Summary – Key Issues

Cooke Aquaculture's SEPA checklist to culture all-female triploid (sterile) steelhead in Puget Sound net-pens

October 1, 2019

Decision: Mitigated Determination of Non-significance (MDNS)

This document is not intended to be a comprehensive discussion of the deliberative process used by Washington Department of Fish and Wildlife (WDFW) to reach our decision. Rather, what follows is a summary of WDFW's perspective on the key issues that were part of that decision process.

Background

In January 2019, Cooke Aquaculture Pacific (Cooke) submitted two applications to WDFW: (1) application to renew an expiring 5-year Marine Aquaculture Permit to continue to culture Atlantic salmon at Cooke's marine net pen facilities in Puget Sound; and (2) an application for a new 5-year Marine Aquaculture Permit to culture all-female triploid (sterile) steelhead at Cooke's marine net pen facilities in Puget Sound. In March 2019, WDFW approved and issued to Cooke a renewal of their 5-year Marine Aquaculture Permit for Atlantic salmon, contingent on the requirement specified in EHB 2957 that farming of nonnative marine finfish in Puget Sound is valid only with a current lease of state-owned aquatic lands. At the same time, WDFW responded to Cooke's second application by informing them that a SEPA process was required to determine the environmental effects of raising all-female, triploid steelhead at their facilities, before WDFW could issue a substantive determination on the permit application. On July 25, 2019 Cooke submitted to WDFW a completed SEPA checklist and a comprehensive set of supporting documents, including information that would add to or complement the 1990 PEIS for environmental impact of fish culture in floating net pens located anywhere in Washington State marine waters. This SEPA analysis is tied to WFDW's substantive decision on the 5-year Marine Aquaculture Permit application for steelhead. This SEPA analysis anticipates and discusses Cooke's planned transfers of juvenile steelhead from its freshwater hatchery to marine net-pens facilities as part of its regular operations. These transfers would require finfish transfer permits from WDFW. This analysis is intended to double as the SEPA analysis for all anticipated transfer permits inherently connected to Cooke's operations approved under the 5-year Marine Aquaculture Permit.

Previous permits issued by WDFW to Cooke

Cooke purchased the existing commercial marine net-pen facilities and freshwater hatcheries in June 2016. Since that time, WDFW has issued to Cooke ten finfish transport permits and one renewal of their 5-year Marine Aquaculture Permit, discussed above. All eleven of these permits were for the transport or culture of Atlantic salmon. WDFW approved these permits based on our understanding that Cooke's marine net-pen operations and their culturing of

Atlantic salmon presented only a low risk to the natural or built environments in and around Puget Sound.

Risk to the natural or built environments

After considering Cooke's SEPA checklist and supporting documents, we concluded that culturing of all-female, triploid (sterile) steelhead at Cooke's marine net-pen facilities in Puget Sound pose a similarly low, but somewhat different risks to the natural or built environments compared with the culturing of Atlantic salmon in Puget Sound. We highlight below a few environmental areas that require a more detailed summary.

WATER AND BENTHIC QUALITY

Water and benthic quality for marine net-pen aquaculture are regulated by the Washington Department of Ecology (Ecology) through their NPDES permit process. Ecology recently reissued NPDES permits to Cooke for their current operations culturing Atlantic salmon at the four net-pen facilities with valid aquatic lands leases with the Washington Department of Natural Resources (DNR). These new permits require more frequent peak biomass sediment and water column dissolved oxygen monitoring than did the previous NPDES permits. Cooke is required by Ecology to submit NPDES permit modifications to their existing permits to culture all-female triploid (sterile) steelhead in their marine net-pen facilities. WDFW defers to Ecology to protect water and benthic quality through their NPDES permit process.

DISEASE TRANSMISSION

Cooke vaccinates Atlantic salmon for IHNV and for a collection of bacterial pathogens prior to fish transport into their marine net-pens. In addition, fish are tested for state-defined regulated pathogens and for exotic North Atlantic variants of PRV. Cooke is required to submit to WDFW each year a revised Regulated Finfish Pathogen Reporting Plan and is required to maintain appropriate biosecurity at all their facilities, including regular fallowing of all net-pen facilities after harvest. Cooke will continue with these same procedures when culturing their all-female, triploid (sterile) steelhead production. In addition, Cooke will be required to implement additional measures, as outlined in the Mitigating Provisions list below.

