Biological Conservation 192 (2015) 74–81

Contents lists available at ScienceDirect

Biological Conservation

journal homepage: www.elsevier.com/locate/bioc

The case of the endangered Kootenai River white sturgeon (*Acipenser transmontanus*) highlights the importance of post-release genetic monitoring in captive and supportive breeding programs

A. Schreier [a,*], S. Stephenson [b], P. Rust [c], S. Young [d]

[a] Genomic Variation Laboratory, Department of Animal Science, University of California Davis, One Shields Avenue, Davis, CA 95616, USA
[b] Ministry of Forests, Lands and Natural Resource Operations, #401-333 Victoria St., Nelson, BC V1L 4K3, Canada
[c] Idaho Department of Fish and Game, 2885W Kathleen Avenue, Coeur d'Alene, ID 83815, USA
[d] Kootenai Tribe of Idaho, 242 Hatchery Road, Bonners Ferry, ID 83805, USA

ARTICLE INFO

Article history:
Received 2 April 2015
Received in revised form 14 July 2015
Accepted 7 September 2015
Available online xxxx

Keywords:
Captive breeding
Genetic monitoring
Post-release mortality
Genetic diversity

ABSTRACT

The success of captive and supportive breeding programs is often determined by abundance criteria but it is also necessary to consider genetic characteristics of reintroduced or supplemented populations as genetic diversity loss can reduce population viability. Genetic analysis of the parent pools is often used to determine whether captive or supportive breeding programs conserve adequate levels of genetic diversity and maximize the effective population size (N_e). This practice assumes that released cohorts reflect the genetic characteristics of parents. Here we provide a case study of how post-release mortality can alter the genetic composition of released cohorts in a supportive breeding program for an endangered population of white sturgeon. Data from ongoing genetic monitoring of wild broodstock in the Kootenai River white sturgeon conservation aquaculture program are combined with multi-year post-release abundance monitoring of captive bred juveniles to reveal high variability in recapture among families. We found that genetic monitoring of broodstock used in supportive breeding overestimates genetic diversity conservation in most year classes due to differential post-release mortality among families. N_e was reduced in most year classes when post-release mortality was considered due to reduced parental representation in released cohorts. Although rarely performed, our results indicate that post-release genetic monitoring is necessary to accurately characterize the genetic composition of released cohorts altered by post-release mortality and should be considered when designing a captive or supportive breeding program.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

Captive breeding and supportive breeding programs have been used to create, enhance, sustain, and restore endangered populations of plants, fish, and wildlife for decades. Captive breeding programs use a pool of parents, isolated in captivity from wild populations, to produce offspring ultimately intended for release into the wild (McLean et al., 2007). In contrast, supportive breeding programs use wild adults to produce offspring that will be reared in captivity and released to supplement vulnerable populations. Although success or failure of these programs is often judged by abundance criteria (i.e. number or size of new populations established, abundance increases in supplemented populations), it is equally important to consider the genetic characteristics of reintroduced or supplemented populations (Laikre et al., 2010; IUCN/SSC, 2013). Genetic diversity can affect the viability and adaptive potential of a wild population (Reusch et al., 2005; Zeisset and Beebee, 2012) as well as influence the behavior and abundance of other species in its community (Bailey et al., 2004; Crutsinger et al., 2006). Mating between close relatives, which may occur in small captive breeding programs, can produce highly homozygous inbred offspring exhibiting lowered fitness (Saccheri et al., 1998; Hedrick and Kalinowski, 2000; Woodworth et al., 2002). Therefore maintaining adequate levels of genetic diversity and minimizing inbreeding in both captive and wild populations is a high priority for captive and supportive breeding programs.

