Attachment B

Additional Information:
Response to WDFW Questions

Attachment B to SEPA Checklist
Additional Information: Response to WDFW Questions

Cooke Aquaculture Pacific Marine Aquaculture Permit Application to Transition from Raising Atlantic Salmon to Raising Sterile All-Female Triploid Rainbow Trout/Steelhead at the Cooke Existing Marine Net Pen Sites in Puget Sound, Washington

INTRODUCTION

Cooke Aquaculture Pacific, LLC (Cooke) proposes to change the fish species being farmed at its seven (7) existing marine net pen aquaculture sites from Atlantic Salmon (*Salmo salar*) to domesticated stocks of mono-sex (all-female) sterile (triploid) Rainbow Trout (*Oncorhynchus mykiss*). While the common names Rainbow Trout and steelhead are often used interchangeably, this environmental review document and permit applications will refer to Rainbow Trout, which is the single official common name given to the species (*O. mykiss*) by the American Fisheries Society several years ago. All-female triploid Rainbow Trout proposed to be raised in the marine net pen facilities are a sterile, mono-sex stock of fish which reduces the risks of genetic interference to native populations. Sterile mono-sex fish stocks are also used in numerous public agency recreational fish stocking programs for this same reason.

Four of the existing Cooke Puget Sound marine net pen sites have valid permits for the commercial rearing of Atlantic Salmon in marine net pens. The salmon farms in Washington have been raising almost exclusively Atlantic Salmon for the past 30 years. A recent change in Washington State law (RCW 77.125.050), however, requires phasing out non-native finfish in marine net pen aquaculture. This change means commercial marine net pen production in Washington will need to find a commercially viable native species of fish to grow in order stay in the business of growing seafood for human consumption. Rainbow Trout are a native species to the Pacific Northwest region and have been commercially farmed in Washington, primarily in freshwater facilities, for more than 80 years, if not longer. Cooke is requesting re-approval of their Marine Finfish Aquaculture Permit (WAC 220-370-100) from the Washington Department of Fish and Wildlife (WDFW) that will allow the company to start growing domesticated stocks of a mono-sex, sterile Rainbow Trout (*Oncorhynchus mykiss*) at their marine farms. Cooke received the renewal of their Marine Finfish Aquaculture Permit from WDFW on March 19, 2019 allowing Cooke to continue raising Atlantic Salmon at the four farm sites (Clam Bay, Fort Ward, Orchard Rocks and Hope Island) with a valid Aquatic Use Permit from the Washington Department of Natural Resources (WDNR). Other than transitioning to the commercial farming of a different species of fish, the company is not planning any alteration to the existing fish pen physical structures, site locations, supporting equipment, or general current practices, methods, maintenance and cultivation techniques being used for growing Atlantic Salmon at the farms. Domesticated stocks of triploid Rainbow Trout/steelhead have very similar physiological and metabolic requirements to those of domesticated stocks of Atlantic Salmon. The basic difference is that all-female triploid Rainbow Trout are known to be reproductively sterile, and thus convert their energy almost entirely to growth. By comparison, diploid populations of Rainbow Trout and Atlantic Salmon will reach a certain age and begin to expend growth energy toward the production of gametes and secondary sexual characteristics (sexual maturation).

Raising native-stock fish species in marine net pens is consistent with the recommendations of the *Final Programmatic Environmental Impact Statement: Fish Culture in Floating Net-Pens* (Washington Department of Fisheries, January 1990). The Preferred Alternative in the Programmatic EIS (PEIS) also concludes that *"In areas where WDF determines there is a risk of significant interbreeding (with indigenous species) or establishment of harmful self-sustaining populations, the agency should only approve the farming of sterile* ***or*** *monosexual individuals, or genetically incompatible species."* By farming a mono-sex (all-female) and sterile (triploid) stock of Rainbow Trout/steelhead, the proposed species change in Cooke Puget Sound net pens incorporates both methods of reducing the risks of genetic interference to indigenous populations or from escaped farmed fish establishing self-sustaining feral populations. WDFW requested that a SEPA Checklist be prepared along with Additional Information (e.g., information to augment the 1990 Programmatic EIS) to compare the change in environmental impacts that may occur as a result of the change of species to be farmed in Cooke Aquaculture marine net pens, and to update the 1990 PEIS with information derived from studies that have been conducted since that time on elements of the environment that are relevant to the analysis of effects that may result from the Cooke species change proposal. The 1990 *Final Programmatic Environmental Impact Statement: Fish Culture in Floating Net-Pens* is adopted herein by reference, in accordance with WAC 197-11-630. This SEPA Additional Information document addresses the potential environmental effects of the species change proposal on the subjects of Escapement, Potential Interactions with Other Salmonid Species in the Natural Environment, Genetic Interactions, and Operational Differences. Potential effects on threatened, endangered, and candidate animal species Federally-listed under the Endangered Species Act since the 1990 PEIS was issued, as well as potential effects on State-listed and candidate species, are provided in SEPA Checklist Attachment D.

A project-specific SEPA Checklist was submitted to WDFW on April 25, 2019, and subsequently revised concurrent with preparation of this Additional Information document to respond to comments and questions identified by WDFW. Because the existing Cooke Aquaculture net pens have previously obtained the facility construction permits as well as Finfish Aquaculture Permits, Clean Water Act Section 402 NPDES Waste Discharge Permits, and Aquatic Land Leases, there is no site selection or construction required to implement the Cooke species change proposal. There will be only minor operational differences to farm triploid Rainbow Trout/steelhead rather than Atlantic Salmon, with insignificant impacts to elements of the Natural Environment. There will be no change in impact to elements of the Built Environment as a result of implementing the species change proposal.

Cooke will use local stocks of Rainbow Trout/steelhead produced by Troutlodge hatcheries in Pierce County. Troutlodge brood stock are cultivated in Washington specifically for the production of ova to supply both private and public aquaculture operations. Troutlodge, a Washington-based company, has been producing Rainbow Trout/steelhead eggs for sale to private fish farms and public enhancement hatcheries since 1945. Brood fish are raised in regulated pathogen-free conditions for their entire life cycle. The company utilizes a comprehensive health testing and disease-free certification program at their Washington facilities which exceed World Organization of Animal Health (OIE) standards, allowing them to export live salmonid eggs throughout the world.

Troutlodge has been producing mono-sex (all-female) populations of Rainbow Trout/steelhead eggs since the mid-1990s. The all-female (XX only) ova are subsequently fertilized with X-only mono-milt. Triploidy is induced by mechanical pressure shock. For a short period of time, a high-pressure hydrostatic shock is applied to the newly fertilized eggs at a specified time point, post-fertilization. The post-fertilization pressure treatment forces the fertilized egg to retain the third set of chromosomes that is normally ejected at this time. Pressure is then released and the triploid (3N) eggs are allowed to continue development. Ploidy is confirmed using a fluorescent nucleic acid label on either embryo or blood tissue using a flow cytometer at the Washington State University School of Veterinary Medicine. Testing results of Troutlodge triploid fish and eggs over a period of five (5) years (from 2013 to 2018) demonstrate a high rate of success in triploid induction (99.83% − 2,950 of the 2,955 fish and/or eggs sampled); see SEPA Checklist Attachment A. Further discussion on the production, testing and physiology of female triploid Rainbow Trout/steelhead is provided in response to Genetic and Interbreeding questions (Section C) below.

Cooke operates two freshwater hatcheries in the Scatter Creek area of Thurston County. The hatcheries raise and produce the juvenile fish that are eventually transferred to the marine net pens for final cultivation to the desired harvest size. Eyed all-female triploid Rainbow Trout/steelhead eggs would be supplied to the Cooke hatcheries from the Troutlodge hatchery under a WDFW Fin Fish Transport permit. The eggs would be hatched and cultured to a certain size in the Cooke hatcheries, and then transferred to the marine net pens after undergoing the necessary fish pathogen screening protocols, subject to review and approval by WDFW. Thereafter, a Fin Fish Transport Permit would be required from WDFW for each specified lot of fish to be transferred from Cooke hatcheries to the marine net pens. Additional information on disease biosecurity practices, disease control and health certifications for Fin Fish Transport Permits is provided in response to Disease Risk questions (Section B, Questions B.7 and B.8) below.

Marine net pen cultivation and production protocols for Rainbow Trout/steelhead will be basically the same as those used for Atlantic Salmon. Maximum cage density levels are expected to be managed at the same levels (approximately 0.9 to 1.2 pound/ft^3 or 15 to 20 kg/m^3), resulting in comparable maximum biomass levels that have historically been attained at each of the existing Cooke Aquaculture sites. Depending upon fish size at harvest (targeted mean weights of approximately 7 to 9 pounds or 3.5 to 4.2 kg); the fish population sizes at each marine net pen site are expected to be similar to stocking levels for Atlantic Salmon. The fish feed composition for marine-reared Rainbow Trout/steelhead diets will be the same or similar to the currently-used marine salmon diets. Modern salmon and trout feeds are composed of highly digestible ingredients that are specifically formulated for optimal growth and feed conversion rates. No differences in water quality or sediment quality are expected to result from this species change or the accompanying Rainbow Trout/steelhead-specific feeds that would be used. Additional information on feed composition, expected feed conversion rates, projected growth rates, projected pen densities and production cycles is provided in response to Operational Differences questions (Section D, Question D.2) below.

Equipment and all net pen support structures, stock nets, and predator exclusion nets will be the same or similar to what has been used for the cultivation and production of Atlantic Salmon at these farms. If approved, the Rainbow Trout/steelhead produced in the marine net pens will be harvested, processed, packaged and shipped fresh to seafood customers throughout the United States. Aquaculture is a form of agriculture, and is designated as a preferred use of the aquatic environment by the State's Shoreline Management Act ([RCW 90.58.020, WAC 173-26-201[2][d] and WAC 173-26-241[3][b][i][A]). Cooke is dedicated to producing high quality seafood in a sustainable and environmentally-sound manner.

PURPOSE OF THE ADDITIONAL INFORMATION DOCUMENT

Additional Information provided in this document supplements the project-specific SEPA Checklist and updates the 1990 Programmatic EIS. If WDFW decides to publish this information as an addendum to the PEIS, certain SEPA procedures will apply. An Addendum is an environmental document used to provide additional information or analysis that does not substantially change the analysis of significant impacts and alternatives in the existing environmental document. … An addendum may be used at any time during the SEPA process (WAC 197-11-706). An addendum shall clearly identify the proposal for which it is written and the environmental document it adds to or modifies. An agency is not required to prepare a draft addendum, or to circulate an addendum for public review and comment, though it is encouraged to do so.[1]

This Additional Information document addresses issues originally evaluated in the *Final Programmatic Environmental Impact Statement: Fish Culture in Floating Net-Pens* (Washington Department of Fisheries, January 1990), as well as responding to comments and questions identified by WDFW during the Cooke Marine Finfish Aquaculture Permit renewal process. The 1990 PEIS provides a detailed analysis of all issues considered relevant to net-pen aquaculture at the time it was published. Additional

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1 WAC 197-11-625(1), (2), and (5).

information provided herein is focused on updating sections of the PEIS applicable to the Cooke Aquaculture species change proposal with more recently available scientific information, and addresses the proposed change of farmed species at the existing marine net pen aquaculture facilities from Atlantic Salmon to an all-female triploid Rainbow Trout/steelhead stock.

SEQUENCE OF EVENTS IN THE MARINE FINFISH AQUACULTURE PERMIT RENEWAL PROCESS

On November 28, 2018, Cooke Aquaculture Pacific (Cooke) submitted application materials to the Washington Department of Fish and Wildlife (WDFW) for the renewal of their existing Marine Finfish Aquaculture Permits for Cooke Puget Sound salmon farms in Washington State. The application materials submitted included information necessary for the renewal of Atlantic Salmon *(Salmo salar)* Marine Aquaculture Permits and for the approval to start farming the all-female triploid Rainbow Trout/steelhead (*Oncorhynchus mykiss)* at the same net pen facilities.

Cooke received an email message from Dr. Kenneth Warheit (WDFW Supervisor, Fish Health Section) on January 2, 2019, requesting additional information on the proposal to begin farming all-female triploid Rainbow Trout (aka steelhead trout) in the marine net pens. Cooke provided additional information to the Department on January 9, 2019, in the form of a Fact Sheet on all-female triploid Rainbow Trout/steelhead production, including stock origin and other information. After submitting the All-Female Triploid Rainbow Trout Fact Sheet, Cooke was advised by WDFW that in order for the Department to process the renewal of the Atlantic Salmon Marine Aquaculture Permit in a timely manner, Cooke would need to revise their original application for rearing both *S. salar* and *O. mykiss* and resubmit it as two separate applications, one for approval of growing *S. salar* and one for growing *O. mykiss* in the marine net pens. Revised applications, one to farm Atlantic Salmon and one to farm all-female triploid Rainbow Trout, were subsequently submitted to WDFW on January 18, 2019.

On March 19, 2019 Cooke received correspondence from WDFW informing the company that the Department had renewed and issued a Marine Aquaculture Permit to raise Atlantic Salmon, but the application for a permit to raise a new species (*O. mykiss*) in the net pens would require the Department to perform an environmental review through the SEPA process. Under the State Environmental Policy Act (SEPA), local governments and State agencies use the SEPA environmental checklist to help determine whether a proposal's impacts are likely to be significant. This helps determine:

- If an applicant can put measures in place to avoid, minimize, or counter adverse effects.
- Whether compensatory mitigation measures can offset significant impacts.
- If an environmental impact statement (EIS) needs to be prepared for further analysis.

On April 25, 2019 Cooke submitted their SEPA Environmental Checklist and preliminary outline of additional support information to Ms. Lisa Wood, WDFW SEPA Lead Coordinator.

On May 21, 2019, Cooke received e-mail correspondence from Dr. Kenneth Warheit and Ms. Lisa Wood, after the Department had reviewed the SEPA documents. Dr. Warheit and Ms. Wood listed comments and additional questions to be addressed by Cooke for the species change proposal in the Additional Information documents. Those May 21 requests have been integrated into the Cooke original outline for the Additional Information and supporting documents to be provided to augment the SEPA Checklist and 1990 PEIS. Responses are provided below on the subjects of Escapement Issues, Potential Interactions with Other Salmonid Species in the Natural Environment, Genetic Interactions, and Operational Differences.