FISH ESCAPE FROM NET-PENS

Perhaps the largest concern with open net-pen aquaculture is the escape of fish into open waters of Puget Sound. We consider four components to fish escapes: (1) small scale leakage of fish that could occur during the transfer of fish to and from transport vessels, and from other causes such as net failures due to predators, maintenance error, floating debris, or vandalism; (2) large-scale escapes resulting from infrastructure failure, such as the 2017 accident at Cooke Aquacultures' Cypress #2 facility; (3) ecological consequences of escaped fish; and (4) genetic consequences of escaped fish. Unlike Atlantic salmon that cannot interbreed with Pacific salmon, net-pen cultured of steelhead in Puget Sound represents a potential elevated risk to Pacific salmon, in particular native steelhead. Cooke has greatly reduced this risk by cultivating mono-sex sterile steelhead, rather than fertile fish of both sexes as they do with Atlantic salmon. Cooke will be required to implement procedures and monitoring activities to further

reduce and document risk – see the Mitigating Provisions section below. Here we focus on two elements related to fish escapes: infrastructure integrity and maintenance, and the triploidy procedure error rate, which will affect the number of diploid (fertile) fish in each lot of triploid fish.

Infrastructure maintenance and integrity – Since the Cypress #2 accident, Cooke has implemented a series of procedures to reduce the risk of both small-scale leakage and large-scale escape of fish. These activities are documented in Cooke's Fish Escape Prevention, Response and Reporting Plan, which requires updating at least once per year. In addition to these activities, since 2017, each of Cooke's facilities have been inspected and evaluated by Mott MacDonald, a civil engineering company with offices in Seattle and elsewhere. As part of their evaluations Mott MacDonald provided a list of maintenance and repair activities needed at each of the facilities. Cooke conducted those repairs and provided documentation of those repairs to DNR. Furthermore, DNR, Ecology, and WDFW have inspected each facility at least once since 2017. Finally, as part of the new law that sunsets nonnative finfish marine aquaculture in Washington State (EHB 2957), Cooke is required to have each net-pen facility inspected above and below waterline by an engineering firm approved by WDFW, roughly every two years when the facilities are fallow. Transport of fish into the net-pens is contingent on the findings from that inspection. In other words, WDFW will not approve transport permit applications for net-pen facilities whose structural integrity is inadequate.

Triploidy error rates – The efficacy of the methods used to create triploid and therefore sterile fish is not 100%. This means that in every batch of triploid fish there may be fish that are fertile and can interact reproductively and spawn with wild individuals of the same or similar species. The presence of fertile domesticated fish increases the risk of interbreeding with wild populations, but that risk is directly related to the actual number of fertile fish produced and the degree to which those fish are domesticated. Cooke provided a historical triploidy error rate of 0.17%. Knowing the number of diploid-fertile fish that will be transported into each net-pen raft is essential when assessing risk to native steelhead from the Cooke's cultured fish. Therefore, we evaluate Cooke's assessment of the triploidy error rate, and provide an estimate of the risk of hybridization between Cooke's all-female steelhead and native steelhead in Puget Sound.

Triploid-sterile fish are created when a batch or lot of eggs (anywhere between roughly 500,000 and a couple of million eggs; J. Parsons pers. comm. 2019) are placed within a vessel at a specific time after the eggs have been fertilized and water-hardened, and high-pressure hydrostatic shock is applied to the eggs for a specified length of time. The shock forces each egg to retain an otherwise extruded polar body, creating an extra set of chromosomes (three sets - triploidy, rather than the normal two sets – diploidy).

Appendix A in Cooke (2019) is a table provided to Cooke by Troutlodge, the Washington State-based supplier of the all-female triploid eggs to Cooke, that indicates that Troutlodge's proprietary procedure to create triploid-sterile fish has an error rate (i.e., rate of diploidy, and

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

Troutlodge used sample sizes ranging from 43 to 100 fish (mean = 82) per lot to calculate the triploidy error rate (Cooke 2019; Appendix A). Cooke suggested that for future lots to be transported into Puget Sound net-pen facilities, sampling rate be 100 fish per lot, reducing that sample size down to 60 if results appeared consistent with the data in Appendix A (Cooke Aquaculture 2019; B-26). If the rate of diploidy (i.e., triploidy error rate) is indeed 0.0017 (0.17%) then the probability of drawing at least one diploid fish from a lot of 1,000,000 fish using a sample of 60 or 100 is 0.10 and 0.16, respectively (Table 1). That is, given an unbiased draw from the lot, there is a low expectation that a diploid fish will be selected using sample sizes of 60 or 100, even when there are diploid fish present in that lot. To achieve high confidence that at least one diploid fish is selected (probability > 0.90), a sample equal to 1500 is required[1]. Sample sizes of 60-100 are more appropriate for rate of diploidy equal to 5% rather than 0.17% (Table 1).

