Smolt parental lineage assessment to evaluate successful spawn of returned 2009 Alouette sockeye spawners

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SUMMARY

The extirpation of a natural sockeye population in Alouette Reservoir following the 1920s construction of a hydroelectric dam may be reversible by the lake's now-resident kokanee population's indicated ability to revert to anadromy. In the spring with the release of water over the dam, juvenile resident kokanee fish have been observed migrating downstream in numbers up to 63,000 (called juvenile downstream migrants, JDM); in the late summer, sockeye have been returning to the Alouette in numbers of up to 110 adult upstream migrants (AUM). To determine if these returning adults are successfully spawning, and if their progeny are more likely to emigrate, we carried out parentage analyses (using 14 microsatellite loci and the programs Colony 1.2, PASOS 1.0, and Cervus 3.0) of 896 1-year-old JDM against the 15 sampled 2009 AUM, and of 14 2-year-old JDM caught in 2011 combined with 888 yearling JDM caught in 2010 against the 53 2008 AUM. While no progeny were assigned perfectly to a parental pair, 1 and 5 JDM from the 2009 and 2008 brood years, respectively, were allocated with a single mismatching allele to AUM. Given that genotyping error rates indicate a possibility of up to 3 in 900 fish being incorrectly completely genotyped, one or more of these fish may actually be a perfect match. Furthermore, 48 2011 JDM were matched perfectly to 11 2009 AUM, 134 2008 brood JDM were matched perfectly to 39 of the 2008 AUM, all on a single parent basis. Otolith microchemistry of 5 out of the 48 DJM revealed that their maternal origin was freshwater, and hence might have been the progeny of male sea-run kokanee. Based on these results, it seems likely that the returning sockeye adults may have mated with the resident kokanee.

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

Despite this utter disappearance, it appears that Alouette's non-anadromous sockeye salmon are contributing to the re-establishment of an anadromous sockeye salmon run in the Alouette Reservoir. Experimental release of surface water over the Alouette dam from 2005 to 2011 has resulted annually in between 5,000 and 63,000 juvenile downstream migrants (JDM) from the reservoir (Baxter and Bocking 2006, Humble et al. 2006, Mathews and Bocking 2007, 2009, 2010). Following the first release, a total of 28 adult upstream migrants (AUM) returned to Alouette River in 2007. Since then, more AUM have returned each year, numbering 54, 45, 115, and 11 in 2008, 2009, 2010, and 2011, respectively (Backle 2008, Cruickshank and Crowston 2011). 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 non-anadromous sockeye salmon (kokanee) that inhabit the reservoirs formed by the dam (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 the project has been successful in demonstrating that Alouette kokanee can revert to anadromy, it was not known if these re-anadromized AUM (hereafter called 'sea-run kokanee') can successfully reproduce when released in the reservoir and if their progeny are more likely to migrate to sea and survive than those of typical lake-resident kokanee. To address these questions we took advantage of the fact that the 1-, 2-, and 3-year-old progeny of the 2009, 2008, and 2007 AUM were expected to migrate to sea in the spring of 2011. However, as it is not possible to visually identify the JDM as

progeny of AUM or kokanee, we employed two complementary techniques to identify the fish: genetic parentage assignment and otolith microchemistry.

According to Mendelian inheritance for diploid organisms, parent and progeny must share at least one allele for each locus. Therefore, progeny of the transplanted AUM can be identified by the unique DNA sequence of individual 2009 AUM parents, 15 of which were sampled before release into the reservoir. Complementarily, the chemistry at the core of an otolith will reflect the chemistry of the mother which spawned the fish, as the otolith core is formed from the yolk of the egg, and can hence be used to determine whether the mother inhabited a marine (indicating anadromy, and therefore AUM) or freshwater (kokanee) environment (Kalish 1990). Because removing an otolith would require killing the fish, otolith microchemistry analysis was restricted to fish that died during enumeration and that were identified by genetic parental analysis as potential progeny of an anadromous parent.

Our plan was therefore to perform parental assignment analysis and to use otolith microchemistry of potential AUM progeny as a supplementary test to cross-validate the parental analysis of the 2009 AUM. We also revisited the parental analysis of the 2008 AUM using an updated list of potential progeny.

Goals and objectives

The main goal was to assess the reproductive success of the 2009 AUM that were returned to the reservoir. This goal was achieved through

- ✓ Microsatellite DNA analysis of the tissues sampled from the 2011 JDM parental analysis to determine if any of the JDM sampled in 2011 were the progeny of sea-run kokanee (i.e., AUM transplanted into the reservoir in 2009).
- ✓ Measurements of the stable isotopes ratio of strontium in otoliths of up to 20 of the JDM that were accidentally killed during enumeration and that could be identified by genetic parental analysis as potential progeny of AUM.
- ✓ Estimation of the total number of JDM originating from AUM.
- ✓ Preparation of a technical report summarizing findings.

A secondary goal was to update a previous parental analysis of the 2008 AUM in assigning a revised list of 2010 1-year-old JDM (based on available fish size data) which was updated to also include 2-year-old JDM caught in 2011.

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 at100-026700-06000.

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. 2000a). 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. 2000a). 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 (LLO, 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 125.5m, but such spills have been very rare. Based on BC Hydro records from 1984, 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 2011

BC Hydro experimentally released water via the crest gate over the spillway during the period of 15 April to 14 June, 2011. Flow at the gate ranged from 2.1 to 5.4 $cm^3 \bullet s^{-1}$ and averaged 3.8 $cm^3 \bullet s^{-1}$ except during the period of 2 June to 8 June, when the flow averaged 6.2 $cm^3 \bullet s^{-1}$ (Brent Wilson BC Hydro, personal communication). The low level outlet was closed during this surface water release.

Fish Collection and DNA samples

LGL Limited monitored the JDM using a rotary screw trap (RST) located approximately 1.5 km downstream of the Alouette Dam from 16 April, 2011 to 8 June, 2011. A mark-recapture experiment initiated on 18 April consisted on each day of caudal fin clipping up to 110 JDM from the RST catch, which were released upstream, and recaptured downstream by the RST (see Mathews and Bocking 2011 for the details). The RST was checked daily in the morning, and the number of live and dead JDM, both clipped and unclipped, were counted.

An unbiased estimate of the total catchable population size (N) was obtained using a pooled Peterson estimator with a Chapman modification as

$$N = (M+1)(C+1)/(R+1) - 1$$

where

C= total number of fish caught in the second sample (including recaptures)

M= number of fish caught, marked and released in the first sample

R= Number of recaptures in the second sample (first marked and released in the first sample)

The variance of *N* was estimated as:

$$\text{var}(N) = [(M+1)(C+1)(M-R)(C-R)] / [(R+1)^2 (R+2)]$$

The variance was used to estimate an approximate 95% confidence interval for the population estimate based on the following equation:

$$N \pm 1.965 \text{ SQRT}(\text{Var}(N))$$

Parental Analysis

Sampling design

Because the transplanted AUM were so few in number relative to the resident spawning kokanee, a fairly large sample size was necessary to have a reasonable probability of detecting AUM progeny. To determine an appropriate target sample size, we first estimated that the 15 AUM transplanted in 2009 could have produced up to 480 JDM if half were female (rounded up to 8), all spawned successfully producing an average of 60 yearlings[1] each, and all the yearlings migrated out of the reservoir in 2011. Our estimate of 480 clearly represents an upper bound for the number of progeny of AUM that could be expected to migrate downstream in 2011. Based on past smolt migration, this upper bound represents between <0.08% (480/62, 923 JDM observed in 2007) to 9.48% (480/5065 JDM observed in 2006) of all JDM. We determined that a sample size of 900 JDM could be expected to yield a maximum of between 7 and 85 progeny of AUM-origin, and would be sufficient to test whether any of the returning sockeye had reproduced successfully.

To obtain a sufficient sample of the 2011 JDM, up to 40 fish were randomly selected from the RST catch each day, sampled for DNA, measured for fork length (FL) and released. We created a representative sample of the total outmigration by first calculating the proportion of JDM migrating each week and then randomly subsampling from daily samples so that weekly proportions were preserved when daily subsamples were pooled to create the 'seasonal sample.'

[1] 3000 eggs/female x 0.10 egg-to-fry survival x 0.2 fry-to-smolt survival (e.g., Foerster 1968)

To enhance the possibility of detecting progeny of the 2008 AUM, JDM sampling rate on age-2 JDM was increased by collecting a 'targeted' sample of larger fish from the RST.

DNA analysis:

Tissue samples were analyzed to obtain a multilocus genotype at 14 microsatellite loci (*Ots2, Ots3, Ots100, Ots103, Ots107, Ots108, Oki1a, Oki1b, Oki6, Oki10, Oki16, Oki29, One8,* and *Omy77)* (Beacham et al. 2005).

Parental allocation

A number of computer programs have been developed to identify the most likely parents of individual progeny from 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 only the genotypes of the AUM, not those of all the possible kokanee parents of the JDM; moreover, none of the known genotypes can be associated with gender.

To perform a parental analysis in an open system, three additional sets of data are relevant: the number of uncollected parents (those for which no genotype data are available), the frequency of genotyping errors, and allele frequencies in the population(s).

