at the spawning facility where embryos are disinfected prior to shipping. Fish are tested for Regulated Pathogens at 30 days post swim-up after hatching, and again prior to transport to marine net-pens. Biosecurity measures continue while the fish are reared in the net-pens, and there are routine fish health exams administered by Cooke. WDFW will inspect each facility at least once per year, but more optimally twice per year. During these inspections, fish will be sampled for the presence of Regulated Pathogens and PRV.

Cooke maintains single generation stocking of their net-pens, which is a biosecurity measure that reduces stress and breaks pathogen transmission chains (see Section 4.2.4). Net-pens are also fallowed for at least 42 days after harvest and before restocking. This will allow time for the containment and predator nets to be cleaned and repaired, and contributes to breaking pathogen transmission chains.

#### 4.2.3.2. Vaccination

The purpose of a vaccine is to provide immunological protection against a specific pathogen to prevent the onset of disease. Vaccines work by providing an initial or primary immunization – a response to an antigen (i.e., the vaccine) that results ultimately in the production of antibodies (Newman 1993). Vaccines prime the

immune response by creating B-cell lymphocytes (plasma cells) that produce the antibodies that are specific to the antigen presented by the vaccine. When an individual fish encounters the pathogen for which the vaccine was produced, the immune system is already primed to secrete antibodies specific to that pathogen. This can result in a range of responses from the amelioration of clinical signs to a rapid immunological response and the prevention of infection and disease. The efficacy of a vaccine varies depending on the type of vaccine, the immunological response, and the pathogen itself. Not all vaccines are 100% efficacious, and when they are effective, that effectiveness may not last through the life of the individual fish.

**Cooke vaccinated each juvenile Atlantic salmon by injection prior to their transport to salt water, targeting Infectious Hematopoetic Necrosis virus (IHNV) and the following bacteria: *Vibrio anguillarum*, *V. ordali*, *Aeromonas salmonicida*, *Tenacibaculum maritimum*, *Piscirickettsia* sp., *Moritella viscosa*, and *Allivibrio wodanis***. Vaccines have not been developed for all pathogens, and which vaccines are administered is based on the experience and knowledge of the veterinarian of record (VOR) who is licensed in Washington and has established a veterinary-client-patient-relationship (VCPR) with Cooke and the fish. Cooke anticipates using a subset of the suite of vaccines used for Atlantic salmon for their production of all-female triploid steelhead trout, focusing initially on IHNV, *V. anguillarum*, *V. ordali*, *A. salmonicida*, *and M. viscosa*.

### 4.2.4. Pathogen Amplification and Transmission

Net-pen aquaculture can present a variety of disease risks to wild populations (McVicar 1997, Kurath and Winton 2011). Left unmitigated, these risks may have negative effects on these populations. Aquatic farms are monocultures where fish may be handled extensively and are crowded into unnaturally high densities in environments that are not optimal for the fish. These conditions may lead to immune suppression, placing net-pen fish at risk of infection and disease (Murray and Peeler 2005, Kurath and Winton 2011). When fish are moved from the freshwater hatchery environment to the marine net-pens, they are subjected to a new environment that contributes to stress. These fish also are exposed to "wild" pathogens. The monoculture, high densities, suppressed immune systems, and the presence of wild pathogens to which these fish are naïve are conditions that can promote the amplification and transmission of these pathogens among the cultured fish (Kurath and Winton 2011). These conditions can lead to disease outbreaks, placing the farm fish population at risk.

Any disease outbreak is detrimental both to fish farms and to the aquaculture industry. Diseased individuals require treatment and treatment is expensive. Some fish will die, further eroding the business' profit margins. For these reasons the aquaculture industry is motivated to reduce the incidence of disease. For example, in Norway, risk of salmon alphavirus (SAV) and infectious salmon anemia virus (ISAV) transmission was mitigated by coordinating among neighboring farms the stocking, harvesting, and net-pen fallowing. Vaccination and early pathogen detection programs were implemented, as were veterinary prescribed treatments (Jones et al. 2015). As discussed above, Cooke runs their Atlantic

salmon net-pens as single generation operations, limiting the number of times fish are handled thereby reducing some stress that may promote infection and disease. Net-pens are fallowed, and nets are cleaned following harvest eliminating potential sources of pathogens and breaking pathogen transmission chains. Fish are vaccinated for a set of pathogens, reducing the risk of infection and disease. At the onset of certain diseases, fish are treated with antibiotics (see Section 4.2.7), and WDFW regulations and Cooke's biosecurity protocols reduce the introduction of certain pathogens into net-pen populations. These mitigating operations reduce the risk of infection and disease within Cooke's Atlantic salmon program, and these same mitigating operations will also be in place for Cooke's steelhead trout program.

Despite these mitigating operations, farm fish will become infected by wild pathogens transmitted from wild populations. If fish farms amplify these pathogens, are individuals from the wild populations at increased risk if the pathogens spill back into the wild environment? Kurath and Winton (2011:73) demonstrated that "viruses move from wild fish reservoirs to infect domestic fish in aquaculture more readily than 'domestic' viruses move across the interface to infect wild stocks." They also showed 15 examples of pathogens moving from wild populations to domestic populations, and only five examples for the reverse transmission. Taranger et al. (2015:1008) state "[f]or most pathogens, clear evidence for transmission from farmed to wild fish is limited . . . . [and that] [m]ost of the diseases that currently cause problems in fish farms are likely enzootic, originating from wild fish stocks." Taranger et al. (2015) focused on four viruses and their associated diseases that result in outbreak conditions in the Atlantic salmon industry in Norway. Included in this study was heart and skeletal muscle inflammation (HSMI) and its etiological agent piscine orthoreovirus (PRV). In each of these four cases, the viruses do occur in the wild populations, to varying degrees, and may have been transmitted from the farm fish back to the wild fish (and from farm population to farm population), but the incidence of disease was either extremely low or non-existent in the wild populations. Overall, although there may be a few documented cases of bacterial or viral transmission from fish culture to wild populations, only a small subset of those involve marine net-pens (Kurath and Winton 2011), and there is limited evidence that these transmissions result in disease in the wild populations, even if the transmission is associated with disease outbreaks in the net-pens (Wallace et al. 2017).

The net-pen environment differs from the wild environment, which affects pathogen transmission, and the incidence of infection and disease. This helps explain why the amplification of wild pathogens by farmed fish does not appear to put wild populations at increased risk of disease. Wild salmonid populations, for example, would be exposed to net-pen pathogens as they migrate from fresh- to marine-water as juveniles and when they return to freshwater as adults. These populations are not subjected to the stress-inducing net-pen environment, as they travel in densities considerably lower than what occur in net-pens (Kennedy et al. 2016). The pathogens themselves do not stay concentrated in halos around net-pens, as water movement diffuses the pathogens (Brooks 2005, Brooks and Stucchi 2006), and solar radiation and microbial activity may further reduce pathogen numbers (Garver et al. 2013). Disease is intermittent within the net-pen environment, and

net-pens are not a continual source of pathogens. There is evidence that pathogens can remain in sufficient concentrations to cause infection as they are dispersed from their source net-pen, but the evidence is based only on farm to farm transmission, not farm to wild transmission, and that transmission is limited by distance and time (e.g., Gustafson et al. 2007, Salama and Murray 2011, Murray 2013, Salama and Murray 2013). Compared with farmed fish, wild fish are not immune compromised, and they travel through environments that are not favorable for the transmission of pathogens. Except perhaps in freshwater spawning aggregations, and in freshwater hatcheries, wild fish are exposed to pathogen densities that are lower than that within net-pen facilities, even in wild environments in the vicinity of farms that are experiencing a disease outbreak.

### 4.2.5. Pathogen Virulence and Emergence of New Pathogens

Similar to the amplification of wild pathogens within aquatic farms discussed above, stocking densities and aquacultural practices can lead to the emergence of new diseases and the increase in virulence of existing pathogens (Murray and Peeler 2005, Mennerat et al. 2010, Pulkkinen et al. 2010, Walker and Winton 2010, Kennedy et al. 2016). Based on the evolution of virulence theory, Kennedy et al. (2016) outlined factors related to aquaculture operations that may lead to the increase in virulence of existing pathogens. These factors include rearing at high densities, compression of the rearing cycle, use of broodstock with limited host genetic diversity, and accepting endemic disease in cultured populations. These factors together can contribute to unbroken pathogen transmission chains, which can lead to increase in virulence or the emergence of new pathogens (e.g., Breyta et al. 2016b). For example, high rearing densities can occur in both WDFW hatchery programs and in healthy wild populations spawning naturally. However, in both cases, the high densities are not sustained and only exist during one part of the life cycle, thereby breaking pathogen transmission chains associated with high densities. In aquatic farms, transmission chains are maintained by immediately stocking after harvest the empty net-pens with new smolts from freshwater hatcheries, resulting in continuous occupancy of the aquatic farm.

As discussed above, Cooke maintains their Atlantic salmon net-pens as single generation operations, net-pens are fallowed for at least 42 days, and nets are cleaned following harvest. This process maintains fish health and breaks pathogen transmission chains. In addition, prior to transport into net-pens, each lot of fish is tested for regulated pathogens and PRV, and fish are vaccinated. While in the net-pens, when necessary, fish are treated with antibiotics to remedy disease and reduce mortality, with a secondary benefit to prevent the transmission of endemic pathogen infections. These processes maintain fish health and break pathogen transmission chains. Cooke will be required to continue these operations when culturing all-female triploid steelhead trout. **Therefore, WDFW considered Cooke's culturing of Atlantic salmon in Puget Sound to be of low risk to promote the evolution of pathogen virulence or of new pathogens and considers there to be no change in that risk when culturing of all-female triploid steelhead trout.**

Mordecai et al. (2019) describe three newly discovered viruses that occur in out-migrating juvenile Chinook and sockeye salmon, and in hatchery and commercial aquaculture production of Chinook salmon in British Columbia. The authors discussed the potential link

between salmonid declines and the presence of viruses, and suggested that farmed Pacific salmon "may pose some transmission risk to their wild counterparts" (Mordecai et al. 2019:2). In the paper's abstract, the authors also connect dead or dying farmed fish, the presence of these new viruses in those fish and in wild fish, and the health of wild fish populations. Unfortunately, by suggesting a connection among the occurrence of the new viruses in both farmed and wild fish, disease (e.g., death and dying) in farmed fish, and the health of wild fish populations, the authors opened the door for others to make a causal link among these elements (e.g., Our Sound, Our Salmon[4]). Mordecai et al. (2019) suggest, but do not show, that these viruses are the etiological agent for any disease. Furthermore, only one of these viruses (SPAV-1) was associated with symptoms consistent with disease, and this occurred only in the farmed Chinook salmon, not in wild Chinook or sockeye salmon. Based on the data provided by the authors in a supplemental file associated with their Figure 2[5], prevalence of each virus, summing across species and origin, is 1.63% for SPAV-1, 1.78% for SPAV-2, 2.85% for PsNV, and 0.39% for CAV. CAV occurs only in farmed Chinook salmon, while SPAV-2 effectively occurs only in wild Chinook salmon (one fish each in aquaculture and hatchery, out of a combined total of 5716 fish). As Mordecai et al. (2019) indicate, the transmission of these viruses among aquaculture, hatchery, and wild fish is not known. These viruses are not common, have not been shown to cause disease, and are not associated with outbreaks. Considering all four viruses together, prevalence in wild fish is negatively correlated with prevalence in farmed fish; that is, the higher the virus's occurrence in farm fish the lower its occurrence in wild fish, and *vice versa*. **Mordecai et al. (2019) is a sound scientific publication, but it provides no evidence for either pathogen amplification within farmed fish and disease transmission from farm fish to wild fish; or viral evolution (virulence or new species) associated with net-pen aquaculture.**

### 4.2.6. Summary Discussion of Three Pathogens: IHNV, PRV, and Sea Lice

#### 4.2.6.1. IHNV

Infectious hematopoietic necrosis virus (IHNV) is part of the Rhabdoviridae family; can cause acute infection, disease, and mortality in salmonids, especially in juvenile fish; affects both cultured and wild fish in fresh- and salt-water; and is endemic throughout the Pacific Northwest from Alaska to California and east to Idaho (Morzunov et al. 1995, Anderson et al. 2000, Kurath et al. 2003). The virus (IHNV) is listed by Washington State as a Regulated Pathogen (WAC 220-370-050(20)(a)(i)), and the disease (IHN) is recognized by the World Organization of Animal Health (OIE) as a Notifiable Disease[6]. WDFW is required by policy[7] to test for IHNV in broodstock for all anadromous salmonids at their hatchery facilities. In addition, embryos or live fish transported by private or commercial entities into or through Washington must be tested for IHNV; Finfish Transport Permit applications will be denied by WDFW for any lot that tests positive for IHNV. Lots of fertilized Atlantic salmon or steelhead trout

[4] https://static1.squarespace.com/static/5898d1b3cd0f689b98657619/t/5ddc152426c4ae3e67cd892b/1574704429197/OSOS_Final_SEPA_Comments.pdf

[5] DOI: https://doi.org/10.7554/eLife.47615.015

[6] https://www.oie.int/animal-health-in-the-world/oie-listed-diseases-2020/

[7] The Salmonid Disease Control Policy of the Fisheries Co-Managers of Washington State (July 2006)

embryos transported from a spawning facility to Cooke's freshwater hatchery must be free of IHNV to receive a Finfish Transport Permit from WDFW. Likewise, any lot of Atlantic salmon or steelhead trout smolt transported into Cooke's marine net-pens must be free of IHNV to receive a Finfish Transport Permit from WDFW.

There are three genogroups of IHNV in North America (U, M, and L) based on phylogenetic analyses of the middle portion of the G-gene (mid-G) (Kurath et al. 2003). The genogroups or clades have different primary hosts and different geographic distributions. In Washington, only the U and M clades exist, with the U clade divided into two subgroups (UP and UC) (Breyta et al. 2016a).

The UP clade occurs primarily along the outer coast, Puget Sound, and the Columbia River watershed upriver of the confluence with the Snake River. The UP clade is the dominant group in Puget Sound and the upper Columbia River (i.e., found in at least 75% of detections). Its primary host is sockeye salmon, with approximately 90% of its occurrence, and there is high mortality associated with this subgroup.

The UC clade occurs throughout the Columbia and lower Snake River watersheds and is the dominant group in most of this area, except in the lower and upper Columbia River. The UC clade appears to be a generalist, occurring in both Chinook salmon and steelhead trout, and it has low pathogenicity and is associated with low mortality.

The M clade in Washington is represented by the MD subgroup and throughout its range it is sympatric with either the UP or UC clades. The MD clade is the dominant group only in the lower Columbia River and it occurred briefly on the outer coast in 2007-2013. The primary hosts for the M clade are steelhead and rainbow trout, and as with the UP clade there is high mortality associated with this subgroup (Breyta et al. 2016a).

In Puget Sound only the UP clade currently exists, its primary host is sockeye salmon, and mortality can be high. The UP clade can infect steelhead trout and Chinook salmon, but there are only a few detections (adults) in both species and the virus is not associated with disease in these species (G. Kurath, pers. comm 2019).

Kurath et al. (2016) conducted laboratory challenges with Atlantic salmon, immersing juvenile fish for 1 hour in water containing IHNV. They used different variants of the U, M and L clades, and found that in Atlantic salmon, unlike sockeye and Chinook salmon, or steelhead trout, all variants caused mortality to a varying degree (20-100% for the U clade, 30-63% for the M clade, and 41-81% for the L clade). Similar studies were conducted on sockeye salmon (Garver et al. 2006, Purcell et al. 2009, Penaranda et al. 2011b), Chinook salmon (Hernandez et al. 2016), and steelhead and rainbow trout (Garver et al. 2006, Penaranda et al. 2009, Penaranda et al. 2011b, Breyta et al. 2014), with results consistent with the field observations discussed above: U clade has high virulence in sockeye salmon but low virulence in Chinook salmon, and steelhead and rainbow trout; and M clade has high virulence in steelhead and rainbow trout, low virulence in Chinook and sockeye salmon, but can replicate in sockeye salmon.