Cooke effectively achieves a high sample size by pooling the results from all 36 lots in their Appendix A (N = 2955). To test the efficacy of this method, we modeled the procedure used by Cooke. That is, we assumed an actual rate of diploidy = 0.17%; drew from a hypergeometric distribution[2]; sampled cumulatively from 36 independent lots, each with a total of 1 million eggs; and used sample sizes equal to 60, 150, 600, and 1500. We modeled each sample size 100,000 times to generate frequency distributions of calculated rates of diploidy (Figure 1). The

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

[2] Equivalent to a binomial (e.g., triploid v. diploid) distribution, but sampling is without replacement, changing the probability after each trial.

mean rate of diploidy from 100,000 separate cumulative samples from 36 lots, for each sample size was unsurprisingly 0.17%; however, the rate of diploidy ranged from 0% to nearly 0.7% for the sample size = 60, with a large overall variance (Figure 1). That is, with a sample size of 60 for each of the 36 lots, there is a reasonable probability of calculating a rate of diploidy = 0.09%, 0.12%, or 0.19%, all close to 0.17%, and all quite low, but none equal to 0.17% (Figure 1). As we increased sample size from 150 to 1500, the precision of our estimate of the rate of diploidy increased.

If the probability of selecting one or more diploid fish from a pool of 1 million fish, with a triploidy error rate = 0.17% and a sample size = 100 is 0.16 (Table 1), then we would expect to draw at least one diploid fish from 16 lots out of 100 lots, or 5.8 lots out of 36 lots. Troutlodge's data showed one diploid fish from 5 lots out of 36 lots, with an average sample size per lot = 82. These results are what would be expected if the triploidy error rate was indeed close to 0.17%. We used the same modeling framework described above for Figure 1 to determine the percentage of times the calculated rate of diploidy from a cumulative sample from 36 lots was less than 0.2% out of 100,000 iterations, using a sample of only 60 fish, and actual rates of diploidy equal to 0.02%, 0.17%, 0.2% and 0.5%[3]. One hundred, 69, and 57 percent of the calculated rates of diploidy were less than 0.2% (0.002) for actual rates of diploidy equal to 0.02%, 0.17%, and 0.2% respectively. The percentage of times the calculated rate of diploidy was less than 0.2% dropped dramatically to 2% for an actual rate of diploidy = 0.5%. This suggests that the rate of diploidy for the Troutlodge triploidy procedure averages less than 0.5% and is probably close to 0.2%, essentially the same as the 0.17% provided by Cooke Aquaculture. However, drawing sample sizes of 60 or 100 from a lot of one million fish will not produce a precise measure of the triplody error rate.

Estimating risk to native steelhead from escaped steelhead from net-pens - To estimate risk of hybridization between Cooke's net-pen steelhead and native steelhead in Puget Sound, we estimated the following parameters. In general, we agree with the logic Cooke presented on pages B-28 through B-29 (Cooke 2019).

- **Number of fish in net-pens: 1,000,000** [Clam Bay facility; Cooke Aquaculture (2019: B-33). Represents the largest facility and therefore the worst-case scenario]
- **Triploidy error rate: 0.2%** [From above]
- **Proportion of the fish that escape: 0.82** [Based on the proportion of Atlantic salmon that escaped from Cypress #2 net-pen failure (Clark et al. 2017). We consider this to be a near worst-case scenario.]
- **Proportion of the escaped fish that elude recovery efforts: 0.77** [Based on the number of Atlantic salmon that were recovered following the Cypress #2 net-pen failure (Clark et al. 2017). Depending on the number of fish that escape, age of fish that escape, when the fish escape, and the behavior of the fish when they escape, this proportion can be much different than what we present here.]