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 0.02 for all simulations, which should be large enough to account for all possible sources of error (primarily allelic dropout, but also lab mistyping and mutation) (Beaumont 1999, Koskinen et al.2002[2]). Knowing allele frequencies for both groups of parents (AUM versus kokanee) would help to constrain realistic parental genotypes in simulations upon which likelihood of parentage is calculated, but we know only the genotypes of AUM while the bulk of the candidate parents are kokanee. As we have no reason to believe that the AUM and kokanee are reproductively isolated, we estimated allele frequencies for the *O. nerka* in the reservoir by combining genetic data from all available samples the JDM (2010 and 2011) and AUM (2007, 2008, 2009, and 2010).

[2] $2.0 \times 10^{-4} – 6.0 \times 10^{-4}$ mutation rates reported in Beaumont and Koskinen; 3×10^{-3} typing error at PBS (R. Withler unpublished data)

RESULTS

JDM population estimate and size distribution of JDM

A total of 35,542 (±769, 1 se) JDM traveled downstream between 15 April and 14 June, 2011 (Petersen mark-recapture estimation described by Mathews et al. 2012, in prep.). Roughly 50% of the JDM emigrated within the first three weeks, and the maximum daily migration (2757 fish) occurred on 14 May (Figure 2).

Weekly samples of 60 to 300 JDM were measured (FL) and sampled for DNA analysis, yielding a total sample of 1736 JDM. The size distribution of the sampled JDM suggests that most JDM were 1 year old with an average FL of 7.29 cm (Figure 3a). Mortalities ('morts') among the JDM tended to be smaller than the randomly sampled JDM. By design, the targeted samples comprised considerably larger 2-year-old (defined as FL 9.2 to 13.5 cm, n=14) and 3-year-old (FL > 13.5cm, n=16) JDM (Figure 3b).

Sample selection for Genotyping

A representative seasonal sample of 847 JDM was selected for genotyping by randomly subsampling the random daily samples in proportion to the weekly estimate of migration numbers. The weekly migration estimates of JDM and the weekly composition of the seasonal sample selected for genotyping are illustrated in Figure 4a, b. Tissues from the 30 targeted samples of older fish were also chosen for genotyping to search for progeny of AUM transplanted in 2008 and 2007 (Figure 4c). The total sample of 69 morts was also genotyped to provide an opportunity to use otolith microchemistry to validate any DNA parental assignments to AUM (Figure 4c). Thus, a total of 946 samples was selected for genotyping; their size distribution is shown by type in Figure 5.

DNA analysis

Genotypes could be determined for 926 (98%) of the JDM samples including 827 of the 847 randomly sampled yearling JDM, all 69 morts, and all 30 of the targeted 2- and 3-year-old JDM. Of these cases, genotypes were successfully determined at all 14 loci examined in 92% of 926 samples, and at 13 loci in 5% of samples. The remaining cases (all morts) were genotyped at 10, 11 or 12 loci (Figure 6).

All 15 AUM transplanted (i.e., transferred into the reservoir) in 2009 were successfully genotyped at all 14 loci. After reanalysis, 52 of the 53 AUM transplanted in 2008 were successfully genotyped at all 14 loci; the remaining AUM could be genotyped at only 12 loci.

Parentage analysis

We carried out parentage analyses for the 896 1-year-old JDM (827 sampled randomly + 69 morts) that were the potential progeny of the AUM transplanted in 2009. We also performed parallel analyses for the 14 2-year-old JDM caught in 2011 and the 888 1-year-old JDM caught in 2010[3], all of which were the potential progeny of the AUM transplanted in 2008. Details of the parentage analyses are presented in Appendix 2 but the main findings are summarized here by brood year.

2009 Brood Year: Only 15 of the 45 AUM transplanted in 2009 were sampled for DNA (Cruickshank and Crowston 2011) and could be genotyped for inclusion in this analysis. None of the 896 JDM samples could be perfectly matched to any pairing among the 15 genotyped AUM (Table A2.2). However, 48 JDM could be perfectly matched to a single AUM, and 11 of the 15 AUM were matched in this way (Table A2.2). After allowing one trio mismatch in 14 loci (i.e., assuming some genotyping error), one JDM could be matched to one parental pair with 86% confidence (Table A2.3a). Minimum estimates for the number of 'uncollected parents' (i.e., non-genotyped fish of either type) ranged from 503 (PASOS) to 650 (COLONY).

2008 Brood Year: As for the 2009 brood year, none of the 902 JDM samples could be perfectly matched to any pairing of the 53 genotyped AUM in 2008. Allowing one trio mismatch in 14 loci created potential matches of five JDM to five different parental pairs, but confidence levels were only 53% or less (Table A2.3b). 134 JDM could be perfectly matched to single parents (39 of the 53 AUM, Table A2.2). Minimum estimates for the number of uncollected parents (all kokanee) ranged from 622 (COLONY) to 691 (PASOS).

[3] 467 JDM with FL< 9.5cm and 421 JDM sampled at random which were likely 1-year old as most of the randomly-sampled fish were <9.5 cm – see Appendix 1).

Otolith microchemistry

Because none of the 69 JDM mortalities were assigned to any pairing of the 15 genotyped AUM, no suitable otoliths were available for microchemistry analysis to verfiy DNA assignments. However, 5 of the JDM mortalities were perfectly matched to single AUM parents, and it now seems worthwhile to analyze otolith microchemistry in these individuals. JDM ID 1922, 1923, 1924, 1802, and 1927 were assigned to AUM ID 692, 693,699,688 and 699, respectively. All 5 JDM had a freshwater signature from the core to the outer edge of the otolith. Hence if the AUM were the true parents, they would have to be male. It should be noted that because an anadromous maternal signature could be detected only if the AUM parent were female (which is not known), failure to detect a marine maternal signature in any of these 5 JDM could not refute the parentage assignment based on DNA.

DISCUSSION

Have transplanted sea-run kokanee produced smolts?

Given the sampling rates of JDM in 2011 and 2010, there is a reasonable chance of detecting any AUM progeny emigrating downstream in these years (see Methods). The weakest link in the investigation of AUM spawning success in 2009 was that only15 of 45 AUM were sampled for tissues needed to support the genetic parental analysis. Also, the power of the parentage and otolith microchemistry analyses would have been increased substantially if the AUM had been sexed at the time of genetic sampling (i.e., if genotypes had been associated with gender). In part because of these deficiencies, this study has not demonstrated conclusively that any of the transplanted sea-run kokanee produced sea-run smolts. Nevertheless, the results strongly suggest that some did.

No parental pairings among the AUM transplanted in 2009 perfectly matched any of the sampled JDM. (Note however that only 15 out of a total of 45 AUM were sampled for DNA). Similarly, no parental pairings among the AUM transplanted in 2008 (all 53 were sampled for DNA) perfectly matched any of the JDM sampled in 2010 (at age 1) or 2011 (at age 2).

The genotyping error rate at the PBS Molecular Genetics Lab for assays of microsatellite DNA in sockeye salmon has been measured at 0.003 (R. Whitler unpublished data). This measured rate implies that a genotyping error will occur for about 3 out of 900 fish. Previous observations also suggest that typing errors tend to occur at just one locus per fish (R. Whitler unpublished data). We therefore

expanded our consideration of potential parental pairs to include those with a single trio mismatch. In 2009, the parental pair formed by AUM 687 and AUM 694 had a high probability (86%) of having produced JDM 988, given that this match exhibits a trio mismatch of only 1 and a MOT of 1. In 2008, the parental pair formed by AUM 41 and AUM 6 had a moderate probability (53%, one trio mismatch and. MOT of 1) of having produced JDM 695, a 2 year-old fish (fork length 11.4cm) caught in 2011.

Even without invoking genotyping errors, 48 JDM could be perfectly matched to 11 of the AUM in 2009 when they were considered as single parents. Given that most AUM were not genotyped in 2009, it seems plausible that some of the 15 genotyped AUM mated with other AUM or with kokanee for which no genotypes were available. More surprisingly, 134 JDM could be perfectly matched to 39 of the 53 AUM in 2008 when they were considered as single parents. Given that all AUM were genotyped in 2008, these matches, if real, must have involved mating with smaller uncollected kokanee. Alternatively, uncollected kokanee with similar genotypes could also account for these matches.

Sockeye salmon are known to mate assortatively by size and previous behavioural experiments have demonstrated that given a choice, small males are more likely to mate with larger females than large males are to mate with small females (Foote and Larkin 1988). Because the transplanted AUM were larger than lake-resident kokanee, we would expect ‘hybrid’ matings between these size classes to be more common between female AUM and male kokanee than vice versa. Because the average size of eggs and emergent fry (Wood and Foote 1990) and lake-resident juveniles (Wood et al. 1999) is typically larger in sockeye than kokanee, we might reasonably expect any yearling progeny of sea-run kokanee to be larger on average than the progeny of lake-resident kokanee. However, the mean fork length of age-1 JDM that matched AUM genotypes was not significantly larger than those that did not ($n= 48$, $P > 0.237$, ANOVA). These negative results do not support the hypothesis that the JDM with matching genotypes are hybrid progeny of sea-run and lake-resident kokanee, but the test is not a strong one.

Ways to improve future studies

Collecting additional information could improve the ability to assess whether transplanted sea-run kokanee can spawn successfully in the Alouette Reservoir, and whether their offspring have a greater propensity to emigrate than the progeny of lake-resident kokanee. Knowing the sex of the returning adults would increase the statistical power of the parental analysis. For example, the likelihood estimates that

2008 AUM 46 mated with AUM 10, AUM 24 and AUM 53 would have been greatly improved by knowing which of the putative mating pairs involved different sexes.