A. ESCAPEMENT ISSUES

Behavior

A.1. What is known about the species-specific behavior of cultured Rainbow Trout; in particular, cultured all-female (AF) triploid Rainbow Trout?

Cooke Aquaculture Pacific Response: The behavior of triploid all-female Rainbow Trout/steelhead raised in captivity differs from that of naturally or enhancement hatchery-produced Rainbow Trout/steelhead because the captive rearing experience alters physical and phenotypic traits. Reinbold, D., G. H. Thorgaard, and P. A. Carter. (2009) found domesticated populations of Rainbow Trout selected for high growth rates and large body size, resulted in reduced sprint swimming performance. They found significantly higher body masses and significantly slower swim speeds in highly domesticated progeny groups compared to semi-wild hybrid progeny groups.

Effects of domestication on the behavioral traits of Rainbow Trout/steelhead in natural conditions has been extensively studied to determine the degree of divergence of hatchery from their wild donor populations. Steelhead bred in captivity quickly (within one to two generations) lose reproductive fitness (Araki et al. 2008). The magnitude of this fitness loss has been estimated as 40% per generation in captivity (Araki et al. 2007). Johnsson and Abrahams (1991) compared the foraging behavior of laboratory-reared juvenile steelhead and steelhead/domesticated Rainbow Trout hybrids. Both strains suffered identical mortality rates and therefore were considered to be equally susceptible to predation. This confirmed that the hybrid trout were significantly more willing to take risks than the wild steelhead. Cultivated stocks raised in aquaculture facilities are protected from the selective predatory pressures that naturally-produced fish would experience almost constantly in the natural environment. As a result, captive stocks have generally been found to exhibit less predator avoidance behavior than wild origin stocks. In a study by Alvarez et al, (2003) on brown trout *(S. trutta)* second generation hatchery fish and the offspring of wild fish raised under hatchery conditions were insensitive to predation risk. This indicates that behavioral divergence between wild and domesticated individuals can arise from a process of direct or indirect selection on reduced responsiveness to predation risk, or as a lack of previous experience with predators (Alvarez et al. 2003).

The farming experience of the captive-reared Rainbow Trout/steelhead is also much different from that of naturally-produced Rainbow Trout/steelhead in that the captive-reared fish experience only pelleted food nourishment during their cultivation and are not reliant on finding natural prey items for their survival and growth. This results in fish that do not readily recognize their normal natural prey items and a reduced ability to effectively capture wild prey items. It is generally thought that farmed fish are maladapted to feeding in the wild (Blanchfield 2009) and the stomach contents of adult Rainbow Trout/steelhead that had escaped from marine farms consisted primarily of indigestible items such as small pieces of wood and other articles that resembled commercial food pellets (Rikardsen and Sandring 2006).

The triploid genetics of the captive all-female Rainbow Trout/steelhead eliminates the reproductive physiology of these fish due to the absence of a functional ovary. Studies of all-female triploid Rainbow Trout/steelhead released into streams showed that female triploids did not exhibit any migratory or reproductive behavior, while some of the male triploid trout would still exhibit reproductive behavior despite being physiologically sterile (Piferrer et al. 2009). Hatchery reared and aquaculture facility reared trout would also have no experience with a natural migration from their natal freshwater rearing location or marine experience other than at the aquaculture location. Thus, the cultivated triploid Rainbow Trout/steelhead would have none of the imprinting, migrating, and prey experience that provides wild and hatchery-released steelhead the experience that supports spawning migrations to their natal streams. Most escaped farmed fish have low fitness for the wild and quickly become easy victims of predators such as marine mammals, other fish, and birds (Amos et al 2002).

A.2 How do cultured Rainbow Trout behave in the wild if they escape from a marine net pen? To what extent do they exhibit "site fidelity" behavior to remain in proximity to the net pen?

Cooke Aquaculture Pacific Response: Rainbow Trout/steelhead reared in net pens have been shown to initially exhibit "site fidelity" that is, to remain near their rearing site when released. After several days, they begin to disperse into the surrounding environment, but they do not have an imprinted natal site, and tend to disperse over a substantial area. However, Bridger et al. (2001) found high site fidelity among triploid steelhead experimentally released during the growing season within a Newfoundland bay. Bridger found 75% of the released triploid steelhead remained within a 500 m radius of the summer grow-out site 32 days after release.

In an experiment to determine the degree to which released domesticated stocks of all-female triploid steelhead exhibited fidelity to an aquaculture facility, Bridger et al. (2001) released all-female triploid steelhead from a cage towed approximately 1 km away from the original rearing site. They observed strong site fidelity among the domesticated steelhead stocks released during the growing season. Fidelity was only slightly larger for the on-site releases (fish released directly from the rearing site) compared to the off-site releases. Off-site released domesticated steelhead made a rapid return to their rearing sites, suggesting imprinting to the rearing site and homing behavior. Released steelhead eventually dispersed from the release site. Bridger found that 65% of AF triploid steelhead released approximately 1,000 m outside of the summer rearing site returned to the site within two days of release. Dispersed triploid steelhead were also detected in the vicinity of other aquaculture sites throughout the Canadian bay. Their observations indicate that the domesticated steelhead will actually return to the rearing site and that some level of orientation exists based on cues or imprinting that was established while growing on the site Bridger et al. (2001).

Westley et al. (2013) found that naturally-produced steelhead stray at rates of 0.30 to 2.3%. They conclude that these patterns are largely the result of species-specific behavioral and endocrine factors during the juvenile life stages, but analyses also suggest that environmental factors can influence straying rates during the adult upstream migration. All-female triploid Rainbow Trout/steelhead reared in net pens do not have the opportunity to encounter behavioral experiences that are gained by wild and hatchery-reared juveniles during their downstream out-migration. They also do not have the capacity to develop the same endocrine factor as hatchery and wild steelhead due to the absence of functional gonads. Lacking both the environmental and hormonal cues, an aquaculture facility all-female triploid Rainbow Trout/steelhead would not be imprinted for a particular natal stream.

Rikardsen and Sandring (2006) found that hatchery Rainbow Trout/steelhead at post-smolt (120 to 340 g) and adult stages (800 to 3,400 g) adapted differently to natural marine prey after escaping from two fish farms in northern Norway. Young domestic Rainbow Trout/steelhead more easily adjusted to natural feeding after escape than the older, larger fish, which often fed on indigestible items similar in shape to the commercial pellets to which they were accustomed. Lindberg et al. (2009) simulated the escape of 48 adult diploid Rainbow Trout/steelhead from a net cage fish farm in Lake Övre Fryken, Sweden. The post release dispersal of Rainbow Trout/steelhead was fast, showed long range dispersal behavior, low winter survival and the fish lacked the ability to find suitable spawning habitats. These results suggested that reproducing for the first time may be an obstacle to the establishment of escaped farmed Rainbow Trout/steelhead.

A.3 In the event of incremental or significant escape, what is the potential for cultured all-female (AF) triploid Rainbow Trout to interact/co-mingle with wild and/or hatchery-reared Rainbow Trout in the natural environment?

Cooke Aquaculture Pacific Response: The effect is determined in part by the age of the triploid Rainbow Trout/steelhead at release and their absence of rearing/migratory experience that both hatchery and wild steelhead encounter during their migration to estuarine and marine habitats (see the response to Question A.1 and 2 above). The Cooke triploid Rainbow Trout/steelhead will be raised from eggs to smolt size on ground water (well water) at the Cooke freshwater hatchery. The smolt will are carried in fish transport trucks from the hatchery and then taken by a fish transport vessel to the marine net pens. The fish will be reared in the marine net pens from smolt to adult size in about 14 to 16 months.[2] During their early rearing freshwater phase, they have no opportunity to imprint on the migratory path to a natal stream.

Selective breeding of salmonids for commercial aquaculture has resulted in domesticated strains possessing a divergent physiological and behavioral phenotype from that of wild conspecifics (Martens et al. 2014). This is particularly true for triploid Rainbow Trout/steelhead that have been intentionally produced to have different physiology and behavioral characteristics than wild steelhead. Martens et al. (2014) concluded that rapid growth of domestic-strain Atlantic Salmon was achieved through the combination of enhanced feed consumption (increased by ~40%) and feeding efficiency (up to 60% improved feed conversion ratio) relative to the conspecific wild fish.

Scott et al. (2014) found that triploid Rainbow Trout/steelhead released into native environments (e.g., protected lakes) had reduced survival and suggested that a lower tolerance to hypoxia in triploids could explain part of the lowered survival. Intentional releases of hatchery-reared diploid Rainbow Trout/steelhead have been shown to result in substantial portions of naturally-spawning fish to be from hatchery-reared parents. Kostow et al. (2003) found that hatchery-produced fish made up 60 to 82% of the natural spawners in the Clackamas River, Oregon. This high percentage of hatchery fish naturally spawning in the wild would not be possible from a net pen release of the all-female triploid Rainbow Trout/steelhead that have a mean sterility rate of 99.8 %.

A.4 What is their chance of survival in the wild?

Cooke Aquaculture Pacific Response: Substantial numbers of Rainbow Trout/steelhead have been reared in hatcheries and released in Washington waters since early in the 20th century. In recent years, roughly 6 million hatchery-produced juvenile steelhead have been released annually by State, Tribal and Federal hatcheries in Washington State waters (Puget Sound, Coastal, and Columbia Basin). Nearly all of these fish are reproductively competent (e.g., fertile) fish that can spawn with either wild or other hatchery-produced steelhead. Many of these hatchery-produced steelhead return to spawn naturally, either with other hatchery-produced fish, or some with wild fish. However, the offspring of hatchery-produced adults have reduced fitness for survival and reproduction in natural environments as compared to wild fish (Chilcote et al. 1986; Kostow et al. 2003; McLean et al. 2008; Araki et al. 2007 and 2008; Blouin et al. 2014; and Berntson et al. 2011). Kostow et al. (2006) found that surviving hatchery females produced only 4.4 to 7.0% the number of offspring produced per wild female.

Araki et al. (2008) concluded that diploid hatchery fish have lower fitness in natural environments than wild fish. This fitness decline can occur very quickly, sometimes following only one or two generations of captive rearing. The all-female Rainbow Trout/steelhead produced by Troutlodge have been reared in hatchery conditions for many years (and multiple generations) with the specific purpose of producing fish that survive and grow rapidly in artificial rearing conditions. The production of triploid all-female Rainbow Trout/steelhead produces fish that grow rapidly, have slower swimming speed, and do not have

[2] The freshwater phase from egg to smolt takes about 10 to 12 months, after which the smolts are reared in saltwater pens for another 14 to 16 months before being harvested. Total time from egg to harvest is approximately 24 to 28 months. These fish have no chance to imprint on a river system since the hatchery runs on well water and they are taken directly from the hatchery to the net pens by transfer vessel.

reproductive capability (average sterility rate of 99.83%). The absence of functional reproductive organs eliminates their capacity to produce the hormones that stimulate spawning migration and behavior (Kobayashi et al. 1998, Piferrer et al. 2009). The life history that involves only pellet food and eliminates exposure to feeding on wild prey renders the triploid Rainbow Trout/steelhead unfit for long-term survival under wild conditions. Escaped fish may survive for months on fat reserves but are likely not capable of out-competing wild or hatchery fish for prey resources, or reproductive habitat.

Chilcote et al. (1986) found that the success of diploid hatchery fish in producing smolt offspring was only 28% of that for wild fish. With the triploid Rainbow Trout/steelhead having a sterility rate of greater than 99.8%, and most or all escaped fish not reaching spawning habitat, only a few escapes would likely produce any smolt offspring.

The proposed aquaculture production of triploid Rainbow Trout/steelhead will use only fish that are produced in a disease-free facility. All-female triploid Rainbow Trout/steelhead are not functionally reproductive in that they do not produce viable gametes. Thus, the all-female triploid steelhead that might be inadvertently released from existing aquaculture facilities have little risk of contributing to the reproducing population of steelhead in Washington waters or diminishing the fitness of offspring that already includes a large component of hatchery-produced fish. If 10,000 triploid all-female triploid steelhead were released and survived to the spawning period, they would potentially contribute 20 fish to a spawning population that already includes several million hatchery-produced fish, if they were released during the period of natural spawning and if they did successfully migrate to a stream in which wild and/or hatchery fish spawn.

A several-year study (Blanchfield 2009) using radio telemetry on open-cage freshwater-raised Rainbow Trout/steelhead intentionally released out of a net pen and into a lake environment showed that the fish suffered high annual mortality after the first year, with none surviving beyond 3 years. The study also found that upon initial release from the net pen, most fish tended to spend a significant amount of time near the cage site. A main finding of this study was the low survival of escaped farmed fish (Blanchfield 2009).

Previous telemetry studies of farmed Rainbow Trout/steelhead and Atlantic Salmon in the marine environment have shown a mix of post-escape movement patterns (Blanchfield 2009). Rainbow Trout/steelhead in coastal Newfoundland exhibited fidelity to the cage sites, movement among the various cages sites within the area, and gradual dispersal (Bridger et al. 2001); while Atlantic Salmon showed rapid dispersal away from the farm sites (Whoriskey et al. 2006). There appears to be some attraction to the cage sites by escaped fish, but a wider geographic dispersion of some of the fish is likely to occur. Tidal currents, site-specific geography, marine mammal and other predator interactions and the initial fitness of the escaped fish to begin with, would all play a part in how widely and quickly escaped fish disperse.

Initial survival outside the protection of the net pen environment is dependent on the level of predation experienced by the escaped stocks. Whole-lake experimental comparisons have shown that domesticated Rainbow Trout/steelhead are more often found in high-risk pelagic habitats and as a result suffer greater mortality compared to their wild counterparts (Biro et al. 2004, 2006). Farmed salmon stocks have not had to utilize predator avoidance traits in order to survive and reproduce. Brood fish spend their entire life in the protected environment of the brood stock hatchery before being artificially propagated to create the next generation of production eggs. With selective pressures removed, there is no competitive advantage for beneficial phenotypic predator avoidance traits to be passed on to the next generation.