As discussed in section 4.2.3.2, Cooke vaccinates their Atlantic salmon for IHNV, and will be vaccinating their all-female triploid steelhead trout for IHNV prior to transport to marine net-pens. Cooke uses a DNA vaccine that encodes the virus's transmembrane glycoprotein (Kurath et al. 2006; H. Mitchell, pers. comm. 2019). This vaccine was derived from IHNV WRAC (039-82) strain from rainbow trout in Southern Idaho (Corbeil et al. 1999, Corbeil et al. 2000). This strain is part of the M clade genogroup (Penaranda et al. 2011a), and the vaccine is highly efficacious in steelhead and rainbow trout (Corbeil et al. 1999, Corbeil et al. 2000, LaPatra et al. 2000, LaPatra et al. 2001, Purcell et al. 2004). The vaccine confers homologous (M clade) and cross-genogroup (U clade) protection (Penaranda et al. 2011a) for up to two years (Kurath et al. 2006). The vaccine also is efficacious when administered to Chinook and sockeye salmon, but with lower relative percent survival values than in steelhead and rainbow trout (Garver et al. 2005). The vaccine appears efficacious in Atlantic salmon where Cooke's Puget Sound net-pens have tested negative since the 2012 IHN outbreak of unvaccinated Atlantic salmon.

Based on the epidemiology of the 2012 IHN outbreak in the Rich Passage net-pens (prior to ownership by Cooke), the virus was transmitted from wild fish to farmed fish (G. Kurath, pers. comm 2019), and there is no evidence that the virus was transmitted back to the wild fish and resulted in disease in wild fish.

In summary, the UP clade is the only IHNV genogroup that occurs in Puget Sound. This genogroup has high virulence in Atlantic and sockeye salmon, but low virulence in Chinook salmon and steelhead trout. Cooke will continue to vaccinate using an M clade DNA vaccine, which is efficacious against both U and M clade IHNV. **Based on the phylogeography and relative virulence of the IHNV genogroups, the risk of viral transmission from the farmed fish back to wild fish, and vaccination status of the farm fish, Cooke's net-pen facilities in Puget Sound present low risk of transmission of IHNV to wild salmonid populations. In addition, since the UP clade is of low virulence in steelhead trout, by switching from Atlantic salmon to all-female triploid steelhead trout Cooke is lowering the already low risk to wild populations.**

#### 4.2.6.2. PRV [8]

Piscine orthoreovirus (PRV), originally named piscine reovirus (Palacios et al. 2010) but renamed by Markussen et al. (2013) to reflect the virus's phylogenetic relationships within the Reoviridae family, is a double stranded RNA virus endemic to the North Atlantic and the North Pacific, but also occurs in Chile. There are three PRV genogroups, defined genetically: PRV-1, PRV-2, and PRV-3 (Palacios et al. 2010, Olsen et al. 2015, Takano et al. 2016). PRV-1 was initially identified in Atlantic salmon farms in Norway (Palacios et al. 2010). It is now known to occur in Atlantic salmon farms throughout the North Atlantic (Garseth et al. 2013, Kibenge et al. 2013, Marty et al. 2015, Adamek et al. 2019; for other references see Table 1 in Polinski and Garver

[8] As part of Fisheries and Oceans Canada's pathogen transfer risk assessment of PRV in British Columbia, Polinski and Garver (2019) provided an excellent summary of the virus. The summary presented below follows Polinski and Garver (2019).

2019), and in Chile (Kibenge et al. 2013, Godoy et al. 2016). Retrospective analyses show that the virus has been commonly present in both the North Atlantic and eastern North Pacific Ocean regions since the mid-1980s or earlier (Marty et al. 2015, Polinski and Garver 2019). PRV-1 also occurs in:

(1) farmed Chinook salmon in British Columbia (Di Cicco et al. 2018),
(2) farmed coho salmon in Chile (Godoy et al. 2016),
(3) wild sockeye, Chinook, coho, pink, and chum salmon, and steelhead, cutthroat, and Dolly Varden trout in the eastern North Pacific (Kibenge et al. 2013, Miller et al. 2014, Morton et al. 2017, Purcell et al. 2018), and
(4) wild Atlantic salmon and sea (brown) trout in the North Atlantic (Garseth et al. 2013, Vendramin et al. 2019).

PRV-2 is known only from coho salmon in Japan, is the etiological agent for erythrocytic inclusion body syndrome (EIBS), and is associated with anemia (Takano et al. 2016).

PRV-3[9] was first discovered in farmed rainbow trout in Norway (Olsen et al. 2015). Later it was found in

(1) farmed rainbow trout throughout the North Atlantic (Dhamotharan et al. 2018, Adamek et al. 2019, Polinski and Garver 2019),
(2) brown trout in Germany (Kuehn et al. 2018), and
(3) coho salmon in Chile (Godoy et al. 2016).

PRV-3 is associated with heart and skeletal muscle inflammation (HSMI)-like disease in rainbow trout (Hauge et al. 2017, Dhamotharan et al. 2018) and proliferative darkening syndrome (PDS) in brown trout in Germany (Kuehn et al. 2018), although Fux et al. (2019) argued that PRV-3 is not associated with PDS.

Kibenge et al. (2013), Siah et al. (2015), and others have showed that there are two different subgroups within PRV-1 (PRV-1a, PRV-1b [Group I in Siah et al. 2015]). PRV-1b is restricted to the North Atlantic and in Chile, while PRV-1a occurs in the North Atlantic, eastern North Pacific, and in Chile. Within PRV-1a, genotypes from the eastern North Pacific are a monophyletic group (bold Group II in Siah et al. 2015), derived from a single ancestral viral strain, and are distinct from PRV-1a from the North Atlantic (Warheit, unpublished data). The earliest record of PRV from the eastern North Pacific is from a wild steelhead trout taken in 1977 (Marty et al. 2015). This, plus the monophyly of the eastern North Pacific PRV-1 indicates that there is a distinct evolutionary lineage of PRV-1a that is endemic to the eastern North Pacific and occurs naturally in Pacific salmon.

The viral kinetics of PRV-1 have been studied in detail in laboratories and involves three phases of infection (Polinski et al. 2019, see also Finstad et al. 2014, Wessel et al. 2015, Garver et al. 2016a, Haatveit et al. 2016, Haatveit et al. 2017, Malik et al. 2019, Wessel et al. 2019). The first or early phase includes host entry, viral replication, and

[9] Originally described as PRV-*Oncorhynchus mykiss* or PRV-*Om* since it was described in rainbow trout

dissemination to erythrocytes (red blood cells). The phase lasts 2-3 weeks, and during this time the host's immune system does not appear to recognize the virus, nor does viral shedding into the environment occur, indicating that transmission of the virus is either weak or not occurring. During the second phase, which also lasts 2-3 weeks, viral replication in the red blood cells reaches its peak, viral inclusions bodies within the red blood cells develop, host viral recognition may occur leading to an immune response, and viral shedding and therefore viral transmission occur. Throughout the last or persistent phase, which can go on indefinitely, high viral loads can be maintained but virus replication is reduced, there is no apparent host immune response, viral shedding declines, and there is poor viral transmission.

PRV-1 is infectious during that short 2-3-week second phase when the virus can be transmitted to other fish. If farm fish become infected while in the net-pens, then PRV-1 can amplify resulting in widespread transmission within the farm and most-likely among farms in relatively close proximity, as the virus is robust and can survive in adverse environmental conditions (Aldrin et al. 2010, Lovoll et al. 2012). As such, PRV-1 is ubiquitous among Atlantic salmon farms in Norway (Lovoll et al. 2012), and is pervasive within and among Atlantic salmon farms in British Columbia (Polinski and Garver 2019). During this time the virus can be transmitted to wild populations. If farm fish become infected while in the freshwater hatchery, there is a good chance that these fish enter the marine net-pens when they are already in the third or persistent phase when viral shedding and transmission is low.

The infection dynamics differ between Norway and eastern North Pacific PRV-1 strains (Polinski et al. 2019). First, PRV-1 from the eastern North Pacific is not detected in the blood plasma. However, infection by the Norway PRV-1 resulted in high viral loads in the plasma after one week of initial infection, lasting upwards of nearly eight weeks. Second, host recognition (immune response) was 2-10 times greater in the blood and more than 100 times greater in the heart for PRV-1 from Norway than PRV-1 from the eastern North Pacific. Third, heart inflammation from Norway PRV-1 reached high severity 1-2 weeks after peak viral load, thereafter the inflammation diminished. However, heart inflammation associated with eastern North Pacific PRV-1 occurred later, about four weeks after peak viral load and was maintained at high prevalence, but low severity until the end of the experiment, seven weeks later.

Two diseases have been associated with PRV-1: Heart and skeletal muscle inflammation (HSMI), occurring in farmed Atlantic salmon in Norway, and jaundice syndrome, occurring in farmed Chinook salmon in British Columbia (Wessel et al. 2019). PRV-1b has been shown to be the etiological agent of HSMI in farmed Atlantic salmon in Norway (Wessel et al. 2017). HSMI-like disease, also associated with PRV-1, has also been described from Atlantic salmon net-pens in British Columbia (Di Cicco et al. 2017, Di Cicco et al. 2018), Chile (Godoy et al. 2016), and Scotland (Ferguson et al. 2005). Although PRV-1 is ubiquitous in Atlantic salmon net-pen farms in Norway, HSMI does not occur in all fish, and when it does, it is associated with mortality upwards of 20%, and morbidity as high as 100% (Kongtorp et al. 2004, Kongtorp et al. 2006).

The relationship between PRV-1 infection and disease is complex and may be dependent on the PRV-1 strain. Although PRV-1 occurs in wild, hatchery, and net-pen farmed fish, disease is associated only with farmed fish. In Norway, PRV-1 is widespread and HSMI is common and continues to be a significant problem for the Atlantic salmon industry (Hjeltnes et al. 2019). In laboratory experiments involving PRV-1 from Norway, experimental fish were exposed to PRV, and although HSMI did not develop, moderate to severe heart lesions consistent with HSMI did occur (Hauge et al. 2016, Wessel et al. 2017). There also appears to be an association with viral load and HSMI disease in Norway (Lovoll et al. 2012). In British Columbia, Di Cicco and colleagues established an association between PRV-1 and HSMI-like symptoms in Atlantic salmon net-pen farms (Di Cicco et al. 2017, Di Cicco et al. 2018) and PRV-1 and jaundice/anemia in Chinook salmon net-pen farms (Di Cicco et al. 2018). Although PRV-1 may play a role in these diseases (Polinski and Garver 2019), PRV-1 has not been established as the etiological agent of these diseases in these farms. Furthermore, despite the fact that in laboratory experiments naïve fish exposed to PRV-1 from the eastern North Pacific became infected, neither jaundice/anemia (Atlantic, sockeye, and Chinook salmon) nor HSMI (Atlantic and sockeye salmon) developed in these fish (Garver et al. 2016a, Garver et al. 2016b, Polinski and Garver 2019, Polinski et al. 2019). Unlike HSMI in Norway, jaundice/anemia and HSMI-like diseases are rare in British Columbia. Less than 10% of Atlantic and Pacific salmon farmed fish in British Columbia die within the net-pens, and of these less than 0.5% is associated with heart pathologies. Most of these heart pathologies are idiopathic; and only 0.05% are associated with jaundice in Pacific salmon farms (Polinski and Garver 2019). That is, for every 1000 Atlantic or Pacific salmon in net-pen farms, less than five die with associated heart pathologies, and in Pacific salmon, less than one fish is associated with jaundice.

Morton et al. (2017) reported that the incidence of PRV-1 in wild salmonid populations subjected to high exposure to Atlantic salmon net-pens was statistically greater compared with wild populations with low exposure to Atlantic salmon net-pens. These authors concluded that PRV was being transferred from the net-pens to wild populations and therefore infections in the farms influence infections rates in the wild populations. Garseth et al. (2013) concluded based on a phylogenetic analysis of sequences data that transmission of PRV from Atlantic salmon net-pens to wild populations in Norway is likely. These results are consistent with those of Morton et al. (2017). WDFW compared the prevalence of PRV-1 in Chinook, coho, and sockeye salmon from Alaska (no Atlantic salmon net-pens), Columbia River (no Atlantic salmon net-pens), and Puget Sound (Atlantic salmon net-pens present) (Purcell et al. 2018) with British Columbia prevalence data from Marty et al. (2015) and Morton et al. (2017). Considering only the Marty et al. (2015) and Purcell et al. (2018) data, the highest PRV-1 prevalence for Chinook salmon was from Columbia River fish and for coho salmon from Alaska fish, while for both of these species the two geographic areas with Atlantic salmon net-pens, Puget Sound and British Columbia, showed intermediate prevalence. The prevalence in sockeye salmon was zero for Alaska,

Columbia River and Puget Sound, and 0.3% from British Columbia. PRV-1 prevalence reported in Morton et al. (2017) was considerably greater in all three species than the prevalence documented by Marty et al. (2015) and Purcell et al. (2018). Compared with the highest prevalence in the Marty et al. (2015) and Purcell et al. (2018) data, PRV-1 prevalence in Morton et al. (2017) was – 3x greater for Chinook, 1.5x greater for coho, and 31x greater for sockeye salmon. Although there could be an association between the incidence of PRV-1 in wild populations and exposure to net-pens in British Columbia, the conclusion from Morton et al. (2017) may be affected by how the authors defined prevalence and how they classified wild populations with respect to exposure to Atlantic salmon net-pens.

Morton et al. (2017) also concluded that the PRV infection may lower the fitness of wild fish by decreasing their capacity to complete a difficult migration from marine waters to freshwater spawning areas, thereby impacting population viability. The authors reached this conclusion by showing that "[f]ewer [PRV] infected adults of any species were detected at higher vs. lower elevations in the Fraser River, as well as tributaries of the Skeena and Nass rivers in northern BC. This association points to a cost of infection from PRV to the fitness of wild Pacific salmon" (Morton et al. 2017:12). However, Zhang et al. (2019) found that high PRV viral load had no effect on the oxygen affinity and carrying capacity of the red blood cells even for individuals with minor heart pathology.

In summary, PRV-1 in the eastern North Pacific is phylogenetically different from PRV-1 from the North Atlantic. Although the virus tends to be ubiquitous in both regions, their infection dynamics differ, and disease is rare and the pathogenicity of the virus is low or non-existent in net-pen aquaculture in the eastern North Pacific. Although the virus may be transmitted from the net-pens to wild populations in the eastern North Pacific, the infectious period is short (but the virus may be long-lived in marine waters) and disease does not develop in wild populations. PRV is common in both farmed and wild Atlantic salmon, but its prevalence in wild steelhead trout is low – 1 out of 375 samples (0.3%; Purcell et al. 2018).[10] However, we anticipate that prevalence among all-female triploid steelhead trout in Puget Sound net-pens may be more similar to the high prevalence of farmed Atlantic salmon than that in wild steelhead trout. **Based on these analyses, we considered PRV-1 transmission from Atlantic salmon net-pens to wild salmonid populations in Puget Sound to be a low risk. We consider PRV-1 transmission from all-female triploid steelhead trout net-pens to wild salmonid populations in Puget Sound to be the same as or possibly a lower risk, compared with Atlantic salmon net-pen aquaculture.**

Finally, the August 2017 Cypress #2 accident in Puget Sound resulted in an estimated release of about 250,000 Atlantic salmon. There is a high likelihood that most or all of these Atlantic salmon were positive with a PRV-1 strain from Iceland (Kibenge et al. 2019). The source of the PRV-1 was most likely from the broodstock in Iceland. This means that fish became infected within the freshwater hatchery and were planted in

[10] Morton et al. (2017) documented 4 out of 14 steelhead trout positive for PRV-1 but see above for concerns on how prevalence is defined in this study.

the net-pens when the virus was probably in its persistent non-infectious phase. In 2018 the WDFW Fish Health Unit implemented a surveillance program for PRV-1 at selected hatcheries in Puget Sound, Washington Coast, and Columbia River. To date, we have analyzed 648 samples from Chinook and coho salmon, and steelhead and rainbow trout. Of these samples 37 (6%) tested as strong-positive, 12 (2%) as positive, 34 (5%) as weak-positive, and 564 (87%) tested negative. We obtained readable sequences for 33 samples (5%) and these sequences represented two known strains, both part of the eastern North Pacific clade. **To date there is no evidence that the 2017 Cypress #2 accident resulted in the transmission of the Icelandic PRV-1 to wild populations in Washington.**

#### 4.2.6.3. Sea Lice

Sea lice are ectoparasitic marine copepod crustaceans that are associated with infestations and economic loss in salmonid aquaculture (reviewed in Boxaspen 2006). The copepods undergo a life cycle that starts with a nauplius larva, a planktonic stage that ultimately molt into a planktonic and infectious copepodids. The distribution, abundance, and viability of sea lice is affected by sea temperature and salinity. Bricknell et al. (2006) showed that the survival and parasitic ability of planktonic *Lepeophtheirus salmonis*, a sea louse common in both the North Atlantic and North Pacific oceans, is severely compromised at salinities less than 29 ppt. Similarly, Crosbie et al. (2019) showed that *L. salmonis* nauplii from Norway completely avoided salinities less than 30 ppt, while copepodids tolerated salinities as low as 16-20 ppt. In the eastern North Pacific there are two predominate species of sea lice that affect salmonids, *L. salmonis* and *Caligus clemensi*. At a commercial Atlantic salmon farm near the Broughton Archipelago, British Columbia, the seasonal abundance of plankton larvae for both species of sea lice varied directly with water salinity, and consistent with the North Atlantic studies, larval abundance dropped when salinities fell below 30 ppt (Byrne et al. 2018).