Captive and supportive breeding programs are now designed to minimize the genetic risks associated with genetic diversity loss and inbreeding depression. When possible, these programs include numerous, unrelated parents to preserve genetic diversity and minimize inbreeding (Theodorou and Couvet, 2004). Crosses and family release sizes can be designed to maximize effective population size, N_e, or effective number of breeders, N_b (Fiumera et al., 2004; Cooper et al., 2009), thereby protecting the adaptive potential of the captive population and the wild population into which releases will occur. Equalizing

* Corresponding author.
E-mail addresses: amdrauch@ucdavis.edu (A. Schreier), Sarah.Stephenson@gov.bc.ca (S. Stephenson), pete.rust@idfg.idaho.gov (P. Rust), young@kootenai.org (S. Young).

http://dx.doi.org/10.1016/j.biocon.2015.09.011
0006-3207/© 2015 Elsevier Ltd. All rights reserved.

contributions from parents can also reduce the risk of adaptation to captivity, or domestication, due to relaxed selection pressure or unintentional positive selection on captive reared cohorts (Waples, 1999; Frankham et al., 2000; Chargé et al., 2014). Unequal parental contribution is a particular concern for fish hatchery programs where released cohorts could contain millions of individuals originating from a few families, leading to "genetic swamping" of wild populations with genetically homogenous individuals (Ryman and Laikre, 1991). Indeed, Araki et al. (2007, 2008) documented fitness reductions in wild populations due to introduction of maladaptive alleles from hatchery releases.

Key to minimizing genetic risks of captive and supportive breeding programs is conducting effective genetic monitoring of both the captive and in situ elements of the programs (Seddon et al., 2014a). The in situ component of a captive or supportive breeding program consists of cohorts that have been released in reintroduction or supplementation events. Careful control of matings and monitoring of genetic characteristics of captive bred progeny prior to release can prevent inbreeding and ensure adequate founder representation; however differential survival of individuals due to natural selection or stochastic events in the wild environment may lead to changes in the genetic composition of released cohorts over time. Genetic characteristics of released individuals at the time of recruitment into the adult population may be quite different from characteristics prior to or just after release due to differential post-release mortality (IUCN/SSC, 2013).

Although numerous studies have genetically characterized the captive elements of conservation breeding programs (e.g. Tzika et al., 2008; Ivy et al., 2009; Cooper et al., 2009; Drauch Schreier et al., 2012; Saltzgiver et al., 2012; Seddon et al., 2014b), relatively few studies have examined genetic characteristics of released cohorts over time. Therefore the magnitude of genetic diversity loss due to post-release mortality in captive and supportive breeding programs is poorly understood.

Here we present a case study that illustrates how post-release mortality can alter the genetic composition of released cohorts. We use as a model the Kootenai Tribe of Idaho (KTOI) conservation aquaculture program (CAP) for the endangered Kootenai River white sturgeon population that has operated from 1992 to the present day. Supportive breeding through conservation aquaculture is an important tool to sustain the Kootenai River white sturgeon population as it has experienced virtually no recruitment in over thirty years (Duke et al., 1999; Paragamian et al., 2005). Genetic monitoring of the captive component of the Kootenai River white sturgeon CAP has been ongoing since 2002 and has included tracking broodstock (wild origin parents) genetic diversity over time and developing of a suite of microsatellite markers for parentage analysis (Drauch Schreier et al., 2012). This has led to development of an extensive tissue and genotype archive for the population that includes nearly all broodstock used in the CAP since 2002.

In addition to genetic monitoring of broodstock, post-release monitoring of captive-reared white sturgeon juvenile abundance is conducted by the KTOI, Idaho Fish and Game (IDFG), Montana Fish Wildlife and Parks (MFWP), and British Columbia Ministry of Forest, Lands, and Natural Resources Operations (BCFLNR) scientists. All juvenile encounters are recorded in a recapture database maintained collaboratively by the IDFG, BCMFLNR, and KTOI that also archives family identification for tagged individuals and year class information for all released juveniles. To characterize post-release mortality within and among families and determine whether released cohorts maintain the genetic characteristics of their wild adult parents, we combine data from our genotype archive, the recapture database, and parentage analysis of non-tagged juveniles captured in 2012 and 2013 by IDFG and BCFLNR. We use the number of recaptures from each family as a proxy for the family's persistence in the wild population after release from the CAP. Our objectives were to 1) characterize variance in recapture among CAP families, 2) compare genetic diversity of released cohorts to estimates from CAP broodstock, and 3) determine whether relatedness between parents contributes to variability in recapture among families. We found significant variability in post-release encounters among families both within and among years, which suggests that genetic monitoring focused on captive components of conservation breeding programs may overestimate genetic diversity conservation.