[3] Less than 0.2% is equal to the five left-most bars in Figure 1A.

- **Proportion of diploid fish sexually mature at time of escape: <u>10-50% of fertile fish</u>** [50% is an extreme worst-case scenario, presented by Cooke Aquaculture. Realistically, this proportion should be near zero becuase the fish will be harvested, on average, at less than two years of age, approximately 1-2 years prior to when they would reach sexually maturity (Cooke Aquaculture 2019; J. Parson, pers. comm., 2019). We're using 10% as a low-end estimate, without justification other than it is greater than zero.]
- **Proportion of fish that will survive long enough to attempt to spawn: <u>50% of fertile fish</u>** [Blanchfield et al. (2009) estimated annual mortality of rainbow trout = 50%, while Patterson (2010) estimated that 50% of the rainbow trout died within the first three months; however Patterson had unaccounted fish. Both studies consisted of experimental releases of relatively small sample sizes in freshwater. We estimated that within a year of the Atlantic salmon release from Cypress #2, most fish had either been recaptured or had died.]

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

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

We estimate conservatively that there will be **63 – 316 fertile female steelhead** that would escape and survive to sexual maturity from Cooke Aquaculture's Clam Bay facility following an accident as described above. If the accident were to occur at the Fort Ward site, with a total of 300,000 fish, there would be **19 – 95 fertile female steelhead** that would escape and survive to sexual maturity. Since these fish are all females, they will not spawn with each other, and to genetically affect Washington's steelhead populations, these fish would need to spawn with either hatchery- or natural-origin, natural-spawning steelhead. To do so, these domesticated fish would need to migrate into a steelhead spawning river, without homing instincts or cues to enter a specific river, at the correct time of year, dig redds, and attract mates, all of which we assume would have a low probability of occurrence. Therefore, we consider the risk to be low that domesticated all-female, triploid steelhead stocks cultured in Puget Sound net-pens will affect adversely the genetic structure of Washington's steelhead populations.

Mitigating Provisions

Operations, including future finfish transport permits:

1. Marine net-pen aquaculture must be conducted only where Cooke holds a valid lease on state-owned aquatic lands, issued by the Washington Department of Natural resources.
2. All requirements stipulated by Washington Department of Ecology on NPDES permits must be followed.
3. All activities described in Cooke Aquaculture's Plan of Operation – All-female Triploid Rainbow Trout; Fish Escape Prevention, Response, and Report Plan; and Regulated Finfish Pathogen Report Plan must be followed as written, unless otherwise specified below. All plans must be updated annually and in consultation with WDFW Fish Health and Hatchery programs, with final drafts submitted to WDFW for approval no later than November 30 of the calendar year. The Fish Escape Prevention, Response, and Report Plan must be drafted in consultation with DNR, Ecology, and WDFW.
4. All fish transported into net-pens must contain one or more visual marks, other than the shape of each fish, that unambiguously identifies each fish as commercial aquaculture fish, as opposed to hatchery- or natural-origin free ranging fish of Washington State. For the lot of fish currently being reared in Cooke's freshwater facility, with plans to transport into marine net-pens in fall 2019, that mark can be an adipose fin clip only. However, WDFW considers that commercial aquaculture steelhead marked with adipose fin clip only presents a risk of confusion with the state's hatchery-origin steelhead. Before July 2020 Cooke must present to WDFW an alternate method to visually identify their fish. Before January 2021 Cooke must implement an alternate method, approved by WDFW, to visually identify their fish.
5. For each lot of fish to be transported into marine net-pen facilities, Cooke must provide to WDFW a sample of tissue from 150 fish (or 150 embryos) appropriate for genetic analyses, assuming that the lot is derived from a single brood line. If the lot is composed of more than one brood line, Cooke must provide to WDFW samples of tissue from 150 fish (or 150 embryos) from each brood line. The fish tissue can be from live or lethal sampling. WDFW will genotype samples using their baseline assay of SNP markers and will use the information only to determine if steelhead samples from hatchery- or natural-spawning fish are commercial aquaculture fish or F1 offspring of commercial aquaculture fish.
6. Prior to stocking net pens, Cooke must provide WDFW, DNR, and Ecology the approximate dates for stocking. Within one month after stocking is completed Cooke must provide to WDFW, DNR, and Ecology a report documenting the facility stocked, dates in which stocking occurred, the total number of fish stocked per day, and any complications that may have occurred during stocking. Cooke must report immediately if fish escaped during stocking.
7. Prior to harvest, Cooke must provide WDFW, DNR, and Ecology the approximate dates for harvest. Within one month after harvesting is completed Cooke must provide to WDFW, DNR, and Ecology a report documenting the facility harvested, dates in which harvesting occurred, the total number of fish harvested per day, and any complications that may have occurred during harvesting. Cooke must report immediately if any live fish escaped during harvesting, or if any fish carcass, parts, or offal were discarded into the Puget Sound waters. The discard of carcasses, fish parts, or offal is also a violation of Cooke's NPDES permit.
8. The following monitoring data needs to be reported to WDFW, DNR, and Ecology as part of an expanded Monthly Feed, Biomass, and Disease Control Chemical Use Report, or as separate monthly report(s): (1) the feed conversion rates at each facility, (2) the estimated number of