Farm and wild fish populations in British Columbia have experienced infestations (e.g., Marty et al. 2010, Krkosek et al. 2011), although the link between farm and wild fish infestation is not clear, nor is the link well understood between number of sea lice at farms and wild fish productivity (Morton et al. 2004, Brooks 2005, Beamish et al. 2006, Brooks and Stucchi 2006, Krkosek et al. 2006, Morton et al. 2008, Marty et al. 2010, Krkosek et al. 2011).

Sea lice may be a problem for the salmonid net-pen industry in the North Atlantic and in British Columbia, and sea lice infestations at net-pen facilities in these regions may have a negative effect on wild salmonid populations; however, in Puget Sound, although sea lice do occur in net-pen facilities, and they are monitored; their numbers do not reach a level of concern. Water circulation is complex within Puget Sound, affected by a variety of factors, including the Strait of Juan de Fuca, river discharge, and bathymetry. Nevertheless, on average, through an entire year, surface water salinities with Puget Sound remain at or below 30 ppt (Khangaonkar et al. 2011, Sutherland et al. 2011; see Figure 1 below), which results in high mortality for sea lice

pelagic larvae and minimizes the likelihood of significant sea lice infestations. **Cooke's net-pen facilities are not a nexus for the amplification and transmission of sea lice to native salmonids in Puget Sound**.

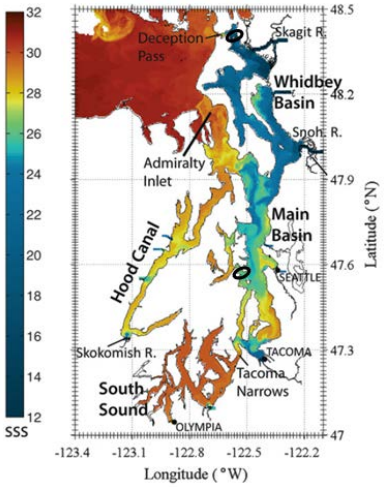


**Figure 1.** From Sutherland et al. (2011:Figure 1).showing sea surface salinity on 21 June 2006. Two ellipses added showing general location of Cooke's Hope Island and Rich Passage net-pen facilities.

### 4.2.7. Antibiotics and Medicated Feed

Antibiotics are administered to net-pen fish usually through medicated feed, referred to as Veterinary Feed Directives (VFDs). These are prescriptions written by licensed veterinarians that have established a Veterinary-Client-Patient-Relationship (VCPR) with the aquatic farmer and the fish. A veterinarian with a VCPR is formally recognized as the veterinarian of record (VOR) for a facility. VFDs, VCPRs, VORs, veterinary licenses, and the drugs that can be used for treatment of specific pathogens are all regulated by both Federal and Washington State rules. It is the VOR's obligation to adhere to these rules (i.e., violations of these rules can result in loss of license and livelihood). The "client" (owner of the fish, or the aquatic farmer) has the freedom to refuse treatment, but only a licensed veterinarian with a VCPR can order a VFD. It is the licensed veterinarian's and the VOR's license that are at risk if VFDs or other chemicals used on the fish are applied improperly or illegally, even if it is without the knowledge of the veterinarian. It is also the veterinarian's responsibility to adhere to the U.S. Food and Drug Administration's (FDA) Judicious Use of Antimicrobials policy.[11]

The most common pathogens (and their associated diseases) of the cultured Atlantic salmon in Puget Sound are: *Tenacibaculum maritimum* (yellowmouth); *Aeromonas salmonicida* (furunculosis); *Vibrio anguillarum* (vibriosis); *Piscirickettsia salmonis* (salmon rickettsia syndrome, SRS); and *Moritella viscosa* (winter ulcer) (J. Parsons, pers. comm 2020)[12]. Farm fish are particularly vulnerable to *T. maritimum* when they first enter salt water and are frequently given antibiotics to treat for yellowmouth. In fact, yellowmouth is the most

[11] See https://www.fda.gov/animal-veterinary/antimicrobial-resistance/judicious-use-antimicrobials

[12] Infectious hematopoietic necrosis virus (IHNV) is also a common pathogen of concern, but IHNV is managed through testing and a vaccine. The last outbreak of IHN in marine net-pens in Puget Sound was in 2012, prior to vaccination.

common disease for which antibiotics are applied to Atlantic salmon in Puget Sound. Experimental trials with culturing triploid steelhead trout in Puget Sound in 2012 showed that steelhead trout are more resistant to yellowmouth than are Atlantic salmon (J. Parson, pers. comm 2020), suggesting that Cooke's proposal to switch from Atlantic salmon to all-female triploid steelhead trout may result in less disease and fewer applications of antibiotics. Each of these bacteria, except for *A. salmonicida* and *P. salmonis,* are obligate marine or brackish water pathogens, and the fish become infected by these "wild" pathogens only after they enter the marine environment.

Love et al. (2020) provided a comparison of antibiotic use rates in Atlantic salmon net-pen in Norway, Scotland, Atlantic Canada, Maine, British Columbia, Washington, and Chile. Overall, from 2013 through 2017, a period prior to Cooke's ownership of the net-pens, antibiotic use based on kilogram of fish was highest in Washington followed closely by Chile. This publication does a good job documenting the relative amounts and trends of antibiotic use; however, the publication does not document the prevalence of specific pathogens in farm, freshwater hatchery, or ambient environments, and the therapeutic need for antibiotics. For example, in their risk assessment of environmental impact of Atlantic salmon farms in Norway Taranger et al. (2015) lists four viruses and sea lice, but no bacterial pathogens. Viral pathogens affect Atlantic salmon fish farms more than bacterial pathogens (see also Johansen et al. 2011), and there is a greater need in Norway to prevent viral outbreaks by using vaccines and biosecurity then there is treating bacterial outbreaks with antibiotics. Washington presents nearly the exact opposite situation than what is encountered in Norway. In the Puget Sound environment, bacterial pathogens are the dominant pathogens of concern, and therefore, we would expect greater use of antibiotics in Washington than in Norway. WDFW provides fish health services at 84 hatchery facilities and approximately 150 salmonid hatchery programs. In maintaining fish health for these programs, we emphasize biosecurity first, but VFDs are an essential part of our toolbox to treat and mitigate bacterial disease outbreaks.

**In overall summary, there would be minimal differences between rearing Atlantic salmon and all-female triploid steelhead trout in Puget Sound net-pens in (1) the contraction, amplification, or transmission of pathogens; (2) their development of disease or their promotion of disease in wild finfish; or (3) their involvement in the increase virulence of existing pathogens or in the development of new pathogens. Furthermore, we consider the overall risk of these hazards to be relatively low**.

### 4.3. Fish Escapes

Large-scale escapes (>10,000 individuals) resulting from infrastructure failure, such as the 2017 accident at Cooke Aquaculture's Cypress #2 facility (Clark et al. 2018), have happened wherever the farming of fish in open net pens is practiced. However, these events are relatively uncommon, accounting for only 19% of the fish escape incidents reported in Norway from 2001 to 2009 (Jensen et al. 2010). Across all species these large-scale incidents have been caused most frequently by mooring failure (e.g., Cypress #2), breakdown and sinking of steel floats, or major tears in the nets (Jensen et al. 2010). In December 2019 a fire destroyed part of a plastic

float system in a pen in British Columbia and nearly all the 21,000 ready-for-harvest Atlantic salmon escaped (https://globalnews.ca/news/6328416/bc-fish-farm-fire-salmon/). There have been four large-scale Atlantic salmon net-pen escape events recorded in Washington; three events in four years, 1996 (107,000 salmon escaped), 1997 (369,000 fish), 1999 (115,000 fish); then no events for 18 years until the accident at Cooke's Cypress #2 net-pen in 2017 (250,000 fish) (Amos and Appleby 1999, Clark et al. 2018).

Other, often small-scale escapes, termed leakage, may occur due to errors during transfer of fish, maintenance errors, or small holes in nets caused by predators, floating debris, or vandalism (Jensen et al. 2010). Leakage of salmon from farms is typically undetectable (Britton et al. 2011, Fisher et al. 2014). There is a growing understanding that more gradual, low-level leakage of fertile fish can have a greater negative demographic and genetic impact on native species than the rarer, large-scale escape events (Baskett et al. 2013, Yang et al. 2019).

### 4.3.1. Structural Integrity of Net-Pen Infrastructure

Most large-scale escapes from salmon marine net-pens are a result of failures in the net-pen infrastructure (Jensen et al. 2010). The collapse of Cooke's Cypress #2 net-pen facility in 2017 that released an estimated 250,000 Atlantic salmon resulted from a failure of the mooring system and structural members of the raft's framing structure (Clark et al. 2018). Excessive biofouling by mussels and other marine organisms increased the drag force on the net-pen array, which likely resulted in the infrastructure failure. Following the Cypress #2 accident, management actions were taken that will lower the risk of net-pen infrastructure failure, compared with the risk that existed prior to the Cypress #2 accident.

- "In early in 2018, DNR and Cooke cooperatively developed a net hygiene monitoring protocol to improve net hygiene and document full compliance with the DNR Aquatic Land Leases. Since June 2018, Cooke has implemented the protocol at the Rich Passage and Hope Island facilities. [Cooke also implemented this protocol at Port Angeles until mid-2019, when rearing operations there concluded.] The protocol requires Cooke to score the cleanliness of each stock net containing fish at each farm on a weekly basis and submit those scores to DNR. Cooke substantiates the reported scores with video footage taken by Cooke divers of two stock nets per array randomly selected by DNR. DNR provides the numbers of the stock nets to be filmed the day before the filming must occur and the video is submitted to DNR within several days of being shot. The video dive footage follows a prescribed path that provides a representative view of biofouling. This video footage is required once per month during the peak vegetation growing season from May to October and every other month from November to April, when there is less vegetative biofouling. DNR's aquatic land manager, usually with a supervisor for a second set of eyes, reviews all video footage and cross checks biofouling observed with the net cleanliness scores submitted the preceding week. To date, the video verification has corroborated the net cleanliness scores reported by Cooke." (Dennis Clark, DNR; pers. communication October 2019).

- EHB 2957 requires that approximately every two-years, when net-pens are fallow, each of Cooke's facilities must be inspected by an independent marine engineering firm, approved by WDFW, and to receive fish the facility must be considered in good working order. In December 2019, a Consent Decree was reached between Cooke and Wild Fish Conservancy, where both parties agreed that before Cooke restocks any of their net-pen facilities, they are required to conduct a load analysis of the mooring and cage systems using environmental condition data that are consistent with the Norwegian aquaculture standard NS 9415. As part of the inspections mandated by EHB 2957, WDFW will require that Cooke provide an engineering analysis certifying that the net-pens conform to the parameters derived from the NS 9415 standard. Each net-pen facility will be evaluated independently as conformity to parameters derived from the NS 9415 standards require evaluation of the environmental conditions (e.g., currents, winds, waves, depth) specific to that net-pen facility. In Norway, the number of escaped Atlantic salmon declined from >600,000 fish per year (2001-2006) to <200,000 fish per year (2007-2009) after enactment of the NS 9415 standards in 2004 (Jensen et al. 2010).

### 4.3.2. Survival of escaped fish

The ability of escaped Atlantic salmon from fish farms in Norway to switch from pelleted feed to wild prey appears to depend upon their life stage at escape. Older, larger fish that escape often do not switch to live feed and survive poorly to sexual maturation (e.g., Skilbrei et al. 2015). Fish from the 2017 Cypress #2 event, that were harvest size at about ten pounds when the incident occurred, were found not to feed in the wild (e.g., only one of 71 fish examined (1.4%) had eaten possibly a small forage fish; WDFW, unpublished data). In contrast, fish that escape at early life stages appear to have a higher likelihood of adapting, feeding, and migrating to return as maturing adults. Jensen et al. (2013) captured Atlantic salmon, that had escaped early in the post-smolt stage, migrating and dispersing through the Arctic Ocean after one winter at sea; the growth and size of the escaped fish were similar to those of wild fish captured at the same time in the same area. Likewise, Skilbrei (2010) and Skilbrei et al. (2015) found that smolt and post-smolt escapees could survive and adopt the marine migratory pattern of their wild conspecifics. Blanchfield et al. (2009) and Patterson and Blanchfield (2013) studied survival of experimentally released diploid rainbow trout from open net-pen aquaculture in freshwater lakes in Ontario, Canada. In both studies annual survival of the released fish was approximately 50%, and although there was movement of fish away from the net-pens, most surviving fish showed continued reliance to the farm site.

Nearly all research on the behavior and survival of escaped farmed fish is based on diploid – fertile Atlantic salmon in Norway. However, in an experimental release of paired diploid and triploid Atlantic salmon from marine net-pens in Ireland, Cotter et al. (2000) and Wilkins et al. (2001) showed that significantly fewer triploid fish returned as adults to the coastal fisheries and to freshwater compared with their diploid siblings. These triploid Atlantic salmon may be less resistant to stressful environmental conditions and have significantly higher occurrence of lens cataracts than the diploid fish (Cotter et al. 2000, Wilkins et al.

2001, Cotter et al. 2002). Wilkins et al. (2001) and others (e.g., Glover et al. 2016) also postulated that the migration behavior of adult female triploid Atlantic salmon to freshwater was reduced by non-normal gonadal development. In the laboratory experiments pairing full-sibling diploid and triploid Atlantic salmon subjected to seawater challenges Leclercq et al. (2011) show that the triploid fish grow a suite of developmental deformities that may compromise their fitness in marine waters, including higher incidence and severity of lens cataracts, jaw malformation, vertebral deformities, and heart deformities possibly related to higher cardiac workloads.

Poorer survival and performance of triploid fish compared with diploid fish are not limited to Atlantic salmon. Scott et al. (2015) compared full-sibling diploid and triploid rainbow trout performance in the laboratory and showed that the triploid trout had significantly poorer hypoxia tolerance than their diploid siblings. The same result was observed in the five different strains of rainbow trout and three different brood years used in the experiment. Similar results were not seen in the adult, lake-reared trout, but Scott et al. (2015) considered that several factors may have confounded the analysis of the adult fish. Johnson et al. (2019) used hatchery rearing of full-sibling diploid and triploid steelhead trout and compared their survivorship and growth in both fresh- and salt-water. After 15 months in saltwater, the survivorship of the triploid fish was only 35% of their starting population, compared with 72% for the diploid fish. Withler et al. (1995) showed results similar to those in Johnson et al. (2019) using coho salmon.

Diploid Atlantic salmon in Norway may not be the best model to predict the survival of escaped all-female triploid steelhead trout from net-pens in Puget Sound. After four large-scale accidental releases and a number of intentional releases of Atlantic salmon in Puget Sound, there is no evidence that these fish survived for any extended period or were successful establishing spawning populations in Puget Sound (Amos and Appleby 1999). The literature on the marine survival of triploid Atlantic salmon and the relative survival of triploid rainbow and steelhead trout, and coho salmon in saltwater experiments suggest that triploid fish do poorly compared with their diploid siblings in marine waters. **We anticipate that in the unlikely event of a large-scale accidental release of all-female triploid steelhead trout from a net-pen in Puget Sound, the relative survival of the steelhead trout would be the same as or less than that previously seen with Atlantic salmon in this region**.