We have used the size (fork length) of the juvenile downstream migrants to partition the juvenile downstream migrants into age classes, and to assign them to brood year. However these data are not available for all fish in all years. Consistent data on the size-at-age would also be useful to validate our partitioning of the juvenile downstream migrants into age-classes.

Finally, having a better estimate of the number of age-1 kokanee in the reservoir, as well as the number and sex ratio of spawning kokanee would be needed to compare the potential number of progeny from lake-resident kokanee to that from transplanted sea-run kokanee. By comparing the ratio of progeny from each source in samples of downstream emigrants (JDM) to that in samples of yearling that remain in the reservoir, it should be possible to determine whether the progeny of transplanted sea-run kokanee have a greater propensity to emigrate than that of lake-resident kokanee such comparison has been proposed for 2012.

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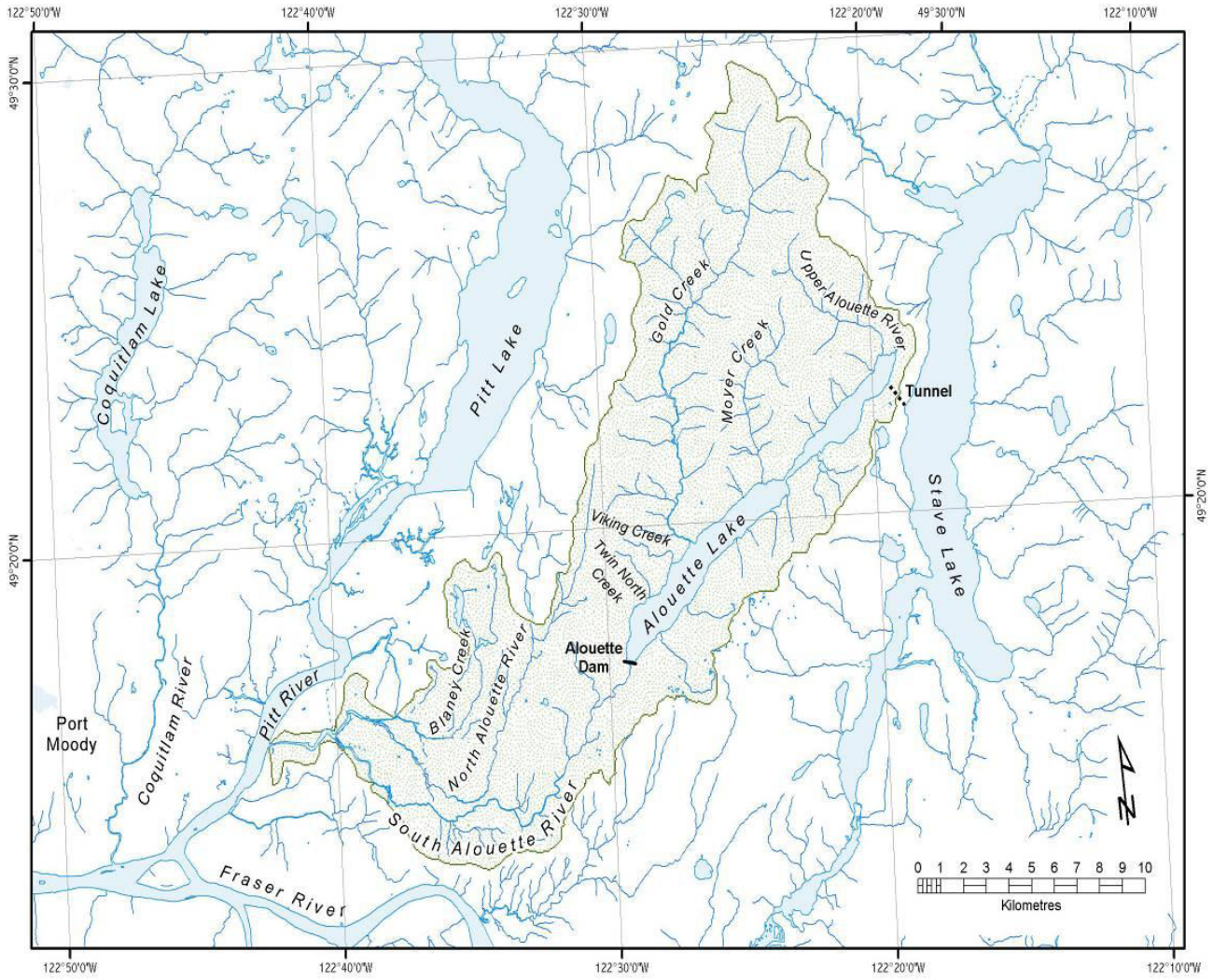

Figure 1. Overview map from the lower mainland of British Columbia showing the location of Alouette Reservoir.

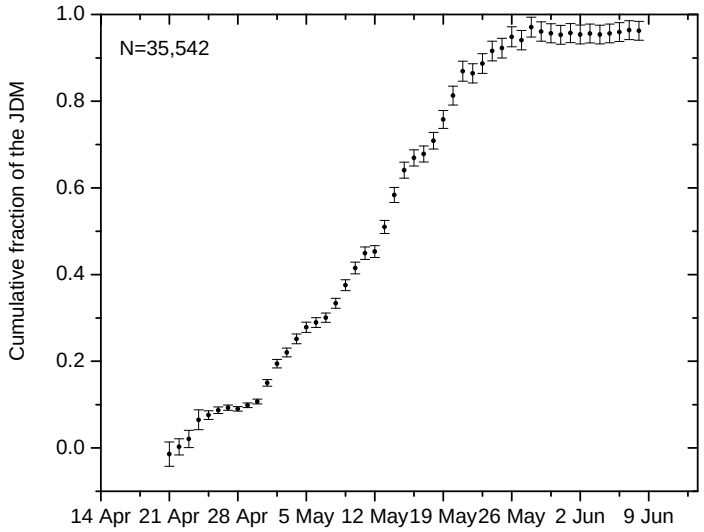

Figure 2. Cumulative fraction (± 1 SE) of the 2011 Peterson's population estimate of juvenile downstream migrants (JDM) in Alouette River (data from M. Mathews LGL Ltd). A 2-day lag from release to recapture was used in the population estimate.

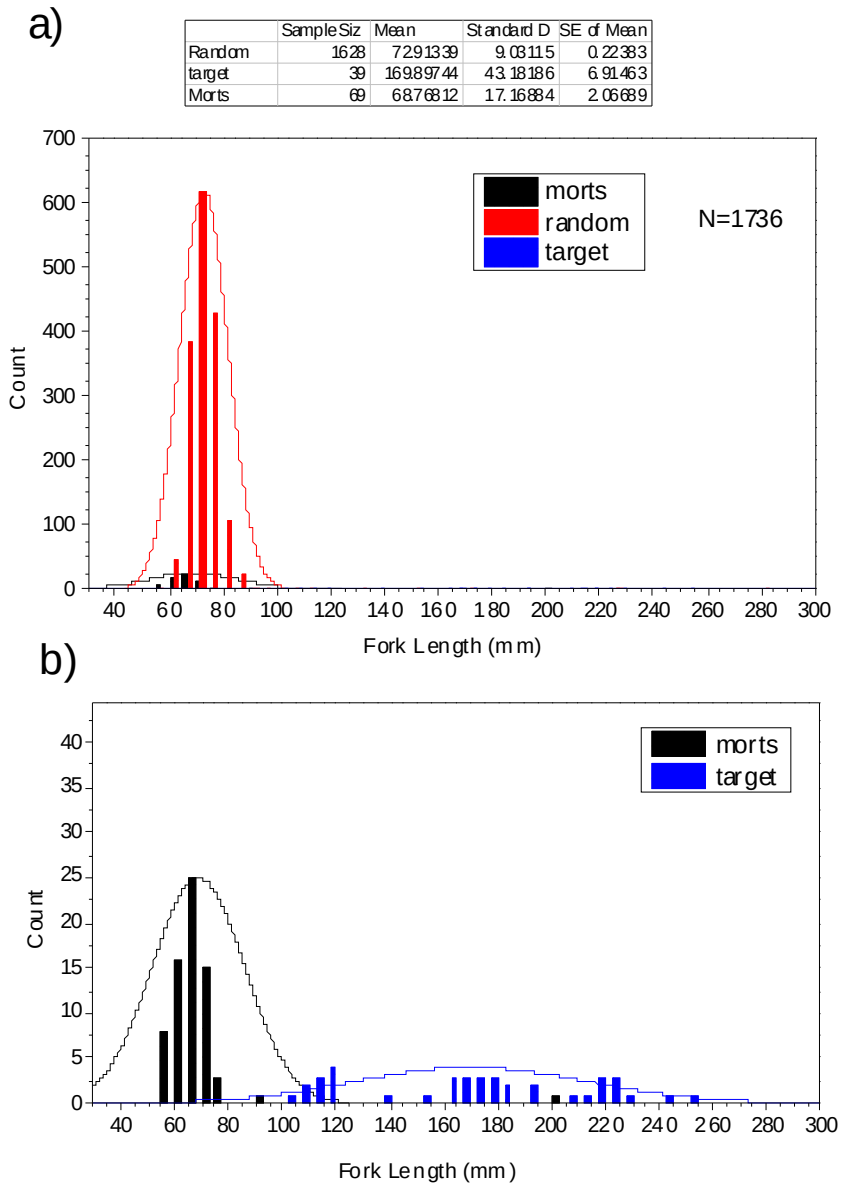

Figure 3. Size distributions of juvenile downstream migrants (JDM) of a) the morts, targeted, and randomly sampled JDM, and of b) the morts and targeted JDM sampled during the course of the water releases in Alouette River in 2011.