In addition, it is generally thought that farmed fish are maladapted to feeding in the wild (Blanchfield 2009). The diets of adult Rainbow Trout/steelhead that had escaped from marine farms primarily consisted of indigestible items such as small pieces of wood and other articles that resembled commercial food pellets (Rikardsen and Sandring 2006). The Spokane Tribal Hatchery and WDFW Sherman Creek Hatchery annually releases approximately 750,000 triploid Rainbow Trout/steelhead into Lake Roosevelt (Peone 2016; WDFW website). In Rufus Woods Lake, where triploid Rainbow Trout/steelhead are raised for aquaculture in net pens, some triploid Rainbow Trout/steelhead are released intentionally and some escape from net pens (Keleher and Cross 2016). Triploid Rainbow Trout/steelhead stomachs (n=409) collected from fish captured in gill nets and creel surveys in Lake Roosevelt and the Rufus Woods area indicated no piscivory with fish primarily consuming *Daphnia*, copepods, ostracods, dipterans, snails, and arthropods (Richards 2011).

Survival and fitness of artificially-propagated salmon and trout in the wild has been studied extensively in the Pacific Northwest. General indications are that salmonids reared in an artificial environment have the potential to rapidly lose fitness characteristics important to their survival in the wild. Accumulating data (Araki et al. 2009) indicate that hatchery fish (Salmonidae) have lower fitness in natural environments than wild fish. This fitness decline can occur very quickly, sometimes following only one or two generations of captive rearing. Although marine aquaculture is a relatively new enterprise, artificial propagation of fish populations has been conducted for centuries, and the major genetic risks associated with salmon hatcheries and marine stock enhancement (loss of fitness, loss of diversity within and among natural populations) have been characterized for several decades (Waples et al. 2010).

Observations and recoveries of escaped pen-reared Atlantic Salmon indicate that escaped fish that manage to survive, do so only temporarily once outside the protection of the net pen environment. Despite being reared in, and having periodically escaped from open net pen aquaculture operations in Washington and British Columbia since the 1980s, escaped Atlantic Salmon have never established a feral population in the Pacific Northwest. Even deliberate releases of Atlantic salmon, eggs, fry and smolts during historical attempts to establish recreational fisheries failed to ever establish self-sustaining populations in the Northern Hemisphere that was outside of their native range, Waknitz et al. (2002). The majority of escaped farmed Atlantic Salmon that have been recovered in Washington and British Columbia are found with empty stomachs and in the state of becoming increasingly emaciated over time outside of the net pen facility (i.e., in the natural environment). Net pen-reared Atlantic Salmon stocks appear to rapidly lose prey recognition and foraging skills, predator avoidance behavior, and reproductive traits. Captively-reared fish stocks become increasingly naïve to the natural selective pressures outside of the artificial rearing environment with each successive generation raised in captivity. Wild fish populations on the other hand, are constantly under the natural selective conditions that encourage adaptive phenotypic and genotypic fitness traits that can be passed on to the next generation.

The Troutlodge stocks of Rainbow Trout/steelhead have been raised in captivity, domesticated and selectively bred for more than 60 years in freshwater raceways and ponds. These stocks are the definition of a domesticated fish stock, having never spawned naturally, fed naturally, or competed in the natural environment for more than 30 generations. Their ability to compete and survive to reproduce in the wild is greatly reduced because of the loss of natural fitness traits resulting from the domestication process. Additionally, triploidy in Rainbow Trout/steelhead is known to reduce the overall tolerance to physiological challenges. Triploid salmonids have been reported to be more sensitive to environmental changes than diploid fish, which may relate to their altered physiology and cellular morphology (Preston 2013). The proposal to use domesticated all-female triploid Rainbow Trout/steelhead is a safeguard that reduces the likelihood of the fish to survive outside of the net pen environment. Using a sterilized, mono-sex stock further reduces the risk of genetic interference from escaped fish that may survive.

With regard to survival and ultimately the genetic risk of escaped fish in the natural environment, Waples et al. (2012) concludes the following; *"These considerations indicate that successful containment of genetic risks associated with marine aquaculture should focus on two general strategies: 1) prevent escapes, and 2) ensure that individuals that do escape have a low probability of surviving to reproduce in the wild. The magnitude of genetic effects of marine aquaculture is determined primarily by how effective* *programs are at these two control points. Regarding the first point, a variety of methods can be used to reduce the probability of escapes, but it is generally recognized that no marine containment system will be 100% effective. The realized genetic effects of marine aquaculture on natural populations, therefore, will depend heavily on the subsequent fate of individuals that escape into natural habitats. According to one view, significant genetic introgression due to escapes from aquaculture is unlikely, because stocks used for aquaculture will either be sterile or so highly domesticated that survival and reproduction in nature is greatly compromised. The extent to which (and time frame over which) this actually occurs, however, is likely to vary considerably among species."* (Waples et al. 2012).

Life Stage and Seasonal Differences

A.5 In the event of an escapement of cultured ALL-FEMALE triploid Rainbow Trout from a marine net pen site, should the amount of recovery effort be influenced by the life stage of the escaped fish in relation to the life stage at the time of wild and hatchery-reared populations in the natural environment?

Cooke Aquaculture Pacific Response: The potential for by-catch of non-target species exists during most fisheries. Fishery managers utilize multiple tools such as spatial-temporal restrictions, gear restrictions, and resource planning to manage the fishery resource and reduce the by-catch of non-target or protected species. Even with perfect execution of these measures by fishery managers and the fishers themselves, unintentional by-catch of non-target species can still occur. The severity of negative consequences from by-catch is dependent on the total amount of by-catch, the survival rates of any released by-catch and the population status of the by-catch species. As with other fisheries where interception of non-target species can occur, gear types and fishing methods play a key role in reducing by-catch impacts.

If a large escapement of all-female triploid Rainbow Trout/steelhead were to occur from a net pen site, fishery managers would need to assess the probability and consequences of ecological impacts of the escaped fish themselves against the potential ecological impacts of those recovery efforts. The same fishery management tools can be used in this instance, and can mitigate the degree of unintended impacts. Similarly, some of the risks of potential negative impacts can be reduced by using a non-reproductively viable (all-female, triploid) stock of fish. This measure reduces the chance of escaped fish from becoming a reproducing feral population and can reduce the probability of genetic introgression on native populations. As discussed later in this document, sterile all-female Rainbow Trout/steelhead stocks have been found to not show signs of sexual maturation or reproductive spawning behavior. It is possible that a small fraction of the farmed population may be diploid females, with the potential to sexually mature and attempt to spawn if they were to survive long enough in the wild. Because the number of those individuals surviving to the point of maturation would likely be low, it can be characterized as a low number of chances for a low probability sequence of events to occur (female diploid > female diploid escapes > female diploid survives to sexual maturation > female diploid locates a spawning stream at the same timing as native fish > female diploid pairs up and successfully mates with a native fish). In making a decision as to whether to recover the escaped fish, the probability of this sequence of events occurring should be weighed against the potential for unintended impacts occurring from the recovery effort itself.

Co-occurrence of similar life stage (adult or juvenile) escaped farmed Rainbow Trout/steelhead with wild or hatchery-stock steelhead, and/or other listed salmonid species, in the marine environment at the same time of year, could occur. As discussed below in response to questions about physiological distinctions and marking techniques, Cooke believes the farm-raised stock of all-female triploid Rainbow Trout/steelhead will have substantial external morphological differences compared to naturally-occurring wild stocks or the enhancement-produced stocks. These physical differences will make an escaped farm raised fish distinguishable if captured in the same environment. Cooke has proposed to externally mark the fish they raise by removal of the adipose fin prior to the fish being transferred to the net pens. This will make the farmed steelhead stocks easily identifiable from wild stocks of salmonids which have intact adipose fins.

Atlantic Salmon that have been raised in Puget Sound net pens over the past 35 years were reproductively viable animals that had the potential to reproduce in the wild and become established. Past large Atlantic Salmon escapement events from net pens in Washington and British Columbia, as well as the intentional planting of millions of Atlantic Salmon eggs, fry and smolt in the past by fishery managers into west coast freshwater systems in their attempts to create sport fisheries, all failed to result in self-sustaining populations.[3] In other words, the probability of successful reproduction was low based on prior knowledge. Despite this fact, the consequences (e.g., the possible establishment of a non-native species) raised the level of concern and effort that was given to remove escaped Atlantic Salmon from the wild by fishery managers. Similarly, the probability of a sterilized mono-sex stock of Rainbow Trout/steelhead establishing a self-sustaining population is also very low, but there is a possibility that some of the escaped Rainbow Trout/steelhead could be diploid females. Based on the efficacy of the triploid process used by Troutlodge and the triploid testing results, the average number of individual diploids in a given population is significantly less than 1% (0.13%); however, some of those fish could survive, mature, find a spawning stream, pair with and then reproduce with native steelhead trout. The likelihood of this sequence of events occurring is low but cannot be dismissed completely. The probability (likelihood) and the consequences (negative impacts) of that type of event must be assessed against the possibility of negative impacts (such as by-catch) occurring from the recapture efforts themselves.

Physiological Distinctions

A.6 Describe potential marking techniques used to specifically identify Cooke Aquaculture fish: fin clipping during vaccination, otolith marking.

Cooke Aquaculture Pacific Response: Cooke proposes to both internally mark the ALL-FEMALE triploid steelhead it raises by thermal marking the otolith at the fry stage and to externally mark the fish by adipose fin removal (clipping) prior to them being stocked into the marine net pens. The adipose fin removal will make the Cooke-reared steelhead visually distinct from any naturally-produced wild steelhead which have an intact adipose fin. The thermal otolith mark will uniquely identify the Cooke Rainbow Trout/steelhead to the hatchery grower (Cooke).

Thermal Otolith Marking

Cooke Aquaculture Pacific uses a thermal otolith marking procedure on 100% of the Atlantic Salmon reared at their hatcheries and marine net pen facilities. Every year-class of Atlantic Salmon fry undergo a brief series of temperature changes that uniquely mark the otolith (ear bones) with a specific series of different sized growth rings. The otolith marks are observable under a microscope. The specific temperature regime for the Cooke hatchery was developed in cooperation with WDFW staff that use the same technique to mark hatchery enhancement fish originating from State and Tribal hatchery programs. Samples of each year-class of fish raised at the Cooke hatchery are sent to WDFW where the otolith marks are both verified and recorded. Washington commercial marine net pen farms growing Atlantic Salmon began the thermal marking program in 2002. It has been a successful technique for identifying an escaped farm- raised Atlantic Salmon to an individual grower. Thermal marking also allows the ability to

[3] WDFW released Atlantic Salmon smolts for the purpose of establishing runs in 1951, 1980, and 1981. Many releases were also made in lakes; however, none of these resulted in the return of adult Atlantic Salmon. For colonization to occur, Atlantic Salmon would need to be successful in each step of a complicated life history, and complete the life history in numbers sufficient to perpetuate the stock. Attempts throughout the United States and world to introduce and establish Atlantic Salmon outside the Atlantic Ocean have failed (Amos and Appleby, WDFW, September 1999).

distinguish offspring if they should arise from a reproductive event in the wild by feral farm fish as those offspring would lack the same thermal markings.

Cooke would use the same thermal marking program for the triploid Rainbow Trout/steelhead stock it is proposing to raise at the company's existing marine net pen facilities. This will individually identify each population of fish being reared as coming from a Cooke Aquaculture farm. The company would again work cooperatively with WDFW staff to develop the unique thermal regime to mark each generation of fry being raised at Cooke hatcheries, destined for transport to the marine net pens. Annual samples would be submitted to WDFW for verification and record keeping.

External Marking by Adipose Clipping

Cooke proposes to externally mark all of the Rainbow Trout/steelhead raised at the hatchery by removing the adipose fins at the time the fish are being vaccinated. The absence of an adipose fin creates a means to identify, by external observation, whether the fish is an escaped farmed Rainbow Trout/steelhead or an unmarked (e.g., adipose fin intact) and presumably wild native steelhead trout during recapture efforts and/or in the midst of any other fisheries that are occurring at the time.

Mass marking by adipose fin clipping is recognized as an effective management tool by State, Federal and Tribal fisheries managers in protecting wild salmon and steelhead and allowing mark-selective fisheries. External morphological differences that are known to occur between farm-raised domesticated stocks of all-female triploid Rainbow Trout/steelhead and presumably wild native steelhead or a fisheries enhancement program steelhead are described below. Genetic records of the Troutlodge stock of Rainbow Trout/steelhead and/or tissue samples for genetic testing would also be provided to WDFW upon request. It is presumed that the DNA analysis would allow the Department to further distinguish between an escaped farmed fish and native, naturally-reproducing fish or fisheries enhancement program fish if necessary.

A.7 Explore a secondary mark, in addition to adipose fin clip, that would distinguish visually Cooke steelhead from tribal and state produced hatchery steelhead.