individuals at each facility, and (3) the number of dead fish collected or observed (the greater of these two numbers) at each facility.

Escape Prevention, Response, and Reporting:

1. As per EHB 2957 and RCW 77.125.060, for each net-pen facility, Cooke must hire, at their own expense, a marine engineering firm approved by WDFW to conduct inspections. Inspections must occur approximately every two years, when net pens are fallow, and must include topside and mooring assessments related to escapement potential, structural integrity, permit compliance, and operations.
2. Cooke must report to WDFW Fish Health Supervisor, Lead Veterinarian, or Aquaculture Coordinator within 24 hours of discovery any fish that has been observed to have escaped from any net-pen facility or during transfer into or out of a net-pen facility, regardless of numbers of fish involved (i.e., the minimum reporting number is one).
3. It is conceivable that an attempt to recover fish after an escape event may negatively affect native Pacific salmonids more than no attempt to recover fish. Cooke is required to work with WDFW, Ecology, and DNR to include a no-recovery option in the 2020 Fish Escape Prevention, Response, and Reporting Plan, to be finalized December 2019. This option should include when, where, and under what conditions a recovery effort should not be attempted. A no-recovery option would be triggered by the state, in consultation with co-managers and federal agencies for the purpose of protecting native Pacific salmonids. A no-recovery option can be triggered by Cooke if the attempted recovery would put the health and safety of its employees at risk.
4. Both the Washington Department of Health and WDFW need to be notified if escaped fish were on medicated feed at the time of their escape or are within the required withdrawal period for the medicated feed used.
5. Before January 1, 2021, Cooke must have engineered mooring and anchoring plans and site-specific engineered drawings stamped by a structural engineer, for each net-pen facility.

Triploidy error rate

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

Finfish Pathogen Reporting and Biosecurity:

1. Net-pen facilities must remain fallow for 42 days after the last fish are harvested and the last containment net is removed for cleaning and repair. This number can be increased per determination of WDFW veterinarian due to disease prevalence just prior to or at the time of harvest.
2. Regulated and Reportable pathogens are found in WAC 220-370 and in The Salmonid Disease Control Policy of the Fisheries Co-Managers of Washington State. These pathogens are:
    a. Regulated Pathogens:
        i. Infectious hematopoietic necrosis virus (IHNV)
        ii. Infectious pancreatic necrosis virus (IPNV)

iii. Infectious salmon anemia virus (ISAV)
iv. *Oncorhynchus masou* virus (OMV)
v. Viral hemorrhagic septicemia virus (VHSV)
vi. *Myxobolus cerebralis* (whirling disease only known in fresh water)

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

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

Literature Cited

Blanchfield, P.J., L.S. Tate, and C.L. Podemski. 2009. Survival and behaviour of Rainbow Trout (*Oncorhynchus mykiss*) released from an experimental aquaculture operation. Canadian Journal of Fisheries and Aquatic Sciences 66(11):1976-1988

Clark, D., K.L. K. Murphy, and A. Windrope. 2017. Cypress Island Atlantic Salmon net pen failure: an investigation and review. Washington Department of Natural Resources, Olympia, WA. 120 p.

Cooke Aquaculture Pacific. 2019. SEPA Checklist with attachments. https://wdfw.wa.gov/licenses/environmental/sepa

Patterson, K. 2010. The fate of farmed Rainbow Trout (*Oncorhynchus mykiss*) released from commercial aquaculture operations in Lake Huron. Thesis, University of Manitoba, Winnipeg, 193 p.