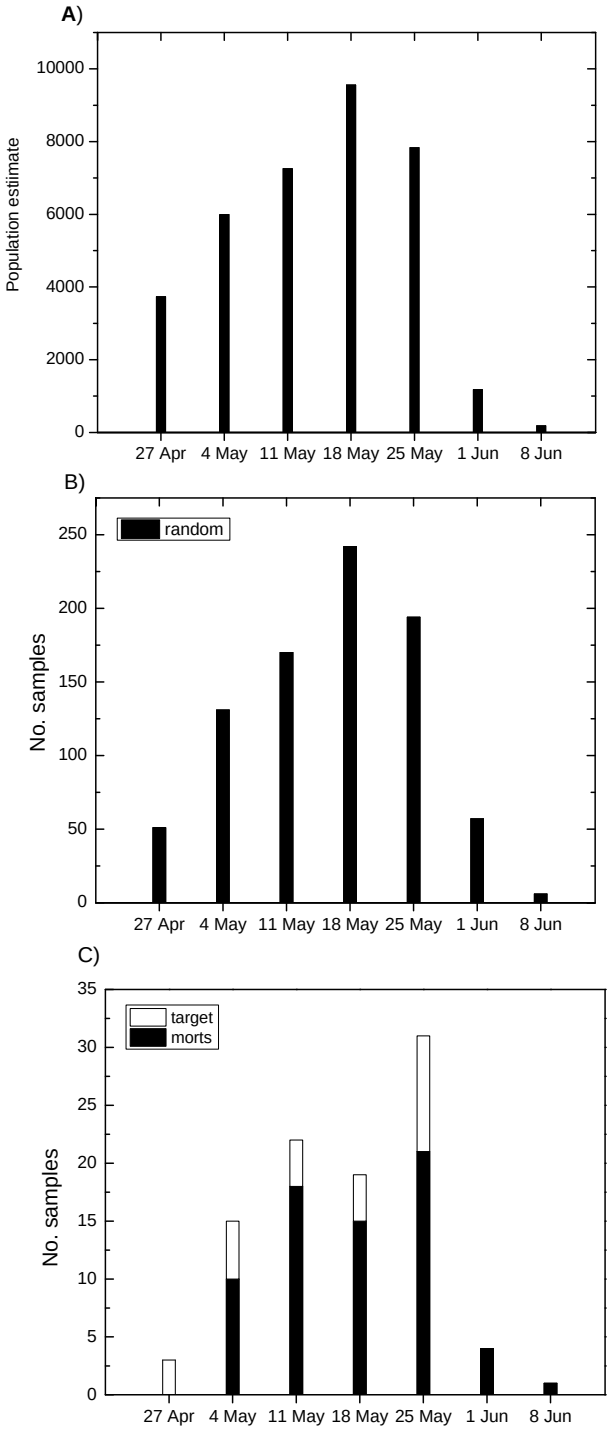

Figure 4. A) Weekly population estimate of juvenile downstream migrants (JDM) and number of tissue samples selected for DNA analysis from JDM collected from B) random sample, and C) morts and targeted samples in the Alouette River in 2011.

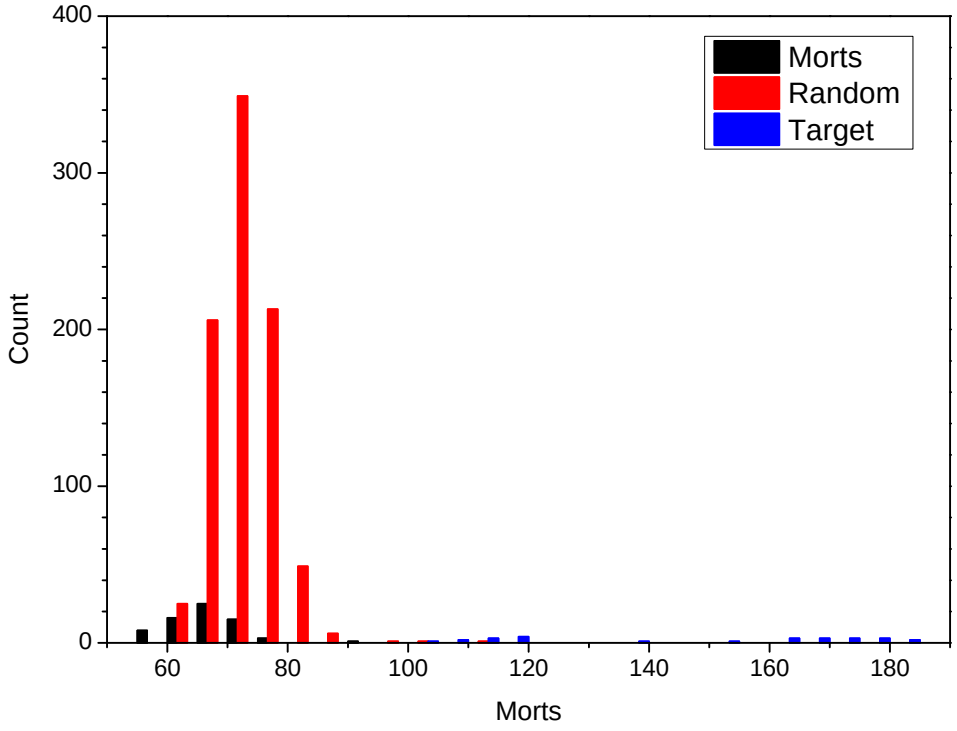

Figure 5. Size distribution of the juvenile downstream migrants (JDM) in the Alouette River in 2011 that were selected for DNA analysis (n=946).

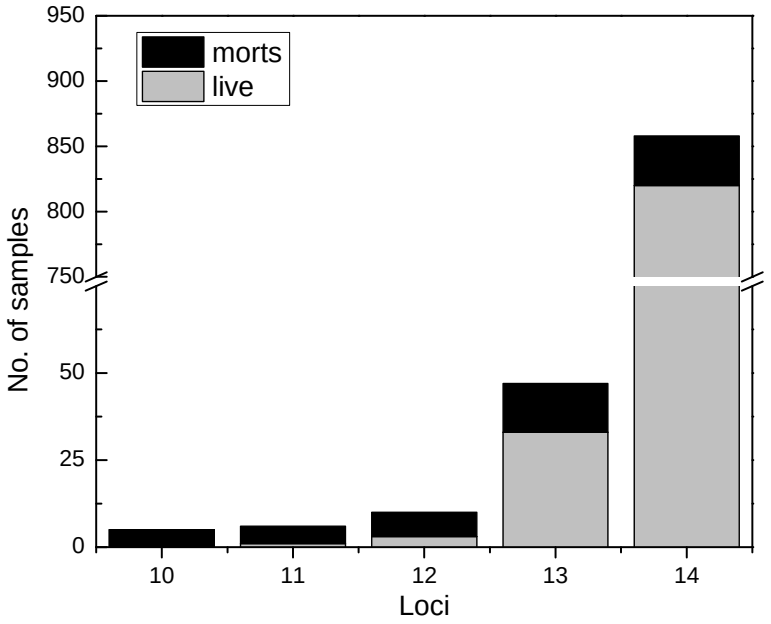

Figure 6. Number of juvenile downstream migrants (JDM) caught in the Alouette River in 2011 that were assigned to a brood year (2008 or 2009) and genotyped with no missing values at 10,11,12,13 and 14 loci.

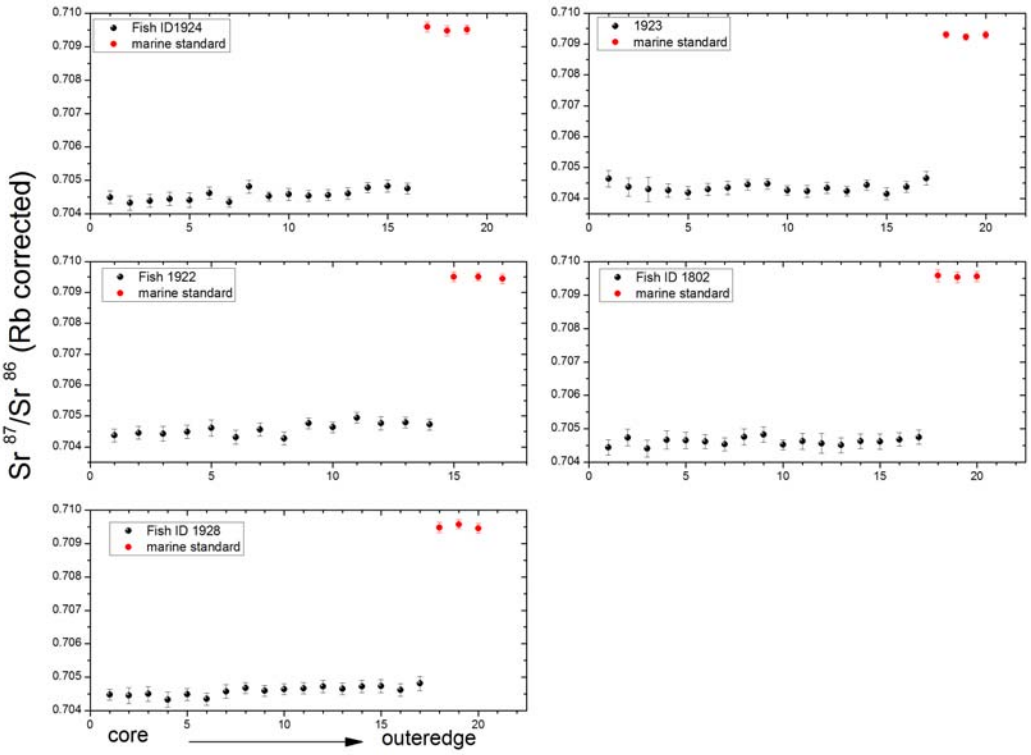

Figure 7. Mean (±1 standard error, SE) ratio of stable isotopes of $^{87}Sr/^{86}Sr$ in the core to the outeredge e of the otolith of 5 JDM that had a perfect match with an AUM as a single parent (black circle) and in marine standard (red circle).