Cooke Aquaculture Pacific Response: Adipose fin clipping is a method that is used to externally mark enhancement hatchery-produced steelhead in Washington. It should be noted that adipose removal is also used on steelhead released by public enhancement hatcheries in California, Oregon, Alaska and British Columbia. Since both the commercially-reared Cooke all-female triploid steelhead and the enhancement-hatchery-reared Rainbow Trout/steelhead would have the same external identification mark (clipped adipose fin), there is the possibility to incorrectly identify the origins of a steelhead recovered in the natural environment. The chance of encountering an enhancement hatchery steelhead and commercially-reared escaped Rainbow Trout/steelhead in the marine or freshwater environment at the same time and; for both types of fish (farm-reared and wild-reared) to be at the same life stage (age); and of similar approximate sizes and exhibiting the same coloration and other external characteristics such as fin condition, body length, body depth, etc., would seem to be unlikely or of very low probability for the following reasons:

The life histories and selective pressures that would occur between a farm-raised Rainbow Trout/steelhead and wild-reared steelhead are extremely different and likely result in many morphological differences (Taylor 1986; Swain et al. 1991; Fleming et al. 1994; Hard et al. 2000; Belk et al. 2008). The early freshwater life stages between the two types of fish result in substantially different juvenile smolts. For instance, the majority of steelhead juveniles reside in fresh water for two years prior to migrating to sea as smolts. Two-year-old naturally-produced smolts range in size from 140 to 160 mm in length (Wydoski and Whitney 1979, Burgner et al. 1992). Commercially-raised all-female triploid Rainbow Trout/steelhead will reach that same length (140 to 160 mm) in 5 months and can be ready for seawater entry within 10 months of hatching. The expected growth rate of the net pen-reared all-female triploid Rainbow Trout/steelhead forecasts the fish reaching the targeted harvest size of 3.5 to 4.5 kilograms (~7 to 10 pounds) in only 14 to 16 months after entry into the marine net pens (~ 24 to 26 months post-hatch). This means the farmed all-female triploid Rainbow Trout/steelhead will be approximately only 2 to 2.5 years in age by the time they are reaching harvestable size, being harvested (e.g., removed from net pens) and taken to the processing plant. Both wild and hatchery-produced, free ranging steelhead typically spend the first 2 years of their life in the freshwater environment and then another 2 years at sea (2/2) before returning to the river at sexual maturation to spawn (see Figure 1 below). Some steelhead stocks take even longer at sea reaching 5 years of age (2/3) prior to their first spawning. Steelhead can spawn multiple times and have been known to reach up to 45 pounds in weight. Typically however, free-ranging naturally produced adult steelhead will be approximately 10 pounds when they return to the river.

Figure 1.

Table 2c. Age structure of Puget Sound steelhead: frequencies of life-history patterns. Age structure indicates freshwater age/ocean age. Reproduced from Busby et al. (1996). Populations in italics are representative of adjacent ESUs.

		Life History (frequency)				
Population	Run	Primary		Secondary		Reference
Chilliwack River	*WSH*	2/2	0.31	2/3	0.31	Maher and Larkin 1956
Skagit River	WSH	2/2	0.48	2.3	0.33	WDFW 1994b
Deer Creek	SSH	2/1	0.95	3/1	0.05	WDF et al. 1993
Snohomish River	WSH	2/2	0.47	2/3	0.36	WDFW 1994b
Green River	WSH	2/2	0.52	2/3	0.17	Pautzke and Meigs 1941
Puyallup River	WSH	2/2	0.61	2/3	0.28	WDFW 1994b
Nisqually River	WSH	2/2	0.51	2/3	0.28	WDFW 1994b
Hoh River	*WSH*	2/2	0.74	2/3	0.14	Larson and Ward 1952

In theory, an escape of the all-female triploid Rainbow Trout/steelhead could occur at the exact same time and location as the migration of naturally-occurring steelhead in the same area. While there is some chance that the escaped fish could be the same size and age class, it is unlikely they would have the same physical characteristics as wild or enhancement hatchery-produced steelhead. As described above, farm-raised all-female triploid Rainbow Trout/steelhead grow very rapidly in comparison to naturally-produced or enhancement hatchery-produced diploid steelhead. The rapid growth rate of all-female triploid Rainbow Trout/steelhead results in a deeper-bodied fish that will have a much higher condition factor (shorter fork length to weight ratio) compared to naturally-occurring or enhancement-produced steelhead stocks.

Triploid Rainbow Trout/Steelhead

Wild Sea Run Steelhead

In the event of an escapement from a farm site and any subsequent targeted recovery efforts, the farmed fish would be distinguishable by their known age class, average weight, and length and condition factor at the time of the escape. This information coupled with the clipped adipose fin would allow the escaped fish to be identified and separated from by-catch of non-targeted wild and/or tribal and state steelhead stocks. Assuming that the escaped farmed fish survived in the wild for an extended period of time, they have been found to become emaciated because of their limited ability to successfully find wild prey items. This could make them less easily recognizable based on the condition factor alone as the undernourished escaped fish becomes more lean and potentially similar in appearance to a natural free-ranging steelhead. However, there are differences in the physical appearance of a fish that is lean and lengthy from surviving in the natural environment, compared to an emaciated fish which becomes disproportionately long (snake-like) in relation to the size of the head and fins.

In addition, creel counts or steelhead enhancement hatchery brood fish collection facilities would likely be capable of distinguishing the external morphological differences in the fish besides just the lack of an adipose fin. While there is a low probability that an escaped all-female triploid Rainbow Trout/steelhead could be misidentified as a Tribal or State enhancement steelhead, the risk of genetic impact to either wild or enhancement stocks would be further mitigated by rearing only all-female triploid stocks of Rainbow Trout/steelhead at the marine net pens. Opportunities for recreational and commercial fishing for steelhead are very limited in Washington. The risk that one of these fisheries would capture extra enhancement hatchery-reared steelhead because they were misidentifying them as being possible escaped farmed all-female triploid Rainbow Trout/steelhead from a net pen facility would not seem to be very high.

Other possible mass marking methods were looked into for a different technique that could further easily distinguish marine net pen escapees from enhancement stocks. There are multiple methods that have been developed over the years by fisheries scientists and enhancement programs to expand the options for externally marking salmonids. Methods such as freeze branding (FB), Visible Implant Filament (VIF), Visible Implant Elastomer (VIE) tags, tattooing and ventral fin removal are methods that have varying levels of success depending upon the type of application. Literature suggests that external marks such as freeze branding, VIF and VIE are only short-term external marks that eventually disappear over the life span of the fish (Figure 2). Ventral fin removal is another option but is less effective than adipose fin removal because of the capability for ventral fin regeneration (up to 47%) after removal (Skalski et al. 2009). Removal of the adipose fin has the most long-term stability with a very low frequency (0 to 4%) of regeneration (Skalski et al. 2009). Of these, only adipose fin removal appears to be the most suitable for mass marking programs, and for the ability for this external mark to be recognizable for the entire life span of the fish.

Figure 2.

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Table 1 Summary of marking techniques for small fish, the availability of unique codes for ease of identifiability, permanency (stability) of the mark, and minimum fish size or life stage requirements

Mark technique	Unique codes possible	Suitable for mass mark	Category of detection*	Stability	Minimum fish size or life stage
External marks					
Fluorescent elastomer (VIE)	240	No	IV or ISD	Variable	26 mm
Fluorescent filament (VIF)	3-character alpha-num	No	IV or ISD	Variable	50 mm
Dye marking	Limited (i.e., ≤30)	Yes	IV or ISD, NS	Variable	25–50 mm
Adipose clip	None	Yes	IV	0–4% regeneration	50 mm
Ventral clip	None	Yes	IV	0–47% regeneration	50 mm
Adipose or ventral clip and CWT (coded wire tag)	Unlimited	Yes	DD	Variable	<2.1 g HLCWT >2.1 g FLCWT
Tattoos	Limited	No	IV, NS	Low	100 mm
Freeze branding	Limited	No	IV, NS	Low	50 mm
Internal marks					
Half-length CWT (HLCWT)	Unlimited	Yes	DD, S	Variable	<2.1 g
Full-length CWT (FLCWT)	Unlimited	Yes	DD	Variable	>2.1 g
Genetic	Unlimited	Yes	DD, NS	Permanent	None
Molecular/laser	Limited	Yes	ISD, NS	30 months	8 days post-yolk absorption
Strontium isotope ratios	None	Yes	DD, NS	Permanent	None
Oxytetracycline	Limited	Yes	DD, S	High	None
Strontium chloride	Limited	Yes	DD, S	High	None
Calcein immersion	Limited	Yes	ISD, S or NS	4–12 months	None
Tetracycline	Limited	Yes	DD, S	High	None
Otolith thermal	Unlimited	Yes	DD, S	Permanent	Emergent fry–advanced yearling
Dry mark otolith (eggs)	Unlimited	Yes	DD, S	Permanent	Only for eggs
PIT	Unlimited	Yes	ISD, NS	85–100%	50 mm

*Detection categories.
IV: Immediate Visual—marks that can be easily and immediately seen by the unaided eye.
ISD: Immediate Specialized Detection—marks that can be immediately detected with the proper equipment. Every fish must be analyzed because these fish do not have a visual identifier.
DD: Delayed Detection—marks that require sacrificing the fish or sampling harvested fish to obtain the tag or tissue for specialized laboratory analysis.
S: Sacrificing the fish is required.
NS: No sacrifice of the fish is required.

A.8 If a recovery effort was implemented to capture escaped cultured all-female triploid Rainbow Trout, what are the observable morphological differences that would distinguish them from wild and/or hatchery-reared populations captured in the natural environment?

Cooke Aquaculture Pacific Response: Initial identification of a potentially escaped all-female triploid Rainbow Trout/steelhead would be made by external observation of the missing adipose fin. Further distinction can then be made by observation of the body shape, general size, fork length and weight of the fish; external markings and coloration; and observation of the condition of the pectoral, dorsal, pelvic and caudal fins. Several external morphological differences are known to occur between farm-raised and wild fish. A range of studies conducted, mainly in temperate countries, has demonstrated the existence of wide morphological differences between wild and farmed fish (Taylor 1986; Swain et al. 1991; Pakkasmaa et al. 1998; Hard et al. 2000; Ojanguren and Brana 2003; Cramon-Taubadel et al. 2005; Berejikian and Tezak 2005; Solem et al. 2006; Belk et al. 2008). The all-female triploid Rainbow Trout/steelhead stocks and the wild or enhancement steelhead stocks found in the natural environment would exhibit distinctive traits based on genotypic and phenotypic differences. In relation to morphology, polymorphisms between alternative environments may occur in the same species, in response to different selective pressures (Robinson and Wilson 1998; Langerhans et al. 2003). The all-female triploid Rainbow Trout/steelhead that Cooke proposes to raise in its existing marine net pens will originate from brood fish that have undergone a selective breeding program over multiple generations, specifically focused on rapid growth and ability to thrive in the hatchery and ultimately in a commercial rearing farm. Wild populations live under the natural processes for survival that continually selects for the ability to successfully forage and compete for prey, avoid predation, thrive and then successfully reproduce in the natural environment. Alternatively, domesticated or farmed stocks of Rainbow Trout/steelhead express traits that are either beneficial or the by-product of being raised in the controlled farm environment, first at the hatchery and then later in net pens, and share few (if any) of the same selective pressures that wild steelhead undergo. Selective pressures present in the fish farming tank are quite different from those existing in the natural environment, which may result in very different morphologies generated by phenotypic plasticity (Taylor 1986; Swain et al. 1991; Fleming et al. 1994; Hard et al. 2000; Belk et al. 2008).

The all-female triploid Rainbow Trout/steelhead proposed to be reared in the Cooke Puget Sound marine net pen facilities would come from fry hatched from incubation trays that are fed a pelletized fish feed from the moment they begin actively feeding. The fry are raised in tanks with controlled flow rates, water temperature, and dissolved oxygen levels. They are protected from predators by the walls of the hatchery building and their rearing tanks, and eventually by the barrier netting around marine net pens. The selective pressures for predator avoidance behavior, physical prowess, body shape or coloration that would increase their chance of survival in the wild are no longer present. Farmed triploid Rainbow Trout/steelhead will exhibit a much higher condition factor (body weight to length ratio) than a wild or hatchery-reared steelhead of the same age. This body shape difference alone would likely make distinguishing a recaptured escaped farm-raised fish from a wild or hatchery origin steelhead captured in the wild, obvious to fisheries managers as well as others. Several studies demonstrate that morphological and physiological differences are observable from the first (F1) generation in a hatchery compared to the same stock of Rainbow Trout/steelhead that reproduced in the wild. Wild juvenile steelhead were found to possess significantly more superficial lateral line neuromasts, normal aragonite-containing otoliths, and significantly larger brains than hatchery-reared steelhead (Brown et al., 2013). Additionally, these differences together predict reduced sensitivity to biologically important hydrodynamic and acoustic signals from natural biotic (predator, prey, conspecific) and abiotic (turbulent flow, current) sources among hatchery-reared steelhead, in turn predicting reduced survival fitness after release (Brown et al. 2013). It should also be pointed out that net pen-reared fish typically show some signs of fin erosion stemming from mechanical damage during their hatchery rearing process and then later the rearing phase in a net pen. This could also be a way for fisheries managers and others to distinguish pen-reared fish from wild or hatchery free-ranging steelhead.

The external appearance of hatchery-raised triploid Rainbow Trout/steelhead (*O. mykiss)* is often compared to a "football" because of their deep body relative to their fork length and the smaller head size compared to diploid Rainbow Trout or sea-run steelhead (*O. mykiss)*. See the photographic comparison in the response to A.7 above. The deep body shape is the result of the rapid growth rates and a tendency for compression of the vertebrae due to the rapid growth rates in comparison to natural or hatchery-origin sea-run steelhead. In the event of an escapement of fish from a marine net pen, information that would be provided by the aquatic farmer to State agencies and Tribes would include the estimated number of fish that had escaped; and the location, age class, average size, health status and medicated feed use history which is required by WAC 220-370-120(b). Cooke proposes that, in the event of an escapement, the Condition Factor (CF) of escaped fish also be provided to State and Tribal fishery managers in the initial fish escape report. The Condition Factor along with the clipped adipose fin would be a way to quickly distinguish between an escaped farmed fish and wild or hatchery-raised steelhead found in the natural environment. Knowledge of the average weight of the escaped fish along with the Condition Factor would 1) help in decisions regarding recapture efforts and coordinating the most effective fishing gear types that could be used, and 2) create a means for the fishers to quickly distinguish an escaped farmed fish from possible by-catch of wild or fisheries enhancement program steelhead stock populations. Photographs would also be provided to the agencies of cohort fish for dissemination to the public that would give further visual characteristics of the escaped fish such as coloration, head shape, fin condition, body shape and scale size.

Lastly, it should be pointed out that all-female triploid Rainbow Trout/steelhead stocks show no signs of ovarian development and maturation compared to normal diploid females (Sumpter et al. 1991; Carrasco et al. 1998). The fact that an escaped fish would be sterile and therefore incapable of reproducing themselves or presenting a risk to the genomics of native stocks, should be considered by fisheries managers in contrast to the possible unintended "take" of protected species and the level and type of escaped fish recovery efforts to be undertaken.

