Assessment of smolt production from anadromous *O. nerka* transferred into the Alouette Reservoir: Brood years 2008-2012

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Prepared with financial assistance from the BC Hydro Bridge Coastal Fish and Wildlife Restoration Program

Table of Contents

List of Tables

List of Figures

List of Appendix Tables and Figures

SUMMARY

A natural sockeye run to Alouette Lake was extirpated by construction of a hydroelectric dam in the 1920s, but the population has persisted in Alouette Reservoir as lake-resident kokanee (the non-anadromous ecotype of *O. nerka*). These kokanee have recently demonstrated an ability to revert to anadromy. Each spring since 2005, BC Hydro has conducted controlled water spill over the dam as part of an experimental program, and each year juvenile *O. nerka* have been observed migrating downstream (called juvenile downstream migrants, JDM) in numbers up to 63,000 per year. Each summer since 2007, up to 115 adult *O. nerka* (called adult upstream migrants, AUM) have returned to the Alouette River. Since 2007, 230 adult AUM have been transferred back into Alouette Reservoir in the hope that they would spawn, produce more sea-run smolts, and help to restore an anadromous sockeye run.

To determine whether these transferred sea-run adults are successfully spawning, and whether their progeny are more likely to emigrate as sea-run smolts than the progeny of lake-resident kokanee, we analyzed parentage of juveniles caught in the river (JDM) and in the reservoir (after the emigration period henceforth called residents, RES) using 14 microsatellite loci and the computer programs COLONY 1.2, COLONY 2.0, PASOS 1.0, and CERVUS 3.0. To cross-validate the results of our parental analysis, we examined otolith microchemistry to determine the life history (anadromous versus non-anadromous) of the female parent. Our findings to date are as follows:

- None of the juveniles sampled have been perfectly matched to any pair of the AUM transferred back into Alouette Reservoir (53 in 2008, 15 in 2009, 112 in 2010, 8 in 2011, and 42 in 2012). Assuming an equal sex ratio, completely successful spawning, typical egg-to-age-1 survival (60 smolts/female), and emigration of all progeny, we would expect the transferred AUM to have produced 95, 10, 38, 20, and 48 age 1 progeny in 2008, 2009, 2010, 2011, and 2012 respectively. These estimates of JDM would be reduced by half if only half the progeny emigrated as JDM. Even so, the non-emigrating progeny should still be detectable by sampling lake-resident juveniles (as was initiated in 2012).

- In absence of perfect matches, we found a few near matches between: 2012 AUM pairs and a 2014 age-1 JDM and a 2013 age-0 RES; 2010 AUM pairs and a 2013 age-2 JDM and a 2012 age-1 JDM; a 2009 AUM pair and a 2011 age-1 JDM; a 2008 AUM pair and a 2011 age-2 JDM; two 2008 AUM pairs and two 2012 age-3 RES; and four more 2008 AUM pairs and four age-1 2010 JDM. In these near matches, a single allele in the progeny cannot be accounted for by the parents' genotypes (called a "trio mismatch"). This mismatch could plausibly be attributed to genotyping error given the known error rate (3 out of 900[1] for microsatellite DNA assay in sockeye salmon at the PBS Molecular Genetics Lab, R. Withler unpublished data).

- A number of progeny (249 JDM, 91 RES and 1 AUM) have been perfectly matched to single sea-run parents (39 AUM transferred in 2008, 13 in 2009, 42 in 2010, 4 in 2011, and 42 and 2012), suggesting that the transferred sea-run kokanee might have mated with resident kokanee. However, the low genetic diversity in the kokanee population raises the likelihood of false positive matches to single parents (i.e., matches by chance rather than parentage); in contrast, false positive matches to pairs of sea-run kokanee are highly unlikely and have not yet been observed in this study.

- Otolith microchemistry was examined for 18 RES (2013), 21 RES (2012), 5 JDM (2011), and 3 JDM (2010) that matched the genotype of a single AUM in the parentage analysis. In every case from 2010-2013 the female parent was non-anadromous, which confirms that none of the RES specimens was produced by mating between female AUM and male resident kokanee. It seems improbable that all 47 specimens could be progeny of (different) male AUM and female resident kokanee, we are led to conclude that most (if not all) of the matches to single AUM are false positives attributable to the low genetic diversity in the resident population.

- An updated parentage analysis with a new program (COLONY 2.0) that incorporated sex data for AUM in brood year 2012 indicated that the near-matches previously reported (without sex data) should be rejected. One of the pairs comprised two females (not possible), and the other comprised two fish of unknown sex (possible but did not meet the likelihood required). With or without constraints on female polygamy, COLONY 2.0 did not find any parental pairs that resulted in a trio mismatch of < 2, and also rejected 63% (when female are considered

[1] 3/900 genotypes having an error at one locus

polygamous) or 67% (when female are considered monogamous) of the perfect matches to a single parent (pair loci mismatch of zero) previously identified.

- In 2010, 6 of the adult sockeye salmon caught and transported into Alouette Reservoir were identified genetically as strays from Weaver Creek. Any progeny of Weaver Creek sockeye would have been genetically distinctive in our samples; however, none were detected, suggesting that the Weaver adults did not produce progeny.

Given the overall discrepancy between the expected and observed number of matches over four consecutive brood years, it continues to seem unlikely that transferred sea-run adults are spawning together successfully, perhaps they died before spawning, did not find one another, or could not find conditions suitable for spawning. Further efforts seem warranted to determine the reasons for this suspected failure.

INTRODUCTION

Alouette River once had sizeable runs of sockeye salmon that were a major food supply for local aboriginal people in the area (Hirst 1991). The main run in the Alouette River was early (April-May to July), but there were also later runs (Bengeyfield et al. 2001, Koop 2001). Anadromous sockeye salmon used to spawn along the shores of Alouette Lake and adjoining tributaries (Hirst 1991, Koop 2001), including Gold Creek, an inlet tributary to Alouette Lake (Hirst 1991). However, by the 1930s, native anadromous sockeye salmon had disappeared entirely from the Alouette River following construction in the late 1920s of a hydroelectric dam located at the outlet of the lake (Bengeyfield et al. 2001).

Despite this complete and well-documented disappearance, it appears that kokanee (the non-anadromous ecotype of sockeye salmon) persisted in the Alouette Reservoir and are now contributing to the re-establishment of an anadromous sockeye salmon run in the Alouette River. Experimental release of surface water over the Alouette dam from 2005 to 2014 has resulted annually in 728 to 63,000 juvenile downstream migrants (JDM) from the reservoir (Baxter and Bocking 2006, Humble et al. 2006, Mathews and Bocking 2007, 2009, 2010, 2011, and Mathews et al. 2013, 2014, 2015 (in prep.). In 2007, two years after the first release, a total of 28 adult upstream migrants (AUM) returned to Alouette River. Since then, more AUM have returned each year, numbering 54, 45, 115, 11, 45, 6, and 2 in 2008, 2009, 2010, 2011, 2012, 2013, and 2014, respectively (Backle 2008, Cruickshank and Crowston 2011, Greta Borick-Cunningham pers. com.). Genetic markers and stable isotope patterns in otoliths of the AUM have confirmed that the 2005 and 2006 JDM, and the 2007 and 2008 AUM, were the progeny of kokanee that now inhabit the Alouette Reservoir (Godbout et al. 2011). Additionally, the combination of genetic evidence of recent population bottlenecks in the Alouette kokanee population, the lack of secondary sexual characteristics of Alouette male kokanee, and an absence of any historical records of kokanee in the reservoir until 1951 suggests that this population is recently descended from the sockeye runs that were extirpated 20 to 25 generations ago (Godbout et al. 2011).

Although monitoring and evaluation to date has successfully demonstrated that Alouette kokanee can revert to anadromy, it is not yet known whether these re-anadromized AUM (also called 'sea-run kokanee') can successfully reproduce when released in the reservoir or would produce more anadromous progeny (i.e., JDM) than lake-resident kokanee. To address these questions we examined juveniles aged 0 to 4 captured in the Alouette River in the spring of 2013 (JDM) or in the Reservoir after the closure of the gate on the spillway (RES) to determine whether any were progeny of AUM transplanted to the Alouette Reservoir in 2012, 2011, 2010, 2009, and 2008, respectively. We also examined 510 age-1 JDM emigrating in 2014 to determine if they were the progeny of the 2012 AUM. However, as it is not possible to visually identify the JDM as progeny of AUM or kokanee, we employed two complementary techniques to identify their provenance: genetic parentage assignment and otolith microchemistry.

Mendelian inheritance in diploid organisms ensures that parent and progeny share at least one allele for each gene (or locus). Therefore, if enough loci are examined, progeny of the transferred AUM can be identified by matching their (virtually[2]) unique DNA sequence to that of their parents. Genetic samples were obtained from 8 and 42 AUM parents in 2011 and 2012 respectively.

In a complementary approach, the chemistry at the core of an otolith, which is derived from the yolk of the egg, can indicate whether the fish's maternal parent inhabited a marine or freshwater environment, (Kalish 1990). Because removing an otolith requires killing the fish, otolith microchemistry analysis was restricted to fish that died during collection, and that were later identified by genetic parental analysis as potential progeny of an anadromous parent.

[2] In practice, the degree of uniqueness depends on the amount of DNA examined (in this case14 microsatellite loci) and the level of genetic diversity in the population (in this case rather low allelic richness 5.8, Godbout et al. 2011).

Our plan was therefore to perform genetic parental assignment analysis, and when possible, to use otolith microchemistry as a supplementary test to cross-validate the genetic parental analysis. We also revisited the parental analysis of the 2012, 2010, and 2009 AUM using an updated list of potential progeny from samples of migrant (JDM) and non-migrant juveniles (RES) nerkids collected in 2013. Hereafter we use the term "nerkid" (from the species name *Oncorhynchus nerka*) to refer collectively to sockeye, sea-run kokanee or resident kokanee.

Goals and objectives:

The main goal was to assess the reproductive success of AUM that returned to Alouette River and were transferred into Alouette Reservoir from 2011 and 2012. This goal was achieved through:

- ✓ Microsatellite DNA analysis of the tissues sampled from JDM and RES collected in 2013 (as potential progeny of 2011 and 2012 AUM).
- ✓ Microsatellite DNA analysis of the tissues sampled from JDM collected in 2014 (as potential progeny of 2012 AUM).
- ✓ Measurements of the ratio of stable isotopes of strontium in otoliths of 18 nerkids that died during collection and were identified by genetic parental analysis as potential progeny of AUM.
- ✓ Preparation of a technical report summarizing findings.

A secondary goal was to update parental analyses of three previous brood years (2010, 2009 and 2008) by assigning a list of potential progeny updated to include age 2, 3 and 4 (sometimes denoted age 2+, 3+ and 4+) JDM and RES collected in 2013. We also undertook the task of determining the sex of the 2013 returning adults, as it provides a basis for estimating the number of progeny from sea-run kokanee and the likelihood of detecting them in future samples. In addition, knowing the sex of potential parents greatly reduces the probability of mis-assignment in parental analysis (as illustrated by a new parental analysis that incorporates sex data determined from photographs for the 2012 returning adults).

MATERIAL AND METHODS

Study Area

The Alouette Reservoir is located in east Maple Ridge at 49.294°N -122.483°W and is catalogued in the BC watershed atlas at 100-026700-06000.

Alouette Reservoir is oligotrophic, relatively small (6 km^2) but deep (maximum depth of 140 m), and located in the steep terrain of the Coast Mountains 72 km from the ocean (Figure 1). It is a coastal reservoir where the majority of the inflow results from seasonal storms and spring snow melt. Alouette Reservoir is formed by a 21-m dam constructed at the south end of the original lake at the natural outlet into the Alouette River, which drains into the Pitt River before entering the Fraser River (Conlin et al. 2000). The 17-km long reservoir comprises two basins, separated by a narrow section, corresponding to the former two lakes Upper and Lower Lillooet Lakes (Conlin et al. 2000). Much of the outflow is diverted through a 1-km tunnel at the north end of Alouette Lake into Stave Reservoir where power generation occurs. Outflow at the original outlet into the Alouette River is controlled by a low level outlet (underwater release), a crest gate (surface water release) and a free crest weir (surface water release) (BC Hydro 2009). Water can spill over the free crest weir if the water level is greater than 125.5 m, but such spills have been very rare. Based on BC Hydro records from 1984, such a spill occurred only in 1986 (3 days in November) and 1995 (5 days in November and December) (Brent Wilson, BC Hydro, Ruskin Dam & Generating Station. 10600 Wilson Street. Mission, BC, V4S 1B4, unpublished data 02/2011). However, as part of a continuing experiment, surface water has been released at the crest gate in early summer every year since 2005.

BC Hydro Operations in 2013 and 2014

BC Hydro experimentally released water via the crest gate over the spillway during the period of 15 April to 14 June, 2013 and XX April to XX June, 2014. In 2013 flow at the gate ranged from 3.3 to 4.3 $m^3 \bullet s^{-1}$ and averaged 3.7 $m^3 \bullet s^{-1}$ (Brent Wilson BC Hydro, pers. comm.).In 2014 it ranged from XX to XX $m^3 \bullet s^{-1}$ and averaged XX $m^3 \bullet s^{-1}$ (Brent Wilson BC Hydro, pers. comm.). The low level outlet was closed during surface water release.

Fish Collection and DNA samples

Migrant *O. nerka* (JDM)

Emigrating *O. nerka* (juvenile downstream migrants, JDM) were captured by LGL Limited using a rotary screw trap (RST), located in the Alouette River 1.5 km below the dam (Figure 2).

In a mark-recapture experiment up to 150 JDM were selected randomly from the RST catch each day and marked by clipping the caudal fin; marked fish were released upstream, and some fraction were later recaptured downstream by the RST (see Mathews et al. 2015 (in prep.) for the details). An unbiased estimate of the total catchable JDM population size was obtained using a pooled Peterson estimator with a Chapman modification, as in previous years.

Non-migrant *O nerka* (RES)

To properly assess the extent and propensity of the *O. nerka* to emigrate to sea, the field work in the reservoir had to be carried out after the emigration period and after the closure of the gate on the spillway. The resident *O. nerka* were captured in the south basin of the Alouette Reservoir using 8 multi-panel

gillnets (85 m long x 3.6 m deep), with mesh ranging in size from 13 mm to 89 mm (Figure 2). To optimize the capture of kokanee, the gillnets were set in the vicinity of the thermocline.

Aging and Classifying Samples by Brood Year

Migrant *O. nerka*

JDM caught in 2013 and 2014 were assigned an age based on their size by using the rules developed for 2012 JDM that had been aged from their scales (Table 1).

Non-migrant *O. nerka*

Resident (RES) nerkids were aged using fish scale samples. RES caught in the fall of 2013 by LGL staff were aged at the Pacific Biological Station, while RES caught in September and July by MOE were aged at the MOE lab.

It is sometimes difficult to age *O. nerka* from scales, and ageing error seems common (judging from discrepancies between fork length and age estimated from scales). To ensure that no potential progeny were mistakenly excluded from the parental analysis, we assigned fish of uncertain age to more than one brood year during parental analysis; fish whose fork length was in a zone of overlap between age groups were assigned to both age groups. For example, a juvenile collected in 2013, whose fork length fell within the specified interval of overlap for length distributions of age-1 and age-2 fish, was considered both as the potential age-1 progeny of 2011 AUM and as the potential age-2 progeny of 2010 AUM (Table 1).

DNA analysis:

As in previous years (Godbout et al. 2012), tissue samples were analyzed to obtain a multi-locus genotype at 14 microsatellite loci (*Ots2, Ots3, Ots100, Ots103, Ots107, Ots108, Oki1a, Oki1b, Oki6, Oki10, Oki16, Oki29*, *One8*, and *Omy77)* based on procedures described by Beacham et al. (2005).

Sex determination of the 2013 returning adults in Alouette River

Parental analysis is significantly improved when the sex of potential parents is known. Unfortunately, when the adults are returning in the river they are immature and it is not possible to identify their sex based on the usual morphometric characteristics. After confirming the sex for AUM that had died before they could be transplanted in 2010 (n=11), we identified attributes of the vent that could be used for sexing *O. nerka*. These attributes and sexing criteria are described in Appendix 2 (Figure A2.1, A2.2). Sex was determined for 3 of the 4 AUM that were photographed in 2013.

Parental allocation

A number of computer programs have been developed to match individual progeny to the most likely parents based on a list of genotypes of progeny and potential parents. We used three complementary programs: COLONY (version 1.2), PASOS (version 1.0), and CERVUS (version 3.03). In this case (an unsexed open system) we know the genotypes of the AUM, but not those of all the possible kokanee parents of the JDM or RES; moreover, prior to 2011, the AUM genotypes cannot be associated with sex. To perform a parental analysis in an open system, three additional sets of information must be estimated: the number of uncollected parents (those for which no genotype data are available − the resident kokanee in our case), the frequency of genotyping errors, and the allele frequencies in the population(s) (assumed to be in Hardy-Weinberg equilibrium).