Appendix 1. Supplementary data on 2010 downstream migration

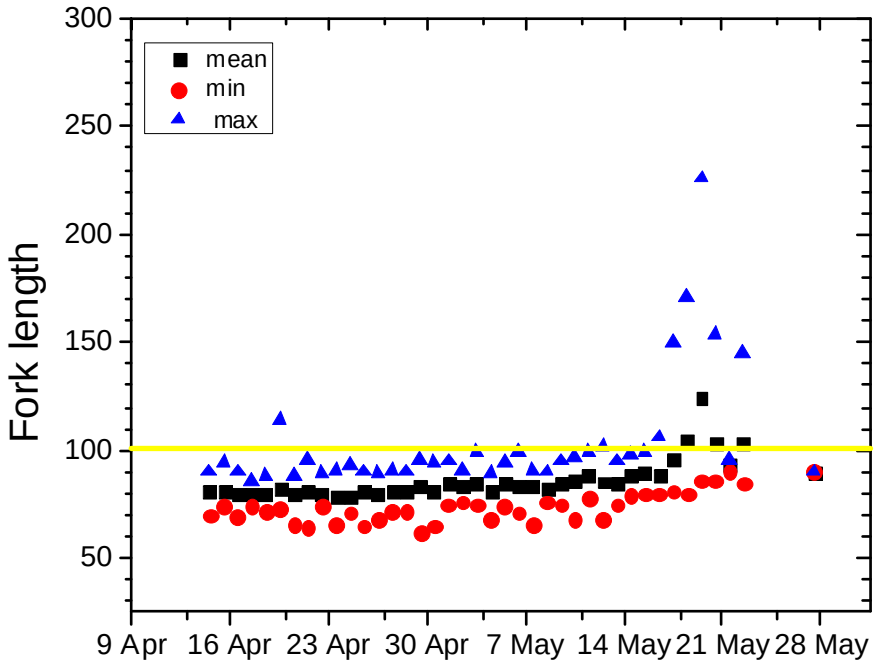

Figure A1.1. Daily statistics of the fork length (mm) of the 2010 juvenile downstream migrants (JDM) in the Alouette River selected at random (Data provided by Megan Mathews, LGL Ltd.).

Appendix 2. Parental Assignments for JDM from brood years 2009 and 2008

COLONY

COLONY is a sibling relationship reconstruction program that partitions progeny into full-sibling and half-sibling relationships that are statistically most probable (Jones and Wang 2009). Parental genotypes are simulated based on information about allele frequency and typing error (Wang 2004). The sibling groups and associated parental genotypes required to explain the sibling groups are then used to estimate the number of unsampled ('uncollected') parents that produced the progeny. The version (1.2) of COLONY that we used cannot assign individual progeny to specific parents.

2009 brood year: The genotyped sample of 896 1-year-old JDM collected in the Alouette River in 2011 were progeny of parents spawning in 2009. COLONY partitioned this sample into 531 full-sib families nested within 119 half-sib families. This result suggests that 650 parental genotypes, likely comprising at least 531 females and 119 males, contributed to the production of the 896 JDM.

2008 brood year: A total of 902 genotyped JDM could be attributed to spawners returning in 2008, by combining the sample of 888 1-year-old JDM collected in 2010 with the 14 2-year-old JDM collected in 2011. COLONY partitioned this sample into 498 full-sib families nested within 124 half-sib families. This result suggests that 622 parental genotypes, likely comprising at least 498 females and 124 males, contributed to the production of the 902 JDM.

PASOS

To assign progeny to specific parental pairs, we used PASOS (Duchesne et al. 2005) which begins by identifying parents among those collected that could best account for the genotype of each progeny. In the case of open systems (which PASOS is designed for), the initial parental allocation algorithms often include false parents which are then eliminated if allelic deviations exceed a user-defined error tolerance called the 'maximal offset tolerance' (MOT). For example, if MOT is restricted to 1 and a

progeny has an allele with 33 motif repeats at a particular locus, PASOS would not eliminate a potential parent having 32, 33, or 34 motif repeats, but would eliminate a potential parent with 31 or fewer, or 35 or more motif repeats. PASOS can handle non-sexed parents, but requires genotypes without allelic dropouts (no missing alleles).

If none of the collected parents is considered an adequate match, progeny genotypes are allocated to either one or two missing parents (i.e., parents that are inferred to be uncollected (UC)). The allocation rate is defined as the number of allocations to collected parents as a proportion of the number of possible allocations (collected + uncollected). Allocation success declines dramatically as the proportion of uncollected parents increases because the progeny from uncollected parents are increasingly misallocated to collected parents ('overallocation'). If genotyping error is negligible, overallocation could be avoided by examining more loci, but in practice, genotyping error rates accumulate over loci and constrain the improvement that can be achieved. Thus, graphing the estimated allocation rate against the cumulative number of loci examined (an 'allocation rate curve') provides a way to improve estimates of the number of uncollected parents (e.g., Figure A2.1). As more loci are examined, the estimated allocation rate usually drops sharply to a break point and then declines asymptotically to a stable level that corresponds to the best estimate of the true allocation rate. To assess 'correctness,' an allocation curve is also derived from simulations based on the genotypes of the progeny and collected parents, and on the estimated total number of parents (collected and uncollected parents). A tight fit between the two allocation curves implies that parents have been correctly identified, and that one can proceed with the parental allocations. This condition is shown in Figure A2.1.

2009 brood year: With MOT set to 0, PASOS generated an allocation rate curve that indicates 503 uncollected parents (Figure A2.1, Table A2.1). The correctness rate was estimated at 0.57 and 5.9% of the JDM were assigned to collected parents (i.e., the AUM spawning in 2009) (Table A2.1). Fifty genotype allocations were made to 11 AUM with 96% (48/50) to a single parent and 4% (2/50, progeny ID 798) to a parent pair (ID 690 and 692) (Figure A2.2a, Table A2.1). It is worth noting that parent ID 688 was assigned nearly a third of all the 48 allocated JDM (Figure A2.2b). With MOT set to 1 the correctness level shrank to 0.22, with ~90% of the allocations going to a single parent and about 10% to 14 parental pairs (Table A2.1).

2008 brood year: With MOT set to 0, PASOS generated an allocation curve that reached an equilibrium that indicates 691 uncollected parents (Figure A2.3, Table A2.1). The correctness rate was calculated as 0.52 with 13.9% (106/758) of the JDM assigned to a collected parent (AUM spawning in 2008). The 106 allocations were to 37 AUM with 92.5% (98/106) to a single parent and 7.5% (8/106) to 4 parental pairs (Table A2.1). A pedigree and the number of progeny assigned to each AUM are illustrated in Figure A2.4a and b. With MOT set to 1, PASOS allocated 247 progeny genotypes to 45 collected parents at a correctness value of 0.29; of these, 82.2% (203/247) were assigned to single parents and 17.8% (44/247) to parental pairs.

CERVUS

CERVUS assigns parentage by exclusion after accounting for genotyping error and incomplete sampling (Marshall et al. 1998). If at least one of the two alleles at each locus in an individual progeny matches corresponding alleles in the genotypes of two potential parents, the parental pair is said to be a perfect match. However, it is possible for both potential parents to perfectly match the progeny when they are considered separately, but for a mismatch to occur when both parents are considered simultaneously. For example, a ‘trio mismatch’ occurs when both parents share the progeny’s first allele at a locus, but neither share the progeny’s second allele, which then cannot be accounted for. CERVUS records the number of loci at which trio mismatches occur, which provides an index of confidence in parentage assignments. In addition, every parent-progeny match is given a likelihood of transmission for each locus, taking into account genotyping error rate (here set at 0.02) and allele frequencies calculated from the genotypes provided. This likelihood is the probability that the candidate parent is the true parent, divided by the probability that a randomly-generated parent is the true parent. The likelihoods at each locus are multiplied together and the natural logarithm is taken to produce a logarithm of the odds (LOD) score (Marshall et al. 1998). The most likely parent is identified as the one with the most positive LOD score and individuals with negative LODs are excluded as parents. A confidence level is also estimated for each parental assignment based on simulated parentage analyses in which uncollected parental genotypes are generated from allele frequencies (calculated from the genotypes provided), and the specified proportion of parents that were collected (which we estimated using COLONY and PASOS).