B. Potential Interactions With Other Salmonid Species in the Natural Environment: Both Federally-Listed Threatened and Endangered Salmon Species, and Non-Listed Species

Competition

B.1 In the event of incremental or significant escape of cultured ALL-FEMALE triploid Rainbow Trout into the natural environment, to what extent would they compete with wild, Federally-listed salmonid species for food, space and other habitat requirements?

Cooke Aquaculture Pacific Response: It is unlikely that incremental or significant escape of triploid steelhead would result in detectable competition with wild and the numerous hatchery-reared diploid steelhead. Younger all-female triploid Rainbow Trout/steelhead would have no experience with the wild prey resources consumed by wild and hatchery-reared Rainbow Trout/steelhead. The all-female triploid steelhead would be small in number compared to the approximately six million hatchery diploid fish released into Washington waters each year. The all-female triploid steelhead would tend to be surface feeders due to their feeding experience with pellets rather than natural prey. The small number of net pen escapees that would be sexually functional are unlikely to have many individuals that would successfully find spawning habitat that is currently underutilized. Although all-female triploid steelhead are likely to be of larger size than wild and hatchery steelhead of similar age, they tend to be weaker swimmers and thus not competitive with the wild and hatchery fish.

Predation

B.2 In the event of incremental or significant escape of cultured AF triploid Rainbow Trout into the natural environment, what would be the potential for them to prey upon the young of wild, Federally-listed salmonid species?

Cooke Aquaculture Pacific Response: Studies to date suggest that escaped cultured fish do not easily transition to feeding on native fauna (Abrantes et al. 2011). Cultured Rainbow escapes often feed on pellets, leaves, and woody debris and only approximately 0 to 2% have been observed to have fed on fish. The potential for escaped all-female triploid Rainbow Trout/steelhead to feed on young of the wild, Federally-listed salmonid species is exceedingly low. Escapes would have to occur when listed young of the year were in their vicinity, which is only a fraction of the year. In addition, the relative abundance of large numbers of hatchery releases of non-listed fish would further reduce the likelihood that a federally listed fish would be consumed.

By-Catch

B.3 In the event of incremental or significant escape of cultured all-female triploid Rainbow Trout, what are the potential risks of accidental "take" of wild, Federally-listed salmonid species as by-catch in a recovery effort?

Cooke Aquaculture Pacific Response: Recovery methods could be modified to reduce the likelihood of accidental take of wild, Federally listed salmonid species. For example, if an escape event occurred during juvenile outmigration or adult spawning migration of listed species, then recovery gear could be limited to more selective methods, e.g., hook and line, to reduce by-catch.

Also see the response to Questions A.5, A.6 and A.7, above.

B.4 What mitigation measures and plans can be incorporated to reduce the risk of accidental take of listed salmonid species in escaped fish recovery efforts?

Cooke Aquaculture Pacific Response: The prevention of any escapement from the pens in the first place is the best method of reducing the risk of accidental take of listed salmonid species since no recovery effort would be necessary. After the Cypress Site 2 fish escapement, Cooke worked closely with both WDFW and the Washington Department of Ecology (Ecology) to update and improve the company's fish escape prevention, response and reporting plans. These improvements were developed from the lessons learned in the Cypress incident, and include an improved and expanded emergency communications plan, improved inspection programs, increased reporting responsibilities for Cooke that include a list of individual Tribal resource managers to be contacted in the event of an escapement, Incident Command Training for key Cooke staff members, and an emergency response vessel contact list.

Cooke has also worked with the Department of Natural Resources to develop a net hygiene scoring program that tracks the net cleaning progress from week to week at each of the farm sites and is reported to WDNR. Improved containment technologies reduce escapement risks and carry obvious economic, environmental and sociological incentives for the aquatic farmer.

B.5 Please estimate rate of escapes during stocking and through small tears in net.

Cooke Aquaculture Pacific Response: The rate of escapes during stocking is zero. The risk of this type of event occurring has been reduced substantially by the incorporation of the single-generation stocking program. There are few live fish transport or handling events with this type of production compared to the prior multi-generation production strategy which had fish being pumped from pen to pen, periodically size-graded and transferred between different farm sites. Single-stocking means the young fish are size-graded into groups at the hatchery, vaccinated and then transferred to an individual pen at the marine site where they remain until they are harvested. The employees are trained in Fish Escape Prevention, and the proper techniques for inspecting the stock net for holes, securing the transport vessel and discharge pipe during fish stocking and harvesting events. The transport vessel is securely tied to the side of the net pen facility immediately adjacent to the pen that is going to be stocked or destocked. During stocking, the fish are pumped from the hold of the vessel by a vacuum fish pump. The discharge hose from the pump chamber is mechanically clamped to the fish pump chamber and the hose is supported by the vessels' crane. The outlet end of the discharge hose is placed into the fish pen designated to receive the fish, and is secured in place with mooring lines. The vacuum pump has to be running for fish to be pumped through the pipe and into the pen. The reverse is true when fish are harvested from the pen. They are pulled through the vacuum pump chamber and discharged into a machine that humanely dispatches them. The dispatched harvested fish slide down an enclosed chute and into the hold of the vessel. Employees at the farm site assist and supervise these handling events. For all of these reasons, there is low risk of fish escapes during stocking or destocking of the fish pens.

The estimated rate of escapes through small tears in the net is zero. There is no constant rate of escapement of fish through the netting or a leakage rate as suggested by the B.5 information request. There is, however, always going to be some potential risk of escapements from accidents and damage to the fish containment nets. The job of a fish farmer is to minimize the probability of accidents by using multiple prevention procedures. Risks are identified and plans are developed and implemented to reduce those risks. Employees are trained in the company's Fish Escape Prevention Plans. There are various methods, materials and procedures that are incorporated that reduce the risk of small holes or breaches occurring in the netting that could lead to an escape. Stronger stock netting materials, improved predator barrier nets, surface chafe guards on the stock and predator nets, improved net designs, testing break strength, repair and replacement procedures, routine net inspections by divers during mortality dives, and many other practices are incorporated in the procedures to prevent both large and small fish escapes.

B.6 Describe in detail planned recovery actions in the event of a spill. Steelhead do not school to the extent that salmon school, which may mean recapture efforts may not the same as for Atlantic salmon. The Fish Escape Prevention, Response, and Reporting Plan submitted with your marine finfish aquaculture permit application was designed for Atlantic salmon. What are the risks of negatively affecting federally-listed salmonids during recovery after a spill event?

Cooke Aquaculture Pacific Response: The response to this information request is provided above in Section A and in prior Section B responses. Cooke worked with both WDFW and Ecology to update the Cooke Fish Escape Prevention and Response Plans in October 2018, taking into account lessons learned from the Cypress Site 2 fish escapement and response. Specifically, the plans were updated with improvements to the communication channels and identifying the potential resources that could be used for an escape recovery process. The updated plans have been approved by WDFW and Ecology. These plans, actions, procedures and policies are directly applicable to raising any salmonid species from smolt to adult in the Cooke Aquaculture Puget Sound marine net pens. The basic principles of risk identification of the facilities, procedures and equipment, and the subsequent development and implementation of accident prevention procedures are all very much the same. Copies of these plans were submitted with the original Marine Finfish Aquaculture Permit Application materials. The company will be working with both WDFW and WDOE in reviewing and updating those plans with respect to the new NPDES permit condition requirements that are expected to be issued in July 2019. In addition, any permit conditions specific to raising all-female triploid Rainbow Trout/steelhead that are required by WDFW to issue a new Marine Finfish Aquaculture Permit would also be incorporated into updated versions of the plan.

Briefly, if a fish escapement were to occur, the company must notify WDFW, Ecology, WDNR, NMFS and individual Tribal resource managers and begin implementation of the Cooke Aquaculture − Fish Escape Prevention, Reporting and Response Plans (dated October 12, 2018). Excerpts from the current plan are inserted below, describing fish recovery procedures and actions that would be implemented in the event of an escape.

EMERGENCY PROCEDURES FOR DETERMINING AND REPORTING A SIGNIFICANT FISH RELEASE

Investigation

- Site Managers, Assistant Site Managers and/or the General Manager will investigate to determine whether a Significant Fish Release has occurred based on one or more of the following factors: (1) observations of fish behavior in the net-pen; (2) observations of fish stocks outside the containment net; (3) unexplained decrease in daily feed intake; (4) review of fish inventory information; (5) observations of a large breach in the containment net at the surface or by the dive team below the surface; and (6) an unusually high presence of seal or sea lion activity nearby a fish pen.
- If a pen is suspected of having a breach in net integrity, the Area Manager, Site Manager and/or Assistant Site Manager will deploy the dive team as immediately as possible to inspect the pen for a breach and make any repairs if necessary. If immediate deployment conflicts with the safety of the dive team because of night fall, hazardous weather, tidal or other unusual conditions, the safety of the dive team is given priority. The dive inspection is to be carried out as soon as safer conditions allow.
- If a breach in the containment net is discovered in a pen by the divers or from surface observations that is large enough to have allowed a large number of fish to potentially escape then the pen will be suspected of possibly having had an accidental fish escape. The accidental fish escape reporting, recovery and re-inventory procedures will be initiated by the General Manager, Site Manager and/or the Permit Coordinator.

- If an accidental fish escape is suspected, state agencies are to be notified within 24 hours (see Emergency Reporting and Contact Lists).
- The following sections cover the emergency response and reporting procedures in the case of a significant escapement.

Emergency Reporting Procedures

A positive determination that a significant fish release has occurred will initiate the emergency reporting and recovery procedures. An Accidental Fish Release Report will be submitted to the Washington Departments of Fish and Wildlife and the Washington Department of Ecology within 24 hours in the format below.

Accidental Fish Release Report Information

Fish Release Report Date: ______	Age Class of Fish: _______________
Location of Escape: ___________	Disease History: ________________
Date of Release: _____________	Medicated Feed History: __________
Number of Fish: ______________	Species of Fish: ___________
Avg. Weight of Fish: ___________	Cause of Release: ____________
Employee Name: _____________	Employee Position: ___________

Incident Command Structure (ICS) Training and Cooke Staff Responsibilities

In the event of a significant emergency situation at the fish pens that requires the activation of an Incident Command Structure (ICS), an ICS certificated Cooke employee or representative will actively participate with the ICS. The employee will have technical knowledge of the net pen operations and be a resource to the ICS. The following members of the Cooke Emergency Management Team will have received training in the Incident Command process by January 1, 2019, as described below.

- ICS Training: The Area Managers and Site Managers from each marine farm location, the Business Support Analyst and the Permit Coordinator will complete the online ICS-100 training course and have received the appropriate ICS-100 course completion certificate. The ICS-100 online course is available at:

    https://training.fema.gov/is/courseoverview.aspx?code=IS-100.c

Cooke Communications and Responsibilities During a Significant Emergency Event

General Manager and/or Permit Coordinator will contact the following and provide the preliminary Accidental Fish Release Report information described below:

> **Key Contacts** – WDOE, WDNR, WDFW, NWIFC and nearby tribal Natural Resource Managers
>
> **Information** – Compile and provides the fish inventory information for Emergency Reporting Procedures as described below including: species of fish, age, size, fish health history, date of last medicated feed use, estimated number of escaped fish and other pertinent information.

Contact the Washington Department of Health − if the escaped fish are within the required withdrawal period for recent medicated feed use. Information to the Department of Health will include the type of medication and the date when withdrawal will be met.

<u>**General Manager and Site Managers contacts the following:**</u>

Emergency response vessels, tug-boats, harvest vessels, and any other support vessels as needed to respond to the situation.

PROCEDURES FOR RECOVERY OF ESCAPED FISH

<u>Recapture Procedures</u>

- The first priority shall be to determine and attempt to correct the cause of the accidental fish release through the repair of the breach.
- In the event of a catastrophic failure of the equipment, securing of the net pen structure or other appropriate immediate response actions may be necessary first in order to stabilize the site before other actions on the fish pen site can be taken. The safety of Cooke employees and contractors takes priority at all times.
- Cooke management maintains an Emergency Work Vessel Contact list. Copies of the contact list are attached to the end of this plan. The vessel contact list will be updated annually along with other parts of this plan.
- Concurrently with the actions to stop and reduce further fish escapements from the facility, the Cooke Emergency Management Team will begin the process of implementing the rapid recovery of escaped farmed fish. This will include utilizing internal resources as well as contacting outside resources to aid in the recapture process.
- If there is reason to suspect an accidental fish escape has occurred (see above section − Emergency Procedures for Determining and Reporting a Significant Fish Release), the Cooke Emergency Management Team will contact the Washington Department of Fish and Wildlife (WDFW) regarding the feasibility and approval of possible fish recovery measures in the area of the escapement.
- Recovery efforts are dependent upon approval from WDFW and written authorization by WDFW must be obtained by the Cooke Emergency Management Team before commencing any recapture efforts.
- Upon receiving authorization from WDFW, the company will commence recovery of escaped fish through one or more of the following actions: (1) use of company skiffs and seine nets; (2) contacting the Northwest Indians Fishery Commission and nearby tribal Natural Resource managers to help facilitate the recapture of escaped fish; (3) contacting and engaging the services of local commercial fishing boat operators to facilitate the recapture escaped fish.

Recapture Gear, Boats and Methods

The company owns and operates several large work vessels with cranes. These vessels have the capacity to pull and refit anchors, transport supplies/equipment to the farm sites, lift stock nets to the surface and swap stock nets with new ones. These vessels can be relocated to any site in an emergency situation. The name of the vessel and their home port location is as follows: <u>F/V Clam Digger −Anacortes, Cypress and Hope Island; F/V Elsie Em and F/V Supplier − Bainbridge Sites; F/V Farm Hand − Port Angeles</u>.

Gear − Each farming area typically has 2 to 3 braided nylon seine nets approximately 15 fathoms long and 9 fathoms deep with 1" mesh. These nets are used for harvesting fish but can be used as beach seine nets in an emergency. Company work skiffs would be used to deploy the beach seines and crew along the nearby shorelines for attempted escaped fish recovery.

Methods − The most effective method to recover escaped fish would be to hire outside contractors with commercial fishing gear and vessels designed for capturing and killing wild and hatchery raised fish. There are multiple factors that would go into the decision process for which type of gear and fishing methods could be used to recapture escaped fish. The time of year, location and the size of the escaped fish will all need to be considered before deploying commercial fishing boats which could result in by-catch of non-target species.