### 4.3.3. Genetic Issues

The genetic consequences of escaped diploid-fertile native species of farmed fish into open waters is a major concern with marine net-pen aquaculture (Hindar et al. 1991, Amos and Appleby 1999, Bolstad et al. 2017, Forseth et al. 2017, Glover et al. 2017, Yang et al. 2019). It is important to note that a wide variety of outcomes, ranging from no detectable effects (Glover et al. 2012) to substantial genetic introgression and even total displacement of wild populations by escaped farmed fish (Saegrov et al. 1997, Glover et al. 2012), were initially observed following escapes of Atlantic salmon from open net pens in Europe (reviewed in Hindar et al. 1991, Glover et al. 2017).

Escapes of fertile Atlantic salmon from open net pen aquaculture in the North Atlantic have been shown to have damaging impacts on the genetic variability both within and between native populations (Fleming et al. 2000, Houde et al. 2010, Karlsson et al. 2016, Bolstad et al. 2017, Glover et al. 2017). The most comprehensive data originate from Norwegian waters where five decades of farming Atlantic salmon was punctuated with escapes of millions of fish at different life stages (Diserud et al. 2019, Glover et al. 2019). Escaped, fertile and domesticated farm fish interbred with wild Atlantic salmon, thereby reducing fitness and placing more pressure on sometimes already dwindling wild populations (Fleming et al. 2000). Results show that invasions of escaped farm fish reduce reproductive fitness, reduce population productivity, disrupt local adaptations, and reduce the genetic diversity of wild salmon populations (Fleming et al. 2000, Bourret et al. 2011, Karlsson et al. 2016, Bolstad et al. 2017, Glover et al. 2017).

The impacts of escapes may vary depending on the status of the native stocks. In one example, Glover et al. (2012) studied introgression in 21 native populations of Atlantic salmon that had been exposed to large numbers of escaped farm fish and found that some populations were heavily introgressed (one native population was completely replaced with farm fish) while other populations were genetically intact. The authors concluded that healthy stocks of native fish that densely populated streams were resistant to introgression while depleted populations were much more vulnerable (see similar conclusions in Sylvester et al. 2018). This finding suggests that depleted stocks of steelhead trout and Chinook salmon in Puget Sound would be similarly vulnerable to genetic impacts from fertile con-specific farm escapees.

**The use of triploid fish is recognized as normal aquaculture procedure that mitigates for the potential risks to the genetic structure and viability of wild populations from escaped farmed fish (e.g., Amos and Appleby 1999, Cotter et al. 2000, Naylor et al. 2005, Waples et al. 2012, Baskett et al. 2013, Rust et al. 2014, Glover et al. 2016, Hawkins et al. 2019). Cooke is proposing to use all female triploid – sterile fish**

### 4.3.4. All female and Triploidy (Sterile) Fish

The most effective strategy to mitigate the risk of aquaculture large- or small-scale (leakage) escapes from open net pens is to limit farms to the use of sterile all-female fish (Thorgaard 1992, Cotter et al. 2000, Baskett et al. 2013, Lerfall et al. 2017). Sterile females are preferred because sterile males in many species may undergo sexual maturation and attempt to spawn even though these males produce no viable offspring (Hindar et al. 1991, Oppedal et al. 2003, Tiwary et al. 2004, Feindel et al. 2010). Such behavior from escaped males could lower the spawning success of native fish. For example, the release of sterile males has been used to reduce reproductive potential of wild populations in order to suppress populations of unwanted pests (Twohey et al. 2003, Bergstedt and Twohey 2007, Siefkes 2017). **Cooke's proposal is to use all-female triploid fish**.

Sterile females will be unable to successfully breed with native males and will eventually senesce and die (Tiwary et al. 2004, Lerfall et al. 2017). Introgression between escaped triploid females and the native populations will no longer be a risk to those native

populations. Some results suggest that sterile salmon have a reduced instinct to enter freshwater (Cotter et al. 2000, Wilkins et al. 2001, Glover et al. 2016); thus escaped sterile females will also be less likely to compete for the spawning of wild males or dig up the redds of wild females. **Cooke's proposal is to use all-female triploid – sterile fish**.

Sterile lots of fish are most frequently produced by inducing triploidy--producing fish with three sets of chromosomes rather than the normal two. Biological regulation of chromosome sets is not as rigorously controlled in fish as in other vertebrates (Miller et al. 1994): triploidy is naturally common in some species (Qin et al. 2016, Zhigileva et al. 2017, Wu et al. 2019) and triploidy has been seen at low rates in wild salmonids (Thorgaard et al. 1982). Inducing triploidy, as in done in watermelons or bananas or oysters, for example, renders the organism largely "seedless" – that is, sterile.

The technology for producing triploid lots of fish is simple and easily applied on a commercial scale (Lerfall et al. 2017). Inducing triploidy to produce sterile Pacific salmon was optimized at Washington State University (Thorgaard et al. 1982, Parsons et al. 1986, Seeb et al. 1986, Thorgaard 1992). Triploids were raised in growth trials in net pens by the Squaxin Tribe more than 30 years ago (Seeb et al. 1993).

Triploidy can be induced at rates approaching 100% by shocking newly fertilized eggs with heat or pressure (Benfey and Sutterlin 1984); induced triploidy is practiced by some aquaculturists to reduce product loss due to precocious maturation prior to harvest (Janhunen et al. 2019) and used by management agencies who require sterile fish for sportfish stocking programs (e.g., over 9 million triploid rainbow trout have been stocked in freshwater by WDFW since 1995).

The efficacy of the methods used to create triploid fish is not 100%. This means that in every batch of triploid fish there may be fish that are fertile and can spawn with wild individuals of the same species. **Cooke's proposal is to receive all-female triploid fish from Troutlodge, a Washington State company based out of Bonney Lake. Troutlodge estimates that their average triploidy success rate is 99.83%, or a failure (error) rate of 0.17%. For the purpose of the following exercise, we will use a triploidy error rate = 0.20%**. Section 4.3.5 for discussion of triploid rates

#### 4.3.4.1. Estimating genetic risk to native steelhead from steelhead escaping from Cooke's net-pens following a catastrophic failure of the net-pen infrastructure

To estimate risk of introgression between Cooke's net-pen steelhead and wild steelhead in Puget Sound, we estimated the following parameters and scenarios. These scenarios assume no mortality within the net-pens prior to escape. Mortality within the net-pens would reduce the number of both sterile and fertile fish that would escape.

- **Number of fish in net-pens: 1,000,000** [Clam Bay facility; Cooke Aquaculture Pacific (2019: B-33). Represents the largest facility and therefore the worst-case scenario.]
- **Triploidy error rate: 0.2%** [From above]
- **Proportion of the fish that escape: 0.82** [Based on the proportion of Atlantic salmon that escaped from Cypress #2 net-pen failure (Clark et al. 2018). We consider this to be a near worst-case scenario.]

- **Proportion of the escaped fish that elude recovery efforts: 0.77** [Based on the number of Atlantic salmon that were recovered following the Cypress #2 net-pen failure (Clark et al. 2018). Depending on the number of fish that escape, age of fish that escape, when the fish escape, and the behavior of the fish when they escape, this proportion can be much different than what we present here.]
- **Proportion of diploid fish sexually mature at time of escape: 10-50% of fertile fish** [50% is an extreme worst-case scenario, presented by Cooke Aquaculture Pacific (2019). Realistically, this proportion should be near zero because the fish will be harvested, on average, at less than two years of age, approximately 1-2 years prior to when they would reach sexually maturity (Cooke Aquaculture Pacific, 2019; J. Parson, pers. comm., 2019). We're using 10% as a low-end estimate, without justification other than it is greater than zero.]
- **Proportion of fish that will survive long enough to attempt to spawn: 50% of fertile fish** [Blanchfield et al. (2009) estimated annual mortality of rainbow trout = 50%, while Patterson (2010; see also Patterson and Blanchfield 2013) estimated that 50% of the rainbow trout died within the first three months; however, Patterson and Blanchfield (2013) had unaccounted fish. Both studies consisted of experimental releases of relatively small sample sizes in freshwater. See section 4.3.2 for discussion of survival of escaped fish. We estimated that within a year of the Atlantic salmon release from Cypress #2, most if not all fish had either been recaptured or had died.]

Number of mature diploid-fertile steelhead from Cooke's net-pens that may be present in Puget Sound following an accidental release like that which occurred with Atlantic salmon at Cypress #2 in August 2017:

- 1,000,000 fish x 0.002 = 2,000 diploid-fertile fish in net-pen out of 1 million fish
- 2,000 fish x 0.82 = 1,640 diploid-fertile fish that will escape
- 1,640 x 0.77 = 1,263 diploid-fertile fish that elude recovery efforts
- 1,263 x 0.50 = 632 diploid-fertile fish that are sexually mature (higher estimate)
- 1,263 x 0.10 = 126 diploid-fertile fish that are sexually mature (lower estimate)
- 632 x 0.50 = 316 sexually mature diploid-fertile fish that survived (higher estimate)
- 126 x 0.50 = 63 sexually mature diploid-fertile fish that survived (lower estimate)

Number of mature diploid-fertile steelhead from Cooke's net-pens that may be present in Puget Sound following an accidental release like that which occurred with Atlantic salmon at Cypress #2 in August 2017, but without a recovery attempt (see Section 4.3.6 and Mitigating Provisions):

- 1,000,000 fish x 0.002 = 2,000 diploid-fertile fish in net-pen
- 2,000 fish x 0.82 = 1,640 diploid-fertile fish that will escape
- ~~1,640 x 0.77 = 1,263 diploid-fertile fish that elude recovery efforts~~
- 1,640 x 0.50 = 820 diploid-fertile fish that are sexually mature (higher estimate)
- 1,640 x 0.10 = 164 diploid-fertile fish that are sexually mature (lower estimate)
- 820 x 0.50 = 410 sexually mature diploid-fertile fish that survived (higher estimate)
- 164 x 0.50 = 82 sexually mature diploid-fertile fish that survived (lower estimate)

We estimate conservatively that there will be 63 – 316 fertile female steelhead that would escape and survive to sexual maturity from Cooke Aquaculture's Clam Bay facility following an accident as described above (82 – 410 fertile female steelhead if a no recovery option was employed, or recovery was unsuccessful). If the accident were to occur at the Fort Ward site, with a total of 300,000 fish, there would be 19 – 95

fertile female steelhead that would escape and survive to sexual maturity (25 – 123) fertile female steelhead if a no recovery option was employed, or recovery was unsuccessful). Since these fish are all females, they will not spawn with each other, and to genetically affect Washington's steelhead populations, these fish would need to spawn with either hatchery- or natural-origin, natural-spawning steelhead. To do so, these domesticated fish would need to migrate into a steelhead spawning river, without homing or cues to enter a specific river, at the correct time of year, dig redds, and attract mates. These estimates are contingent on a catastrophic failure of the net-pen infrastructure, which are relatively uncommon (see Section 4.3). **Considering both the frequency of net-pen infrastructure failure and the low error rate producing triploid fish, we consider the risk to be low that domesticated all-female, triploid steelhead stocks cultured in Puget Sound net-pens will affect adversely the genetic structure of Washington's steelhead populations.**

### 4.3.5. Triploidy Rates

Appendix A in Cooke Aquaculture Pacific (2019) is a table provided to Cooke by Troutlodge, (the Washington State-based supplier of the all-female triploid eggs) that indicates that Troutlodge's proprietary procedure to create triploid-sterile fish has an error rate (i.e., rate of diploidy, and therefore rate of fertile fish) of 0.17%. This means that out of a lot of 1 million fish processed, there will be 998,300 triploid-sterile fish and 1,700 diploid-fertile fish. Troutlodges's table (Cooke 2019: Appendix A) shows 36 different lots of fish, each of unspecified size, that were subjected as eggs to the Troutlodge's triploidy procedure, one procedure per lot, from August 2013 through April 2018. The table includes the number of fish tested (N = 43 – 100), the number diploid results, number of triploid results, and the percent triploidy for each individual lot. Instead of treating each triploidy procedure as independent events Troutlodge treated the 36 triploidy procedures as a single continuous process, and therefore summed the total number of diploid results (five; one each in five lots) and divided that sum by the sum of all fish tested, across all 36 lots (2955), to produce an estimate of the error or diploidy rate of 0.17%. Cooke (2019: B-25) has adopted this error rate and justified Troutlodge's analysis stating that the "results in Appendix A are additive." We agree with Cooke Aquaculture that the procedure used by Troutlodge to generate triploid fish has a low error rate; perhaps as low as 0.17%. However, we disagree that the 36 lots, each containing an undisclosed number of eggs, subjected to Troutlodge's triploidy procedure over a 56-month period can be treated a single continuous process. The estimate is particularly problematic since potential variables in the procedure that may affect the procedure's efficacy were not provided (e.g., lot size; time between fertilization, water-hardening, and pressure treatment; duration and amount of high-pressure hydrostatic shock). In other words, we disagree with the method Cooke provided to estimate triploidy error rate.

Troutlodge used sample sizes ranging from 43 to 100 fish (mean = 82) per lot to calculate the triploidy error rate (Cooke 2019; Appendix A). Cooke suggested that for future lots to be transported into Puget Sound net-pen facilities, sampling rate be 100 fish per lot, reducing that sample size down to 60 if results appeared consistent with the data in Appendix A

(Cooke Aquaculture 2019; B-26). If the rate of diploidy (i.e., triploidy error rate) is indeed 0.0017 (0.17%) then the probability of drawing at least one diploid fish from a lot of 1,000,000 fish is 0.10 (n = 60 fish sampled) or 0.16 (n = 100 fish)[13]. That is, given an unbiased draw from the lot, there is a low expectation that a diploid fish will be selected using sample sizes of 60 or 100, even when there are diploid fish present in that lot. These sample sizes might underestimate the triploidy error rate, thereby underestimating the number of diploid or fertile fish in the lot. To achieve high confidence that at least one diploid fish is selected (probability > 0.90), a sample equal to 1500 is required[14]. Sample sizes of 60-100 are more appropriate for rate of diploidy equal to 5% rather than 0.17%.

Troutlodge effectively achieves a high sample size by pooling the results from all 36 lots in Cooke (2019:Appendix A) (n = 2955). To test the efficacy of this method, we modeled the procedure used by Troutlodge. That is, we assumed an actual rate of diploidy = 0.17%; drew from a hypergeometric distribution; sampled cumulatively from 36 independent lots, each with a total of 1 million eggs; and used sample sizes to test for triploidy success equal to 60, 150, 600, and 1500. We modeled each sample size 100,000 times to generate frequency distributions of calculated rates of diploidy (Figure 2). The mean rate of diploidy from 100,000 separate cumulative samples from 36 lots, for each sample size was unsurprisingly 0.17%; however, the rate of diploidy ranged from 0% to nearly 0.7% for the sample size = 60, with a large overall variance (Figure 2). That is, with a sample size of 60 for each of the 36 lots, there is a reasonable probability of calculating a rate of diploidy = 0.09%, 0.12%, or 0.19%, all close to 0.17%, and all quite low, but none equal to 0.17% (Figure 2). As we increased sample size from 150 to 1500, the precision of our estimate of the rate of diploidy increased.

If the probability of selecting one or more diploid fish from a pool of 1 million fish, with a triploidy error rate = 0.17% and a sample size = 100 is 0.16 (see above), then we would expect to draw at least one diploid fish from 16 lots out of 100 lots, or 5.8 lots out of 36 lots. Troutlodge's data showed one diploid fish from 5 lots out of 36 lots, with an average sample size per lot = 82. These results are what would be expected if the triploidy error rate was indeed close to 0.17%, as calculated by Troutlodge. We used the same modeling framework described above for Figure 2 to determine the percentage of times the calculated rate of diploidy from a cumulative sample from 36 lots was less than 0.2% out of 100,000 iterations, using a sample of only 60 fish, and actual rates of diploidy equal to 0.02%, 0.17%, 0.2% and 0.5%[15]. The calculated rates of diploidy were less than 0.2% (0.002) 100% of the time for actual rates of diploidy = 0.02%, 69% of the time for actual rates of diploidy = 0.17% (Troutlodge's estimate), and 57% of the time for actual rates of diploidy = 0.2%. The percentage of times the calculated rate of diploidy was less than 0.2% dropped dramatically to 2% for an actual rate of diploidy = 0.5%. **This suggests that the rate of diploidy for the Troutlodge triploidy procedure averages less than 0.5% and is probably close to 0.2%, essentially the same as the 0.17% provided by Cooke. However, drawing sample sizes of**

[13] Based on a hypergeometric distribution, which is equivalent to a binomial (e.g., triploid v. diploid) distribution, but sampling is without replacement, changing the probability after each trial.