Assignment success deteriorates as the proportion of uncollected parents increases, and more quickly with CERVUS than PASOS (Duchesne 2005). Note that in PASOS, parental pairs are eliminated from consideration by the MOT in the individual parent-progeny matches of each pair. A parental pair identified in PASOS with MOT set at 0 does not necessarily indicate that the progeny has inherited both alleles at each locus from one of the parents (i.e., does not preclude a trio mismatch). Thus, PASOS and CERVUS complement one another in the way they accommodate restricted error tolerances; PASOS focuses on the offset tolerances that preclude potential matches, whereas CERVUS focuses on the number of loci that preclude potential matches.

2009 brood year: CERVUS found that no parental pair of AUM could be perfectly matched (trio mismatch of 0 at all 14 loci) to any of the 896 JDM from spawning in 2009 (Table A2.2). However 11 of the 15 AUM could be perfectly matched to 50 of the JDM on a single parent basis (Table A2.2). Of these 50 matches, AUM ID687 was assigned parentage to JDM ID988 and AUM ID688 to JDM ID1419 with > 95% confidence (Figure A2.5a). Additionally, despite having a trio mismatch of 1, CERVUS assigned one parental pair (AUM ID687 and AUM ID694) to JDM ID988 with 86% confidence (Table A2.3a). Lastly, 15 AUM could be matched as a single parent with 286 JDM allowing one pair mismatch of 1 in 14 loci (Table A2.2).

2008 brood year: Again CERVUS found that no pair of AUM could be perfectly matched to any of the 902 JDM from spawning in 2008. CERVUS did, however, find single-parent matches between 37 AUM and 134 JDM with varying levels of confidence (Figure A2.5b); single-parent matches to 6 AUM (ID6, ID20, ID21, ID34, ID42 and ID46) were assigned a confidence level of > 95% (Figure A2.5b). Allowing a single trio mismatch in 14 loci generated 5 possible matches to parental pairs of AUM, but only one of these (AUM ID41 x AUM ID6 assigned to JDM ID128_695) had a high confidence level (53%) (Table A2.3b).

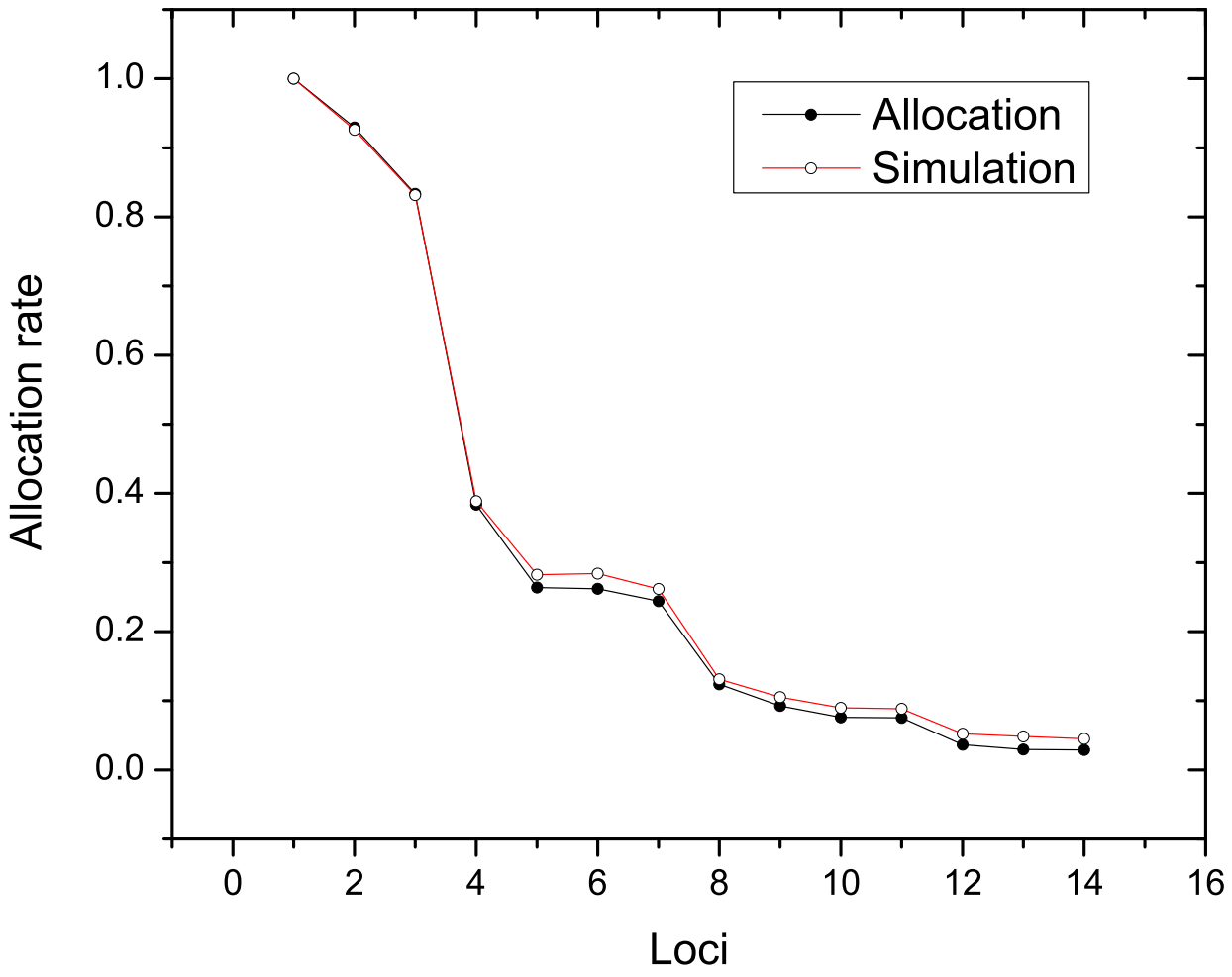

Figure A2.1. Allocation rate CLS (cumulative sequence of sets of loci) curve (closed circles) based on 828 juvenile downstream migrants (JDM) in the Alouette River in 2011 and 15 non-sexed 2009 adult upstream migrants (AUM) transplanted into Alouette Reservoir. This analysis included only genotypes with no missing alleles at 14 loci. The open circles indicate the corresponding curve based on simulations with 830 JDM and 15 AUM. The maximum offset tolerance was set to zero (MOT 0).

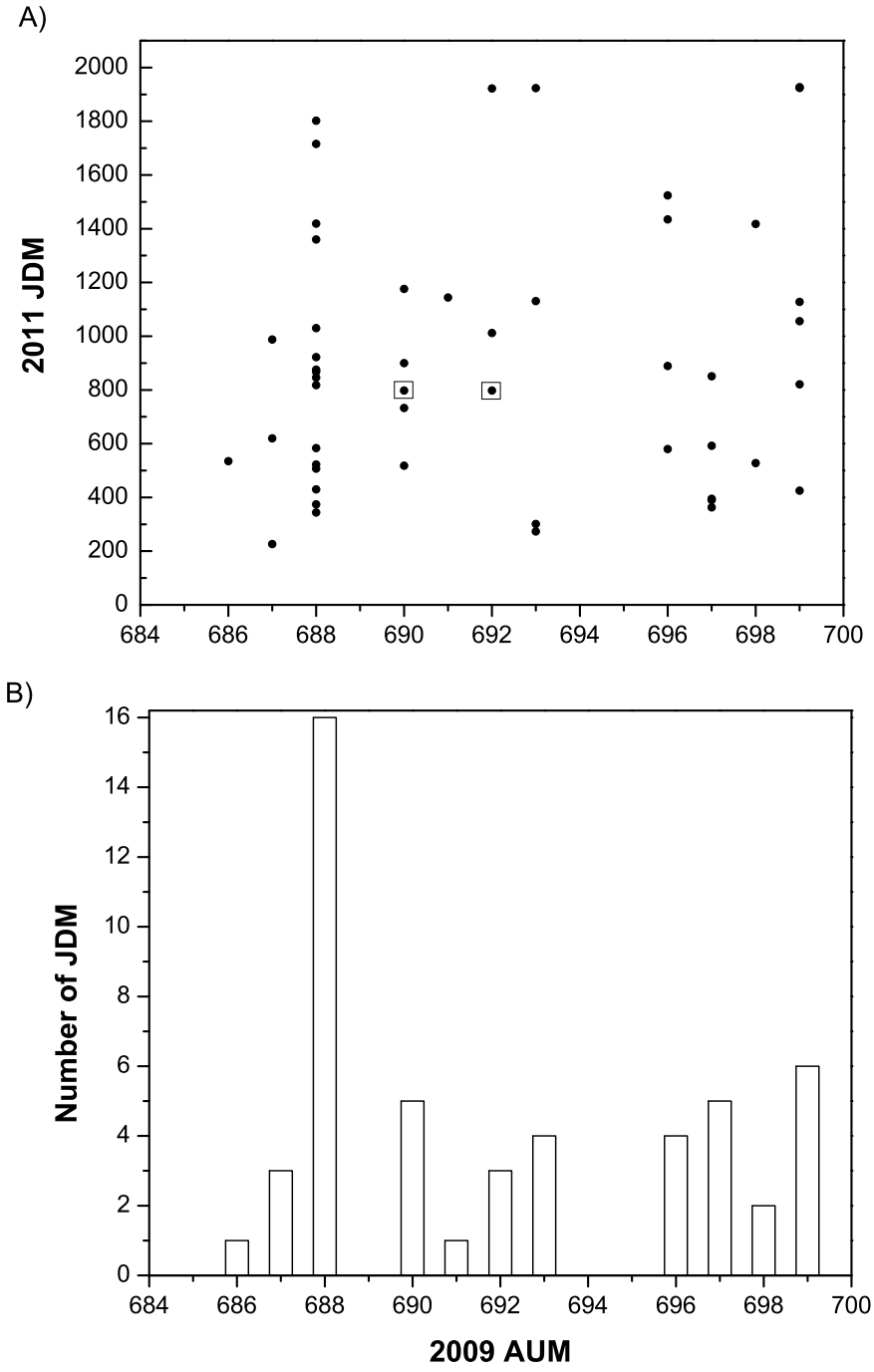

Figure A2.2. Results from PASOS with MOT= 0 showing pedigree based on the 50 allocations of JDM) to 11 AUM in 2009 (ID given on x-axes). A) juvenile assignments (ID given on y-axes) including one to a parental pair of AUM (open squares) and 48 to single parents (closed circles); B) distribution of juvenile allocations across AUM.