Depending on approval from fisheries resource managers however, the immediate area around the farm could be "fenced off" using large purse seine vessel(s). These vessels could be used to reduce the dispersion of the escaped fish and facilitate recovery. Depending on the situation the nets could be pursed and the fish pumped into a harvest vessel using the vacuum pump. Any non-target salmonids could be removed manually by visually observing the fish on a de-watered table and releasing them over the side. The most effective method for capturing and killing escaped fish would be the use of gill-netters if the fish are within the size range that gill net vessels target. Typically gill-nets are made for harvesting fish that are over 3 pounds and up to 15 pounds. While large fish may get tangled in the netting, the size of the heads precludes them from getting gilled in the net. Smaller escaped fish could easily swim through the mesh openings or not be fully gilled and fall off as the nets are retrieved.

- Cooke will work with nearby area Tribes to annually review appropriate fisheries, gear types and identify the key natural resource contacts in the areas near each of the marine net pen farming locations. The tribal contact list has been developed with the names and department contact phone numbers for the nearby tribal entities.
- Within five working days of terminating the fish escape recovery actions, the company will submit a Fish Recovery Response Report to WDFW and WDOE.

We have found very little literature that discusses any behavioral differences in schooling between farm raised Atlantic Salmon and all-female triploid Rainbow Trout/steelhead raised in marine net pens. Both fish school in the pens however, all-female triploid Rainbow Trout/steelhead reared in net pens seem to be especially surface orientated in the net pen, whereas Atlantic Salmon will generally settle into a schooling pattern mid-pen or deeper once they have finished feeding. Both species of net pen reared fish are fed pelletized feed each day, which is distributed across the surface of the pens during the feeding periods. When being fed, the all-female triploid Rainbow Trout/steelhead crowd the surface of the pen to the point that they are substantially breaking the surface or "boiling" the surface water of the pen while they are feeding (personal observation).

Wild steelhead live a more isolated life in comparison while foraging in the freshwater and little is actually known about the social behavior of sea-run steelhead while they are in the marine phase of their life cycle. Likely some schooling and regrouping behavior is exhibited as the fish return to their natal streams at distinct times of year to reproduce. Personal observations and information gathered during previous large escapements of Atlantic Salmon from marine net pens indicate the fish tend to hug the shoreline and congregate, at least initially, in shallow bays and shoreline environments. It is likely that the fish are conditioned to feel more secure when there is an object in their peripheral vision (such as the shoreline) after they initially escape because that is the environment they have been raised in their entire life. Both farmed species of fish are raised for their entire life beginning in fiberglass tanks and then later in the net pens for the production fish which likely leads to substantially different behavior than their wild counterparts in the natural environment.

Disease Risk

B.7 What would be the potential risk of pathogen and/or parasite transmission from cultured AF triploid Rainbow Trout to wild and/or hatchery reared salmonid species when passing by the net pens, or in the event of an escape?

Cooke Aquaculture Pacific Response: Outbreaks of pathogenic virus or bacteria are rare and would be treated, and/or fish would be removed from net pens. During the time of on-site infection with pathogens or parasites, there would be a chance of transmission from cultured fish to wild and/or hatchery salmonids passing by the net pens. However, the transmission of pathogens or parasites would be reduced due to dilution with greater distance from the net pens. The risk of transmission might be greater with escapes from the standpoint of a greater chance for co-mingling of cultured fish with wild/hatchery fish. On the other hand, dispersal of escaped fish would reduce the local concentration of infective organisms being shed.

The use of triploid Rainbow Trout/steelhead would not increase disease concerns relative to the use of Atlantic Salmon. Triploid and diploid Rainbow Trout do not differ in immune responses to infectious diseases (Weber et al. 2013, Wiens et al. 2018).

Should escaped all-female triploid Rainbow Trout/steelhead carry a disease agent, the risk of them being the source of an outbreak in wild fish is low for the following reasons (Amos, Thomas and Stewart 2001, as cited in Rust et al. November 2014):

1) Native pathogens are already a part of the environment where wild fish are routinely exposed and have developed some natural immunity
2) Escapees are unlikely to generate an infectious dose (or infective pressure) sufficient to result in disease in a healthy wild population
3) The mere presence of a pathogen alone will not cause disease without environmental factors that play a large role in triggering disease events (McVicar 1997; Moffitt et al. 1998; and Amos, Appleby et al. 2001 as cited in Rust et al., November 2014), and
4) Most escaped farmed fish have low fitness for the wild and quickly become easy victims of predators such as marine mammals, other fish, and birds.

B.8 Describe disease control methods: Biosecurity of brood stock facilities, eggs, smolt and marine farm sites; disease screening, reportable and regulated pathogen reporting plans, vaccination, and antibiotic use.

Cooke Aquaculture Pacific Response: WDFW and the Washington Department of Agriculture (WDOA) have regulatory authority with regard to aquaculture products. Chapter 220-370 WAC establishes rules promoting the health, productivity and well-being of aquaculture products and wild-stock fisheries. These rules identify the conditions that are required for the transfer and importation of live aquaculture products in Washington and the circumstances when action will be taken to control disease (WAC 220-370-020).

WDFW regulates the movement of live private-sector aquaculture products through the Fish Transport/Import Permit. Private fish hatcheries are required to maintain fish health laboratory screening records and to routinely screen the stocks for regulated pathogens. Fish Transport permits are required for the movement of fish from the freshwater hatchery to the marine net pen facilities. The brood stock producing the AF triploid Rainbow Trout/steelhead stocks are raised in isolation on regulated pathogen-free water. These brood fish are screened for pathogens when they are spawned, and the resulting gametes are incubated on regulated pathogen free ground water at the hatcheries. Negative disease screening results are necessary in order for a transport permit to be issued by WDFW. The smolts will undergo an additional regulated pathogen screening test just prior to transfer to the marine net pens. A Fish Transport Permit is necessary for the movement of the smolts from the hatchery to the marine sites and again, testing requirements for regulated pathogens must be met prior to the issuance of a transfer permit. Additional disease screening requirements may be added by the Department to ensure the health of aquaculture products and wild stock fisheries. Licensed veterinary services and accredited veterinary labs are used by aquatic farmers to certify that brood stock fish and resulting fry are free of regulated pathogens. The movement of live salmonids or gametes across State or international borders (importation) is strictly controlled by the additional Federal regulations enforced by the U.S. Fish and Wildlife Service (USFWS) under Title 50 of the Code of Federal Regulations (Regulation 50 CFR, Part 16.13). A Title 50 accredited fish health inspector must certify that the live aquaculture products have undergone the necessary disease screenings that meet Title 50. Inspection by and approval from USFWS is necessary for the importation or transport of live salmonid products into Washington State. The fish health regulations are designed to control risk and reduce the incidence of serious fish pathogens from negatively affecting private, public and/or Tribal aquaculture facilities, and to protect the wild stock fisheries.

Managers of aquaculture facilities prevent and control the risk of disease events by implementing structured disease prevention programs. The ability to maintain a captive brood stock program in the controlled hatchery environment using pathogen-free ground water is the foundation for growing disease-free stocks. Strict biosecurity measures including isolation of each generation and their life stages at the egg, fry, and smolt stage further enhance the health of the production fish stocks in the marine net pens. Technological advancements in fish farming equipment, feeds and fish rearing procedures have evolved significantly since the 1990 PEIS was written. Facilities routinely utilize ozone and/or UV sterilization at the hatcheries to ensure pathogen-free water supplies at the hatchery. Vaccination of 100% of the smolts prior to transport to the net pens is a routine practice along with the development of improved vaccines.

Effective disease control programs include: 1) routine health exams by aquatic animal health specialists; 2) health inspections prior to movement of fish between regions or health management zones; 3) accurate record keeping by the farmer to include mortalities and presumptive causes, growth rates, and feed rates, and feed conversion rates; 4) implementation of a bio-security plan for each farm site; and 5) use of preventative medicine such as 100% stock vaccination and the use of probiotics (Rust et al., November 2014).

Washington marine finfish farms employ full-time fish health professionals who routinely monitor the health status at the farm sites. Aquatic farmers have economic incentive to maintain the health of their fish stocks through the use of strict biosecurity measures, disease control practices, 100% vaccination of the juvenile fish prior to transfer to marine net pens, and maintaining growing conditions conducive to healthy and fast-growing fish populations. Cooke Aquaculture will use local stocks of triploid Rainbow Trout/steelhead produced by Troutlodge hatcheries in Pierce County. Troutlodge, a Washington-based company, has been producing Rainbow Trout/steelhead eggs for sale to farms and public enhancement hatcheries throughout the world since 1945. Brood stock are raised in regulated pathogen-free facilities for their entire life cycle. The company utilizes a comprehensive health testing and disease-free certification program that exceeds World Organization of Animal Health (OIE) standards at their Washington facilities, allowing them to export live salmonid eggs throughout the world.

Troutlodge has been producing mono-sex (all-female) populations of Rainbow Trout/steelhead eggs since the mid-1990s. Eyed all-female triploid Rainbow Trout/steelhead eggs delivered to Cooke hatcheries from the Troutlodge hatchery under a WDFW Fin Fish Transport permit would be hatched and cultured in isolation on regulated pathogen-free water to a certain size in the Cooke hatcheries, and then transferred to the marine net pens. Prior to transfer to the net pens, the smolts would undergo the required fish pathogen screening requirements subject to review and approval by WDFW and necessary for issuance of a Fish Transport Permit. The Fish Transport Permit is required from WDFW for each specified lot of fish to be transferred from the Cooke hatchery to the marine net pen farms.

C. GENETICS, INTERBREEDING

C.1 Describe production techniques for all-female and triploid stocks of Rainbow Trout and ploidy quality assurance data.

Cooke Aquaculture Pacific Response: High rates of triploid induction in salmonid species are achieved by thermal shocking and pressure shocking the fertilized eggs at a specific stage during early cellular development. Physiological stress is associated with temperature shocks resulting in lower survival to hatch than pressure shocking. It has become the consensus that hydrostatic pressure shock is the methodology of choice for triploid induction in salmonids due to the relative ease to standardize the shock, as compared to temperature, and to obtain consistent results (Piferrer et al. 2009).

Diploid Rainbow Trout/steelhead ova contain two (2) sets of chromosomes while the milt from diploid male trout contains only one (1) set of chromosomes. Under normal post-fertilization conditions between a diploid male and female, the extra set of female chromosomes contained in the ova are ejected during meiosis and the formation of the second polar body. The resulting zygote contains a set of two chromosomes that will develop into diploid organism. A physical or chemical shock applied during meiosis can suppress the formation of the second polar body and the ejection of the extra set of female chromosomes. The resulting zygote contains (3) sets of chromosomes (3n), which will then develop into a triploid organism. In diploid organisms, the condition of triploidy or polyploidy results in the suppression or complete loss of reproductive traits and viability. Ploidy is confirmed using a fluorescent nucleic acid label on either embryo or blood tissue using a flow cytometer at the Washington State University School of Veterinary Medicine. Testing results of Troutlodge triploid fish and eggs over a period of five (5) years (from 2013 to 2018) demonstrate a high rate of success in triploid induction (99.84% − 2,950 of 2,955 fish and/or eggs sampled; see SEPA Checklist Attachment A.

Triploidy in Rainbow Trout/steelhead and other salmonids has also been achieved by developing a population of tetraploid (4n) males that produce 2n milt and crossing them with diploid (2n) females. Survival rates and growth however, has been shown to be severely depressed in the tetraploid individuals in comparison to the diploid controls, and makes brood stock production difficult (Chourrout et al. 1986). It is the Cooke's understanding that hydrostatic pressure treatment on diploid crosses represents the most consistent and technologically available method for the consistent production of triploid Rainbow Trout/steelhead. In addition, the utilization of feminized brood populations (producing X-only gametes) in combination with induced triploidy further ensures that sexual maturation is significantly inhibited. Female triploid Rainbow Trout/steelhead are functionally sterile with no observable development of oocytes (Lincoln and Scott 1984). Sterilization of populations, even if only partially effective, can substantially reduce both genetic and demographic consequences of cultured escapees (Baskett et al. 2013). Studies carried out in Britain on public and private river stocking programs looked carefully at the use of all-female triploids as a means to protect naturally spawning stocks. Results indicated that the stocking of all-female triploids did not noticeably impact wild stocks, and all-female triploids did not exhibit reproductive migration to spawning grounds (Piferrer et al. 2009).

C.2 For the past two years, based on Appendix A of the preliminary outline, your sample size for testing the efficacy of the triploidy procedure was as low as 60 individuals. What would be the sample size to validate triploidy for the lot of fish being moved into a net pen site (n ~ 800,000 for example)? If the number tested is as low as 60, the testing rate would be 0.008%, which seems inadequate to estimate the true triploidy rate.

Cooke Aquaculture Pacific Response: The results provided in Appendix A are additive, and confirm the hydrostatic shock process used at Troutlodge to induce triploidy. Ploidy was confirmed using a fluorescent nucleic acid label on either embryo or blood tissue using a flow cytometer at the Washington State University School of Veterinary Medicine. Testing results of Troutlodge triploid fish and eggs over a period of five years, from 2013 to 2018, and a total of 2,955 fish and/or eggs being tested, demonstrated a mean success rate of 99.84% triploid induction with low variance. Cooke suggests continuing the procedure of randomly sampling 100 fish from each individual lot of eggs/fish from a cohort that is being reared at the Cooke hatchery. Results of the triploid testing for each lot would be submitted to WDFW along with the fish health screening results as part of the Fish Transport Permit Application for that group of fish. If over a period of time, the results of the triploidy testing continues to show a low level of variance (verifying the triploid induction process), then this testing number could possibly be reduced to 60 fish samples.

In addition to the triploid screening process, Cooke suggests that WDFW personnel could perform visual inspections over a large number of adult fish while they are being processed at the processing plant, observing for both external and internal signs of sexual maturation and ovarian development. This could be a cooperative and informational process between Cooke and WDFW that could help give the public confidence that the technology to produce all-female triploid fish results in fish that are not developing reproductive capacity.