[14] Unless you are sampling the entire population, you need to draw at least one diploid fish to assess triploidy error rate.

[15] Less than 0.2% is equal to the five left-most bars in Figure 2A.

**60 or 100 from a lot of one million fish will not produce a precise measure of the triplody error rate. WDFW will require Cooke to have each individual lot they receive from Troutlodge tested for triploidy error rates using a sample size appropriate for the number of eggs in the lot. See Mitigating Provisions**.

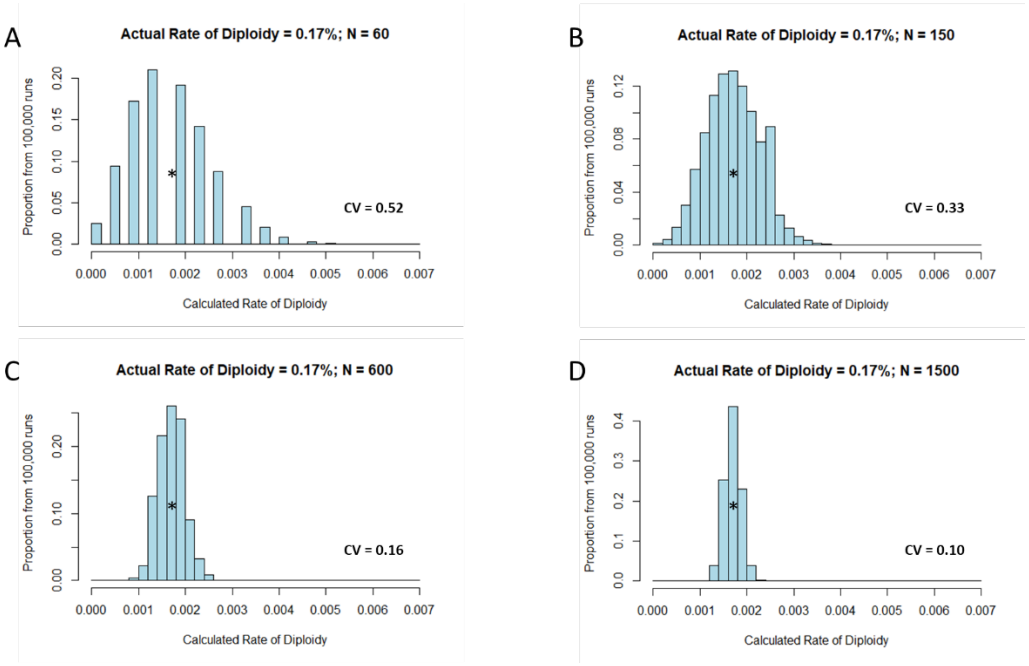


**Figure 2.** Frequency distributions of calculated rates of diploidy, across 100,000 iterations of the model for each of four samples sizes. Asterisk in each plot shows the frequency distribution category for 0.17%. As sample sizes increase there is an increase in the precision of the estimate for rate of diploidy, providing greater confidence that that estimate is accurate.

### 4.3.6. Recapture Efficacy of Escaped Fish

Recapture of fish that escape from net pens has been shown to be relatively ineffective in marine habitats, with rare exception (Dempster et al. 2018). Results show that recapture efforts must be immediate and widespread for best results, but recovery percentages are often still low (Skilbrei and Jorgensen 2010, Chittenden et al. 2011, Dempster et al. 2018). Suggestions that widespread and intense recapture efforts may show some success (Skilbrei and Jorgensen 2010) must be weighed against the risk of bycatch of native non-farm fish. Depending on the method, recapture may cause unacceptable harm in situations where ESA-listed stocks are present. The most effective and least destructive method for recapture is the use of live traps (Chittenden et al. 2011). Recapture efforts after escaped fish disperse, which could occur within hours, days or weeks, are not likely to be effective (Chittenden et al. 2011, Dempster et al. 2018), although the behavior of all-female triploid steelhead trout may differ from those species discussed in the referenced papers. However, the recapture of farmed fish within targeted rivers may provide some mitigation to prevent introgression when fertile fish escape from marine net pens (Glover et al. 2019).

## 4.4. Ecological effects of Net-Pen operations

### 4.4.1. Ecological Interactions

The published literature on the relative risks to wild populations from open net-pen aquaculture emphasizes mostly pathogen and parasite transmission and the effects from escaped farmed fish. The discussions concerning escaped farmed fish focuses on the genetic effects to wild populations, rather than ecological interactions between escaped and wild fish (see discussions above). For example, Forseth et al. (2017) developed a two-dimensional classification system of different anthropogenic factors to assess their relative risk to wild Atlantic salmon populations in Norway. The authors used 15 factors ranging from habitat alteration and hydropower to overpopulation and climate change. Included among the 15 factors were three aquaculture-related factors: sea lice, infections related to fish farming, and escaped farmed fish. The escaped farmed fish factor description was limited to the genetic risk to wild populations (Forseth et al. 2017).

Naylor et al. (2005) attempted a comprehensive assessment of the risks of escaped farmed fish to wild populations, including ecological, genetic, and socioeconomic concerns. Among the ecological risks were competition and predation. However, most of the discussion about competition and predation concerned interactions in the freshwater among juvenile fish, involving escapes from freshwater facilities or offspring from escapes. Naylor et al. (2005) stated that little is known about the competitive interactions between escaped farmed and wild fish in the marine environment, but then speculated that competition may exist since the fish show similar feeding patterns.

Two potential ecological risks to wild populations from net-pen aquaculture that have received limited attention in the literature are (1) the attraction of wild populations to the net-pen facilities, and (2) the potential entrapment and inadvertent harvest of wild fish with the net-pen cages.

Callier et al. (2018) provided a comprehensive review of the relationships between finfish and shellfish aquaculture structures and activities with the attraction (or repulsion) of wild populations. The authors indicated that these relationships are complicated and vary spatially and at several temporal scales. Many of the effects depend on fishery regulations and practices. That is, are the areas around net-pen facilities protected from fisheries, or deliberately avoided by or attract fishing activities? Callier et al. (2018) concluded that there may be effects to wild fish from finfish aquaculture structures and activities related their condition, growth, and reproductive success, and to their population's overall biomass and migratory patterns. However, these factors are poorly understood and the overall effect to population viability is unknown. Callier et al. (2018) reviewed 21 publications involving the aquaculture of eight finfish species, including Atlantic salmon and steelhead trout. The overall conclusions by these authors were consistent among the different farmed fish species. The interaction between Puget Sound net-pen facilities and aquaculture practices and the behavior of wild fish populations have not been studied, but we assume that such interactions occur. We also assume that the interactions in Puget Sound may be similar to those described by Callier et al. (2018), **and that there would be no difference in those**

**interactions between the farming of Atlantic salmon and all-female triploid steelhead trout at Cooke's existing facilities.**

Fish smaller than the mesh size of the net-pen cages can enter and pass through the cages. While in the cages the fish may forage and grow. Fjelldal et al. 2018 document the entrapment of eight wild species within seven Atlantic salmon net-pen facilities in Norway. The seven net-pens held a total of 4,182 Atlantic salmon, and 3,154 entrapped wild fish. The authors did not investigate if this was a normal occurrence in Atlantic salmon farms in Norway, or if there was a negative ecological effect of this bycatch. There exists the possibility that wild fish can become entrapped in Cooke's net-pen facilities in Puget Sound, and become bycatch mortalities when the farmed fish are harvested. The Canadian Government compiles and makes available the incidental finfish bycatch within British Columbia's marine finfish aquaculture farms[16]. From 2011 through September 2019 there were 1256 bycatch incidents reported by the Canadian Government that involved a total of 708,574 fish. However, two of these incidents were the deliberate depopulation of the net-pens to control the spread of IHNV outbreaks. These two incidents involved a single species (Pacific herring) and 406,366 fish, or 57% of the nine-year total. Overall, Pacific herring accounted for 638,950 (90%) of the total bycatch. The median number of fish caught as bycatch was eight fish per incident. A total of 308 Pacific salmon were caught in 87 incidents (median = 9 fish per incident), and no steelhead trout were caught. The population-level effects of this bycatch are not known, but the number of fish caught per incident is small absolutely, and small relative to their population sizes. WDFW will attempt to observe the harvest of fish from Cooke' net-pen facilities (see Section 6, Mitigating Provisions), but **there is no reason to assume that the bycatch, if any, would differ between the farming of Atlantic salmon and the farming all-female triploid steelhead trout at these facilities**.

### 4.4.2. Water Quality and the Benthic Environment

Washington Department of Ecology has the state's regulatory authority to protect water and sediment quality. The U.S. Environmental Protection Agency (EPA) authorized Ecology to administer the Federal Clean Water Act in Washington through National Pollutant Discharge Elimination System (NPDES) permits. RCW Chapter 90.48 defines Ecology's authority and obligations in administering the wastewater discharge permit program. On July 11, 2019, Ecology issued updated NPDES permits for the rearing of Atlantic salmon at four of Cooke's net-pen facilities, Hope Island, Clam Bay, Orchard Rocks, and Fort Ward. These updated permits require increased routine monitoring, inspections, and spill response planning and reporting.

WDFW has consulted with Ecology in making its SEPA determination and issuing to Cooke a Marine Finfish Aquaculture Permit to raise all-female triploid steelhead trout. Once Cooke has submitted to Ecology completed NPDES permit applications to raise all-female triploid steelhead trout at its existing net-pen facilities in Puget Sound, Ecology will begin a multi-step evaluation process with several opportunities for public comment. Ecology will evaluate if changing culture from Atlantic salmon to all-female triploid steelhead trout will

[16] https://open.canada.ca/data/en/dataset/0bf04c4e-d2b0-4188-9053-08dc4a7a2b03

change the character and degree of impact to water and sediment quality. WDFW defers to Ecology on their evaluation of the risk to the water and sediment from Cooke's proposed action. **As a mitigating provision (see Section 6), Cooke's Marine Finfish Aquaculture Permit to raise all-female triploid steelhead trout is contingent on receiving NPDES authorization from Ecology for this activity**.

## 5. NOAA Recovery Plan

The National Marine Fisheries Service (NMFS) published the final ESA Recovery Plan for Puget Sound steelhead trout on December 20, 2019. In the section describing pressures associated with ecological and genetic interactions between hatchery and natural-origin fish, NMFS included a paragraph on "Net pen Operations." The paragraph describes only the net-pen culturing of Atlantic salmon, although the paragraph ends with the mention of the potential replacement of Atlantic salmon with steelhead trout. The paragraph and a bulleted item on page 140 contain unreferenced statements about pollution and pathogen transmission risks from net-pen aquaculture. We have addressed in Section 4 of this document all the net-pen related risks discussed by NMFS in their ESA Recovery Plan.

## 6. Mitigating Provisions

**Operations, including future finfish transport permits:**

1. This Permit is for the marine cultivation of all-female triploid steelhead trout (*Oncorhynchus mykiss*) from embryos originating from Troutlodge, Bonney Lake, Washington.
2. Transgenic fish, as defined in WAC 220-370-100, are not permitted
3. In accordance with Washington State Law (2018 c 179 § 3; RCW 77.175.050) this permit is valid for existing marine net-pen facilities with valid leases of state-owned aquatic lands (Fort Ward, Orchard Rocks, Clam Bay, and Hope Island facilities). This permit will become valid for existing facilities without leases of state-owned aquatic lands (Cypress 1, Cypress 2, and Port Angeles) if these leases are restored, or new leases issued.
4. In accordance with WAC 220-370-100, this permit is valid for a maximum of five years, starting from the date of this correspondence and ending January 21, 2025 or on the date of termination of leases of state-owned aquatic lands, whichever is sooner (RCW 77.175.050).
5. Cooke must receive from the Washington Department of Ecology NPDES authorization to raise all-female triploid steelhead trout in their net-pen facilities in Puget Sound before Cooke can stock facilities with steelhead trout. All requirements and provisions stipulated by Ecology on NPDES permits must be followed.
6. All activities described in Cooke Aquaculture's Plan of Operation – All-female Triploid Rainbow Trout; Fish Escape Prevention, Response, and Report Plan; and Regulated Finfish Pathogen Report Plan must be followed as written, unless otherwise specified below. All plans must be updated annually and in consultation with WDFW Fish Health and Hatchery programs, with final drafts submitted to WDFW for approval no later than November 30 of the calendar year. The Fish Escape Prevention, Response, and Report Plan must be drafted in consultation with DNR, Ecology, WDFW, and effected treaty tribes.

7. All fish transported into net-pens must contain one or more visual marks, other than the shape of each fish, that *unambiguously* identifies each fish as commercial aquaculture fish, as opposed to hatchery- or natural-origin free ranging fish of Washington State. WDFW considers that commercial aquaculture steelhead marked with adipose fin clip only presents a risk of confusion with the state's hatchery-origin steelhead. Before July 2020 Cooke must implement an alternate method, approved by WDFW, to visually identify their fish.
8. For each lot of fish to be transported into marine net-pen facilities, Cooke must provide to WDFW a sample of tissue from 150 fish appropriate for genetic analyses, if the lot is derived from a single brood line. If the lot is composed of more than one brood line, Cooke must provide to WDFW samples of tissue from 150 fish from each brood line. The fish tissue can be from live or lethal sampling. WDFW will genotype samples using their baseline assay of SNP markers and will use the information only to determine if steelhead samples from hatchery- or natural-spawning fish are commercial aquaculture fish or F1 offspring of commercial aquaculture fish.
9. Prior to stocking net pens, Cooke must provide WDFW, DNR, and Ecology the approximate dates for stocking. Within one month after stocking is completed Cooke must provide to WDFW, DNR, and Ecology a report documenting the facility stocked, dates in which stocking occurred, the total number of fish stocked per day, and any complications that may have occurred during stocking. Cooke must report immediately if fish escaped during stocking. If requested by WDFW, DNR, or Ecology, Cooke must allow appropriately trained personnel from these agencies to monitor the stocking activities.
10. Prior to harvest, Cooke must provide WDFW, DNR, and Ecology the approximate dates for harvest. Within one month after harvesting is completed Cooke must provide to WDFW, DNR, and Ecology a report documenting the facility harvested, dates in which harvesting occurred, the total number of fish harvested per day, and any complications that may have occurred during harvesting. Cooke must report immediately if any live fish escaped during harvesting, or if any fish carcass, parts, or offal were discarded into the Puget Sound waters. The discard of carcasses, fish parts, or offal is also a violation of Cooke's NPDES permit. Cooke also must report the number and species of bycatch caught during harvesting. If requested by WDFW, DNR, or Ecology, Cooke must allow appropriately trained personnel from these agencies to monitor the harvesting activities.
11. The following monitoring data needs to be reported to WDFW, DNR, and Ecology as part of an expanded Monthly Feed, Biomass, and Disease Control Chemical Use Report, or as separate monthly report(s): (1) the feed conversion rates at each facility, (2) the estimated number of live individuals at each facility, and (3) the number of dead fish collected or observed (the greater of these two numbers) at each facility during the period since the prior reporting month.
12. For each of their facilities, Cooke must continue the net hygiene monitoring protocol developed cooperatively by Cooke and DNR (see Section 4.3.1 above).
13. WDFW Finfish Transport Permits are required when moving fish from freshwater facilities to marine net pens, or between aquatic farm sites.