COLONY, PASOS and CERVUS can all perform unsexed analyses, and COLONY and PASOS also provide estimates of the number of uncollected parents involved. We assumed a genotyping error of 0.01 for all simulations with CERVUS, which should be large enough to account for all possible sources of error (primarily allelic dropout, but also mutation and lab mistyping) (Beaumont 1999, Koskinen et al.2002[3]). Knowing allele frequencies for both groups of parents (AUM versus kokanee) would help to create realistic parental genotypes in simulations upon which likelihood of parentage is calculated; however, we lack parental kokanee genotypes. As we have no reason to believe that AUM and resident *O. nerka* are reproductively isolated, we have combined all available genotypes of fish originating from Alouette Reservoir to create a representation of the allele frequencies of Alouette nerkids. Samples include JDM (2010, 2011, 2012, 2013, and 2014), resident kokanee (2012 and 2013), and AUM (2007, 2008, 2009, 2010, 2011, 2012, and 2013).

COLONY 2.0 was also used to perform parentage analysis including sex data, which had been determined for most of the 2012 AUM. These results are presented and discussed separately.

Some of the terminology used for statistical results of the parental analysis is potentially confusing and warrants special explanation. For example, in CERVUS, a single parent (A) may be assigned with a particular *confidence level* as the most likely parent of progeny X. The term "most likely" refers to the fact that parent A has a greater likelihood of having produced X than any other parent simulated at random by CERVUS from the specified allele frequency data. The *confidence level* associated with this assignment is the percentage of correct matches made at this level of likelihood in a simulation by CERVUS. This simulation operates as follows: CERVUS generates parental genotypes, hypothetical offspring genotypes, and additional non-parental genotypes using allelic frequency data, the total number of candidate parents, and the proportion of candidate parents sampled versus total candidate parents.

[3] $2.0 \times 10^{-4} - 6.0 \times 10^{-4}$ mutation rates reported in Beaumont and Koskinen; 3×10^{-3} typing error at PBS (R. Withler unpublished data)

CERVUS then introduces to these genotypes random point mutations and typing errors at a rate specified by the user. By running a parental analysis using these simulated genotypes, CERVUS can then calculate the likelihood of individual assignments to the true parent. In our analyses, these simulated confidence intervals should be interpreted with some skepticism because they are based on allele frequencies estimated from samples of AUM and JDM that may not perfectly represent the genetic profile of resident spawners.

RESULTS

Sample Collection

Migrant *O. nerka*

From 16 April 2013 to 23 May 2013, 552 tissue samples and measurements were collected from a total of 6179 JDM (95% confidence interval: 5350-7008) that were estimated to have emigrated downstream (Mathews et al. 2014). Over the course of sampling, 2 JDM were found dead in the RST.

From 15 April 2014 to 27 May 2014, 758 tissue sample and measurements were obtained from the 13,413 JDM (95% confidence interval: 12,423-14,403) estimated to have emigrated downstream.

Non-migrant *O. nerka*

There were 24 gillnet sets done from 7 October 2013-10 October 2013. All RES captured (*n*=168) were numbered, measured (fork length) and weighed, sampled for DNA (a small piece of caudal fin) and scales (for age determination), and then individually frozen. RES thought to be age 2 and older were later dissected to determine sex. The target age class (i.e., age 1 from brood year 2011) was caught in mesh

that was between 13-mm and 89-mm. Most gillnets were set in the vicinity of the thermocline depth of 14m-16m (Table A1.1; Figure A1.1)

We also obtained from S. Harris (MOE) information on 112 RES sampled on 12 July 2013 (44 of which had DNA samples) and from 109 nerkids sampled from 25 and 26 September 2013 (16 of which had DNA) from the north and south basins. These fish were sampled for fork length, weight, maturity sex, tissue samples for DNA analysis, and the heads were preserved in ethanol.

The sex ratio based on all samples for which it was possible to determine sex (*n*=275, DNA sampled or not), was 49% female.

Aging and Classifying Samples by Brood Year

Migrant *O. nerka*

The numbers JDM assigned to brood years 2008-2012 are summarized in Table 2. The size distribution at the age determined by the 2012 rules (Table 1) of the 2013 JDM can be found in Figure 3.

Out of the 758 nerkids caught on 15 April 2014 to 27 May 2014 we were only interested in the potential age 1 based on their fork lengths and the rule developed using scale from the 2012 JDM (FL ≥ 70 and FL ≤100). We randomly selected 510 from a slightly larger range (all fish <120mm FL) so some nerkids were considered potential progeny of the 2012 AUM despite being slightly smaller (n=10) or slightly larger (n=3) than the 2012 cut offs (Figure 4).

In addition, 4 adults returning in 2013 were age 4, and hence were potential progeny of the 2009 AUM (Table 2).

Non-migrant *O. nerka*

Ages were assigned for 268 of 277 scale samples collected from the Alouette Reservoir in September and October 2013 (Table 2), either by DFO (160 aged of 168 collected) or MOE (108 aged of 109 collected). Figure 5 shows the length distribution at age of RES caught in September. Ages were assigned to 108 of the 112 RES caught in July, and to 43 of 44 with DNA samples (Table 2). The size distribution at age of the July RES is in Figure 6.

DNA analysis

Tissues of up to 1299 samples were analyzed for DNA in 2013 and 2014, and genotypes were determined for all except 5 samples.

Genotypes of AUM in 2008 to 2012

All 42 potential parents from 2012 were genotyped at all 14 loci. All 8 parents transferred alive in 2011 were genotyped at all 14 loci (although 4 samples had to be reanalyzed to obtain the full genotypes). Of the 112 AUM transferred into the reservoir in 2010, 103 were genotyped successfully at all 14 loci, 6 at 13 loci, one at 12 loci and 2 at 11 loci. All 15 AUM transferred in 2009 were genotyped at 14 loci. After reanalysis, 52 of the 53 AUM transferred in 2008 were successfully genotyped at all 14 loci; the remaining 2008 AUM could be genotyped at only 12 loci.

Genotypes of Potential Progeny Collected in 2013 and 2014

Genotypes were determined for 547 out of 552 downstream migrants (JDM) captured in 2013; of these, 541 (99%) of these were genotyped successfully at all 14 loci, 5 at 13 loci, and 1 at 12 loci (Figure 7a).

Of the 510 JDM from 2014, 99% were genotyped for all 14 loci (n=508), 0.2% for 13 loci (n=1), 0.2% for 2 loci (n=1) (Figure 8).

Genotypes were determined for all 228 of the resident nerkids (RES) with DNA samples captured in 2013; 224 (98%) of these were genotyped at all 14 loci, 4 (1.7%) at 13 loci, (Figure 7b). One of the 2013 RES (DNA ID 3843) must have been sampled twice as it had a duplicate genotype and was removed from future parental analysis.

In addition, 4 age-4 AUM returning in 2013 (hence the potential progeny of 2009 AUM) were genotyped at 14 loci (Table 3).

When the genotypes of samples collected in 2013 and 2014 are added to data collected in previous years, the total numbers of potential progeny available for parentage analysis are 1183, 1329, 292, 589, and 525 for brood years 2008, 2009, 2010, 2011, and 2012 respectively (Table 4).

Parentage analysis

The number of uncollected parents (UC) estimated using COLONY and PASOS in brood years 2008 and 2009 were similar, estimated to be 807 vs. 712 and 926 vs. 512 parents, respectively (Tables 5 and 6). However, the estimate of UC in brood years 2010 was much higher from PASOS (2010=855) than COLONY (2010=265). In this case, the estimate from PASOS is likely invalid because the allocation rate curve did not stabilize (Figure 9c). In brood year 2012 PASOS had again had a much higher estimate of UC (1178) than COLONY (419), but the allocation curve stabilized so the PASOS estimate was likely accurate (Figure 9e)

2012 Brood year: CERVUS found no parental pair of AUM that could be perfectly matched (trio mismatch of 0 at all 14 loci) to any of the 525 potential progeny. However it did find two potential pairs with a trio mismatch of 1 (Table 7). In addition 40 JDM and 3 RES were matched to a single parent with no mismatches or missing loci (Figure 10).

2011 Brood year: The CERVUS analysis indicated that no parental pair of AUM could be perfectly matched (trio mismatch of 0 at all 14 loci) with a positive logarithm of the odds (LOD) to any of the 589 potential progeny (Table 4)**.** It did find 4 single parents that matched 9 progeny (7 JDM and 2 RES) with no mismatches at any of the 14 loci compared. The most likely matches included JDM offspring ID 13_4448 (95% CL), and RES offspring IDs 13_3813 (70% CL) and 13_3880 (70% CL) (Figure 11).

2010 Brood year: No parental pair of AUM could be perfectly matched (trio mismatch of 0 at all 14 loci) to any of the 279 potential progeny (Table 4). However, three parental pairs were assigned to a migrant nerkid (JDM) with a trio mismatch of 1: AUM ID 2010_ 65 and AUM ID 2010_80 at a confidence level (CL) of 70-80%, AUM ID 2010_07 and 2010_26 at a CL of 50-60%, and AUM ID 2010_12 and AUM ID 2010_35 at a CL of 30-40% (Table 7). Note that one of these assignments involved aan age-2 JDM collected in 2013. We also found 42 AUM assigned as single parents to 64 nerkids (33 JDM and 31 RES), with no mismatches at 14 loci and positive LOD scores (Figure 12). The single parent-progeny matches with highest confidence were to 6 JDM (12_1, 12_20, 12_52, 12_13, 13_4206, 13_4695) and 3 RES (12_893, 13_4695, 13_4833).

2009 Brood Year: Only 15 of the 45 AUM transferred in 2009 were sampled for DNA and could be genotyped for inclusion in this analysis. No parental pair of sea-run kokanee could be matched perfectly (no trio mismatch at any of the 14 loci) to any of the 1212 nerkids. One parental pair (AUM ID 687-AUM ID 694) was assigned to JDM ID 136_988 with 80-90% confidence by allowing 1 trio mismatch (still

using 14 loci) (Tables 4, 7). In addition, 13 AUM were matched perfectly (no mismatch at 14 loci) as a single parent to 70 progeny (52 JDM and 18 RES). Most (50) of the single parent assignations were to age-1 or age-2 JDM (Figure 13).

2008 Brood Year: As with any of the other brood years, none of the 52 AUM could be perfectly matched as a pair (with no trio mismatch using all 14 loci) to any of the 1007 potential progeny. Allowing one trio mismatch at 14 loci revealed seven possible assignments (to 5 JDM and 2 RES) involving different parental pairs and progeny; the best of which was the pair AUM ID 2008_41-AUM ID 2008_6 was assigned to JDM 128_695 with 40-50% confidence (Tables 4, 7). CERVUS also found perfect single-parent matches (no mismatch at 14 loci) between 39 AUM and 160 progeny (121 JDM, 38 RES, and one 2012 AUM) (Figure 14). It is perhaps noteworthy that one of the single parent matches for brood year 2008 involved an adult return in 2012--one of only 15 that returned and that were genotyped at 14 loci.

Proportions of migrants and residents assigned to single parents: To test the hypothesis that the progeny of (transferred) AUM have a greater propensity to migrate downstream than the progeny of lake-resident kokanee, we compared the proportions of JDM and RES assigned to single parents using frequency table analysis (Figure 15, Table 8). Sample sizes were sufficient to compare these proportions only for the 2010 brood year. We found that the proportion of age-1 JDM (39.5%) assigned to a single 2010 AUM parent was not significantly greater than that of age-1 RES (25.3%, chi-square=2.39 prob=0.09). Conversely, the proportion of age-2 JDM (23.7%) assigned to a single 2010 AUM parent was not significantly different than the age-2 RES (31.9%, chi-square=1.36 prob=0.24).

Parental analysis using sexed parents and COLONY 2

2012 Brood year: To improve the power of parental analyses, we previously determined the sex of the AUM collected in 2012 by examining photographs of their vents; 20 were female, 14 were male, and 8

could not be sexed Godbout et al. (2013). We used these sex data in a revised parentage analysis with COLONY 2. The 8 parents of unknown sex were entered as both male and female. Males were considered to be polygamous (McPhee and McQuinn 1998), but females were assumed to be either monogamous or polygamous in separate analyses.

Including these sex data allowed COLONY 2 to reject both parental pairs that CERVUS had previously assigned as possible parental pairs with a trio mismatch of 1 (Table 7). One of these pairs comprised two females (not possible), and the other comprised two fish of unknown sex (possible but did not meet the likelihood required in COLONY 2).

With or without constraints on female polygamy, COLONY 2 did not find any parental pairs that resulted in a trio mismatch of < 2 (Table 9). COLONY 2 also rejected 63% (when female were considered polygamous) and 67% (when female were considered monogamous) of the perfect matches to a single parent (pair loci mismatch of zero) previously identified by CERVUS (Tables 10).

The reduced number of possible matches reported by COLONY 2 is also a consequence of the fact that COLONY 2 considers parentage for all offspring jointly whereas CERVUS considers parentage for each offspring in isolation of other offspring. Therefore, CERVUS may assign the same parent pair to offspring A, B and C separately, without confirming that the genotypes of A, B and C are compatible with full sibship (for example, they may have 5 or more alleles at a locus). COLONY 2 will not generate such incompatible inferences.

Otolith microchemistry

Otoliths from the 18 best matches to single parents for which we had otoliths (highest confidence) were subjected to chemical analysis (Table 11). In all cases, the isotopic signature of strontium ($^{87}Sr/^{86}Sr$) in

both the core and the outer edge of the otolith of the nerkids was typical of a freshwater environment, not a marine environment (Figure 16). We conclude that these nerkids were not the progeny of a female AUM. However, we cannot rule out the possibility that some or all could have been progeny of male AUM that mated with female resident kokanee.

Sex determination of the 2013 AUM

Of the 4 AUM sexed in 2013, 3 were determined to be female and 1 to be male by inspection of their vents (Figure 17).

DISCUSSION

Have transferred sea-run kokanee produced smolts?

We have analyzed DNA samples from juvenile downstream migrants (JDM, 2010-2014) and lake-resident juveniles (RES, 2012-2013) in an attempt to identify progeny of the sea-run kokanee transferred into Alouette Lake in 2008-2012. Our findings to date are as follows:

- None of the juveniles sampled (in the river or lake) can be perfectly matched genetically to *pairs* of adult upstream migrants (AUM) transferred in 2008 (53 AUM), 2009 (15 AUM), 2010 (106 Alouette AUM and 6 Weaver sockeye salmon), 2011 (8 AUM), or 2012 (42 AUM). If the sex ratio was 0.5[4], all sea-run parents survived to spawn, egg-to-age-1 survival had been typical of anadromous sockeye (60 smolts/female), and all progeny had emigrated, then the expected number of detections of progeny (sampled as JDM) would have been 142, 81, 38, 20, and 48 in 2008, 2009, 2010, 2011, and 2012, respectively. These estimates would be reduced by half if

[4] 0.5 seems a conservative estimate as 6 out of the 11 dead 2010 AUM were females and at least 58% of the 2012 AUM were females based on photos of the vent.

only half the progeny emigrated as JDM, but the non-emigrating progeny should still be detectable by sampling lake-resident juveniles (initiated in 2012).

- We did find close (but not perfect) matches between: One JDM and one RES from 2014 to parent pairs from 2012; an age 2 JDM collected in 2013 and an AUM pair in 2010; an age-1 JDM in 2012 and an AUM pair in 2010; an age-1 JDM in 2011 and an AUM pair in 2009; an age-2 JDM in 2011 and an AUM pair in 2008; and an age-3 RES in 2012 with a different AUM pair in 2008. In these cases, the close matches each had only a single trio mismatch which might be interpreted as perfect matches subject to sampling error, a plausible hypothesis given the known (low) genotyping error rate for assays of microsatellite DNA in sockeye salmon (3 out of 900 at the PBS Molecular Genetics Lab, R. Withler unpublished data).

- On the other hand, we did find perfect matches between some juveniles (both downstream migrants and non-migrants) and *single* sea-run parents, which suggests that sea-run kokanee might have mated with resident kokanee. However, the low genetic diversity in the kokanee population also raises the likelihood of a false positive match with a single sea-run parent when the true parent is a resident kokanee with similar DNA sequences at the loci examined. (Note that the odds of a false positive match to a *pair* of sea-run parents are much lower, and such a perfect match has not yet been observed.) Analysis of otolith microchemistry in18 RES in 2013, 21 RES in 2012, 5 JDM in 2011, and 3 JDM in 2010 specimens that perfectly matched the genotype of a single AUM in the parentage analysis indicated that in every case, the female parent was non-anadromous, which confirms that none of the RES specimens was produced by mating between female AUM and male resident kokanee. Because it seems improbable that all 47 specimens could be progeny of mating between (different) male AUM and female resident kokanee, we are led to conclude that most (if not all) of the matches to single AUM are false positives attributable to the low genetic diversity in the resident population.

- An updated parentage analysis with a new program (COLONY 2.0) that incorporated sex data for AUM in brood year 2012 indicated that the near-matches previously reported (without sex data) should be rejected. One of the pairs comprised two females (not possible), and the other comprised two fish of unknown sex (possible but did not meet the likelihood required). With or without constraints on female polygamy, COLONY 2 did not find any parental pairs that resulted in a trio mismatch of < 2, and also rejected 63% (when female were considered

polygamous) and 67% (when female were considered monogamous) of the perfect matches to a single parent (pair loci mismatch of zero) previously identified.