C.3 What is the evidence that triploid female steelhead do not mature, or at least become sexually active?

Cooke Aquaculture Pacific Response: Most species with cells that have a nuclei are diploid (2n), having one set of chromosomes from each parent. A triploid organism contains three sets of chromosomes (3n) instead of the normal number of two. The occurrence of triploidy in normally diploid organism is known to result in organisms that are not reproductively viable because they lack homologous chromosomes, preventing chromosomal pairing and segregation during meiosis. Comparative studies between diploid and triploid Rainbow Trout/steelhead have been carried out demonstrating a lack of sexual maturation occurring in female triploid Rainbow Trout/steelhead. In diploid female controls, the ovaries were packed with oocytes while those from female triploids, although showing the typical lamellar structure of an ovary, contained no oocytes, thus indicating that female triploids are sterile (Lincoln and Scott 1983). Diploid females had large, well developed ovaries containing yolk filled secondary oocytes whereas the triploids had only string like ovaries containing nests of oogonia. No primary oocytes were present (Lincoln and Scott 1984).

Triploid organisms with three sets of homologous chromosomes are found spontaneously in both wild and cultured populations, and can be easily induced in many commercially-relevant species of fish and shellfish (Piferrer 2009). The major consequence of triploidy is gonadal sterility, which is of advantage in the aquaculture of mollusks since it can result in superior growth. In fish, the induction of triploidy is mainly used to avoid problems associated with sexual maturation such as lower growth rates, increased incidence of diseases and deterioration of the organoleptic properties (Piferrer 2009). The induction of triploidy in cultured fish species is a well-known method to prevent the establishment of fertile populations, non-native reproducing populations, and to reduce risks of genetic introgression with naturally-occurring populations. The process is widely used by public fisheries enhancement programs across the United States for creating public fishing opportunities while protecting the genetic integrity of naturally-reproducing fish stocks in lakes and rivers.

The Oregon Department of Fish and Wildlife has incorporated the production of triploid trout for their recreational trout stocking programs in Oregon lakes and river systems. Behavioral studies carried out by the Oregon Hatchery Research Center (OHRC) on the spawning interactions in experimental stream channels between triploid Rainbow Trout/steelhead and control diploid Rainbow Trout/steelhead were completed as part of their review. The ORHC determined that the triploid female trout did not become sexually mature, did not show any indication of spawning activity, and did not interact with control fish. The triploid males in the same study, on the other hand, did show spawning behavior and interaction with the diploid control fish. Some triploid males do not show any indications of sexual maturation, but a significant proportion of triploid males develop sexually dimorphic morphological characters, behave aggressively towards control males, and attempt to spawn with control females (OHRC research summary publication).

C.4 A mean triploidy rate of 99.83% (median = 100%) is excellent, but even with that rate, and with all sites fully operable and stocked (~3.5 M fish), there will be over 6,000 fertile female fish. The lowest triploidy rate in the data you provided was 98.5%, which would generate over 50,000 fertile female fish. If the last escape event (~250,000 fish) occurred with triploid steelhead, the number of fertile female (425 – 3,750, based on the rates discussed above), would have exceeded the number of wild steelhead returning to spawn in many rivers in Puget Sound. You state on page 2 of your preliminary outline that using all-female, triploid fish would "eliminat[e] risks of genetic interference." Based on the above, these methods would reduce, but not eliminate the risk. Please evaluate this risk in a manner that doesn't simply dismiss the risk.

Cooke Aquaculture Pacific Response: Cooke has initiated many new improvements with regard to preventing escapes over the past two years and believes that by changing to cultivating sterilized all-female stocks of Rainbow Trout/steelhead, the company is taking additional steps to reduce the risk of effects related to escaped farmed fish. Answers provided by Cooke in the response to SEPA Checklist questions state, *"All-female triploid Rainbow Trout have been selected to be raised in the existing net pen facilities because the use of mono-sex and sterile stocks of fish is recognized as means to significantly reduce potential genetic interaction with natural populations."* The draft SEPA Checklist has been revised to clarify that Cooke does not believe all risk would be eliminated by the use of all-female triploid Rainbow Trout/steelhead, nor does the company believe that zero risk is possible. There is always some level of uncertainty, especially when dealing with highly variable and complex ecological systems. Assessing risks involves evaluating how likely potential problems are to occur (probability); how severe those potential problems might be (negative consequences); and what measures can be taken to keep the problems from occurring or correct them if they do occur (safeguards/mitigation). Risk management is an essential tool that is used in almost every human activity, from just crossing the road to building a skyscraper. WDFW uses a risk management strategy when they perform disease screening protocols for regulated fish pathogens at State-operated hatcheries. The Department manages fish health risks by screening the returning adult salmon brood stock fish for regulated fish pathogens and other common pathogens; however, there is always some level of risk for a disease occurrence. For this reason, other biosecurity measures are taken to further reduce risks and pathogen contingency plans are developed to address potential negative consequences if they occur.

As stated on page 3 of the SEPA Checklist:

> "Raising native-stock fish species in marine net pens is consistent with the recommendations of the *Final Programmatic Environmental Impact Statement: Fish Culture in Floating Net-Pens* (Washington Department of Fisheries, January 1990). The Preferred Alternative in the Programmatic EIS (PEIS) concludes that *"In areas where WDF determines there is a risk of significant interbreeding (with indigenous species) or establishment of harmful self-sustaining populations, the agency should only approve the farming of sterile* ***or*** *monosexual individuals, or genetically incompatible species."*

Cooke points out in Section A.11 of the SEPA Checklist that the proposal to use an all-female triploid stock of native fish combines both methods identified in 1990 by WDF for reducing risks of genetic introgression on native species;["By farming a monosex (all-female) and sterile (triploid) stock of Rainbow Trout/steelhead, Cooke's proposed change of species incorporates both methods of eliminating risks of genetic interference to indigenous populations or the establishment of self-sustaining runs."] The company's request to raise a sterile and monosex population of fish is consistent with the 1990 PEIS Preferred Alternative. Both of these measures were recognized (by WDF at the time) to reduce risks of negative impacts from interbreeding with indigenous species. Public fisheries managers have been for many years and are currently using these same methods (e.g., the use of all-female triploid trout stocking programs) in public recreational fisheries throughout the world (including in Washington State) as means to eliminate the risks of genetic introgression on naturally-reproducing populations. We have replaced the term "eliminating" with the word "reducing" in the revised SEPA Checklist (July 2019) to clarify that risks will not be completely eliminated by the species change proposal, but will be reduced by implementing this risk management action.

While we agree that 99.83% is not the same as 100%, and leaves the potential for the occurrence of a comparatively small number of diploid females in a given population, there are several factors that would reduce the probability of escaped diploid female fish interbreeding and thereby negatively impacting local stocks. As suggested in the WDFW concern that hypothetically, there could be from 6,000 to 50,000 fertile females in the population of approximately 3.5 million fish stocked in Cooke Puget Sound marine net pens, this is an oversimplification. It is not reflective of the biological reality of the fish or the physical reality of the operations– factors that need to be considered to assess actual potential risks of genetic interference from an escapement. There is low to no risk that either of these calculated numbers (6,000 or 50,000 fertile females escaping, surviving, and interbreeding) would ever occur. First, it is very unlikely that there would be 3.5 million fish (total) in the Cooke Puget Sound marine net pens all at the same time. Production is staggered so that some sites are harvesting fish while others are fallow, or in the process of planting or growing the next crop. This operational procedure also means that the fish at the different farm sites will be of different year classes compared to one another; therefore, there would be no risk that all of them would reach sexual maturity at the same time.

More importantly, there is low to no likelihood even if there were 3.5 million fish (total) in all Cooke Puget Sound marine net pens, that all of them would escape at the same time or over a short time span. The different net pen sites are located in spatially distant areas of Puget Sound (Rich Passage, Hope Island, Strait of Juan de Fuca and northern Puget Sound – see SEPA Checklist Figure 1). This reduces the risk of a single catastrophic event from releasing the entire population of fish from all the net pen facilities at the same time. Additionally, the WDFW calculation does not take into consideration that the farmed fish are contained within two layers of netting (the stock growing net and the predator exclusion net) on five sides of their pen (sides and bottom), and one layer of netting over the surface of the pen (the bird deterrent net). Even in a catastrophic event, the likelihood of fish escaping without incurring physical damage as they egress the pen structure is very low. It is more likely that a large percentage of the fish that would escape in a catastrophic event would become trapped in the netting structures and either be killed outright or suffer injuries to the point that their survival time outside of the pens would be reduced. This was observed during the Cypress Island Site 2 event when the pen structures where significantly distorted by tidal forces and a large number of the fish were found dead in the bottom of pens. Salmonids rely on a skin barrier system composed of mucus cells scattered in the epidermis that secretes products with antimicrobial activities (K. Shepard 1994). Removal of this outer layer of fish skin may increase susceptibility to infections (Y.S. Svendsen 1997). Abrasions to the skin mucus layer and especially the loss of scales can quickly result in bacterial infections in the skin and the formation of ulcerations, a general decline in the physical condition, and an increased mortality rate.

From experience with past escapes of farmed fish, the percentage of fish that survive in the wild only do so temporarily, as they do not thrive outside of the net pen environment. Escaped farmed Atlantic Salmon recovered in the wild typically are found to not have been actively feeding, becoming more emaciated over time. Hatchery enhancement stocks as well as commercially-domesticated stocks have been shown to rapidly lose foraging, predator avoidance, and reproductive traits in comparison to naturally-produced stocks. The WDFW statement that *"there will be over 6,000 fertile female fish"* is an over simplification that does not account for the other physical and biological factors that substantially change this hypothetical scenario. While a small percentage of the farmed population may be fertile, the risk of those fish all escaping, surviving, reaching sexual maturity, and then successfully reproducing would be reduced by the reality factors described in this response. Cooke understands agency concerns regarding both escaped fertile fish as well as sterile fish, which is why the company will continue to improve upon farming practices and containment measures, and invest in new technology and materials that help to minimize the risks of stock loss from their farms.

WDFW posed a hypothetical scenario of escapement similar to the 2017 Cypress Island Site 2 escape, postulating that had *"the last escape event (~250,000 fish) occurred with triploid steelhead, the number of fertile females (425 – 3,750, based on the rates discussed above)"* would have escaped. Although 3,750 may be a large number of fertile fish relative to spawning wild steelhead, the biological reality of this hypothetical scenario needs to be taken into consideration. Not all escaped fertile fish would mature and interbreed with wild steelhead due to a number of factors.

First, the fertile farmed fish would not all mature in captivity by the time of escape. Normal age of Rainbow Trout/steelhead maturation is 4 years, whereas the total culture duration for Cooke operations is 9 months in fresh water and 14 months in seawater. Thus, the age at harvest is approximately 2 years less than the normal maturation age. Even in a worst-case scenario if 50% of the fertile fish were to be mature at the time of escape, this would leave approximately 213 to 1,875 mature escapees.

Second, the fertile escaped fish would need to feed, survive, and mature in the wild. Studies of the transition of escaped farmed salmonids to feeding on native fauna show low rates from 2 to 13% (Morton and Volpe 2002, Abrantes et al. 2011). Feeding is critical for both survival and maturation. Survival of released farmed Rainbow Trout/steelhead in a protected lake was only 40% within one year, even with access to excess food near the net pens (Blanchfield et al. 2009). Thus, the likelihood of escaped fertile female Rainbow Trout/steelhead in Puget Sound transitioning to feeding and surviving to maturation is low (10%). In the hypothetical situation of 425 to 3,750 fertile escapees, the survival rate prediction (40%) would be that approximately 43 to 375 fertile fish might survive to sexual maturity.

Third, if escaped fertile female farmed fish survived and matured, they would need to migrate to a river during the spawning season in order to interbreed with native steelhead. This would require reproductive synchrony with native steelhead as well as precise navigation to a specific river, which is highly unlikely due to diverse genetic backgrounds and the lack of homing imprinting in the farmed Rainbow Trout/steelhead. Steelhead bred in captivity quickly (within one to two generations) lose reproductive fitness (Araki et al. 2008). The magnitude of this fitness loss has been estimated as 40% per generation in captivity (Araki et al. 2007). The Rainbow Trout/steelhead proposed for Cooke Puget Sound marine net pens have been reared in captivity for approximately 30 generations. Even within just two generations of captive rearing, applying the estimated 40% fitness loss per generation results in the fertile Rainbow Trout/steelhead with only approximately 20% of their original reproductive fitness. If this 20% reproductive fitness loss were applied to the likelihood of 43 to 375 fish surviving to successfully mate with wild steelhead, the number of reproductively viable females would be reduced to 9 to 75 fish as a most conservative estimate. If the actual 30 generations of captive rearing and reproductive fitness loss were applied to the likelihood of 43 to 375 fertile fish surviving a marine net pen escapement, the ability of those fish to achieve reproductive success would be extremely low.

C.5 It is important to note that we are also concerned with biological, ecological, and social effects of escaped fish on non-listed but valued species in the Puget Sound area.

Cooke Aquaculture Pacific Response: See the response to information requests in Sections A and B above.

Cooke believes that the application materials previously submitted to WDFW for re-approval of the Marine Finfish Aquaculture Permit; the revised SEPA Checklist information; and the additional information provided in this document and literature review (Attachment C) on the proposed species change to native Rainbow Trout/steelhead, in conjunction with the 1990 *Programmatic EIS on Fish Culture in Floating Net Pens*, previous NMFS risk assessments and literature reviews regarding Puget Sound net pen salmon farming, and the numerous prior Federal ESA reviews and biological opinions on net pen aquaculture projects in Puget Sound, have looked at the potential effects from many perspectives, over many years.

In addition, previous decisions by the Pollution Control Hearings Board on net pen aquaculture in Puget Sound, along with the continued development of new and improved regulations by local, State and Federal agencies all help to minimize and mitigate risks of significant impacts that might otherwise occur as a result of escaped farmed fish in the environment. Attachment C is an updated scientific literature review and bibliography with regard to salmon and Rainbow Trout/steelhead aquaculture, prepared by technical experts as part of this SEPA Additional Information document. Curriculum vitae for the technical experts are provided in Attachment E.