2. Materials and methods

2.1. Study population

The Kootenai (spelled Kootenay in Canada) River, a tributary to the Columbia River, contains a small, declining population of white sturgeon that has been isolated from other white sturgeon populations in the Columbia Basin by Bonnington Falls for 10,000–12,000 years (Fig. 1). Listed as federally endangered in both the US and Canada (USFWS, 1994; DFO, 2007) the Kootenai River white sturgeon population currently consists of ~1000 wild adults (Beamesderfer et al., 2014). Threats to the Kootenai River white sturgeon population include land use changes, dyking and channelization of the Kootenai River between Bonners Ferry to Kootenay Lake, decreased water quality from agricultural and mining inputs, and ecological modifications to the Kootenai River from impoundment by Libby Dam, such as hydrograph inversion and reduced nutrient inputs (Duke et al., 1999; Paragamian et al., 2005). Recruitment failure in the Kootenai River white sturgeon population began during the 1950's to mid-1960's (Partridge, 1983; Paragamian et al., 2005); the CAP is a necessary stopgap measure until natural recruitment is restored. Although the specific causes of recruitment failure remain unclear, research to date suggests that egg and/or larval suffocation, predation, and/or other factors of early life mortality contribute to persistent recruitment failure (Kock et al., 2006).

The KTOI CAP operates on a gamete capture model (Crossman et al., 2011), where eggs and sperm are collected from wild adult broodstock either brought into the hatchery for spawning, in the case of females, or handled in the field, in the case of spermiating males. Eggs obtained from females in the hatchery are placed into bowls and mixed with milt collected from individual males in the field. Although each female may be mated with more than one male, sperm is not pooled so each bowl produces a full sibling family. Each full sibling family is hatched into a separate 8 ft. raceway and reared in a separate 8 ft. circular tank. From 1999–2014, a proportion of fertilized eggs was transported to the Kootenay Trout Hatchery (KTH) in British Columbia as a failsafe against equipment failure or other events that could lead to catastrophic losses of families at a single facility. For most families, individuals were released at ages 8–28 months (mean = 15 months), although from 2008–2012 the KTOI conducted "river releases" of large numbers of free embryos (2–4 days post-hatch) from a proportion of families produced each year. Individuals released at ≥30 g receive a passive integrated transponder (PIT) tag and all individuals are marked by a pattern of scute removal unique to their year of birth. Hatchery releases of the KTOI and KTH families are conducted within Kootenay Lake (river kilometer; rkm 75–120) and in the Kootenai River from Kootenay Lake upstream to rkm 307 (Justice et al., 2009). At the time of our analysis, more than 222,000 hatchery-reared progeny have been released from the CAP since experimental releases began in 1992 (Justice et al., 2009).

2.2. Sample collection

2.2.1. Kootenai River white sturgeon recapture database

We searched the IDFG Kootenai River white sturgeon recapture database (version updated 12/17/2013) for individual recapture events that could be assigned unambiguously to a full-sibling family via PIT tag. The database was filtered by year class to include recapture records from captive-reared juveniles produced from 1995–2012. We counted the number of unique progeny recaptured for each full-sibling family. Only a single recapture event was recorded for individuals recaptured more than once. In six instances, half sibling families were combined