- In 2010, 6 of the adult sockeye salmon caught in the trap at Alouette River and transported into Alouette reservoir were later identified (genetically) as strays from Weaver Creek. Any progeny of Weaver Creek sockeye would have been genetically distinctive in our samples, but none were detected, which suggests that the Weaver adults did not produce progeny.

It is interesting to note that the proportion of 2010 single parent matches tended to be greater for age 1 JDM (39% vs 25%) .than age 1 RES (although not significant) while the proportion of age 1 JDM assigned to a sea-run kokanee as a single parent was positively correlated with the number of AUM (r-pearson=0.97, P > F 0.0058) (1.5% with 8 AUM (2011), 6% with 15 AUM (2009), 8% with 42 AUM (2012), 15.1% with 52 AUM (2008), and 39% with 103 AUM (2010). Both relationships would be predicted by our hypothesis that the progeny of (transferred) AUM have a greater propensity to migrate downstream than the progeny of lake-resident kokanee, (provided total fry recruitment to the reservoir from both sea-run kokanee and resident kokanee is constant, or if progeny of lake-resident kokanee are overwhelmingly abundant and have a much lower propensity to emigrate). However, these relationships must be spurious if the single parent matches are actually false positives, as suggested by the otolith chemistry results.

The proportion of the JDM that were assigned an 2010 AUM as a single parent was higher than that of the RES, but the difference was not statistically significant. So we are not yet rejecting the null hypothesis of no difference in the assignment of an AUM to JDM and to RES.

Given the overall discrepancy between the expected and observed number of matches over four consecutive brood years, it seems unlikely that many (if any) of the transferred sea-run adults are spawning successfully together, perhaps because they died before spawning, did not find one another, or could not find conditions suitable for spawning. Thus, further efforts seem warranted to determine the reasons for this suspected failure.

To date, factors most limiting our ability to identify progeny are:

- The number of transferred sea-run kokanee is low relative to the number of resident kokanee. This issue is exacerbated when sea-run parents are not sampled for DNA (For example, in 2009 only 15 out of the 45 sea-run kokanee were sampled for DNA).

- The genotypes of the kokanee spawners are unknown.
- Sex of potential parents transferred in 2008-2011 remains unknown. This uncertainty greatly reduces the statistical power of the parental analysis, making false positives more likely in those brood years.

Ways to improve future studies

There are a few options to improve future assessments of spawning success in transferred sea-run kokanee, and the propensity of their progeny to emigrate to sea. First, we have now determined sex for 34 of the 42 AUM in 2012, which should greatly improve the statistical power of future parental analyses involving this brood year. More accurate sex determination could be achieved using an ultrasound technique (Frost et al. 2014).

Second, to overcome the difficulties describe above, we are proposing a hatchery-based experiment to simultaneously advance the objective of restoring an anadromous sockeye run by increasing the number of sea-run smolts (JDM), while further investigating the suspected poor spawning success of the sea-run adults (AUM) transferred back into the reservoir. We suggest that instead of transferring all sea-run adults back into Alouette Reservoir, approximately 10 returning sea-run kokanee females be spawned in a hatchery to produce 25,000-30,000 fry. These would be marked (by clipping the adipose fin) and released into Alouette Reservoir the following year once the spring emigration period and the water release is over. Total juvenile abundance in the reservoir would be estimated by age class in collaboration with MOE. Based on recent estimates of the resident kokanee population, 25,000 hatchery-reared fry would constitute about 20% of the total nerkid fry population in the lake (based on the average number of fry, Harris et al. pers. comm.), and should be readily detectable the following year, either as downstream migrants or as resident juveniles.

Hatchery-reared juveniles (recognized both genetically and by external adipose fin clips) and unmarked juveniles from natural spawning of sea-run adults (recognized genetically) would be enumerated in samples of downstream migrants (JDM) in spring and non-migrants (RES) in summer. Fry-to-JDM and

fry-to RES survival rates could be calculated if, as proposed, known numbers of hatchery-reared fry were planted into the reservoir. Detection of expected numbers of hatchery-reared JDM would confirm that sea-run kokanee can produce viable progeny, and that these progeny can survive within the reservoir to become smolts. The ratio of detections in the samples of JDM and RES would provide a direct estimate of the emigration rate for progeny of sea-run kokanee. Continued failure to detect progeny of sea-run kokanee from natural spawning, despite confirmed survival of hatchery-reared progeny, would indicate that natural spawning of transferred adults is unsuccessful, and currently an obstacle to restoring anadromy. The experiment could also confirm (or refute) the effectiveness of the genetic methods for detecting progeny used to date, and provide a means to assess egg viability and egg size of the fully-mature sea-run kokanee, which has been difficult to do in the field. Perhaps most importantly, the experiment would be expected to increase smolt production from returning adults, consistent with the primary objective of the program.

If natural spawning of transferred adults has been unsuccessful, then continuing to transfer sea-run adults may simply squander opportunities to resolve barriers to recovery. If the propensity for anadromous migration is heritable (as expected), then genes for anadromous migration will be disproportionately carried by JDM, and total mortality of JDM (when transferred sea-run adults fail to spawn) represents selection *against anadromy* in the lake-resident kokanee population. Selection against anadromy has likely not yet occurred (at least via this mechanism) on the kokanee populations in the Alouette and Coquitlam reservoirs because juveniles trapped behind the dam have been unable to escape the reservoir in most years (i.e., no spill since dam construction until 2005). For this reason, these kokanee populations may be unusual in that they likely still retain much of their original potential to revert to anadromy.

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ACKNOWLEDGEMENTS

The cooperation of many people was essential in meeting the objectives of this study. We are very grateful to LGL Limited, particularly to Elmar Plate for sampling *O. nerka* in the reservoir, to Megan Mathews and Jason Smith (LGL Limited) for assistance with sampling in the river, George and Roxanne Moody from the Katzie First Nation for their daily operations of the traps and data collection. Thanks also goes to James Bruce of Creekside Aquatic Sciences, for his help with the sampling in the reservoir. Huge thanks are due to Shannon Harris (BC Ministry of the Environment) for her tremendous assistance in filling in missing information and for contributing samples that were integral to this study. Special thanks go to Brent Wilson (BC Hydro), Geoff Clayton and Greta Greta Borick-Cunningham of the Alouette River Management Society (ARMS) for providing information. Thanks are also due to Darlene Gillespie and Judy McArthur for help on ageing, ALLCO hatchery staff for monitoring and sampling the returning adults, and Ted Sweeten for his help with sex determination. Funding for this project was provided by BC Hydro Bridge Coastal Fish and Wildlife Restoration Program and in-kind contributions from DFO.

	Fork Length Range (mm)		
Age Class	JDM	RES (July)	RES (September)
Age 0	<70		<85
Age 1	70-95	<119	85-155
Age 1-2	95-100	119-131	156-198
Age 2	101-184		199-226
Age 2-3		179-217	227-240
Age 3	>184	>217	241-267
Age 3-4			268-289

Table 1. Size ranges for assigning age to juvenile downstream migrants (JDM) and non-migrants (RES). Ages 1-2, 2-3, and 3-4 are assigned for fork length (FL) intervals that overlap between consecutive age classes, such that assigned ages are uncertain. Size ranges are based on FL distributions of fish aged from scales that were collected in 2012 (JDM) and 2013 (RES).

Brood year	Age class	JDM 0	JDM 1	JDM 1-2	JDM 2	JDM 3	AUM 4	Total
2012	0	1	511					512
2011	1		446	20				466
2010	2			20	79			99
2009	3					6	4	10
2008	4							

Brood year	Age class	RES 0	RES 1	RES 1-2	RES 2	RES 2-3	RES 3	RES 3-4	Total
2012	0	15							15
2011	1		100	24					124
2010	2			24	22	24			70
2009	3					24	34	8	66
2008	4							8	8

Table 2. Sample sizes from 2013 and 2014 analyzed as potential progeny of AUM in brood years 2012, 2011, 2010, 2009 and 2008 by age class. Upper panel includes migrants (JDM) caught in 2013 and 2014, and one AUM (potential progeny) returning in 2013; lower panel includes non-migrants (RES) caught in 2013. Assigned ages 1-2, 2-3, and 3-4 indicate that the potential progeny were analyzed by assuming they belonged to both age classes. Note that numbers in this table include all aged JDM for which microsatellite DNA data are available.

		JDM		RES		AUM	
Brood year	Age	All	No. at 14 loci	All	No. at 14 loci	All	No. at 14 loci
2008	1	884	739	0	0		
2008	12	2	2	0	0		
2008	2	12	12	0	0		
2008	23	0	0	120	107		
2008	3	0	0	138	124		
2008	34	0	0	8	8		
2008	4	0	0	0	0	19	15
2009	1	892	823	0	0		
2009	12	7	7	20	17		
2009	2	42	38	172	147		
2009	23	0	0	144	130		
2009	3	6	5	34	33		
2009	34	0	0	8	8		
2009	4	0	0	0	0	4	4
2010	1	33	33	65	58		
2010	12	25	25	44	41		
2010	2	79	77	22	22		
2010	23	0	0	24	23		
2011	0	0	0	3	3		
2011	1	442	439	100	98		
2011	12	20	20	24	24		
2012	0	0	0	15	15		
2012	1	510	508	0	0		

Table 3. Total numbers of *O. nerka* analyzed as potential progeny in parental analyses by brood year (2008-2012) and type (JDM, RES and AUM refer to juvenile downstream migrants, non-migrants, and returning adults, respectively). Number at 14 loci denotes the number with complete genetic data (i.e., no missing values at all 14 loci).

Brood year	AUM	Progeny	MM	Number of Parents allocated			Progeny allocated to single parents			Progeny allocated to parental pair		
				JDM	RES	AUM	JDM	RES	AUM	JDM	RES	AUM
2012	42 (42)	525 (523)	0	21	4	0	40	3	0	0	0	0
			1	40	8	0				1	1	0
2011	8(8)	589 (581)	0	3	2	0	7	2	0	0	0	0
			1	0	0	0				0	0	0
2010	111(103)	292 (279)	0	28	28	0	33	31	0	0	0	0
			1	8	2	0				3	1	0
2009	15(15)	1329 (1212)	0	11	7	0	52	17	0	0	0	0
			1	2	0	0				1	0	0
2008	53(52)	1183 (1007)	0	39	23	1	117	38	1	0	0	0
			1	9	4	0				5	2	0

Table 4. Results of parental analyses with CERVUS by brood year. Columns are brood year, number of adult upstream migrants (AUM) and potential progeny (with numbers genotyped at all 14 loci shown in parentheses), number of mismatches allowed (MM), number of adult upstream migrants (AUM) assigned as parents to downstream migrants (JDM) and resident (RES) nerkids, and the number of JDM and RES assigned to single or paired parents.

AUM year	Half sib	Full sib	Total Number of Parents
2008	157	650	807
2009	164	762	926
2010	52	213	265
2011	86	365	451
2012	81	338	419

Table 5. Estimated number of half-sib and full-sib families in the returning adults (AUM) for each brood year based on the parental analysis with COLONY 1.

Brood Year	Number of				Number of AUM allocated			To single parent			To parental pair			Correct-ness
	AUM	UC	PP	MOT	JDM	RES	AUM	JDM	RES	AUM	JDM	RES	AUM	
2012	42	1178	523	0	19	3	0	33	3	0	0	0	0	0.45
2011	8	1030	584	0	3	2	0	7	2	0	0	0	0	0.49
2010	103	855	279	0	19	23	0	27	23	0	2	3	0	0.87
2009	15	512	1212	0	11	6	0	49	16	0	2	0	0	0.57
2008	52	712	1007	0	36	17	1	97	27	1	4	2	0	0.49

Table 6. Results of parental analyses with PASOS by brood year. MOT refers to the maximum allowable number of offsets between a parental and an offspring allele; only the results for MOT=0 are shown. Columns show brood year, number of adult upstream migrants (AUM), estimated number of uncollected parents (UC), number of potential progeny (PP which includes both juvenile downstream migrants, JDM and residents, RES), MOT, number of AUM allocated to JDM and RES offspring, the number of JDM and RES assigned to single or paired parents, the correctness level of the allocations, and the total number of allocations made.

Progeny ID	Age	Type	AUM 1	Pair Loci MM 1	CL 1	AUM 2	Pair Loci MM 2	CL 2	Trio Loci MM	Trio CL
A) 2012										
3881	0	RES	2119	1	0.2	2132	1	0.3	1	0.216
8104	1	JDM	2130	0	0.5	2145	1	0.2	1	<0.1
B) 2010										
13_4076	2	JDM	2010_07	0	0.8-0.9	2010_26	0	0.8-0.9	1	0.5-0.6
12_1	1	JDM	2010_65*	0	0.6-0.7	2010_80	0	0.95-0.99	1	0.7-0.8
12_36	1	JDM	2010_12	0	0.7-0.8	2010_35	0	0.5-0.6	1	0.3-0.4
C) 2009										
136_988	1	JDM	2009_687	0	0.99	2009_694	1	0.5-0.6	1	0.8-0.9
D) 2008										
128_695	2	JDM	2008_41	0	0.5-0.6	2008_6	0	0.9-0.95	1	0.4-0.5
405	1	JDM	2008_46	1	0.1-0.2	2008_53	0	0.7-0.8	1	0.1-0.2
432	1	JDM	2008_14	1	0.1-0.2	2008_31	1	0.2-0.3	1	0.1-0.2
897	1	JDM	2008_10	1	0.5-0.6	2008_46	1	0.1-0.2	1	0.2-0.3
1144	1	JDM	2008_28	1	0.1-0.2	2008_7	0	0.8-0.9	1	0.2-0.3
12_379	23	RES	2008_2	1	0.4-0.5	2008_53	0	0.5-0.6	1	0.2-0.3
12_882	23	RES	2008_37	1	0.1-0.2	2008_4	1	0.5-0.6	1	0.2-0.3

Table 7. Confidence levels for assignments of parental pairs in CERVUS that had a trio mismatch of 1 or 2 based on 14 loci (or 13 loci if marked by asterisk). Columns are ID, age, and type of progeny, parent ID (AUM1, AUM2), pair loci mismatch (Pair Loci MM 1 and 2) and confidence level (CL1, CL2) for each parent, and trio loci mismatch (Trio Loci MM) and confidence level (Trio CL) for the parental pair assignment.

		Total number of progeny			Number of progeny assigned to an AUM		
Brood Year (N)	Age	JDM	RES	AUM	JDM	RES	AUM
2008 (52)	1	739	-	-	114 (15.26%)	-	-
	2	14	-	-	3 (21.4%)	-	-
	3	-	231	-	-	38 (16.45%)	-
	4	-	8	15	-	1 (12.50%)	1 (6.67%)
2009 (15)	1	825	-	-	49 (5.94%)	-	-
	2	43	271	-	4 (9.30%)	14 (5.17%)	-
	3	5	64	-	-	10 (15.63%)	-
	4	-	-	4	-	-	-
2010 (103)	1	38	75	-	15 (39.47%)	19 (25.33%)	-
	2	97	69	-	23 (23.71%)	22 (31.89%)	-
2011 (8)	0	-	3	-	-	-	-
	1	459	122	-	7 (1.53%)	2 (1.64%)	-
2012 (42)	0	-	15	-	-	3 (20.00%)	-
	1	510	-	-	40 (7.84%)	-	-

Table 8. Numbers by age and brood year of juvenile downstream migrants (JDM), residents (RES) and returning adults (AUM) assigned with no mismatch to a single sea-run parent (AUM) in parental analyses with complete genotype data at all 14 loci. Columns are brood year (with total number of AUM shown in parentheses), age of the potential progeny, number of JDM, RES, and returning AUM, and the numbers (and percentages in parentheses) of JDM, RES and AUM assigned to a single AUM parent.

Offspring ID	Inferred father	Inferred mother	Female	CL father	CL mother	trio CL	Trio MM
7829	2141	2116	P-M	0.5	0.3	0.1	2
8157	2144	2146	M	0.1	0.3	<.01	4
8549	2134	2122	M	0.3	<.01	<.01	3

Table 9. Best assignments of potential progeny to parental pairs of sea-run kokanee (AUM) in sexed-parent analysis with COLONY 2 assuming males are polygamous, and females are either strictly monogamous (M) or can be polygamous (P). P-M means the match was found with both monogamous and polygamous females. Confidence level of the father and mother, trio confidence level (trio CL), and trio mismatch (Trio MM) from CERVUS (shaded portion of the table).

Monogamous female		Polygamous female		
Offspring ID	Parent ID	Offspring ID	Parent ID	Parent sex
		3863	2120	F
		3945	2115	F
		7808	2147	F
		7809	2115	F
		7826	2126	F
7829	2141	7829	2141	M
		7832	2115	F
7867	2157			M
		7894	2131	F
7909	2153			F
7917	2157	7917	2157	M
7925	2125			F
8022	2141			M
8028	2141			M
8066	2139			F
8076	2136	8076	2136	F
8106	2145	8106	2145	F
8125	2147			F
		8186	2153	F
		8215	2119	F
8186	2153			F
8363	2155			M
8406	2119			F
		8363	2155	M
		8452	2115	F
		8533	2153	F

Table 10. Assignment of age-1 juvenile migrants (JDM) collected in 2014 and age-0 residents (RES) collected in 2013 to adult sea-run kokanee (AUM) that returned in 2012. Possible matches from parental analysis with COLONY 2 are reported only if there was no mismatch at any of the 14 loci. Parental sex data are included when available (M is male, F is female), and two scenarios are considered for females (strict monogamy versus possible polygamy).