**Escape Prevention, Response, and Reporting:**

1. In accordance with Washington State Law (2018 c 179 § 12; RCW 77.175.060) for each net-pen facility, Cooke must hire, at their own expense, a marine engineering firm approved by WDFW to

conduct inspections. Inspections must occur approximately every two years, when net pens are fallow, and must include topside and mooring assessments related to escapement potential, structural integrity, permit compliance, and operations. Analyses of the mooring and cage systems of each net-pen facility must use environmental condition data that are consistent with the Norwegian aquaculture standard NS 9415 (see Section 4.3.1 above).

2. Cooke must report to WDFW Fish Health Supervisor, Lead Veterinarian, or Aquaculture Coordinator within 24 hours of discovery of any fish that has been observed to have escaped from any net-pen facility or during transfer into or out of a net-pen facility, regardless of numbers of fish involved (i.e., the minimum reporting number is one).
3. It is conceivable that an attempt to recover fish after an escape event might negatively affect native Pacific salmonids more than no attempt to recover fish. Cooke is required to work with WDFW, Ecology, DNR, effected treaty tribes, and NOAA to include a no-recovery option in the 2021 Fish Escape Prevention, Response, and Reporting Plan, to be finalized December 2020. This option should include when, where, and under what conditions a recovery effort should not be attempted. A no-recovery option would be triggered by the state, in consultation with co-managers and federal agencies for the purpose of protecting native Pacific salmonids.
4. Both the Washington Department of Health and WDFW need to be notified if escaped fish were on medicated feed at the time of their escape or are within the required withdrawal period for the medicated feed used.
5. Before January 1, 2021, Cooke must have engineered mooring and anchoring plans and site-specific engineered drawings stamped by a structural engineer, for each net-pen facility.

**Triploidy error rate**

1. Cooke is to work with Troutlodge and WDFW to develop or implement an alternative method or employ a different sampling and statistical design to estimate the triploidy error rate. This method will be implemented on each lot of fish to be transported into marine net-pen facilities and provide the state with an estimated number of diploid-fertile fish in that lot. This alternative method or design must be implemented no later than July 2020, unless stated otherwise by WDFW. In the absence of the alternative method Cooke will be required to sample 600 fish from each lot to determine triploidy error rate (see Section 4.3.5 above)

**Finfish Pathogen Reporting and Biosecurity:**

1. Cooke must ensure that all state and federal Veterinary-Client-Patient-Relationship (VCPR), Veterinarian of Record (VOR), and Veterinary Feed Directive (VFD) rules and laws are followed (e.g., WAC 246-933-200, 21 CFR 514, 21 CFR 558).
2. In accordance with WAC 220-370-080 and 220-370-130 authorized WDFW employees shall have access to freshwater hatchery facilities and marine net-pen facilities to conduct inspections, to collect samples for disease surveillance, and to inspect net-pen infrastructure.
3. Net-pen facilities must remain fallow for 42 days after the last fish are harvested and the last containment net is removed for cleaning and repair. This number can be increased per determination of WDFW veterinarian due to disease prevalence just prior to or at the time of harvest.
4. Net-pen facilities must be managed as single-generation stocking.

5. Broodstock (parents) of embryos or fish going to Cooke Aquaculture freshwater rearing facilities will be sampled and tested at a certified lab for Washington Regulated Pathogens (see Table 1 below) at the 2% APPL annually within three months of transfer from Troutlodge to Cooke's freshwater facility.
6. Lots of pre-marine smolts prior to transfer from Cooke's freshwater faculties to marine net-pens will be sampled and tested at a certified testing lab for Washington State Regulated and Reportable pathogens (see #2 above) at the 2% APPL.
7. Cooke's freshwater and marine facilities are subject to inspections by WDFW to ensure proper biosecurity, fish health, and pathogen sampling. Sampling levels can be modified by WDFW in response to pathogen findings.
8. Under no conditions should fish carcasses be removed from the net-pens and returned into waters of Puget Sound. The discard of carcasses is also a violation of Cooke's NPDES permit.
9. All disease outbreaks, unexplained mortality, regulated, reportable, or exotic pathogen findings must be reported to the WDFW Fish Health Supervisor, Lead Veterinarian, or Aquaculture Coordinator within 24 hours.
10. A fish health evaluation report written by a certified fish health inspector must be submitted to WDFW each year, no later than January 31, summarizing fish health inspections, laboratory tests, and the presence of pathogens, for the previous calendar year, at each net-pen facility (one report that includes all net-pen facilities).

**Table 1.** Regulated and Reportable pathogens described in WAC 220-370 and in The Salmonid Disease Control Policy of the Fisheries Co-Managers of Washington State.

a. Regulated Pathogens:
   i. Infectious hematopoietic necrosis virus (IHNV)
   ii. Infectious pancreatic necrosis virus (IPNV)
   iii. Infectious salmon anemia virus (ISAV)
   iv. *Oncorhynchus masou* virus (OMV)
   v. Viral hemorrhagic septicemia virus (VHSV)
   vi. *Myxobolus cerebralis* (whirling disease only known in fresh water)

b. Reportable Pathogen:
   i. All viral replicating agents other than those listed as Regulated pathogens that are found on cell culture using procedures outlined in the AFS-USFWS Specific Procedures for Aquatic Animal Health Inspections or OIE Aquatic Code.
   ii. Strains of pathogenic bacteria resistant to antimicrobial agents approved for use in fish or used through an extra-label prescription or INAD permit.
   iii. *Piscirickettsia salmonis*
   iv. *Nucleospora salmonis*
   v. North Atlantic variants of PRV 1, and all variants of PRV 3

## 7. Literature Cited

Adamek, M., J. Hellmann, A. Flamm, F. Teitge, N. Vendramin, D. Fey, K. Risse, F. Blakey, E. Rimstad, and D. Steinhagen. 2019. Detection of piscine orthoreoviruses (PRV-1 and PRV-3) in Atlantic salmon and rainbow trout farmed in Germany. Transboundary and Emerging Diseases **66**:14-21.

Aldrin, M., B. Storvik, A. Frigessi, H. Viljugrein, and P. A. Jansen. 2010. A stochastic model for the assessment of the transmission pathways of heart and skeleton muscle inflammation, pancreas disease and infectious salmon anaemia in marine fish farms in Norway. Preventive Veterinary Medicine **93**:51-61.

Amos, K. H., and A. Appleby. 1999. Atlantic Salmon in Washington State: A Fish Management Perspective. Washington Department of Fish and Wildlife. Olympia, Washington.

Anderson, E. D., H. M. Engelking, E. J. Emmenegger, and G. Kurath. 2000. Molecular epidemiology reveals emergence of a virulent infectious hematopoietic necrosis (IHN) virus strain in wild salmon and its transmission to hatchery fish. Journal of Aquatic Animal Health **12**:85-99.

Baskett, M. L., S. C. Burgess, and R. S. Waples. 2013. Assessing strategies to minimize unintended fitness consequences of aquaculture on wild populations. Evolutionary Applications **6**:1090-1108.

Beamish, R. J., S. Jones, C. E. Neville, R. Sweeting, G. Karreman, S. Saksida, and E. Gordon. 2006. Exceptional marine survival of pink salmon that entered the marine environment in 2003 suggests that farmed Atlantic salmon and Pacific salmon can coexist successfully in a marine ecosystem on the Pacific coast of Canada. ICES Journal of Marine Science **63**:1326-1337.

Benfey, T. J., and A. M. Sutterlin. 1984. Triploidy induced by heat shock and hydrostatic pressure in landlocked Atlantic salmon (Salmo salar L.). Aquaculture **36**:359-367.

Bergstedt, R. A., and M. B. Twohey. 2007. Research to support sterile-male-release and genetic alteration techniques for sea lamprey control. Journal of Great Lakes Research **33**:48-69.

Blanchfield, P. J., L. S. Tate, and C. L. Podemski. 2009. Survival and behaviour of Rainbow Trout (*Oncorhynchus mykiss*) released from an experimental aquaculture operation. Canadian Journal of Fisheries and Aquatic Sciences **66**:1976-1988.

Bolstad, G. H., K. Hindar, G. Robertsen, B. Jonsson, H. Saegrov, O. H. Diserud, P. Fiske, A. J. Jensen, K. Urdal, T. F. Naesje, B. T. Barlaup, B. Floro-Larsen, H. Lo, E. Niemela, and S. Karlsson. 2017. Gene flow from domesticated escapes alters the life history of wild Atlantic salmon. Nature Ecology & Evolution **1**.

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

Boxaspen, K. 2006. A review of the biology and genetics of sea lice. ICES Journal of Marine Science **63**:1304-1316.

Breyta, R., A. Black, J. Kaufman, and G. Kurath. 2016a. Spatial and temporal heterogeneity of infectious hematopoietic necrosis virus in Pacific Northwest salmonids. Infection Genetics and Evolution **45**:347-358.

Breyta, R., A. Jones, and G. Kurath. 2014. Differential susceptibility in steelhead trout populations to an emergent MD strain of infectious hematopoietic necrosis virus. Diseases of Aquatic Organisms **112**:17-28.

Breyta, R., D. McKenney, T. Tesfaye, K. Ono, and G. Kurath. 2016b. Increasing virulence, but not infectivity, associated with serially emergent virus strains of a fish rhabdovirus. Virus Evolution **2**.

Bricknell, I. R., S. J. Dalesman, B. O'Shea, C. P. Campbell, and A. J. Mordue Luntz. 2006. Effect of environmental salinity on sea lice *Lepeophtheirus salmonis* settlement success. Disease of Aquatic Organisms **71**:201-212.

Britton, J. R., J. Pegg, and R. E. Gozlan. 2011. Quantifying imperfect detection in an invasive pest fish and the implications for conservation management. Biological Conservation **144**:2177-2181.

Brooks, K. M. 2005. The effects of water temperature, salinity, and currents on the survival and distribution of the infective copepodid stage of sea lice (Lepeophtheirus salmonis) originating on Atlantic salmon farms in the Broughton Archipelago of British Columbia, Canada. Reviews in Fisheries Science **13**:177-204.

Brooks, K. M., and D. J. Stucchi. 2006. The effects of water temperature, salinity and currents on the survival and distribution of the infective copepodid stage of the salmon louse (Lepeophtheirus salmonis) originating on Atlantic salmon farms in the Broughton Archipelago of British Columbia, Canada (Brooks, 2005) - A response to the rebuttal of Krkosek et al. (2005a). Reviews in Fisheries Science **14**:13-23.

Byrne, A. A., C. M. Pearce, S. F. Cross, S. R. M. Jones, S. M. C. Robinson, M. J. Hutchinson, M. R. Miller, C. A. Haddad, and D. L. Johnson. 2018. Planktonic and parasitic stages of sea lice (Lepeophtheirus salmonis and Caligus clemensi) at a commercial Atlantic salmon (Salmo salar) farm in British Columbia, Canada. Aquaculture **486**:130-138.

Callier, M. D., C. J. Byron, D. A. Bengtson, P. J. Cranford, S. F. Cross, U. Focken, H. M. Jansen, P. Kamermans, A. Kiessling, T. Landry, F. O'Beirn, E. Petersson, R. B. Rheault, O. Strand, K. Sundell, T. Svasand, G. H. Wikfors, and C. W. McKindsey. 2018. Attraction and repulsion of mobile wild organisms to finfish and shellfish aquaculture: a review. Reviews in Aquaculture **10**:924-949.

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

Clark, D., K. Lee, K. Murphy, and A. Windrope. 2018. Cypress Island Atlantic Salmon net pen failure: an investigation and review. Page 120 *in* W. D. o. N. Resources, editor., Olympia, WA.

Cooke, A. P. 2019. SEPA Checklist with Attachments.*in* S. t. W. D. o. F. a. Wildlife, editor.

Corbeil, S., S. E. LaPatra, E. D. Anderson, J. Jones, B. Vincent, Y. L. Hsu, and G. Kurath. 1999. Evaluation of the protective immunogenicity of the N, P, M, NV and G proteins of infectious hematopoietic necrosis virus in rainbow trout Oncorhynchus mykiss using DNA vaccines. Diseases of Aquatic Organisms **39**:29-36.

Corbeil, S., S. E. LaPatra, E. D. Anderson, and G. Kurath. 2000. Nanogram quantities of a DNA vaccine protect rainbow trout fry against heterologous strains of infectious hematopoietic necrosis virus. Vaccine **18**:2817-2824.

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

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

Crosbie, T., D. W. Wright, F. Oppedal, I. A. Johnsen, F. Samsing, and T. Dempster. 2019. Effects of step salinity gradients on salmon lice larvae behaviour and dispersal. Aquaculture Environment Interactions **11**:181-190.

Dempster, T., P. Arechavala-Lopez, L. T. Barrett, I. A. Fleming, P. Sanchez-Jerez, and I. Uglem. 2018. Recapturing escaped fish from marine aquaculture is largely unsuccessful: alternatives to reduce the number of escapees in the wild. Reviews in Aquaculture **10**:153-167.

Dhamotharan, K., N. Vendramin, T. Markussen, O. Wessel, A. Cuenca, I. B. Nyman, A. B. Olsen, T. Tengs, M. K. Dahle, and E. Rimstad. 2018. Molecular and Antigenic Characterization of Piscine orthoreovirus (PRV) from Rainbow Trout (Oncorhynchus mykiss). Viruses-Basel **10**.

Di Cicco, E., H. W. Ferguson, K. H. Kaukinen, A. D. Schulze, S. R. Li, A. Tabata, O. P. Gunther, G. Mordecai, C. A. Suttle, and K. M. Miller. 2018. The same strain of Piscine orthoreovirus (PRV-1) is involved in the development of different, but related, diseases in Atlantic and Pacific Salmon in British Columbia. Facets **3**:599-641.

Di Cicco, E., H. W. Ferguson, A. D. Schulze, K. H. Kaukinen, S. Li, R. Vanderstichel, O. Wessel, E. Rimstad, I. A. Gardner, K. L. Hammell, and K. M. Miller. 2017. Heart and skeletal muscle inflammation (HSMI) disease diagnosed on a British Columbia salmon farm through a longitudinal farm study. Plos One **12**.

Diserud, O. H., P. Fiske, H. Saegrov, K. Urdal, T. Aronsen, H. Lo, B. T. Barlaup, E. Niemela, P. Orell, J. Erkinaro, R. A. Lund, F. Okland, G. M. Ostborg, L. P. Hansen, and K. Hindar. 2019. Escaped farmed Atlantic salmon in Norwegian rivers during 1989-2013. ICES Journal of Marine Science **76**:1140-1150.

Feindel, N. J., T. J. Benfey, and E. A. Trippel. 2010. Competitive spawning success and fertility of triploid male Atlantic cod Gadus morhua. Aquaculture Environment Interactions **1**:47-55.

Ferguson, H. W., R. T. Kongtorp, T. Taksdal, D. Graham, and K. Falk. 2005. An outbreak of disease resembling heart and skeletal muscle inflammation in Scottish farmed salmon, Salmo salar L., with observations on myocardial regeneration. Journal of Fish Diseases **28**:119-123.

Finstad, O. W., M. K. Dahle, T. H. Lindholm, I. B. Nyman, M. Lovoll, C. Wallace, C. M. Olsen, A. K. Storset, and E. Rimstad. 2014. Piscine orthoreovirus (PRV) infects Atlantic salmon erythrocytes. Veterinary Research **45**.

Fisher, A. C., J. P. Volpe, and J. T. Fisher. 2014. Occupancy dynamics of escaped farmed Atlantic salmon in Canadian Pacific coastal salmon streams: implications for sustained invasions. Biological Invasions **16**:2137-2146.

Fjelldal, P. G., M. F. Solberg, K. A. Glover, O. Folkedal, J. Nilsson, R. N. Finn, and T. J. Hansen. 2018. Documentation of multiple species of marine fish trapped in Atlantic salmon sea-cages in Norway. Aquatic Living Resources **31**.

Fleming, I. A., K. Hindar, I. B. Mjolnerod, B. Jonsson, T. Balstad, and A. Lamberg. 2000. Lifetime success and interactions of farm salmon invading a native population. Proceedings of the Royal Society B-Biological Sciences **267**:1517-1523.

Forseth, T., B. T. Barlaup, B. Finstad, P. Fiske, H. Gjoaester, M. Falkegard, A. Hindar, T. A. Mo, A. H. Rikardsen, E. B. Thorstad, L. A. Vollestad, and V. Wennevik. 2017. The major threats to Atlantic salmon in Norway. ICES Journal of Marine Science **74**:1496-1513.