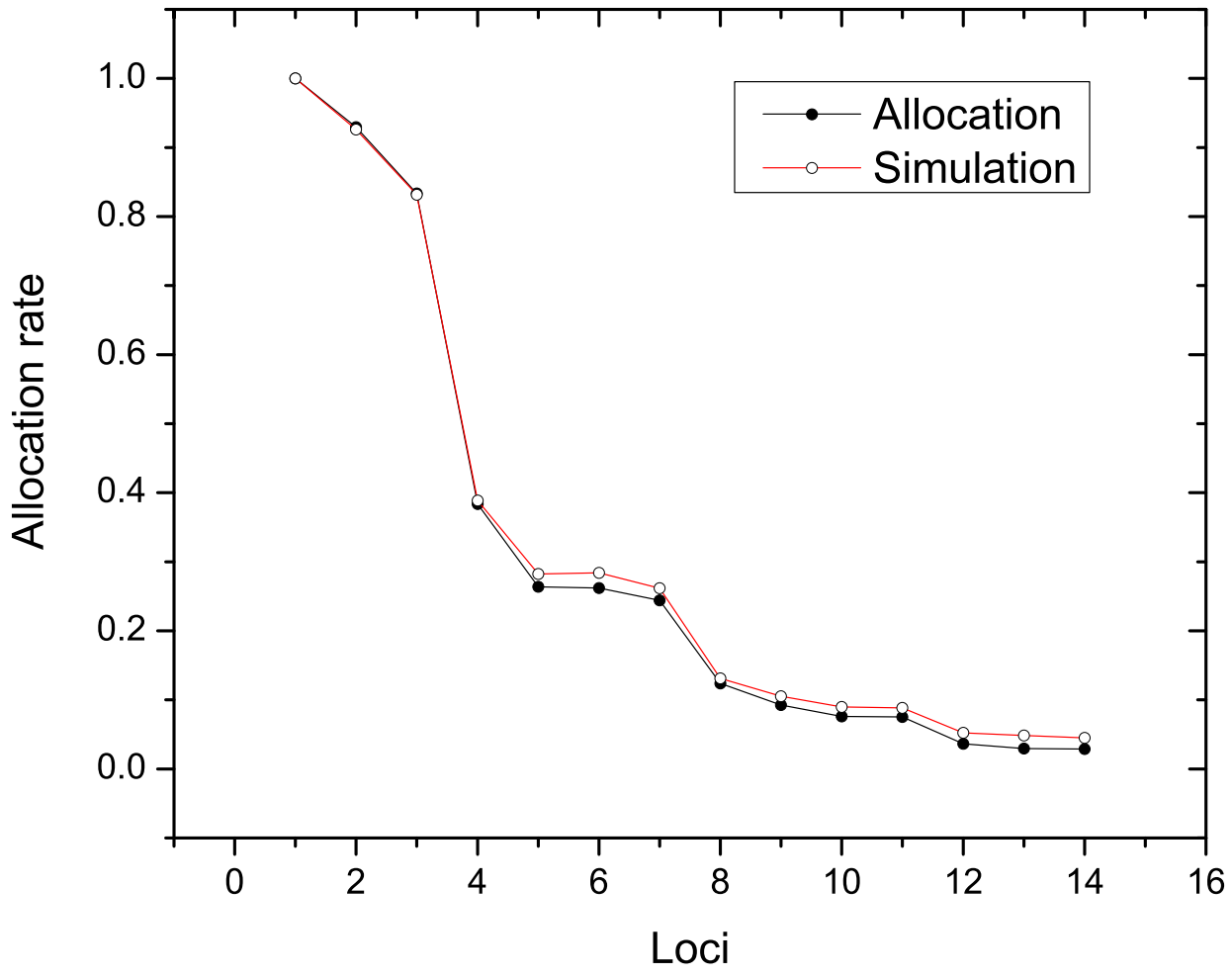

Figure A2.3. Allocation rate CLS (cumulative sequence of sets of loci) curve (closed circle) based on 758 juvenile downstream migrants (JDM) in the Alouette River in 2010 and 52 non-sexed 2008 adult upstsream migrants (AUM) transplanted into Alouette Reservoir. This analysis included only genotypes with no missing alleles at 14 loci. The open circles indicate the corresponding curve based on simulations with 830 JDM and 52 AUM. The maximum offset tolerance was set to zero (, i.e., MOT **0).**

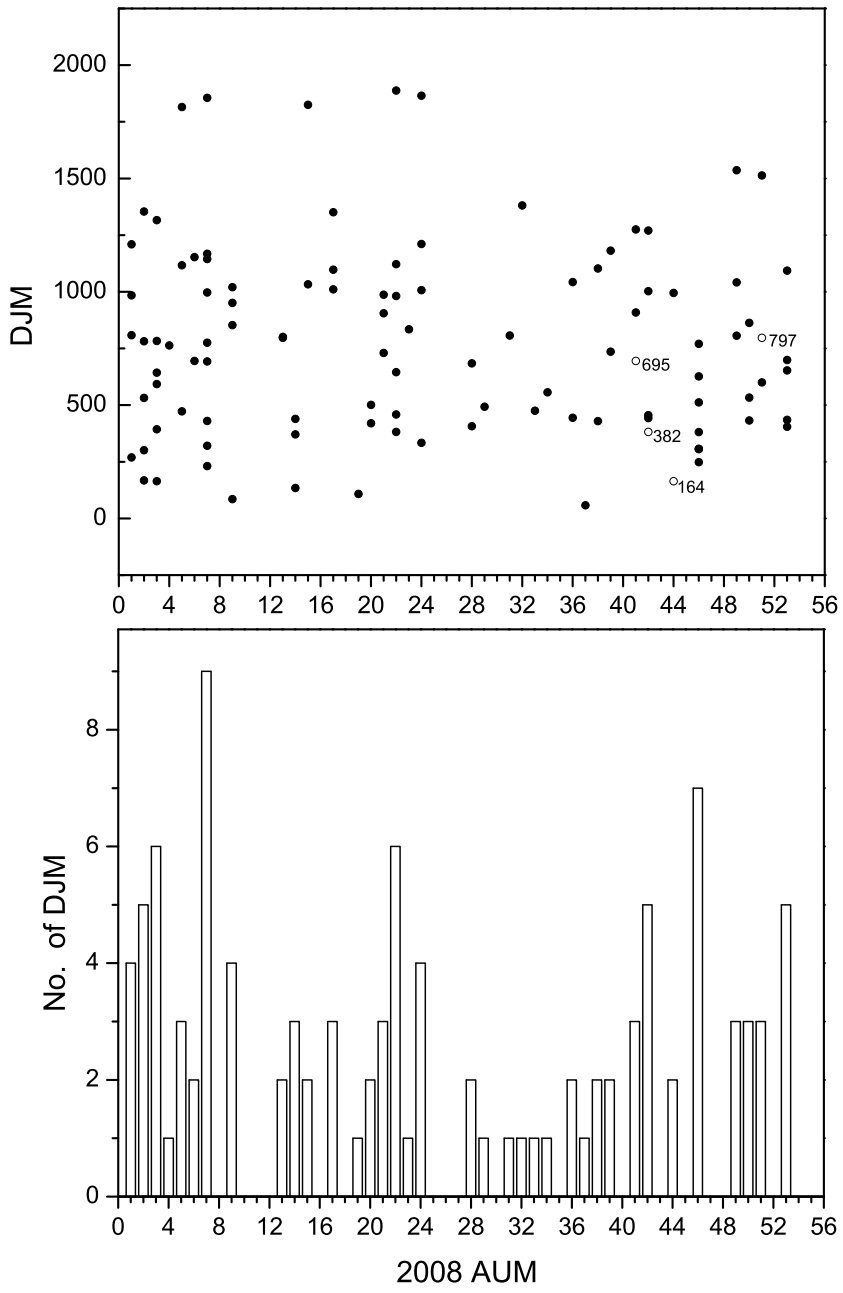

Figure A2.4. Results from PASOS with MOT= 0 showing a) pedigree based on the 106 allocations of JDM (ID given on y-axes) to 36 AUM in 2008 (ID given on x-axes). A) juvenile assignments including four JDM (695,797,382, and 164) to four parental pairs of AUM (6-41,13-51,22-42,3-14) (open circles) and 98 to single parents (closed circles) ; and B) distribution of juvenile allocations across AUM.