Vial No.	Source	Fish ID	CL	FL	Origin	Year Caught	Age	AUM Year
13_3813	LGL	13	0.7	133	RES	2013	1	2011
13_3819	LGL	19	0.4	239	RES	2013	2-3	2010
13_3847	LGL	47	0.7	186	RES	2013	1-2	2010
13_3865	LGL	65	0.4/0.4	240	RES	2013	2-3	2009/2010
13_3866	LGL	66	0.8	208	RES	2013	2	2010
13_3870	LGL	70	0.5	218	RES	2013	2	2010
13_3880	LGL	80	0.8	127	RES	2013	1	2011
13_3898	LGL	98	0.4	266	RES	2013	3	2009
13_3903	LGL	103	0.7	288	RES	2013	3-4	2009
13_3906	LGL	106	0.6	205	RES	2013	2	2010
13_3911	LGL	111	0.4	255	RES	2013	3	2009
13_3920	LGL	120	0.2	285	RES	2013	3-4	2008
13_3963	LGL	163	0.7	240	RES	2013	2-3	2010
13_4802	MOE	549	0.6	210	RES	2013	2	2010
13_4829	MOE	13	0.4	200	RES	2013	2-3	2010
13_4833	MOE	17	0.99	168	RES	2013	2	2010
13_4851	MOE	62	0.7	185	RES	2013	2-3	2010
13_4854	MOE	66	0.7	185	RES	2013	2-3	2010

Table 11. Otoliths selected for otolith microchemistry in 2013. Columns are otolith vial number (Vial No.), source of the sample (source), DFO Fish ID, (Fish ID), confidence level (CL) of the juvenile assignment to a single AUM parent, fork length (FL) fish), origin of the juvenile (RES indicates a fish was resident in Alouette Reservoir), and the year the fish was caught (Year caught) followed by its age (Age) and the year the potential parent spawned (AUM year).

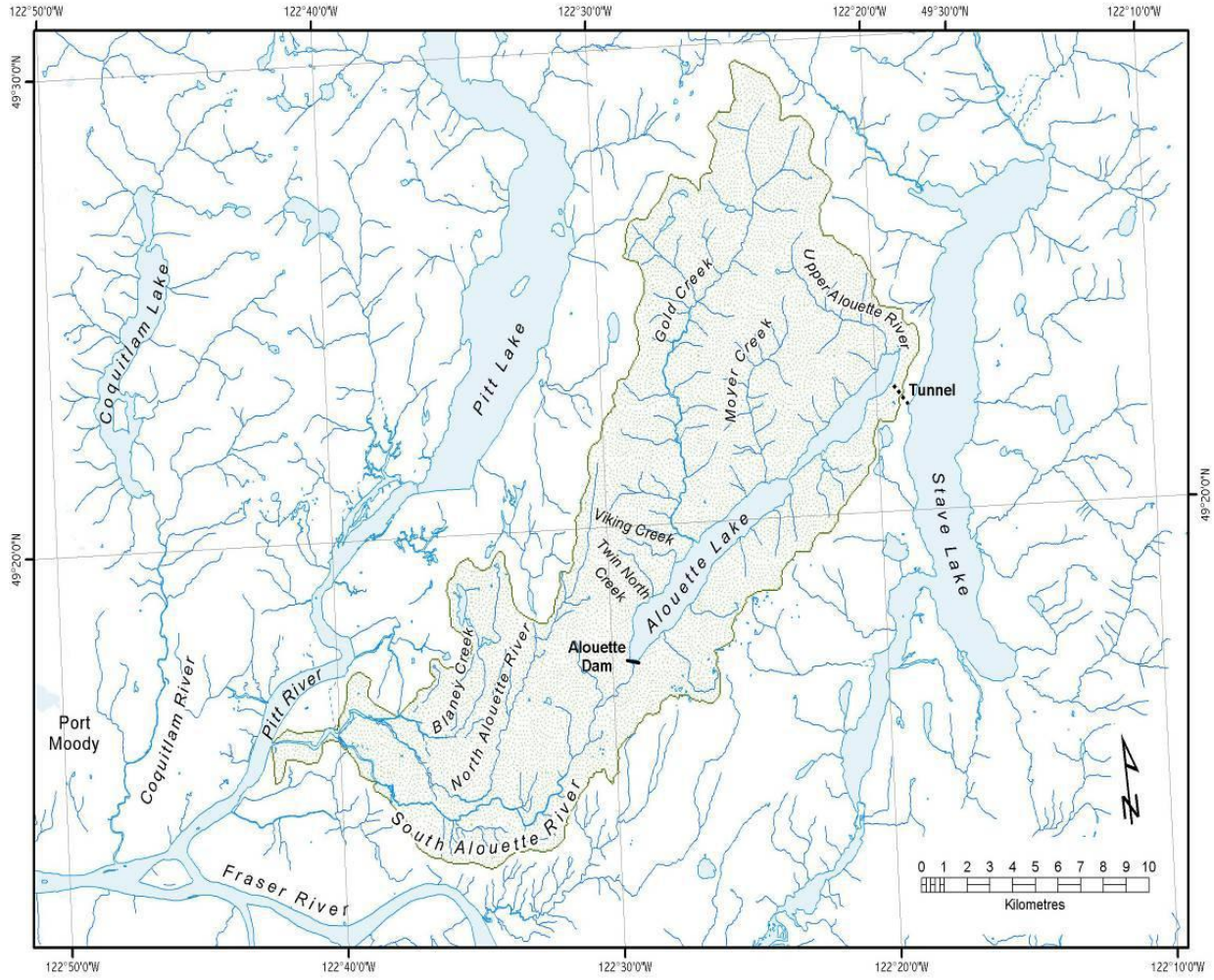


Figure 1. Overview map from the lower mainland of British Columbia showing the location of Alouette Reservoir.

Figure 2. Map of Alouette Reservoir showing sampling locations in the southern basin (1 to 3) and in the northern basin (Stations 4 and 5), DFO gillnetting locations (sets 1-24), and the location of the rotary screw trap (RST) in the Alouette River. (Map is from S. Harris et al. 2010).

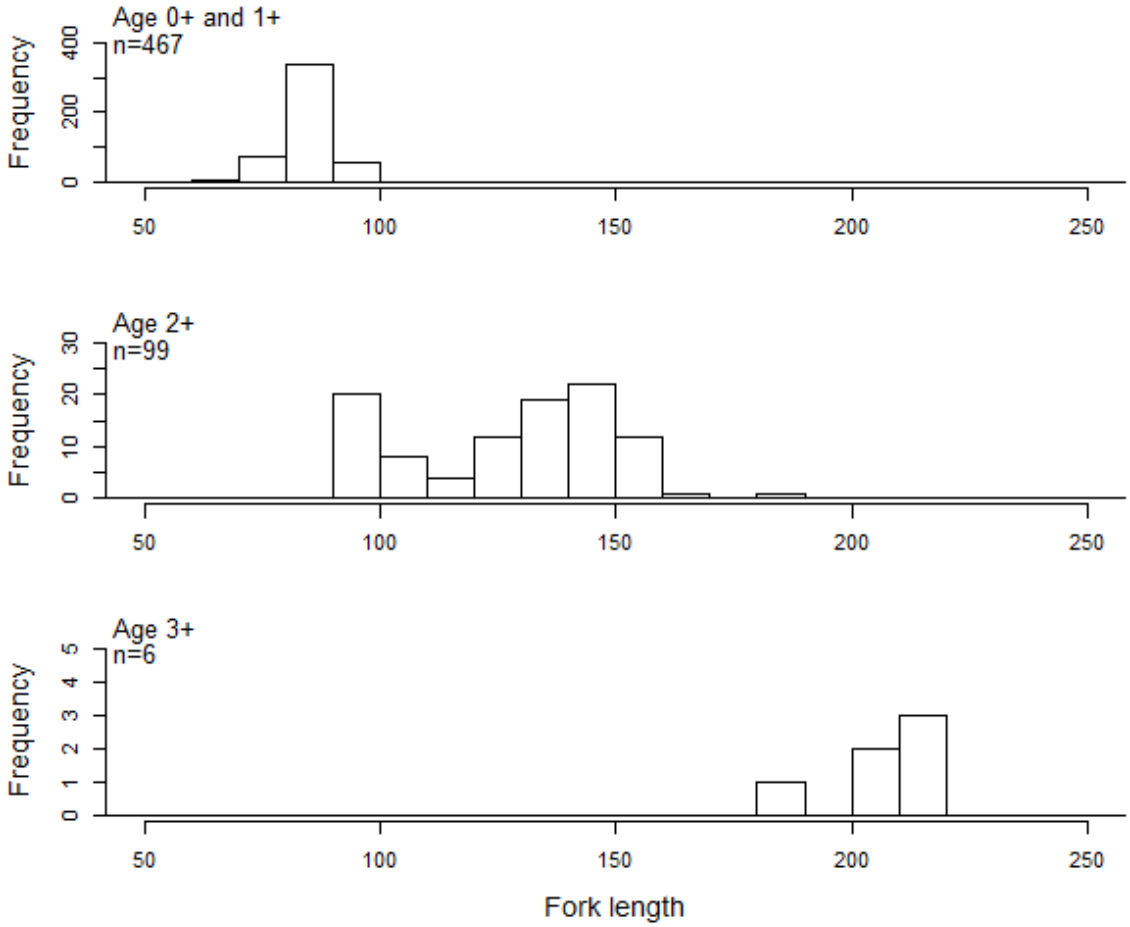


Figure 3. Fork length distributions (mm) of JDM *O.nerka* caught in 2013 by age class where age was assigned based on length cut offs estimated for JDM caught and aged from scales in 2012. N=552. Note that 20 fish in the transitional age 1-2 category are shown in both the age 1^+ and 2^+ panels (Table 2a). Note that in this and subsequent figures, the + after an age designation indicates that the fish were captured mid-way through the year designated.

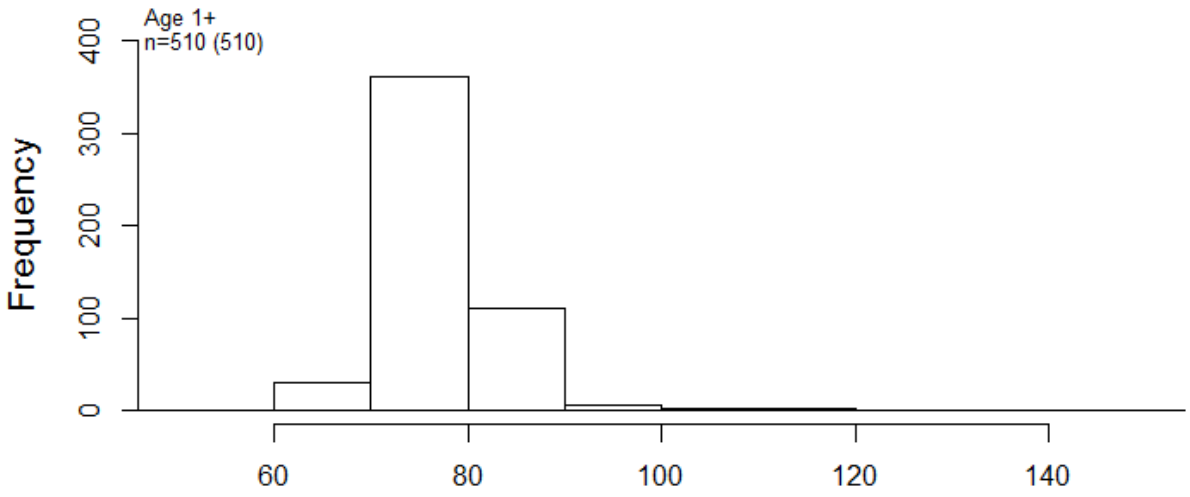


Figure 4. Fork length distributions (mm) of age 1 JDM *O.nerka* caught in 2014. Age was assigned based on length cut offs estimated for JDM caught and aged from scales in 2012. N=510.

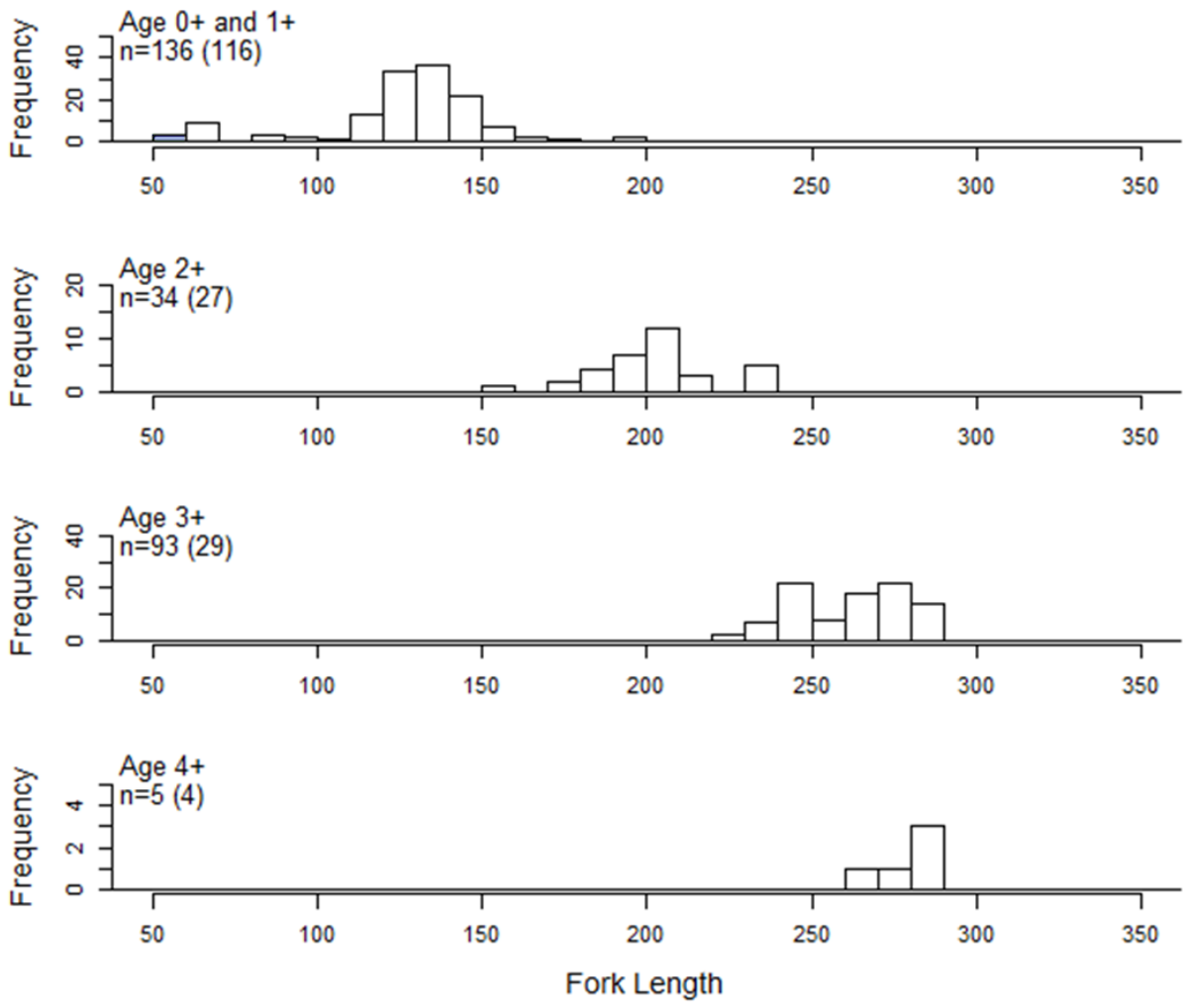


Figure 5. Fork length distributions (mm) by age class of RES *O.nerka* caught in September-October 2013. Sample sizes refer to all fish aged from scales by DFO or MOE (total =268), and in parentheses, the subset also sampled for DNA tissues. Note that the length distributions of age 0 (n=12, shade bar) and age 1 residents do not overlap.