Table 1. Probabilities of selecting one or more diploid individuals following a triploidy procedure on 1,000,000 eggs, using a sample of N individuals and a given true Rate of Diploidy (i.e., true triploidy error rate). Yellow highlighted cells indicate samples sizes used by Cooke Aquaculture (N = 60 and 100)[1] and Cooke Aquaculture's stated triploidy error rate of 0.0017 (triploidy rate = 99.83%), based on data from Troutlodge. Probabilities based on hypergeometric distribution.

N	Rate of Diploidy						
	0.0005	**0.0017**	**0.005**	**0.01**	**0.02**	**0.05**	**0.1**
30	0.01	0.05	0.14	0.26	0.45	0.79	0.96
60	0.03	0.10	0.26	0.45	0.70	0.95	1.00
100	0.05	0.16	0.39	0.63	0.87	0.99	1.00
150	0.07	0.23	0.53	0.78	0.95	1.00	1.00
250	0.12	0.35	0.71	0.92	0.99	1.00	1.00
500	0.22	0.57	0.92	0.99	1.00	1.00	1.00
750	0.31	0.72	0.98	1.00	1.00	1.00	1.00
1000	0.39	0.82	0.99	1.00	1.00	1.00	1.00
1250	0.47	0.88	1.00	1.00	1.00	1.00	1.00
1500	0.53	0.92	1.00	1.00	1.00	1.00	1.00

[1] Data from Attachment A in Cooke Aquaculture's SEPA documents. Original sample sizes ranged 60 – 100, but when samples not run were removed from analyses, sample sizes ranged 43 – 100, with mean sample size across 36 separate triploidy procedures = 82.

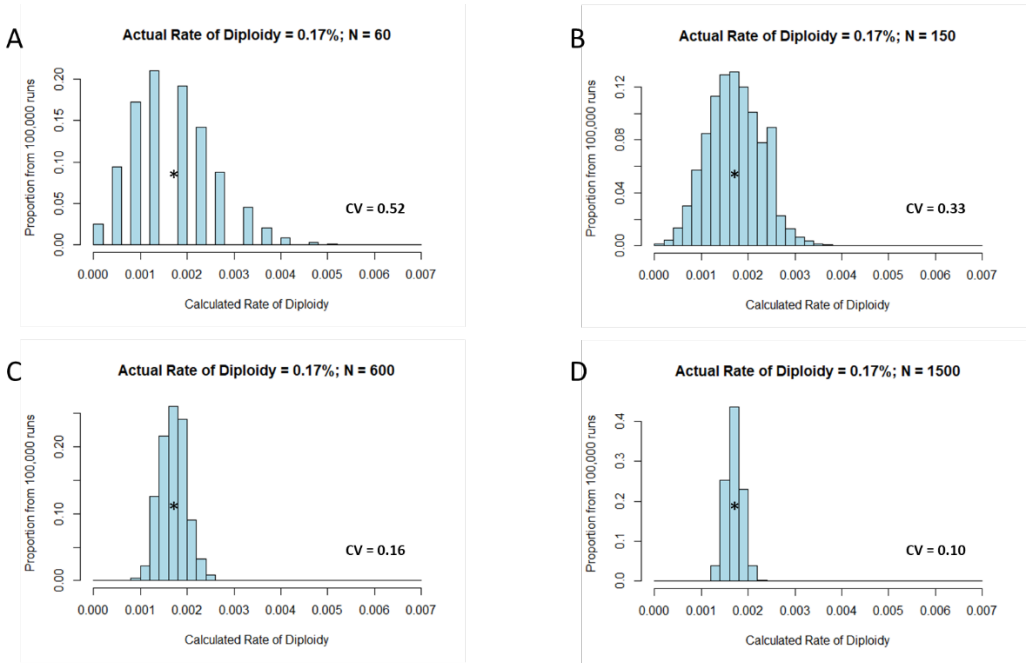

Figure 1. Frequency distributions of calculated rates of diploidy, across 100,000 iterations of the model for each of four samples sizes. Asterisk in each plot shows the frequency distribution category for 0.17%. As sample sizes increase there is an increase in the precision of the estimate for rate of diploidy, providing greater confidence that that estimate is accurate.