Fux, R., D. Arndt, M. C. Langenmayer, J. Schwaiger, H. Ferling, N. Fischer, D. Indenbirken, A. Grundhoff, L. Dolken, M. Adamek, D. Steinhagen, and G. Sutter. 2019. Piscine Orthoreovirus 3 Is Not the Causative Pathogen of Proliferative Darkening Syndrome (PDS) of Brown Trout (Salmo trutta fario). Viruses-Basel **11**.

Garseth, A. H., T. Ekrem, and E. Biering. 2013. Phylogenetic Evidence of Long Distance Dispersal and Transmission of Piscine Reovirus (PRV) between Farmed and Wild Atlantic Salmon. Plos One **8**.

Garver, K. A., W. N. Batts, and G. Kurath. 2006. Virulence comparisons of infectious hematopoietic necrosis virus U and M genogroups in sockeye salmon and rainbow trout. Journal of Aquatic Animal Health **18**:232-243.

Garver, K. A., S. C. Johnson, M. P. Polinski, J. C. Bradshaw, G. D. Marty, H. N. Snyman, D. B. Morrison, and J. Richard. 2016a. Piscine Orthoreovirus from Western North America Is Transmissible to Atlantic Salmon and Sockeye Salmon but Fails to Cause Heart and Skeletal Muscle Inflammation. Plos One **11**.

Garver, K. A., S. E. LaPatra, and G. Kurath. 2005. Efficacy of an infectious hematopoietic necrosis (IHN) virus DNA vaccine in Chinook Oncorhynchus tshawytscha and sockeye O-nerka salmon. Diseases of Aquatic Organisms **64**:13-22.

Garver, K. A., A. A. M. Mahony, D. Stucchi, J. Richard, C. Van Woensel, and M. Foreman. 2013. Estimation of Parameters Influencing Waterborne Transmission of Infectious Hematopoietic Necrosis Virus (IHNV) in Atlantic Salmon (Salmo salar). Plos One **8**.

Garver, K. A., G. D. Marty, S. N. Cockburn, J. Richard, L. M. Hawley, A. Muller, R. L. Thompson, M. K. Purcell, and S. Saksida. 2016b. Piscine reovirus, but not Jaundice Syndrome, was transmissible to Chinook Salmon, Oncorhynchus tshawytscha (Walbaum), Sockeye Salmon, Oncorhynchus nerka (Walbaum), and Atlantic Salmon, Salmo salar L. Journal of Fish Diseases **39**:117-128.

Glover, K. A., J. B. Bos, K. Urdal, A. S. Madhun, A. G. E. Sorvik, L. Unneland, B. B. Seliussen, O. Skaala, O. T. Skilbrei, Y. Tang, and V. Wennevik. 2016. Genetic screening of farmed Atlantic salmon escapees demonstrates that triploid fish display reduced migration to freshwater. Biological Invasions **18**:1287-1294.

Glover, K. A., M. Quintela, V. Wennevik, F. Besnier, A. G. E. Sorvik, and O. Skaala. 2012. Three Decades of Farmed Escapees in the Wild: A Spatio-Temporal Analysis of Atlantic Salmon Population Genetic Structure throughout Norway. Plos One **7**:e43129.

Glover, K. A., M. F. Solberg, P. McGinnity, K. Hindar, E. Verspoor, M. W. Coulson, M. M. Hansen, H. Araki, O. Skaala, and T. Svasand. 2017. Half a century of genetic interaction between farmed and wild Atlantic salmon: Status of knowledge and unanswered questions. Fish and Fisheries **18**:890-927.

Glover, K. A., K. Urdal, T. Naesje, H. Skoglund, B. Floro-Larsen, H. Ottera, P. Fiske, M. Heino, T. Aronsen, H. Saegrov, O. Diserud, B. T. Barlaup, K. Hindar, G. Bakke, I. Solberg, H. Lo, M. F. Solberg, S. Karlsson, O. Skaala, A. Lamberg, O. Kanstad-Hanssen, R. Muladal, O. T. Skilbrei, and V. Wennevik. 2019. Domesticated escapees on the run: the second-generation monitoring programme reports the numbers and proportions of farmed Atlantic salmon in > 200 Norwegian rivers annually. ICES Journal of Marine Science **76**:1151-1161.

Godoy, M. G., M. J. T. Kibenge, Y. W. Wang, R. Suarez, C. Leiva, F. Vallejos, and F. S. B. Kibenge. 2016. First description of clinical presentation of piscine orthoreovirus (PRV) infections in salmonid aquaculture in Chile and identification of a second genotype (Genotype II) of PRV. Virology Journal **13**.

Gustafson, L. L., S. K. Ellis, M. J. Beattie, B. D. Chang, D. A. Dickey, T. L. Robinson, F. P. Marenghi, P. J. Moffett, and F. H. Page. 2007. Hydrographics and the timing of infectious salmon anemia outbreaks among Atlantic salmon (Salmo salar L.) farms in the Quoddy region of Maine, USA and New Brunswick, Canada. Preventive Veterinary Medicine **78**:35-56.

Haatveit, H. M., I. B. Nyman, T. Markussen, O. Wessel, M. K. Dahle, and E. Rimstad. 2016. The non-structural protein mu NS of piscine orthoreovirus (PRV) forms viral factory-like structures. Veterinary Research **47**.

Haatveit, H. M., O. Wessel, T. Markussen, M. Lund, B. Thiede, I. B. Nyman, S. Braaen, M. K. Dahle, and E. Rimstad. 2017. Viral Protein Kinetics of Piscine Orthoreovirus Infection in Atlantic Salmon Blood Cells. Viruses-Basel **9**.

Hauge, H., M. Dahle, T. Moldal, E. Thoen, A. G. Gjevre, S. Weli, M. Alarcon, and S. Grove. 2016. Piscine orthoreovirus can infect and shed through the intestine in experimentally challenged Atlantic salmon (Salmo salar L.). Veterinary Research **47**.

Hauge, H., N. Vendramin, T. Taksdal, A. B. Olsen, O. Wessel, S. S. Mikkelsen, A. L. F. Alencar, N. J. Olesen, and M. K. Dahle. 2017. Infection experiments with novel Piscine orthoreovirus from rainbow trout (Oncorhynchus mykiss) in salmonids. Plos One **12**.

Hawkins, J. L., G. E. Bath, W. W. Dickhoff, and J. A. Morris. 2019. State of Science on Net-Pen Aquaculture in Puget Sound, Washington. Page 219 + viii Unpublished Report to State of Washington.

Hernandez, D. G., M. K. Purcell, C. S. Friedman, and G. Kurath. 2016. Susceptibility of ocean- and stream-type Chinook salmon to isolates of the L, U, and M genogroups of infectious hematopoietic necrosis virus (IHNV). Diseases of Aquatic Organisms **121**:15-28.

Hindar, K., N. Ryman, and F. Utter. 1991. Genetic-effects of cultured fish on natural fish populations. Canadian Journal of Fisheries and Aquatic Sciences **48**:945-957.

Hjeltnes, B., B. Bang-Jensen, G. Bornø, A. Haukaas, and C. S. Walde, editors. 2019. The Health Situation in Norwegian Aquaculture 2018. Norwegian Veterinary Institute.

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

Janhunen, M., H. Vehvilainen, J. Koskela, A. Forsman, and M. Kankainen. 2019. Added value from an added chromosome: Potential of producing large fillet fish from autumn to spring with triploid rainbow trout, Oncorhynchus mykiss. Aquaculture Research **50**:818-825.

Jensen, A. J., S. Karlsson, P. Fiske, L. P. Hansen, K. Hindar, and G. M. Ostborg. 2013. Escaped farmed Atlantic salmon grow, migrate and disperse throughout the Arctic Ocean like wild salmon. Aquaculture Environment Interactions **3**:223-229.

Jensen, O., T. Dempster, E. B. Thorstad, I. Uglem, and A. Fredheim. 2010. Escapes of fishes from Norwegian sea-cage aquaculture: causes, consequences and prevention. Aquaculture Environment Interactions **1**:71-83.

Johansen, L. H., I. Jensen, H. Mikkelsen, P. A. Bjorn, P. A. Jansen, and O. Bergh. 2011. Disease interaction and pathogens exchange between wild and farmed fish populations with special reference to Norway. Aquaculture **315**:167-186.

Johnson, M. A., D. L. G. Noakes, T. A. Friesen, A. H. Dittman, R. B. Couture, C. B. Schreck, C. Banner, D. May, and T. P. Quinn. 2019. Growth, survivorship, and juvenile physiology of triploid steelhead (Oncorhynchus mykiss). Fisheries Research **220**.

Jones, S. R. M., D. W. Bruno, L. Madsen, and E. J. Peeler. 2015. Disease management mitigates risk of pathogen transmission from maricultured salmonids. Aquaculture Environment Interactions **6**:119-134.

Karlsson, S., O. H. Diserud, P. Fiske, and K. Hindar. 2016. Widespread genetic introgression of escaped farmed Atlantic salmon in wild salmon populations. ICES Journal of Marine Science **73**:2488-2498.

Kennedy, D. A., G. Kurath, I. L. Brito, M. K. Purcell, A. F. Read, J. R. Winton, and A. R. Wargo. 2016. Potential drivers of virulence evolution in aquaculture. Evolutionary Applications **9**:344-354.

Khangaonkar, T., Z. Q. Yang, T. Kim, and M. Roberts. 2011. Tidally averaged circulation in Puget Sound sub-basins: Comparison of historical data, analytical model, and numerical model. Estuarine Coastal and Shelf Science **93**:305-319.

Kibenge, M. J. T., T. Iwamoto, Y. Wang, A. Morton, M. G. Godoy, and F. S. B. Kibenge. 2013. Whole-genome analysis of piscine reovirus (PRV) shows PRV represents a new genus in family Reoviridae and its genome segment S1 sequences group it into two separate sub-genotypes. Virology Journal **10**.

Kibenge, M. J. T., Y. W. Wang, N. Gayeski, A. Morton, K. Beardslee, B. McMillan, and F. S. B. Kibenge. 2019. Piscine orthoreovirus sequences in escaped farmed Atlantic salmon in Washington and British Columbia. Virology Journal **16**.

Kongtorp, R. T., M. Halse, T. Taksdal, and K. Falk. 2006. Longitudinal study of a natural outbreak of heart and skeletal muscle inflammation in Atlantic salmon, Salmo salar L. Journal of Fish Diseases **29**:233-244.

Kongtorp, R. T., T. Taksdal, and A. Lyngoy. 2004. Pathology of heart and skeletal muscle inflammation (HSMI) in farmed Atlantic salmon Salmo salar. Diseases of Aquatic Organisms **59**:217-224.

Krkosek, M., B. M. Connors, A. Morton, M. A. Lewis, L. M. Dill, and R. Hilborn. 2011. Effects of parasites from salmon farms on productivity of wild salmon. Proceedings of the National Academy of Sciences of the United States of America **108**:14700-14704.

Krkosek, M., M. A. Lewis, J. P. Volpe, and A. Morton. 2006. Fish farms and sea lice infestations of wild juvenile salmon in the Broughton Archipelago - A rebuttal to Brooks (2005). Reviews in Fisheries Science **14**:1-11.

Kuehn, R., B. C. Stoeckle, M. Young, L. Popp, J. E. Taeubert, M. W. Pfaffl, and J. Geist. 2018. Identification of a piscine reovirus-related pathogen in proliferative darkening syndrome (PDS) infected brown trout (Salmo trutta fario) using a next-generation technology detection pipeline. Plos One **13**.

Kurath, G., K. A. Garver, S. Corbeil, D. G. Elliott, E. D. Anderson, and S. E. LaPatra. 2006. Protective immunity and lack of histopathological damage two years after DNA vaccination against infectious hematopoietic necrosis virus in trout. Vaccine **24**:345-354.

Kurath, G., K. A. Garver, R. M. Troyer, E. J. Emmenegger, K. Einer-Jensen, and E. D. Anderson. 2003. Phylogeography of infectious haematopoietic necrosis virus in North America. Journal of General Virology **84**:803-814.

Kurath, G., and J. Winton. 2011. Complex dynamics at the interface between wild and domestic viruses of finfish. Current Opinion in Virology **1**:73-80.

Kurath, G., J. R. Winton, O. B. Dale, M. K. Purcell, K. Falk, and R. A. Busch. 2016. Atlantic salmon, Salmo salar L. are broadly susceptible to isolates representing the North American genogroups of infectious hematopoietic necrosis virus. Journal of Fish Diseases **39**:55-67.

LaPatra, S. E., S. Corbeil, G. R. Jones, W. D. Shewmaker, and G. Kurath. 2000. The dose-dependent effect on protection and humoral response to a DNA vaccine against infectious hematopoietic necrosis (IHN) virus in subyearling rainbow trout. Journal of Aquatic Animal Health **12**:181-188.

LaPatra, S. E., S. Corbeil, G. R. Jones, W. D. Shewmaker, N. Lorenzen, E. D. Anderson, and G. Kurath. 2001. Protection of rainbow trout against infectious hematopoietic necrosis virus four days after specific or semi-specific DNA vaccination. Vaccine **19**:4011-4019.

Leclercq, E., J. F. Taylor, D. Fison, P. G. Fjelldal, M. Diez-Padrisa, T. Hansen, and H. Migaud. 2011. Comparative seawater performance and deformity prevalence in out-of-season diploid and triploid Atlantic salmon (Salmo salar) post-smolts. Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology **158**:116-125.

Lerfall, J., A. V. Skuland, E. H. Skare, P. R. Hasli, and B. T. Rotabakk. 2017. Quality characteristics and consumer acceptance of diploid and triploid cold smoked Atlantic salmon reared at 5, 10 and 15 degrees C. Lwt-Food Science and Technology **85**:45-51.

Love, D. C., J. P. Fry, F. Cabello, C. M. Good, and B. T. Lunestand. 2020. Veterinary Drug Use in United States Net Pen Salmon Aquaculture: Implications for Drug Use Policy. Aquaculture **In Press**.

Lovoll, M., M. Alarcon, B. B. Jensen, T. Taksdal, A. B. Kristoffersen, and T. Tengs. 2012. Quantification of piscine reovirus (PRV) at different stages of Atlantic salmon Salmo salar production. Diseases of Aquatic Organisms **99**:7-U5.

Malik, M. S., H. Bjorgen, K. Dhamotharan, O. Wessel, E. O. Koppang, E. Di Cicco, E. E. Hansen, M. K. Dahle, and E. Rimstad. 2019. Erythroid Progenitor Cells in Atlantic Salmon (Salmo salar) May Be Persistently and Productively Infected with Piscine Orthoreovirus (PRV). Viruses-Basel **11**.

Markussen, T., M. K. Dahle, T. Tengs, M. Lovoll, O. W. Finstad, C. R. Wiik-Nielsen, S. Grove, S. Lauksund, B. Robertsen, and E. Rimstad. 2013. Sequence Analysis of the Genome of Piscine Orthoreovirus (PRV) Associated with Heart and Skeletal Muscle Inflammation (HSMI) in Atlantic Salmon (Salmo salar). Plos One **8**.

Marty, G. D., D. B. Morrison, J. Bidulka, T. Joseph, and A. Siah. 2015. Piscine reovirus in wild and farmed salmonids in British Columbia, Canada: 1974-2013. Journal of Fish Diseases **38**:713-728.

Marty, G. D., S. M. Saksida, and T. J. Quinn. 2010. Relationship of farm salmon, sea lice, and wild salmon populations. Proceedings of the National Academy of Sciences of the United States of America **107**:22599-22604.

McVicar, A. H. 1997. Disease and parasite implications of the coexistence of wild and cultured Atlantic salmon populations. ICES Journal of Marine Science **54**:1093-1103.

Mennerat, A., F. Nilsen, D. Ebert, and A. Skorping. 2010. Intensive Farming: Evolutionary Implications for Parasites and Pathogens. Evolutionary Biology **37**:59-67.

Miller, G. D., J. E. Seeb, B. G. Bue, and S. Sharr. 1994. Saltwater exposure at fertilization induces ploidy alterations, including mosaicism, in salmonids. Canadian Journal of Fisheries and Aquatic Sciences **51**:42-49.