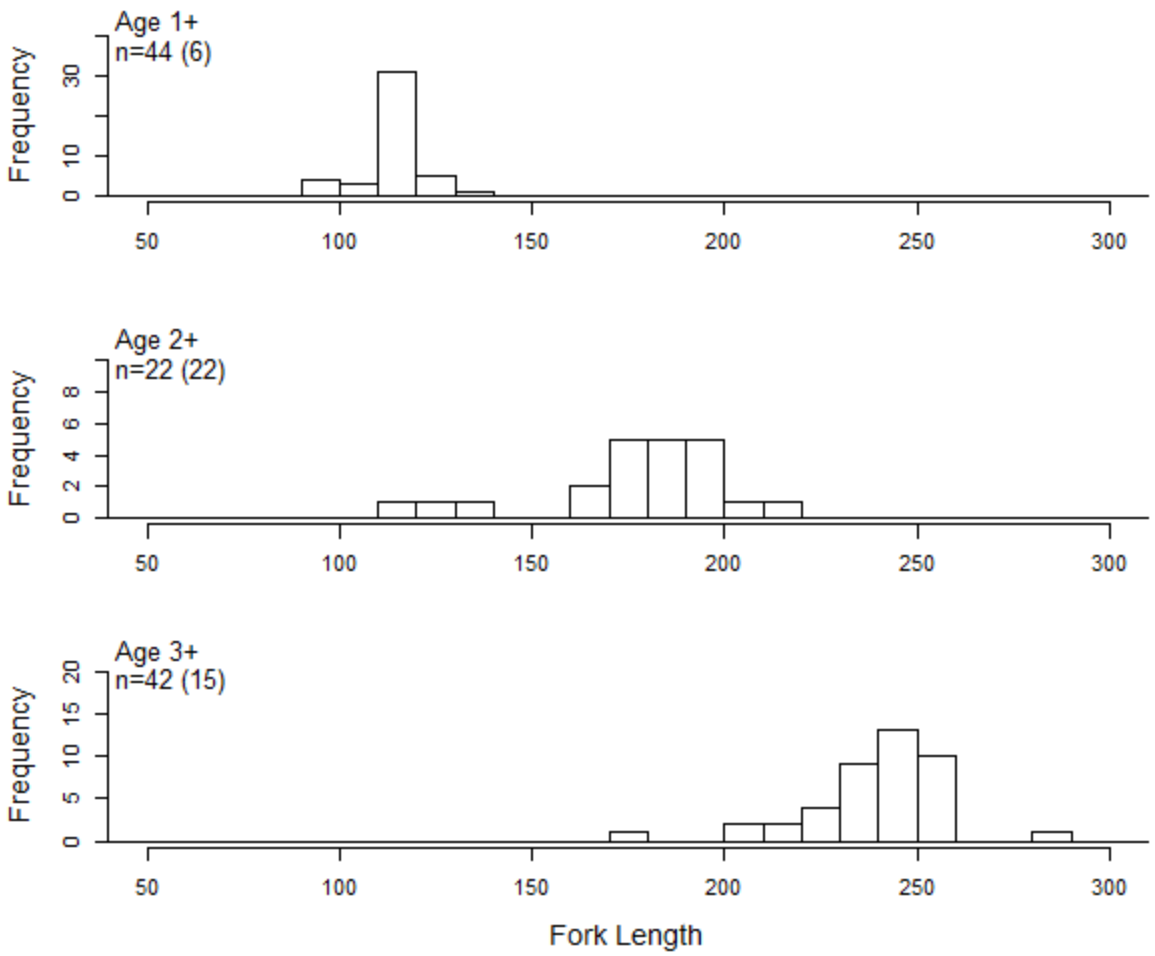


Figure 6. Fork length distributions (mm) by age class of RES *O.nerka* caught in July 2013. Sample sizes refer to all fish aged from scales by MOE (total =108), and in parentheses, the subset also sampled for DNA tissues.

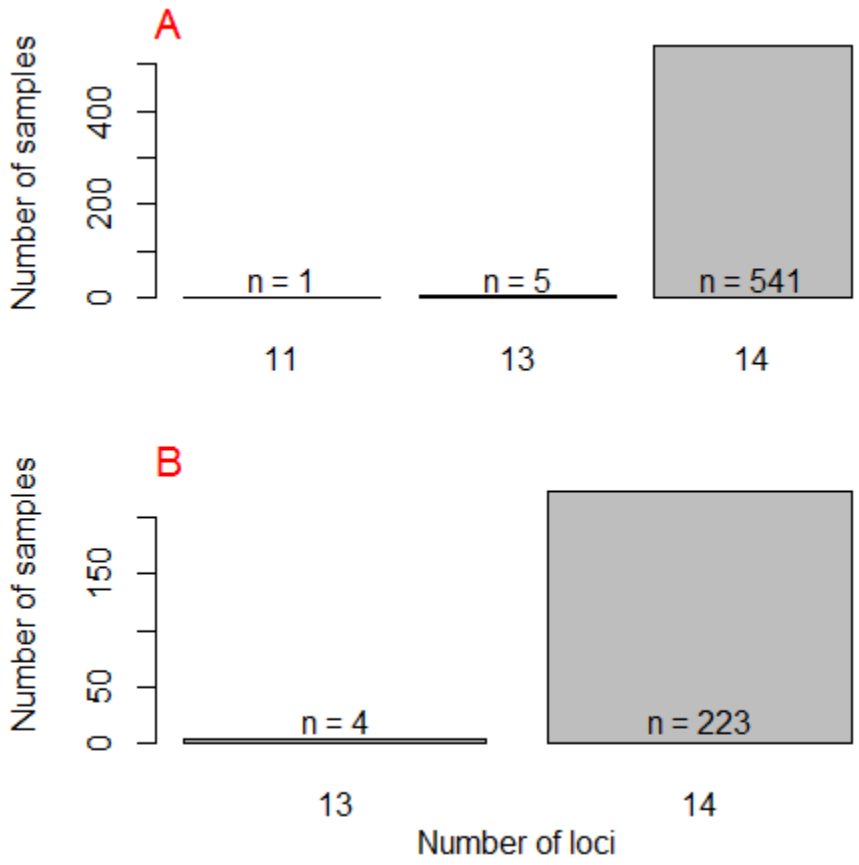


Figure 7. Number of A) Juvenile downstream migrant (JDM) and B) resident (RES) O. nerka caught in the Alouette river and Reservoir in 2013 (total n=775) that were genotyped for 10, 11, 12, 13 and 14 loci

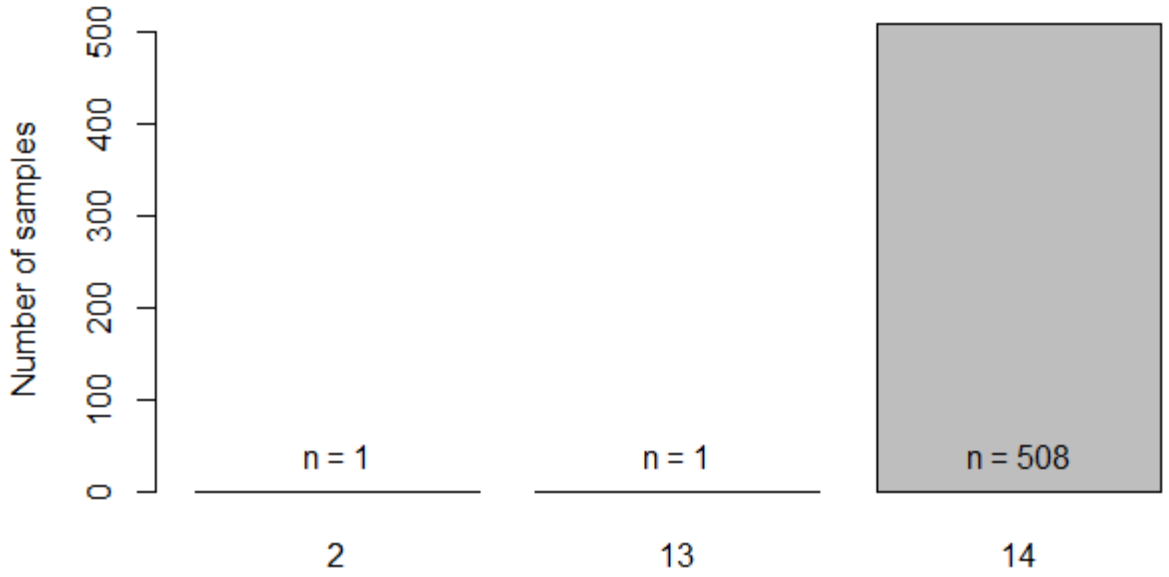


Figure 8. Numbers of juvenile downstream migrant (JDM) caught in the Alouette River or Reservoir in 2013 for which genotype data were available for 2, 13, or all 14 loci.

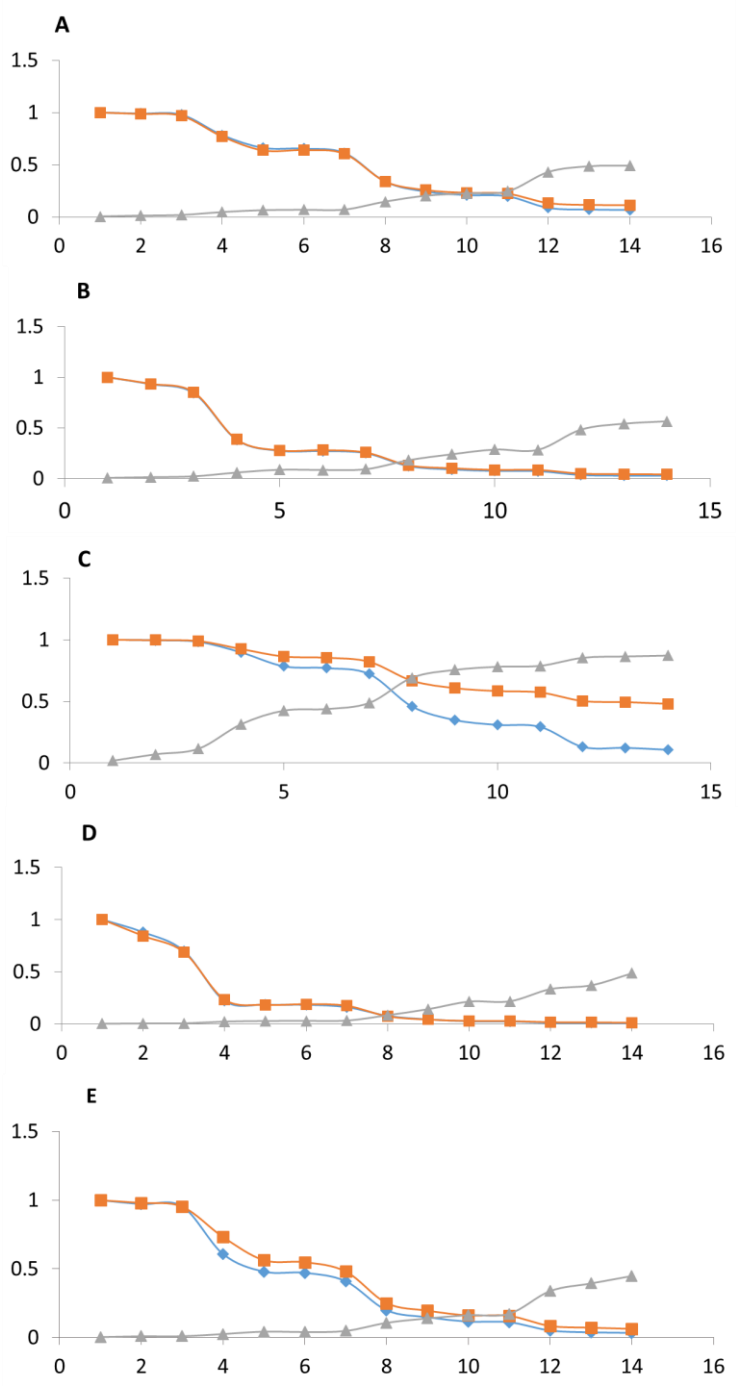


Figure 9. Allocation rate CLS (cumulative sequence of sets of loci) curves for parental analyses with PASOS (MOT=0) for brood years a) 2008, b) 2009, c) 2010, d) 2011, and e) 2012. These analyses included only potential progeny and parents for which complete genotype data were available for 14 loci.

The coloured symbols distinguish the observations (blue diamonds), simulations (orange squares) and correctness (grey triangles) curves.

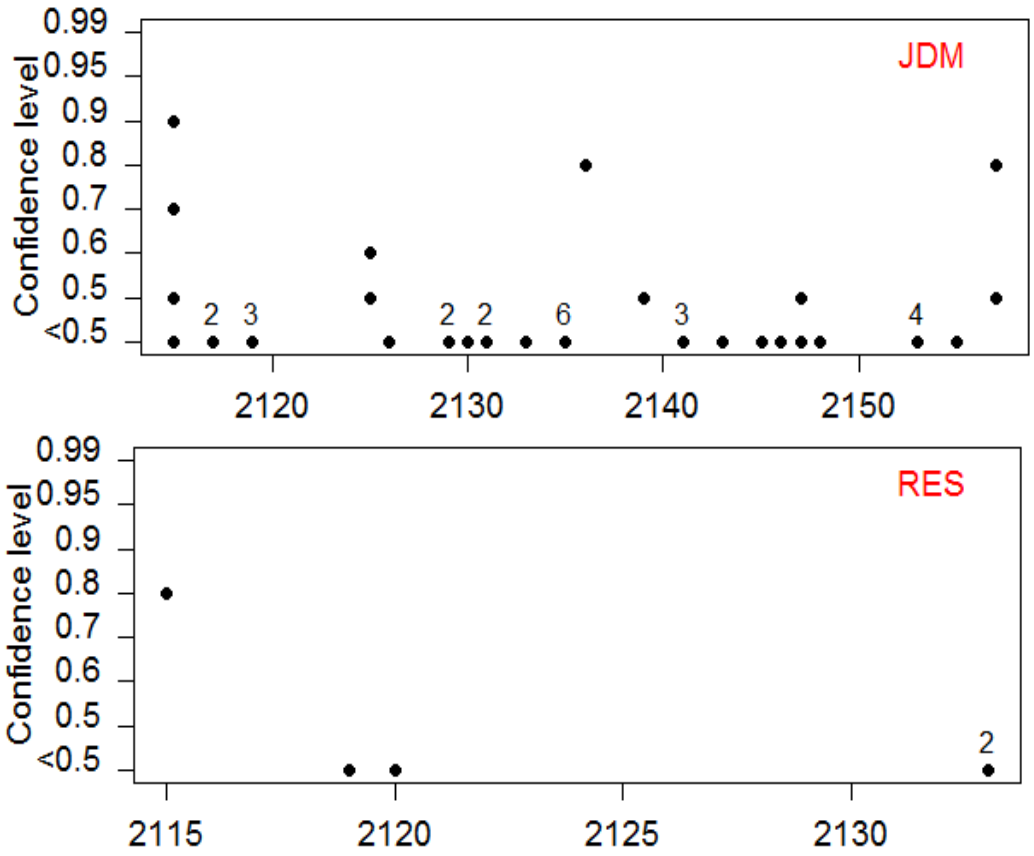


Figure 10. Confidence levels for single-parent assignments in CERVUS with no mismatches at 14 loci for brood year 2012. The x-axis shows the identity number for 25 AUM assigned to 40 JDM (top panel) and 3 RES (bottom panel). Labels beside points indicate the number of progeny assigned at the specified confidence level if greater than 1.

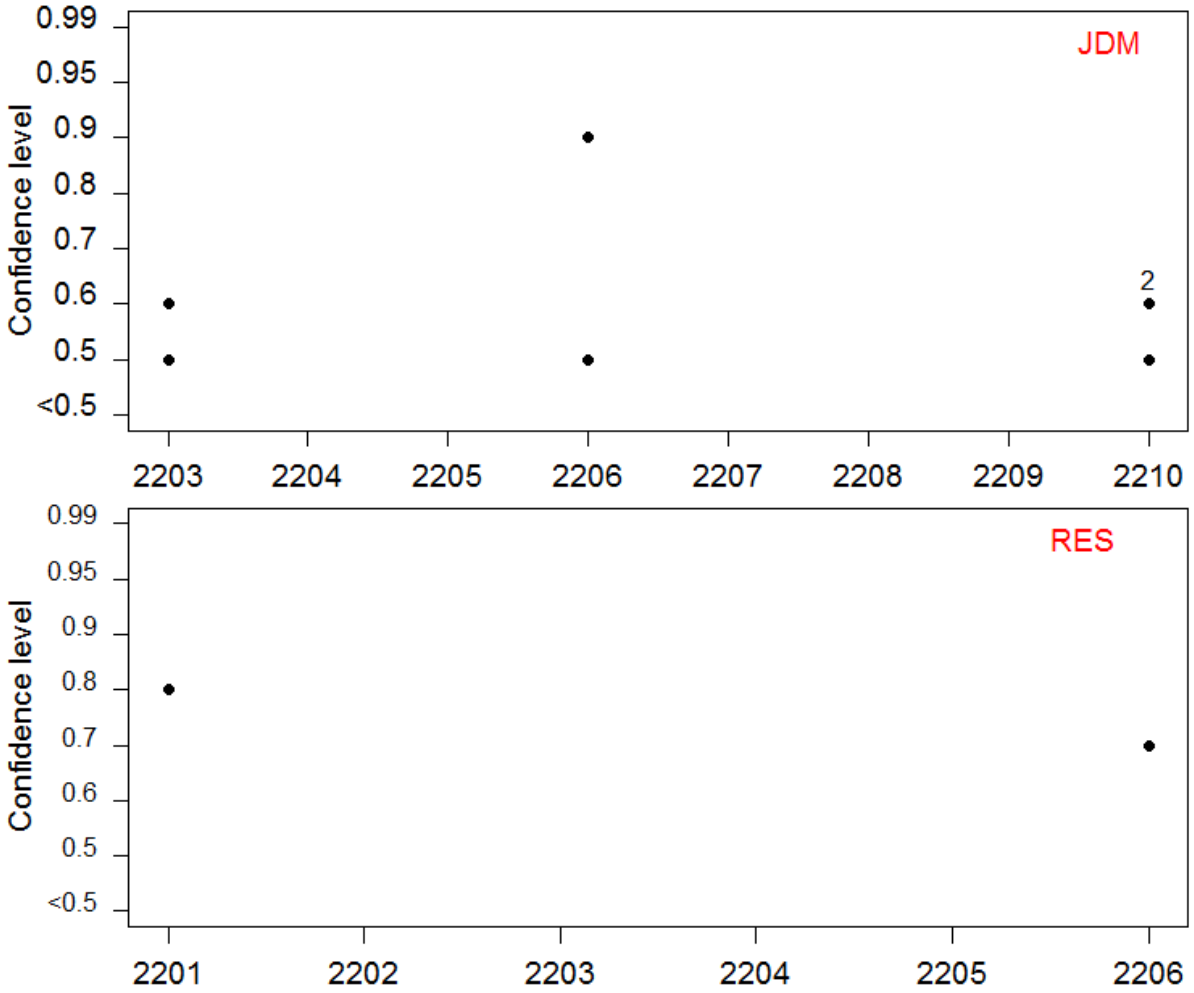


Figure 11. Confidence levels for single-parent assignments in CERVUS with no mismatches at 14 loci for brood year 2011. The x-axis shows the identity number for 4 AUM assigned to 7 JDM (top panel) and 2 RES (bottom panel). Labels beside points indicate the number of progeny assigned at the specified confidence level if greater than 1. All progeny were assigned to a parent only once.