D. OPERATIONAL DIFFERENCES

D.1 Research and industry information regarding no substantive differences in feeding, water quality, benthic impacts, disease and disease control in net pen salmon farming.

Cooke Aquaculture Pacific Response: Commercial salmonid diets for marine-reared Rainbow Trout/steelhead and salmon do not substantially differ. Cooke anticipates utilizing the same or very similar salmonid diets to those currently used to grow Atlantic Salmon in Puget Sound marine net pens. Peak biomass levels will be the same or less than past peak biomass levels for the Atlantic Salmon operations. There will likely be a difference in the time span of peak biomass as the production cycle is expected to be shorter by several months. Current fresh Atlantic Salmon seafood markets are favorable to a certain percentage of larger fish sizes (14 to 16 pounds or larger); therefore, continuing to feed and grow the population to extend the harvesting period can be financially beneficial. On the other hand, the net pen-reared fresh Rainbow Trout/steelhead market is set up primarily around fish of smaller size categories (from approximately 3 to 9 pounds), and there is less market acceptance for farmed Rainbow Trout/steelhead in the 12 pound and up size category. In addition, farmed triploid Rainbow Trout/steelhead are known to have reduced fitness and are less tolerant of physical stressors in comparison to diploid trout. This is due to the tendency of triploid Rainbow Trout/steelhead to have smaller heads relative to their body size, and a subsequent reduction in gill surface area to body mass ratio. Physical exertion from strong currents and hypoxic conditions can cause reduced feeding, loss of growth, and possibly increased mortality. The company anticipates harvesting the fish beginning around the 7 pound size category. By comparison, the company has historically harvested their Atlantic Salmon stocks when the fish reached the 10 to 11 pound average weight category. It is anticipated that the harvesting volumes and frequency would be increased for the triploid Rainbow Trout/steelhead production in comparison to the Atlantic Salmon production based on the smaller targeted average weight and narrower market size acceptance. Based on the tentative growth projections for triploid Rainbow Trout/steelhead, it is likely that the production cycle will be shorter by several months in comparison to raising Atlantic Salmon resulting in the farm sites being fallowed more frequently than past Atlantic Salmon production cycles.

Because there will be no significant differences in feed components, biomass levels, feed volumes or metabolic characteristics between raising Atlantic Salmon or Rainbow Trout/steelhead at the net pen sites, there will likely be little, to no detectable difference in water quality or benthic effects from the change of species. There is industry knowledge about the differences in feeding behavior between the two species as Rainbow Trout/steelhead tend to be more surface-orientated feeders and also do not tend to present as strong of a "stop feeding" signal in general compared to net pen-reared Atlantic Salmon do. However, each group of fish and specific strains within a species can also exhibit different feeding behaviors. The farm feeding technicians need to "learn" the distinct feeding behavior of the Rainbow Trout/steelhead population just like they learned each new generation of Atlantic Salmon which they have raised. There are a numerous variables that can affect the daily feeding behavior of net pen-reared salmon especially when you consider that these farmed fish are being fed for an average of 450 days at the net pen facility before they reach harvest size. Cooke trains and relies on a select number of employees to perform the feeding operation which helps to control and maintain continuity of the process. Those technicians are responsible for detecting the differences in feeding behavior from day to day and for closely watching the feed delivery quantities to prevent over- or under-feeding the pen population. Underwater feed monitoring cameras will be used to observe the feeding behavior of the fish population along with using periodic sample weights, daily expected feed rates, tracking feed quantities, and comparing actual versus expected growth rates to actively monitor the feeding process.

The Hope Island, Clam Bay, Fort Ward and Orchard Rocks sites are all scheduled to receive updated NPDES permits from the Department of Ecology in 2019. The renewed permits call for an increase in benthic monitoring from the current level of once every 2 years to annually. This increased frequency of sediment sampling as well as other water quality monitoring and reporting requirements are all designed to monitor for negative impacts and allow active management to protect the environment.

Cooke does not anticipate any substantial differences in disease control practices or disease control chemicals to be used at the farm sites as a result of the species change proposal. The all-female triploid Rainbow Trout/steelhead stocks will be vaccinated at the hatchery for common bacterial pathogens and IHN virus. The same farm site biosecurity measures will be employed as those currently being used for Atlantic Salmon production.

D.2 Feed types, feed conversion ratios, growth rates, density, biomass, harvest cycles: literature on current net pen salmon and trout farming.

<u>Cooke Aquaculture Pacific Response</u>: Over the past 20 to 30 years, commercial salmon and trout diets have improved substantially. Research into the specific nutritional requirements of salmonids has led to the development of highly digestible nutrients that contribute to rapid and efficient growth and the health of the fish. The fish feed ingredients for marine-reared Rainbow Trout/steelhead diets are the same as that currently used for growing Atlantic Salmon in net pens, formulated for optimal growth and feed conversion rates. The feed conversions for commercially-reared marine net pen Rainbow Trout/steelhead and Atlantic Salmon are similar and dependent on the feed management strategies and techniques used by the individual grower. Significant developments have also been made over the past 10 years in underwater feed and biomass monitoring technologies, such as underwater camera systems, Doppler radar systems, and active sonar capable of real-time biomass growth measurements of the fish. These tools are used by aquatic farmers to monitor feed levels, the growth of the fish stocks, and to calculate the feed conversion rates of the population. As this food production system continues to grow, the aquaculture industry will drive research and development of new and more efficient commercial salmonid diets, feeding techniques, and feed monitoring technologies. Information provided to Cooke regarding marine net pen Rainbow Trout/steelhead production in Chile over the past three years (2016 to 2018) showed a biological feed conversion rate of 1.16, which is similar to some of the best industry standards for marine net pen Atlantic Salmon production (Ron Malnor, Skretting Feeds, personal communication). Cooke would begin by using a commercial salmon diet following the species change to Rainbow Trout/steelhead. Feed trials would likely be carried out using various energy-to-protein ratios to further identify the best site-specific formulations for growth, health, and flesh quality.

Commercial production of Rainbow Trout/steelhead has grown exponentially since the 1950s, especially in Europe and more recently in Chile. This is primarily due to increased inland production in countries such as France, Italy, Denmark, Germany and Spain to supply their domestic markets, and mariculture in cages in Norway and Chile for the export market. Chile is currently the largest producer. Other major producing countries include Norway, France, Italy, Spain, Denmark, USA, Germany, Iran and the UK.

(FAO 2019, Fisheries and Aquaculture). Feeds for Rainbow Trout/steelhead have been modified over the years, and cooking-extrusion processing of foods now provides compact nutritious pelleted diets for all life stages. Pellets made in this way absorb high amounts of added fish oil and permit the production of high-energy feeds, with more than 16% fat. Dietary protein levels in feeds have increased from 35 to 45%, and dietary fat levels now exceed 22% in high-energy feeds. Feed formulations for Rainbow Trout/steelhead use fish meal, fish oil, grains and other ingredients, but the amount of fish meal has been reduced to less than 50% in recent years by using alternative protein sources such as soybean meal. These high-energy diets are efficiently converted by Rainbow Trout/steelhead, often at food conversion ratios close to 1:1 (FAO 2018, Fisheries and Aquaculture).

The planting and production of Cooke all-female triploid Rainbow Trout/steelhead would follow a similar pattern used for the year-around production of Atlantic Salmon at Cooke existing Puget Sound marine net pen facilities. This production cycle utilized two temporally separated saltwater entry groups that originate from a single year-class of brood fish. The groups are typically referred to as spring-entry and fall-entry smolts. Egg production from a single-year class of brood stock can be extended by photo adjustment of the adult fish at the hatchery. Fertilized eggs that are produced from the spring spawn and the fall spawn cohort are transferred into the smolt production hatchery at different time periods. This produces a fall saltwater-ready entry group of fish originating from the spring eggs, and spring saltwater-entry group that comes from the fall-spawned eggs. The seawater-ready all-female triploid Rainbow Trout/steelhead smolts are produced in approximately 9 to 11 months post-hatching. The estimated size of the fish at saltwater entry would be from 100 to 180 gram average weights. The projected marine growth phase from the saltwater-entry smolt to harvestable-sized fish would be approximately 12 to 18 months. Projected average weight at harvest would be from 7 to 10 pounds. Harvesting the fish population typically takes from 2 to 5 months depending upon the number of fish at a site, market conditions, and production plans. By transferring the juvenile fish (smolt) from the hatchery to the marine environment at different times of the year (spring-entry and fall-entry groups), the production of harvestable-sized fish can be spread out during the entire year.

The Fort Ward, Orchard Rocks, Clam Bay and Hope Island net pen sites all have current leases with the Washington Department of Natural Resources. Cooke is requesting approval of a new Marine Finfish Aquaculture Permit for these facilities to raise an all-female triploid Rainbow Trout/steelhead stock, so that the company can begin raising a native stock of fish and continue to produce healthy seafood, local jobs, and economic activity in Washington.

The Cypress Island Site 1 and Site 3, and Port Angeles Harbor sites are also included in the request for approval of a change of species to the all-female triploid stock of Rainbow Trout/steelhead because Cooke is hopeful that the legal issues around the WDNR leases can be resolved. At this time however, there are no plans to plant fish at the Port Angeles or Cypress Island sites until there is further certainty in their legal status. Finally, the Cypress Island Site 2 facility has been completely removed from the water and Cooke has formally asked Ecology to withdraw the Cypress Site 2 NPDES permit. Cooke is therefore not requesting a new Marine Finfish Aquaculture Permit for Cypress Island Site 2 because this site will not be rebuilt.

The list below identifies the existing Cooke marine net pen sites that could eventually transition to raising all-female triploid Rainbow Trout/steelhead, pending agency approvals, and the estimated possible number of fish that could be planted at each site for each generation/production cycle. Planting and the subsequent harvesting of the fish populations would occur at different times of the year at the different sites. The anticipated growth cycle in the marine net pens is approximately 14 to 16 months between first fish in (planted) and last fish out (harvested). Because of this, the various individual sites and/or growing areas may alternate between growing a spring-entry group for one generation, and subsequently be planted with a fall/winter generation.

Site Name	Approximate Number of Fish	Notes
Hope Island	350,000	
Clam Bay	1,000,000	
Orchard Rocks	600,000	
Fort Ward	300,000	
Cypress Site 1	300,000	WDNR lease pending legal status
Cypress Site 3	400,000	WDNR lease pending legal status
Port Angeles	600,000	WDNR lease pending legal status
Cypress Site 2	0	Pens are removed and will not be replaced
Total:	3,550,000*	*Planting cohorts occurs at different sites and times of year. Total fish number would vary depending on the production cycle.

No differences in water quality or sediment quality are expected to result from this change in species or the accompanying Rainbow Trout/steelhead-specific feeds that would be used.

Marine net pen cultivation and production protocols for Rainbow Trout/steelhead are basically the same as those used for Atlantic Salmon. Maximum cage density levels are expected to be managed at the same levels (approximately 0.9 to 1.2 lb./ft^3 or 15 to 20 kg/m^3), resulting in comparable maximum biomass levels that have historically been attained at each of the existing Cooke Aquaculture sites. Depending upon fish size at harvest (targeted mean weights of approximately 7 to 9 lbs. or 3.5 to 4.2 kg), the fish population sizes at each marine net pen site are expected to be similar to stocking levels for Atlantic Salmon.

D.3 What are the differences in feed conversion between steelhead and Atlantic salmon?

Cooke Aquaculture Pacific Response: See the response to Questions D.1 and D.2, above.

D.4 Species differences in net pen aquaculture (comparison between cultured diploid Atlantic Salmon and triploid Rainbow Trout).

Cooke Aquaculture Pacific Response: Within the same or similar rearing environment (mid-latitude temperate marine waters), the commercial culturing of salmonid species such as Atlantic Salmon and Rainbow Trout/steelhead do not differ substantially. Both fish have similar biologic and metabolic requirements as well as temperature and oxygen demands. Atlantic Salmon and Rainbow Trout/steelhead also share very similar life histories. Commercially-domesticated stocks of Atlantic Salmon typically reach sexual maturity at 4 years of age or more; however, a certain percentage (typically males) will mature at 3 years of age. Rainbow Trout/steelhead typically take from 4 to 5 years to reach maturation. Unlike most other Pacific salmon species that die after spawning, both Rainbow Trout/steelhead and Atlantic Salmon are capable of reproducing more than once in their life time, although rates of survival after spawning are typically very low.

Cooke anticipates there will be some production planning differences between culturing Atlantic Salmon and the all-female triploid Rainbow Trout/steelhead. In general, the U.S. fresh farmed Atlantic Salmon seafood market is set up around the harvested fish product being in the 10 to 14 pound dressed “head on, gutted” (HOG) range. Atlantic Salmon farms set their production plans to achieve these targeted average weights within a projected time frame, knowing that the farm will need to be harvested out (e.g., emptied of one generation) within a certain time frame that will allow a fallow period before stocking the farm with the next generation of juvenile fish. In general, growth of Atlantic Salmon to these size ranges can be achieved in the marine net pens in a 14 to 20-month time period.

The commercial market for net pen-grown Rainbow Trout/steelhead in the United States is based on smaller average weights, typically a product in the 7 to 10-pound range. Industry knowledge of the growth rates for domesticated all-female triploid Rainbow Trout/steelhead in net pens indicate the target sizes can be achieved in approximately 14 to 16 months depending mainly upon original stocking size and site-specific growing conditions. This would result in a shorter production cycle and an increased frequency of fallow periods over a given amount of time compared to Atlantic Salmon culture.

Cooke does not anticipate major differences in stocking densities, daily feed requirements, or disease susceptibility compared to current planning for Atlantic Salmon production. Up to the time of this writing, all Atlantic Salmon stocked into Cooke Puget sound marine net pens received an intraperitoneal injection of an inactivated, multivalent vaccine against furunculosis, classical vibriosis, cold water vibriosis, and infectious hematopoietic necrosis. Cooke anticipates the continued use of this vaccine in the production of the all-female triploid Rainbow Trout/steelhead. Biosecurity methods and disease control practices would remain the same, because disease vectors and potential pathogens are similar if not identical for both types of salmonids.

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