Miller, K. M., A. Teffer, S. Tucker, S. R. Li, A. D. Schulze, M. Trudel, F. Juanes, A. Tabata, K. H. Kaukinen, N. G. Ginther, T. J. Ming, S. J. Cooke, J. M. Hipfner, D. A. Patterson, and S. G. Hinch. 2014. Infectious disease, shifting climates, and opportunistic predators: cumulative factors potentially impacting wild salmon declines. Evolutionary Applications **7**:812-855.

Mordecai, G. J., K. M. Miller, E. Di Cicco, A. D. Schulze, K. H. Kaukinen, T. J. Ming, S. R. Li, A. Tabata, A. Teffer, D. A. Patterson, H. W. Ferguson, and C. A. Suttle. 2019. Endangered wild salmon infected by newly discovered viruses. Elife **8**.

Morton, A., R. Routledge, S. Hrushowy, M. Kibenge, and F. Kibenge. 2017. The effect of exposure to farmed salmon on piscine orthoreovirus infection and fitness in wild Pacific salmon in British Columbia, Canada. Plos One **12**.

Morton, A., R. Routledge, and M. Krkosek. 2008. Sea louse infestation in wild juvenile salmon and Pacific herring associated with fish farms off the east-central coast of Vancouver Island, British Columbia. North American Journal of Fisheries Management **28**:523-532.

Morton, A., R. Routledge, C. Peet, and A. Ladwig. 2004. Sea lice (Lepeophtheirus salmonis) infection rates on juvenile pink (Oncorhynchus gorbuscha) and chum (Oncorhynchus keta) salmon in the nearshore marine environment of British Columbia, Canada. Canadian Journal of Fisheries and Aquatic Sciences **61**:147-157.

Morzunov, S. P., J. R. Winton, and S. T. Nichol. 1995. The complete genome structure and phylogenetic relationship of infectious hematopoietic necrosis virus. Virus Research **38**:175-192.

Murray, A. G. 2013. Epidemiology of the spread of viral diseases under aquaculture. Current Opinion in Virology **3**:74-78.

Murray, A. G., and E. J. Peeler. 2005. A framework for understanding the potential for emerging diseases in aquaculture. Preventive Veterinary Medicine **67**:223-235.

Nash, C. E., editor. 2001. The net-pen salmon farming Industry in the Pacific Northwest. U.S. Dept. Commer., NOAA Tech. Memo. NMFS-NWFSC-49.

Naylor, R., K. Hindar, I. A. Fleming, R. Goldburg, S. Williams, J. Volpe, F. Whoriskey, J. Eagle, D. Kelso, and M. Mangel. 2005. Fugitive salmon: Assessing the risks of escaped fish from net-pen aquaculture. Bioscience **55**:427-437.

Newman, S. G. 1993. Bacterial vaccines in fish. Annual Review of Fish Diseases **3**:145-185.

Olsen, A. B., M. Hjortaas, T. Tengs, H. Hellberg, and R. Johansen. 2015. First Description of a New Disease in Rainbow Trout (Oncorhynchus mykiss (Walbaum)) Similar to Heart and Skeletal Muscle Inflammation (HSMI) and Detection of a Gene Sequence Related to Piscine Orthoreovirus (PRV). Plos One **10**.

Oppedal, F., G. L. Taranger, and T. Hansen. 2003. Growth performance and sexual maturation in diploid and triploid Atlantic salmon (*Salmo salar* L.) in seawater tanks exposed to continuous light or simulated natural photoperiod. Aquaculture **215**:145-162.

Palacios, G., M. Lovoll, T. Tengs, M. Hornig, S. Hutchison, J. Hui, R. T. Kongtorp, N. Savji, A. V. Bussetti, A. Solovyov, A. B. Kristoffersen, C. Celone, C. Street, V. Trifonov, D. L. Hirschberg, R. Rabadan, M. Egholm, E. Rimstad, and W. I. Lipkin. 2010. Heart and Skeletal Muscle Inflammation of Farmed Salmon Is Associated with Infection with a Novel Reovirus. Plos One **5**.

Parsons, J. E., R. A. Busch, G. H. Thorgaard, and P. D. Scheerer. 1986. Increased resistance of triploid rainbow-trout x coho salmon hybrids to infectious hematopoietic necrosis virus. Aquaculture **57**:337-343.

Patterson, K. 2010. The fate of farmed Rainbow Trout (*Oncorhynchus mykiss*) released from commercial aquaculture operations in Lake Huron. University of Manitoba, Manitoba, Winnipeg.

Patterson, K., and P. J. Blanchfield. 2013. Oncorhynchus mykiss escaped from commercial freshwater aquaculture pens in Lake Huron, Canada. Aquaculture Environment Interactions **4**:53-65.

Penaranda, M. M. D., S. E. LaPatra, and G. Kurath. 2011a. Specificity of DNA vaccines against the U and M genogroups of infectious hematopoietic necrosis virus (IHNV) in rainbow trout (Oncorhynchus mykiss). Fish & Shellfish Immunology **31**:43-51.

Penaranda, M. M. D., M. K. Purcel, and G. Kurath. 2009. Differential virulence mechanisms of infectious hematopoietic necrosis virus in rainbow trout (Oncorhynchus mykiss) include host entry and virus replication kinetics. Journal of General Virology **90**:2172-2182.

Penaranda, M. M. D., A. R. Wargo, and G. Kurath. 2011b. In vivo fitness correlates with host-specific virulence of Infectious hematopoietic necrosis virus (IHNV) in sockeye salmon and rainbow trout. Virology **417**:312-319.

Polinski, M., and K. Garver. 2019. Characterization of piscine orthoreovirus (PRV) and associated diseases to inform pathogen transfer risk assessments in British Columbia. DFO Can. Sci. Advis. Sec. Res.

Polinski, M. P., G. D. Marty, H. N. Snyman, and K. A. Garver. 2019. Piscine orthoreovirus demonstrates high infectivity but low virulence in Atlantic salmon of Pacific Canada. Scientific Reports **9**.

Price, C. S., and J. A. Morris. 2013. Marine Cage Culture and the Environment: Twenty-first Century Science Informing a Sustainable Industry. Page 158 NOAA Technical Memorandum. NOS NCCOS 164.

Pulkkinen, K., L. R. Suomalainen, A. F. Read, D. Ebert, P. Rintamaki, and E. T. Valtonen. 2010. Intensive fish farming and the evolution of pathogen virulence: the case of columnaris disease in Finland. Proceedings of the Royal Society B-Biological Sciences **277**:593-600.

Purcell, M. K., K. A. Garver, C. Conway, D. G. Elliott, and G. Kurath. 2009. Infectious haematopoietic necrosis virus genogroup-specific virulence mechanisms in sockeye salmon, Oncorhynchus nerka (Walbaum), from Redfish Lake, Idaho. Journal of Fish Diseases **32**:619-631.

Purcell, M. K., G. Kurath, K. A. Garver, R. P. Herwig, and J. R. Winton. 2004. Quantitative expression profiling of immune response genes in rainbow trout following infectious haematopoletic necrosis virus (IHNV) infection or DNA vaccination. Fish & Shellfish Immunology **17**:447-462.

Purcell, M. K., R. L. Powers, J. Evered, J. Kerwin, T. R. Meyers, B. Stewart, and J. R. Winton. 2018. Molecular testing of adult Pacific salmon and trout (Oncorhynchus spp.) for several RNA viruses demonstrates widespread distribution of piscine orthoreovirus in Alaska and Washington. Journal of Fish Diseases **41**:347-355.

Qin, Q. B., J. Wang, M. Hu, S. N. Huang, and S. J. Liu. 2016. Autotriploid origin of Carassius auratus as revealed by chromosomal locus analysis. Science China-Life Sciences **59**:622-626.

Reno, P. W. 1998. Factors involved in the dissemination of disease in fish populations. Journal of Aquatic Animal Health **10**:160-171.

Rust, M. B., K. H. Amos, A. L. Bagwill, W. W. Dickhoff, L. M. Juarez, C. S. Price, J. A. Morris, and M. C. Rubino. 2014. Environmental Performance of Marine Net-Pen Aquaculture in the United States. Fisheries **39**:508-524.

Saegrov, H., K. Hindar, S. Kalas, and H. Lura. 1997. Escaped farmed Atlantic salmon replace the original salmon stock in the River Vosso, western Norway. ICES Journal of Marine Science **54**:1166-1172.

Salama, N. K. G., and A. G. Murray. 2011. Farm size as a factor in hydrodynamic transmission of pathogens in aquaculture fish production. Aquaculture Environment Interactions **2**:61-74.

Salama, N. K. G., and A. G. Murray. 2013. A comparison of modelling approaches to assess the transmission of pathogens between Scottish fish farms: The role of hydrodynamics and site biomass. Preventive Veterinary Medicine **108**:285-293.

Scott, M. A., R. S. Dhillon, P. M. Schulte, and J. G. Richards. 2015. Physiology and performance of wild and domestic strains of diploid and triploid rainbow trout (Oncorhynchus mykiss) in response to environmental challenges. Canadian Journal of Fisheries and Aquatic Sciences **72**:125-134.

Seeb, J., G. Thorgaard, W. K. Hershberger, and F. M. Utter. 1986. Survival in diploid and triploid pacific salmon hybrids. Aquaculture **57**:375-375.

Seeb, J. E., G. H. Thorgaard, and T. Tynan. 1993. Triploid hybrids between chum salmon female x chinook salmon male have early sea-water tolerance. Aquaculture **117**:37-45.

Siah, A., D. B. Morrison, E. Fringuelli, P. Savage, Z. Richmond, R. Johns, M. K. Purcell, S. C. Johnson, and S. M. Saksida. 2015. Piscine Reovirus: Genomic and Molecular Phylogenetic Analysis from Farmed and Wild Salmonids Collected on the Canada/US Pacific Coast. Plos One **10**.

Siefkes, M. J. 2017. Use of physiological knowledge to control the invasive sea lamprey (Petromyzon marinus) in the Laurentian Great Lakes. Conservation Physiology **5**.

Skilbrei, O. T. 2010. Adult recaptures of farmed Atlantic salmon post-smolts allowed to escape during summer. Aquaculture Environment Interactions **1**:147-153.

Skilbrei, O. T., M. Heino, and T. Svasand. 2015. Using simulated escape events to assess the annual numbers and destinies of escaped farmed Atlantic salmon of different life stages from farm sites in Norway. ICES Journal of Marine Science **72**:670-685.

Skilbrei, O. T., and T. Jorgensen. 2010. Recapture of cultured salmon following a large-scale escape experiment. Aquaculture Environment Interactions **1**:107-115.

Sutherland, D. A., P. MacCready, N. S. Banas, and L. F. Smedstad. 2011. A Model Study of the Salish Sea Estuarine Circulation. Journal of Physical Oceanography **41**:1125-1143.

Sylvester, E. V. A., B. F. Wringe, S. J. Duffy, L. C. Hamilton, I. A. Fleming, and I. R. Bradbury. 2018. Migration effort and wild population size influence the prevalence of hybridization between escaped farmed and wild Atlantic salmon. Aquaculture Environment Interactions **10**:401-411.

Takano, T., A. Nawata, T. Sakai, T. Matsuyama, T. Ito, J. Kurita, S. Terashima, M. Yasuike, Y. Nakamura, A. Fujiwara, A. Kumagai, and C. Nakayasu. 2016. Full-Genome Sequencing and Confirmation of the Causative Agent of Erythrocytic Inclusion Body Syndrome in Coho Salmon Identifies a New Type of Piscine Orthoreovirus. Plos One **11**.

Taranger, G. L., O. Karlsen, R. J. Bannister, K. A. Glover, V. Husa, E. Karlsbakk, B. O. Kvamme, K. K. Boxaspen, P. A. Bjorn, B. Finstad, A. S. Madhun, H. C. Morton, and T. Svasand. 2015. Risk assessment of the environmental impact of Norwegian Atlantic salmon farming. ICES Journal of Marine Science **72**:997-1021.

Thorgaard, G. H. 1992. Application of genetic technologies to rainbow-trout. Aquaculture **100**:85-97.

Thorgaard, G. H., P. S. Rabinovitch, M. W. Shen, G. A. E. Gall, J. Propp, and F. M. Utter. 1982. Triploid rainbow-trout identified by flow-cytometry. Aquaculture **29**:305-309.

Tiwary, B. K., R. Kirubagaran, and A. K. Ray. 2004. The biology of triploid fish. Reviews in Fish Biology and Fisheries **14**:391-402.

Twohey, M. B., J. W. Heinrich, J. G. Seelye, K. T. Fredricks, R. A. Bergstedt, C. A. Kaye, R. J. Scholefield, R. B. McDonald, and G. C. Christie. 2003. The sterile-male-release technique in Great Lakes sea lamprey management. Journal of Great Lakes Research **29**:410-423.

Vendramin, N., A. Cuenca, J. Sorensen, A. L. F. Alencar, D. H. Christiansen, J. A. Jacobsen, C. Axen, F. Lieffrig, N. M. Ruane, P. Martin, T. Sheehan, T. M. Iburg, E. Rimstad, and N. J. Olesen. 2019. Presence and genetic variability of Piscine orthoreovirus genotype 1 (PRV-1) in wild salmonids in Northern Europe and North Atlantic Ocean. Journal of Fish Diseases **42**:1107-1118.

Walker, P. J., and J. R. Winton. 2010. Emerging viral diseases of fish and shrimp. Veterinary Research **41**.

Wallace, I. S., P. McKay, and A. G. Murray. 2017. A historical review of the key bacterial and viral pathogens of Scottish wild fish. Journal of Fish Diseases **40**:1741-1756.

Waples, R. S., K. Hindar, and J. J. Hard. 2012. Genetic risks associated with marine aquaculture. Page 149 U.S. Dept. Commer., NOAA Tech. Memo.

Wessel, O., S. Braaen, M. Alarcon, H. Haatveit, N. Roos, T. Markussen, T. Tengs, M. K. Dahle, and E. Rimstad. 2017. Infection with purified Piscine orthoreovirus demonstrates a causal relationship with heart and skeletal muscle inflammation in Atlantic salmon. Plos One **12**.

Wessel, O., A. Krasnov, G. Timmerhaus, E. Rimstad, and M. K. Dahle. 2019. Antiviral Responses and Biological Concequences of Piscine orthoreovirus Infection in Salmonid Erythrocytes. Frontiers in Immunology **9**.

Wessel, O., C. M. Olsen, E. Rimstad, and M. K. Dahle. 2015. Piscine orthoreovirus (PRV) replicates in Atlantic salmon (Salmo salar L.) erythrocytes ex vivo. Veterinary Research **46**.

Wilkins, N. P., D. Cotter, and N. O'Maoileidigh. 2001. Ocean migration and recaptures of tagged, triploid, mixed-sex and all-female Atlantic salmon (Salmo salar L.) released from rivers in Ireland. Genetica **111**:197-212.

Withler, R. E., T. D. Beacham, Solar, II, and E. M. Donaldson. 1995. Freshwater growth, smelting, and marine survival and growth of diploid and triploid coho salmon (Oncorhynchus kisutch). Aquaculture **136**:91-107.

Wu, L. M., Y. J. Li, Y. F. Xu, Y. F. Li, L. Wang, X. Ma, H. F. Liu, X. J. Li, and L. Y. Zhou. 2019. Cloning and characterization of wnt4a gene in a natural triploid teleost, Qi river crucian carp (Carassius auratus). General and Comparative Endocrinology **277**:104-111.

Yang, L. J., R. S. Waples, and M. L. Baskett. 2019. Life history and temporal variability of escape events interactively determine the fitness consequences of aquaculture escapees on wild populations. Theoretical Population Biology **129**:93-102.

Zhang, Y. F., M. P. Polinski, P. R. Morrison, C. J. Brauner, A. P. Farrell, and K. A. Garver. 2019. High-Load Reovirus Infections Do Not Imply Physiological Impairment in Salmon. Frontiers in Physiology **10**.

Zhigileva, O. N., M. E. Kultysheva, A. Y. Svatov, and M. V. Urupina. 2017. Genetic Diversity in Populations of Silver Crucian Carp Carassius auratus gibelio (Cyprinidae, Cypriniformes), Depending on the Type of Reproduction and Reservoir Size. Biology Bulletin **44**:1278-1283.