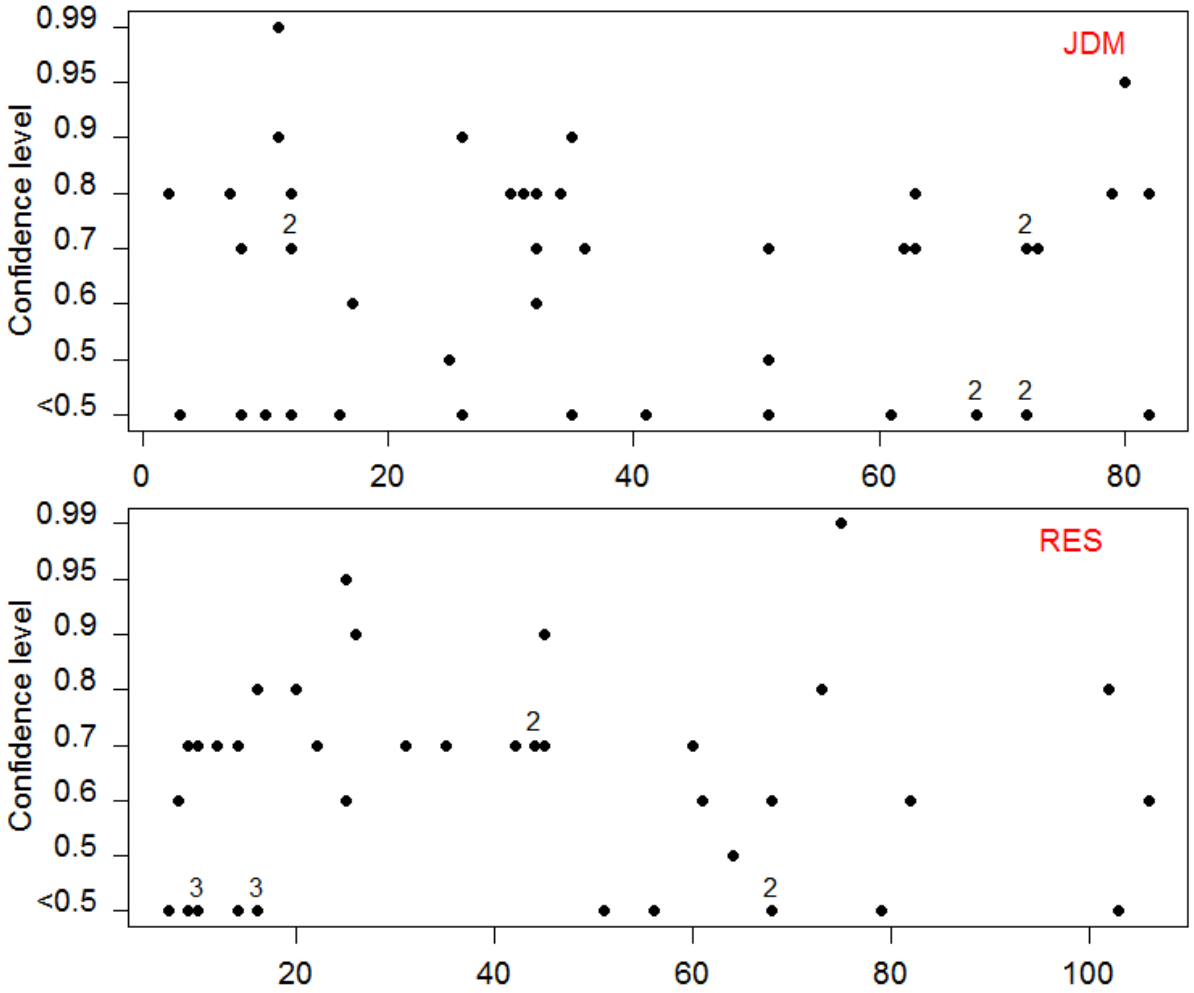


Figure 12. Confidence levels for single-parent assignments in CERVUS with no mismatches at 14 loci for brood year 2010. The x-axis shows the identity number for 42 AUM assigned to 45 JDM (top panel) and 41 RES (bottom panel). Labels beside points indicate the number of progeny assigned at the specified confidence level if greater than 1. There were 33 JDM and 31 RES that were matched to only 1 parent

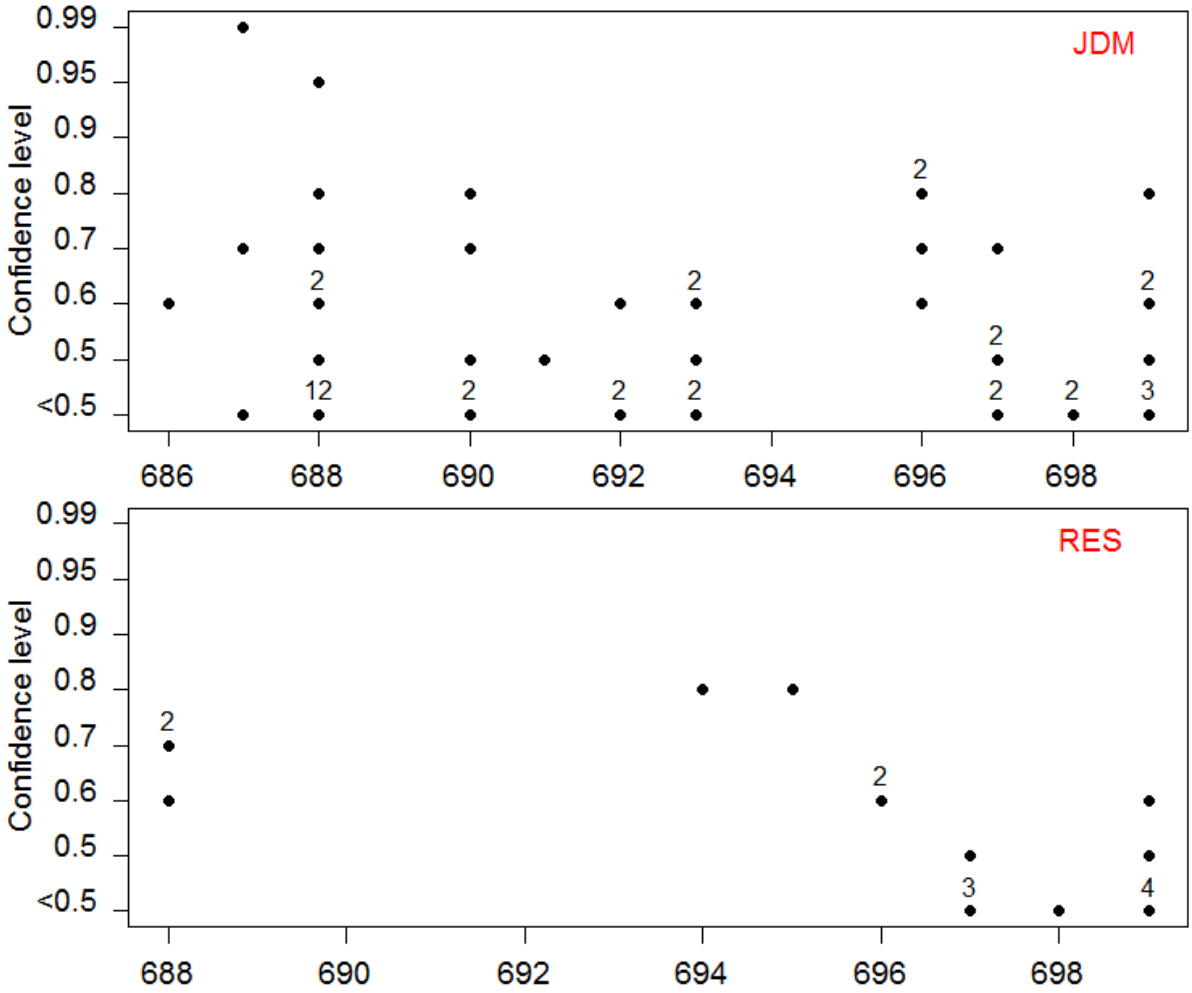


Figure 13. Confidence levels for single-parent assignments in CERVUS with no mismatches at 14 loci for brood year 2009. The x-axis shows the identity number for 13 AUM assigned to 54 JDM (top panel) and 18 RES (bottom panel). Labels beside points indicate the number of progeny assigned at the specified confidence level if greater than 1. There were 52 JDM and 17 RES that were matched to only one parent.

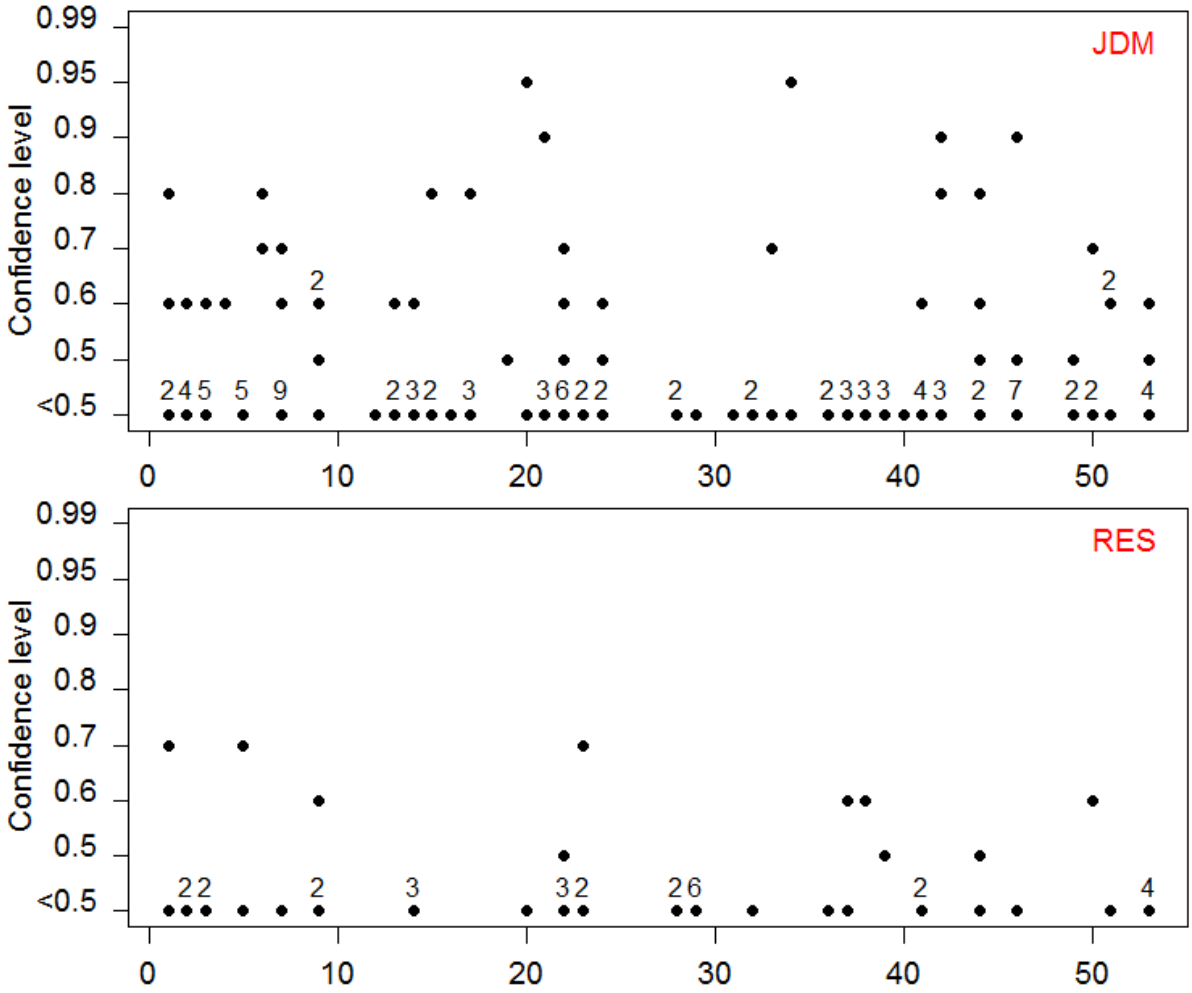


Figure 14. Confidence levels for single-parent assignments in CERVUS with no mismatches at 14 loci for brood year 2008. The x-axis shows the identity number for 39 AUM assigned to 137 JDM (top panel), and to 48 RES (bottom panel). Labels beside points indicate the number of progeny assigned at the specified confidence level if greater than 1. There were 117 JDM and 38 RES that were matched to only one parent.