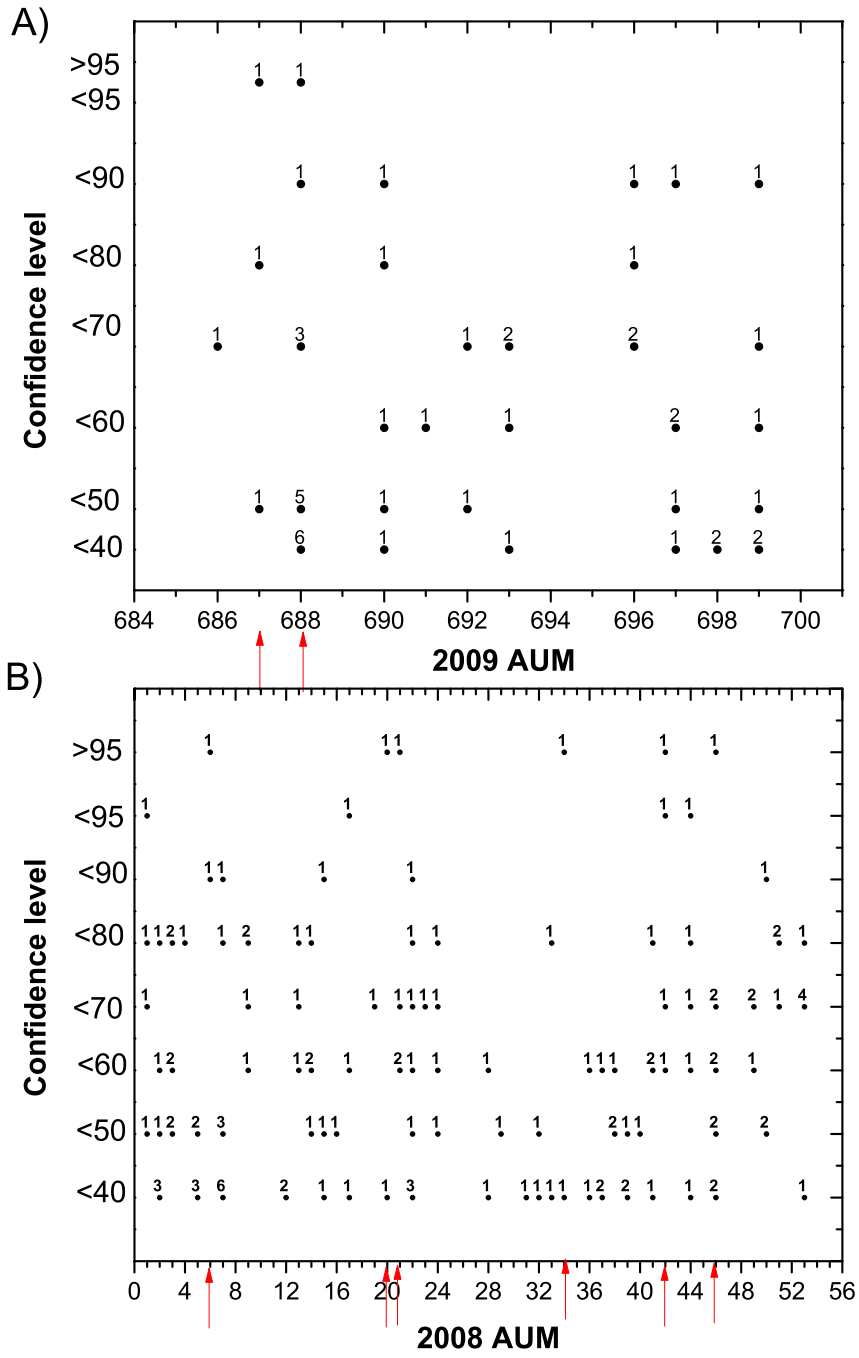

Figure A2.5. Results from CERVUS showing pedigree based on the confidence level (given on y-axes) of A) 50 allocations of JDM to 11 AUM in 2009 (ID given on x-axes), and B) 134 allocations of JDM to 39 AUM 2008. The numbers in the figure refer to the number of JDM. Arrows highlight the AUM assigned to JDM with greater than 95% confidence level.

Brood Year	No. of AUM	No. of UC	No. of JDM	MOT	***No. of allocations***				
					No. of AUM allocated	*to single parent*	to parental pair	*Correctness*	*Total*
2009	15	503	828	0	*11*	*48*	1	*0.57*	*50*
	15	119		1	15	128	14	0.22	186
2008	52	691	758	0	*37*	*98*	4	*0.52*	*106*
	52	267		1	45	203	22	0.29	247

Table A2.1. Results of analyses with PASOS for MOT set to 0 or 1 by brood year. Columns are number of adult upstream migrants (AUM), estimated number of uncollected parents (UC), number of juvenile downstream migrants (JDM) that are allocated to single or paired parents, and the level of correctness of the allocations.

Brood Year	No. of AUM	No. of JDM	No. of MM	***No. of allocations***			
				No. of AUM allocated	*to single parent*	to parental pair	*Total*
2009	15	896	0	*11*	*50*	0	*50*
	15	896	1	15	288	1	290
2008	53	902	0	*39*	*134*	0	*134*
	53	902	1	52	712	5	722

Table A2.2. Results of analyses with CERVUS for a mismatch (MM) of 0 or 1 by brood year. Columns are the number of adult upstream migrants (AUM), number of juvenile downstream migrants (JDM) that are allocated to single or paired parents, and the total number of allocations.

JDM ID	AUM 1	Pair Loci MM	CL 1	AUM 2	Pair Loci MM	CL 2	Trio Loci MM	Trio CL
273**	693	0	74.8	697	4	<28.5	4	<20
458**	688	1	<28.5	696	6	<28.5	6	<20
798*	690	0	86.5	692	3	71.9	3	<20
984**	688	1	<28.5	693	4	<28.5	4	<20
988**	687	0	>95	694	1	53.8	1	86
1092**	690	2	<28.5	698	7	<28.5	7	<20
1309**	693	2	<28.5	700	6	<28.5	6	<20
1504**	688	2	<28.5	699	4	<28.5	4	<20
1908**	691	1	32.5	697	6	<28.5	6	<20
858**	690	2	<28.5	698	6	<28.5	6	<20
1063**	688	2	<28.5	691	5	33.4	5	<20
1144**	688	2	<28.5	691	6	67.5	6	<20
1183**	688	1	<28.5	694	7	44.8	7	<20
1556**	690	3	<28.5	696	7	<28.5	7	<20

Table A2.3a. Paired parentage assignment of 2009 AUM (AUM1 and AUM2) to 14 juvenile downstream migrants (JDM) including 14 pairs from PASOS that had a MOT of 0 or 1 and 1 pair from CERVUS that had a trio mismatch of 1. Statistics from CERVUS included are: pair loci mismatching (Pair Loci MM), confidence level of the individual parent's assignment to the JDM (CL 1 and CL 2), the number of trio mismatches (Trio Loci MM) and confidence level of the trio assignment (Trio CL). Juvenile downstream migrants matched with a MOT of 0 and 1 are shown with a * or **, respectively, and are bolded for a trio mismatch of 1.

.

JDM	AUM 1	Pair Loci MM	CL 1	AUM 2	Pair Loci MM	CL 2	Trio Loci MM	Trio CL
128_695*	41	0	64	6	0	>95	1	53
13**	42	2	<5	9	1	15	3	<5
58**	15	1	11	37	0	49	3	<5
121**	22	1	<32	41	1	<32	4	<5
164*	3	0	80	44	0	90-95	2	18
323	28	1	26	38	1	42	2	12
382*	22	0	89	42	0	74	2	10
405**	46	1	9	53	0	82	1	21
432	14	1	16	31	1	35	1	23
481**	15	1	27	22	1	31	3	<5
483**	1	1	25	37	3	<5	4	<5
533**	33	1	37	50	0	89	3	<10
543**	14	1	38	53	1	13	3	<5
574**	38	1	40	5	3	<5	3	<5
593**	19	1	<32	3	0	56	5	<5
651**	24	1	25	46	2	<5	5	<5
797*	13	0	72	51	0	83	3	<10
810**	2	2	<5	44	1	40	2	<10
863**	44	1	<5	50	0	61	4	<5
897	10	1	64	46	1	12	1	27
984**	1	0	61	26	1	28	4	<5
1122**	2	2	<32	22	0	45	4	<5
1144	28	1	21	7	0	90	1	34
1153**	30	1	11	6	0	90	2	<10
1862**	28	1	45	3	1	11	3	<5

Table A2.3b. Paired parentage assignment of 2008 AUM (AUM 1 and AUM 2) to 25 juvenile downstream migrants (JDM) including 22 pairs from PASOS that had a MOT of 0 or 1 and 5 pairs from CERVUS that had a trio mismatch of 1. Statistics from CERVUS included are: pair loci mismatching (Pair Loci MM), confidence level of the individual parent's assignment to the JDM (CL1 and CL2), the number of trio mismatches (Trio Loci MM) and confidence level

of the trio assignment (Trio CL). Juvenile downstream migrants matched with a MOT of 0 and 1 are shown with a * or **, respectively, and are bolded for a trio mismatch of 1.