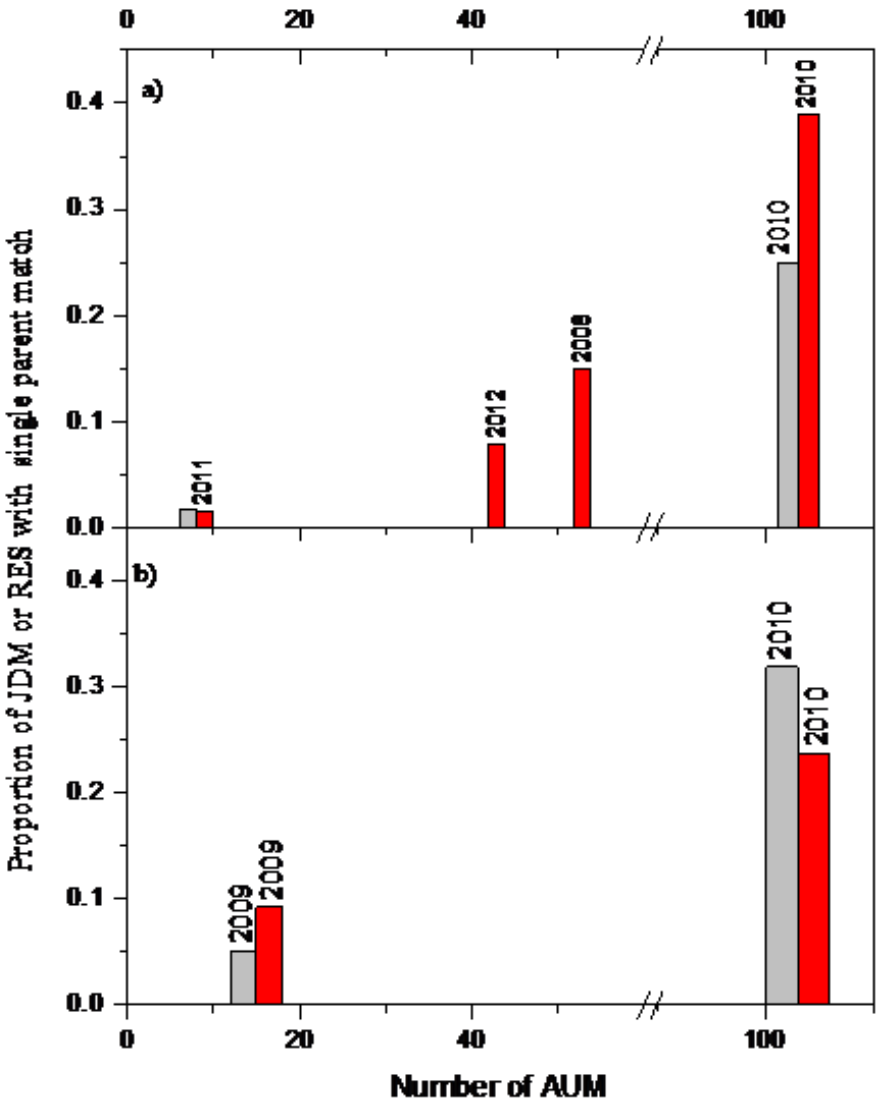
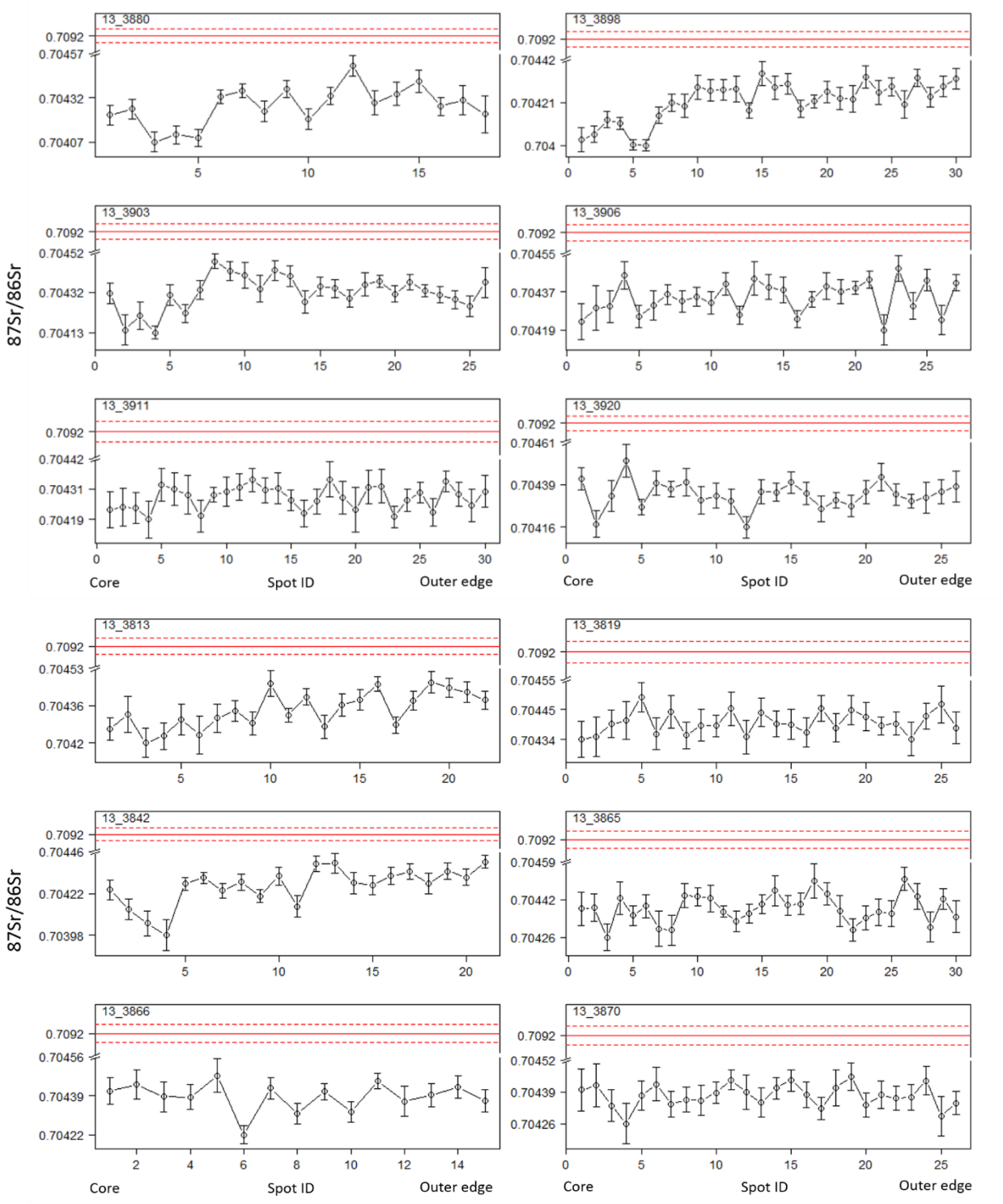


Figure 15. Proportion of the a) age-1 and b) age-2 juvenile downstream migrant (JDM, red) and resident (RES, light grey) *O. nerka* assigned to an AUM as a single parent (based on specimens with no missing genotypes at 14 loci). Numbers above the bar refer to the brood year of the adult upstream migrants (AUM).


13_3880
13_3898
13_3903
13_3906
13_3911
13_3920
13_3813
13_3819
13_3842
13_3865
13_3866
13_3870
87Sr/86Sr
87Sr/86Sr
Core
Spot ID
Outer edge

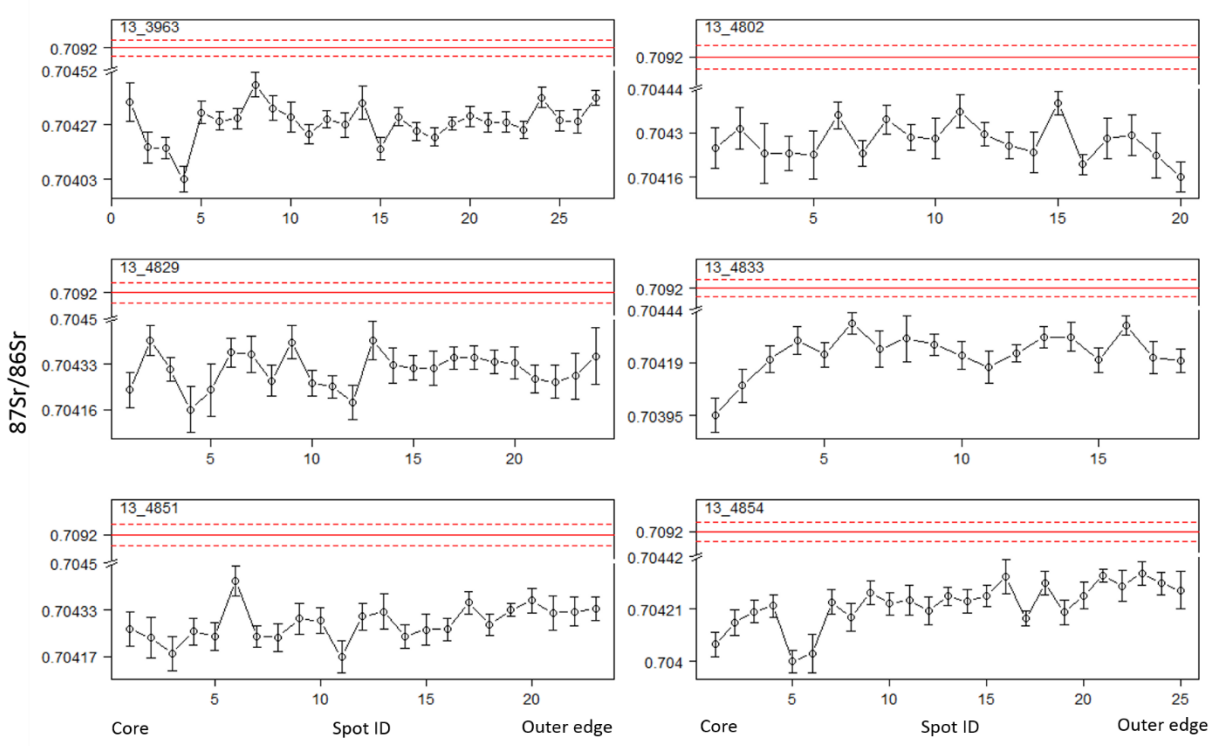


Figure 16. Isotope signature of strontium ratio ($^{87}Sr/^{86}Sr$) of the 18 nerkids that underwent otolith microchemistry analysis (black solid line) (± 1 s.e.). Standard marine signature (solid red line) (± 1 s.e. dotted lines).

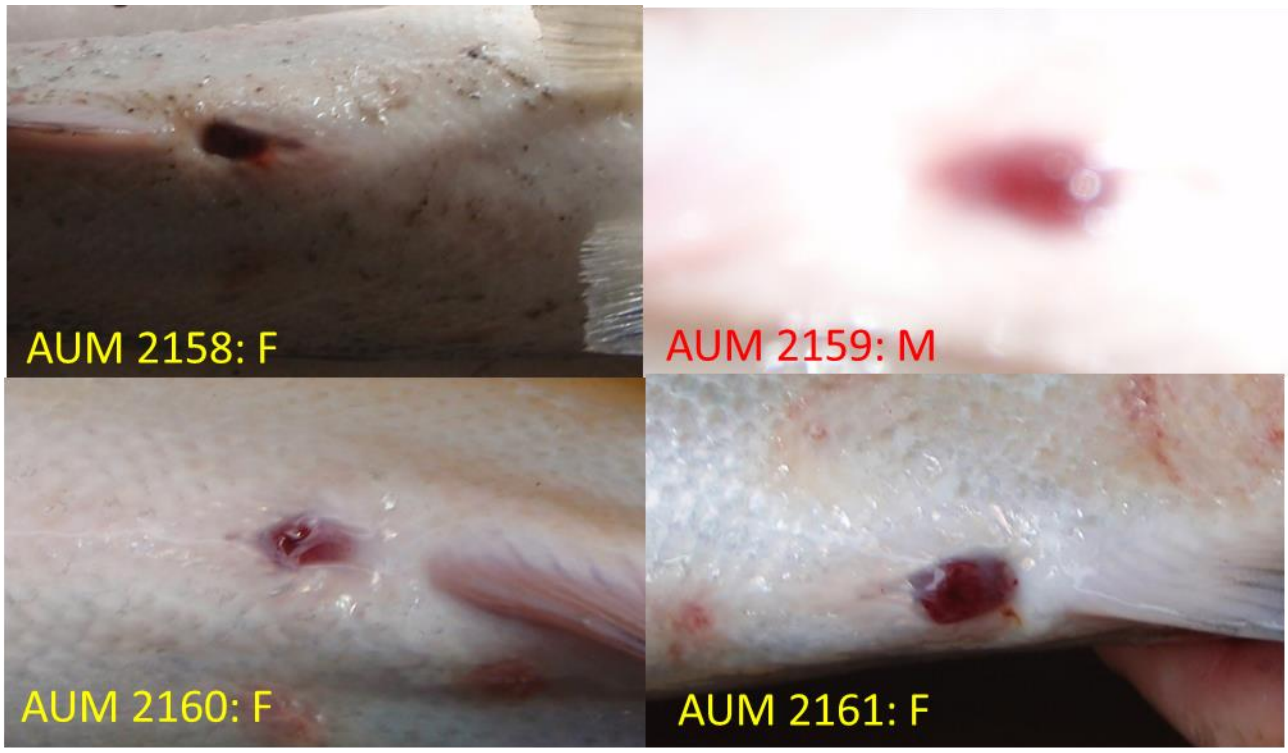


Figure 17. Photos of 2013 AUM used for sex determination. AUM DNA ID number and sex (F, female; M, male) in bottom left of each photo.

APPENDIX 1: Tables and figures describing 2013 field work and samples.

Set #	Net Size (m^2)	Mesh Sizes (mm)	Net Top Depth	Net Bottom Depth	Date & Time In (dd-mm-yy)	Date & Time Out (dd-mm-yy)	Easting	Northing
1	302	19,25, 51, 89, 13	5	8.6	6-10-13 16:30	7-10-13 9:30	538708	5461493
2	302	19,25, 51, 89, 13	10	13.6	6-10-13 16:50	7-10-13 9:40	538708	5461493
3	302	19,25, 51, 89, 13	15	18.6	6-10-13 16:55	7-10-13 10:12	538708	5461493
4	302	19,25, 51, 89, 13	20	23.6	6-10-13 17:30	7-10-13 10:35	538708	5461493
5	216		5	7.4	6-10-13 17:35	7-10-13 10:45	538708	5461493
6	216		10	12.4	6-10-13 17:50	7-10-13 10:55	538708	5461493
7	302	19,25, 51, 89, 13	10	13.6	7-10-13 9:30	8-10-13 12:40	539178	5462153
8	302	19,25, 51, 89, 13	10	13.6	7-10-13 9:40	8-10-13 12:00	539097	5462048
9	302	19,25, 51, 89, 13	15	18.6	7-10-13 10:12	8-10-13 12:30	539178	5462153
10	302	19,25, 51, 89, 13	15	18.6	7-10-13 10:35	8-10-13 12:40	539097	5462048
11	216		10	13.6	7-10-13 10:45	8-10-13 10:30	538708	5461493
12	216		15	18.6	7-10-13 10:55	8-10-13 11:00	538708	5461493
13	302	19,25, 51, 89, 13	15	18.6	8-10-13 12:40	9-10-13 9:30	538708	5461493
14	302	19,25, 51, 89, 13	15	18.6	8-10-13 12:00	9-10-13 9:55	539774	5461985
15	302	19,25, 51, 89, 13	15	18.6	8-10-13 12:30	9-10-13 10:30	538914	5461756
16	302	19,25, 51, 89, 13	15	18.6	8-10-13 12:40	9-10-13 10:40	538914	5461756
17	216		15	18.6	8-10-13 10:30	9-10-13 11:30	538708	5461493
18	216		15	18.6	8-10-13 11:00	9-10-13 14:00	538708	5461493
19	302	19,25, 51, 89, 13	15	18.6	9-10-13 9:30	10-10-13 10:00	538914	5461756
20	302	19,25, 51, 89, 13	15	18.6	9-10-13 9:55	10-10-13 10:20	538914	5461756
21	302	19,25, 51, 89, 13	15	18.6	9-10-13 10:30	10-10-13 7:30	538914	5461756
22	302	19,25, 51, 89, 13	15	18.6	9-10-13 10:40	10-10-13 8:00	538914	5461756
23	216		15	18.6	9-10-13 14:40	10-10-13 9:30	538914	5461756
24	216		15	18.6	9-10-13 14:50	10-10-13 9:45	538914	5461756

Table A1.1. Gillnet setting data, October 2013. UTM values were taken at the midpoint of each set.

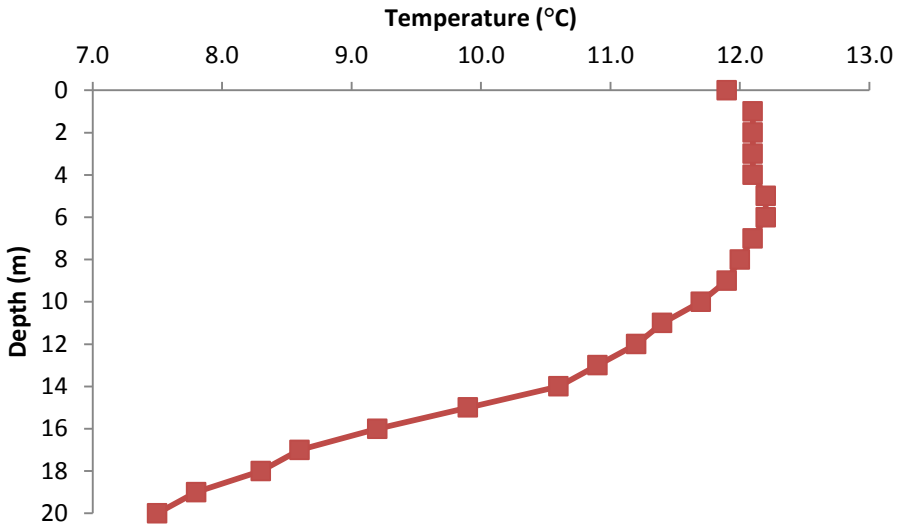


Figure A1.1. Temperature at depth plot for the southern basin of the Alouette reservoir on 15 October 2013. Thermocline found to be between 14m-16m.

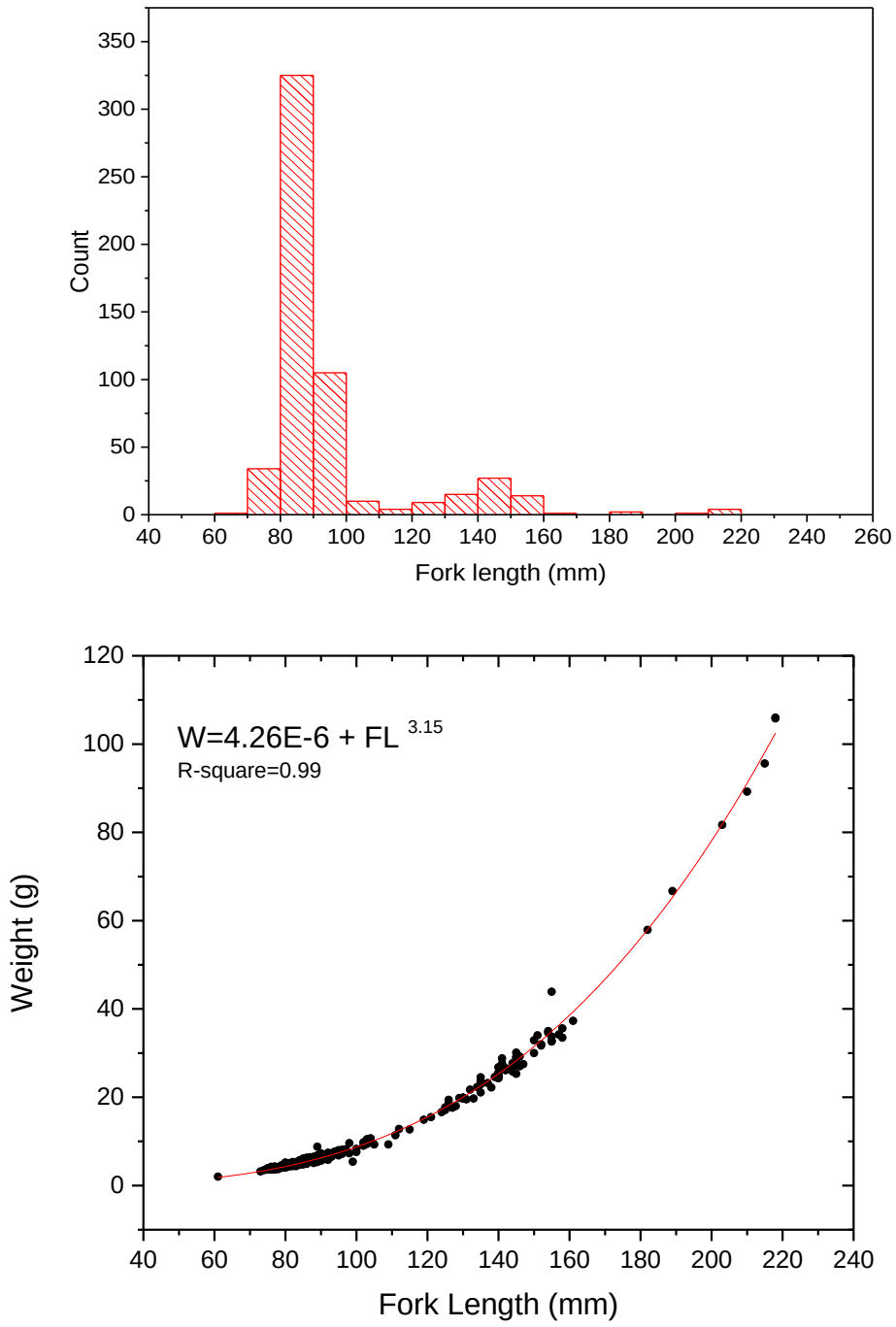


Figure A1.2. Fork-length distribution (top panel) and fork length-weight relationship (bottom panel) of the emigrating *O.nerka* (JDM) in the Alouette River in 2012 (*n*=552)

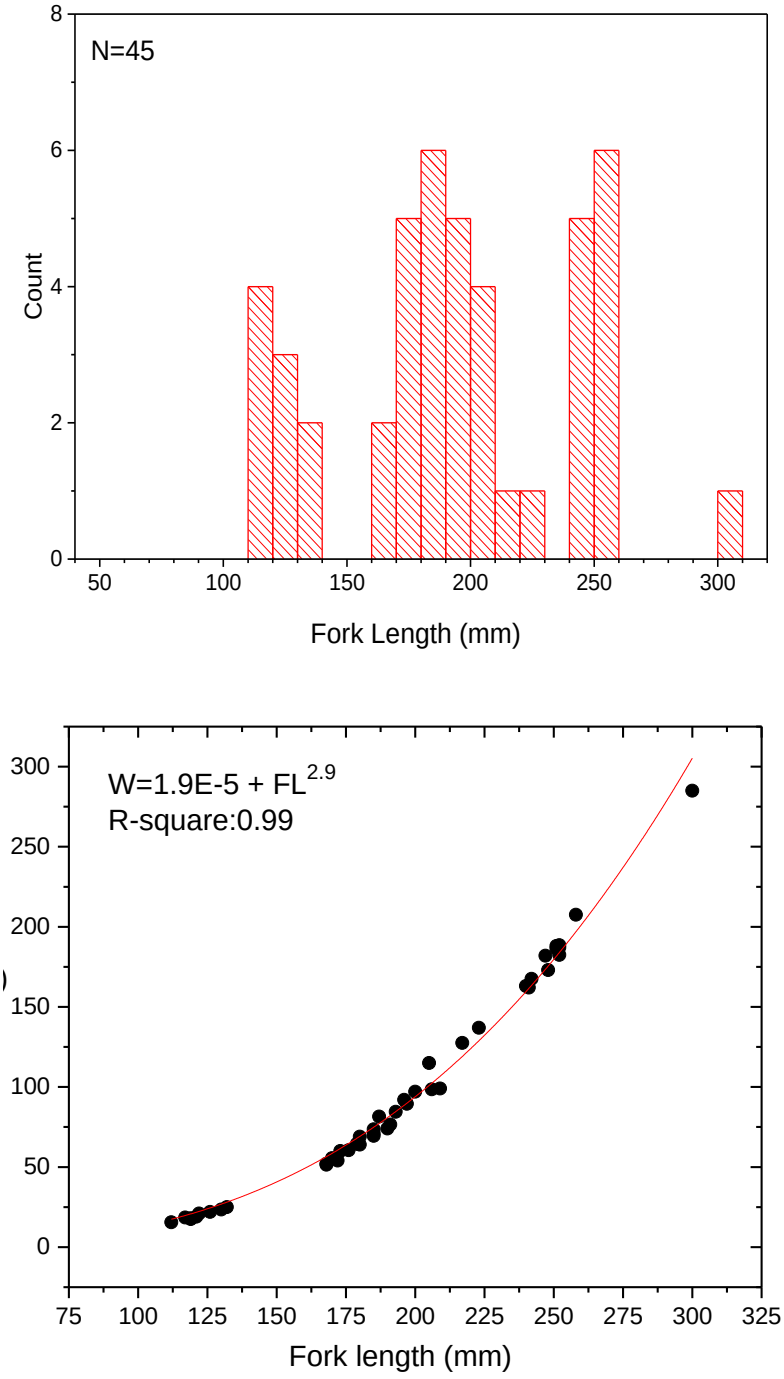


Figure A1.3 . Fork-length distribution (top panel) and fork length-weight relationship (bottom panel) of the residents *O.nerka* (RES) caught in the Alouette Reservoir on 12 July 2013 (*n*=45)

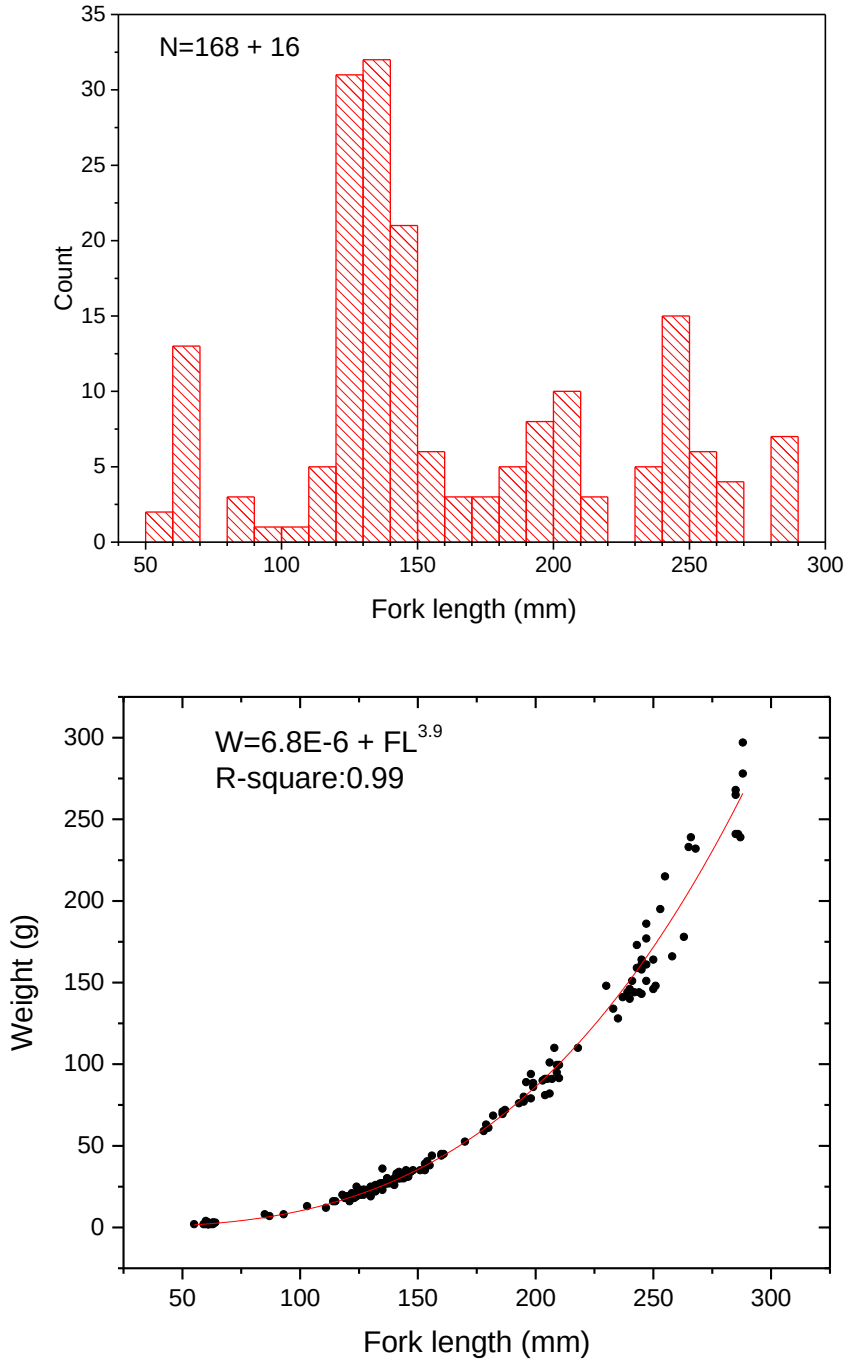


Figure A1.4. Fork-length distribution (top panel) and fork length-weight relationship (bottom panel) of *O.nerka* caught using gillnets in the Alouette Reservoir in September and October and 2013 (*n*=184).

APPENDIX 2: Attributes of the vent used to assign sex to live adult *O. nerka* returning to the Alouette River in July and August.

Sockeye salmon returning to the Alouette River in July and August are not yet mature enough to determine their sex based on typical differences in external morphology (body shape, jaws and head, colour and adipose fin). We therefore attempted to develop criteria for sex determination of live fish based on externally visible attributes of the vent by examining 11 adult sockeye salmon of known sex that were found dead in the Alouette River trap in 2010. The following criteria were used to determine sex of live returning adults:

- Shape: The vents of male salmon tend to be narrower and can appear slit like (Figure a1) whereas the vents of females tend to be rounder (Figure a2). This is the best characteristic of the vent for sexing salmon.

- Depth: Male vents will be more flush with the ventral surface of the fish whereas female vents may protrude slightly and be for furrowed in the middle.

- Colour: Male fish may have pale vents; females tend to have redder vents than males.

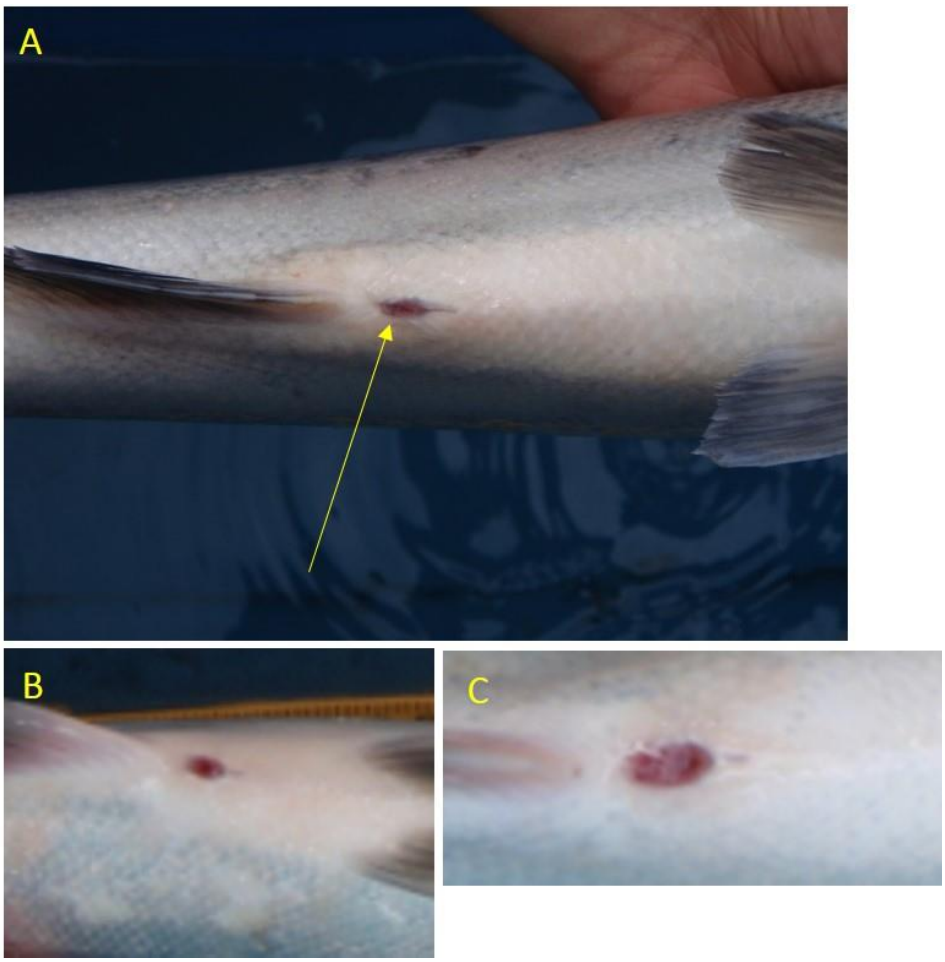


Figure A1.1. Examples of adult male sockeye caught in July 2012 showing vent (indicated by yellow arrow). Notice that the vent is less round than for the female (Figure A.1.2). Note how the vent in A is narrow and the vents in B and C are flush with the ventral surface of the fish.

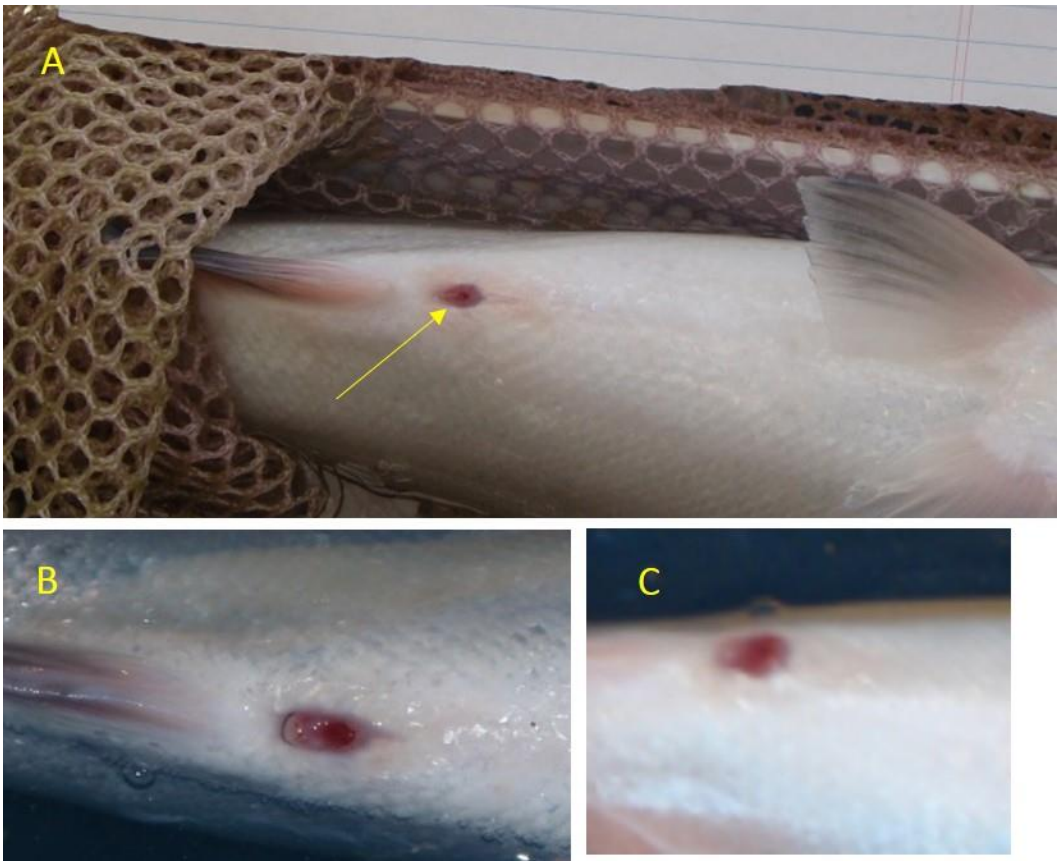


Figure A1.2. Examples of adult female sockeye caught in July 2012 showing vent (indicated by yellow arrow). Notice the round character of the vents in A and C and the furrow in B.