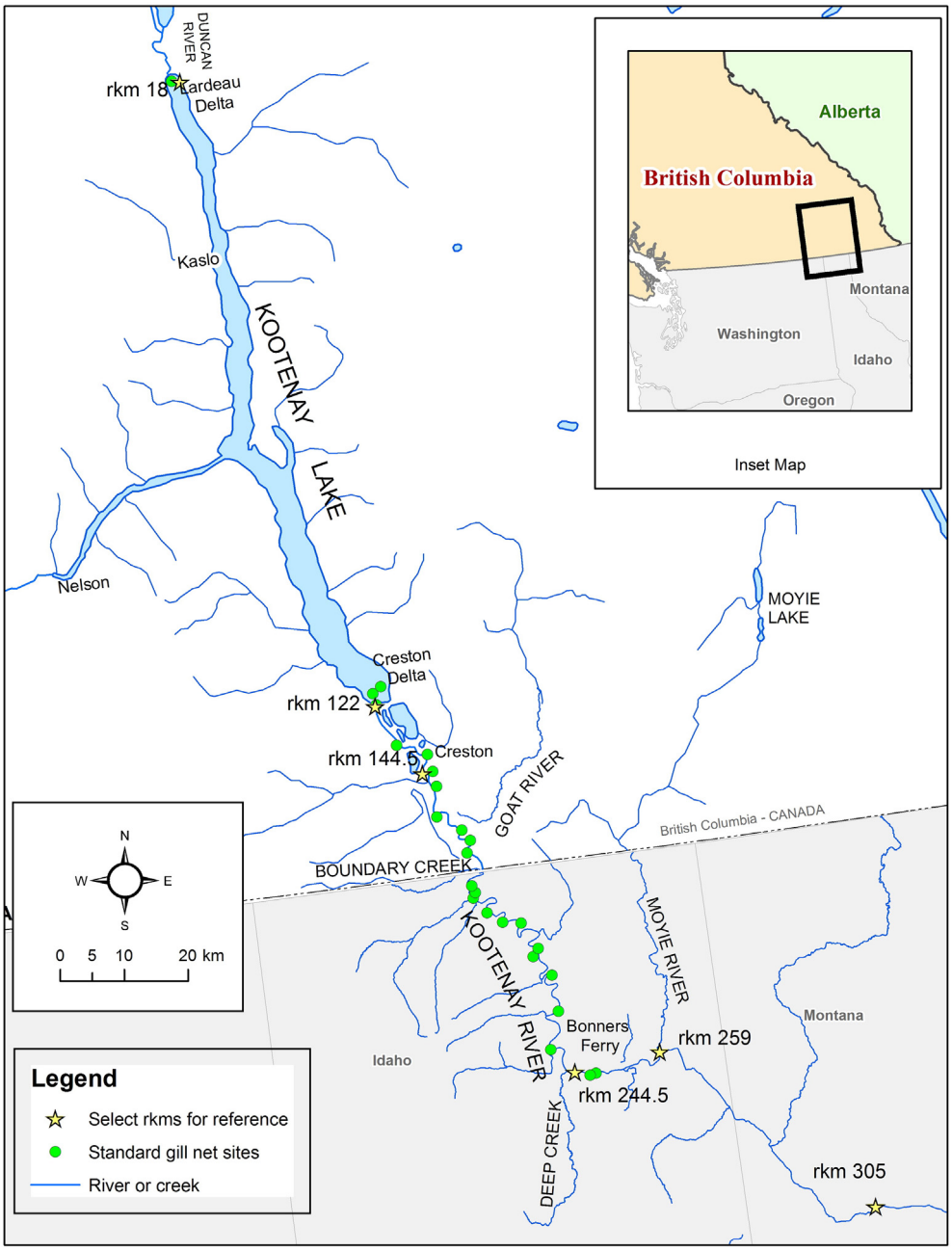

Fig. 1. Juvenile sampling locations within the Kootenai River white sturgeon range.

into single-family units for future analysis due to ambiguous maternity (N = 1) or paternity (N = 5). A total of 4432 juvenile recapture events were identified from the recapture database. The Kootenai River white sturgeon recapture database is curated by the IDFG and accessible after a free account registration at https://fishandgame.idaho.gov/ifwis/portal/opendata/2014-kootenai-river-white-sturgeon-capture-and-release-data.

2.2.2. Kootenai River white sturgeon juvenile sampling

Tissue was collected from non-PIT tagged juvenile white sturgeon encountered by monitoring in two different years so parentage assignment could identify their family of origin. Biologists from the IDFG and BCFLNR collected fin clips from scute-marked juvenile white sturgeon not possessing PIT tags during the summers of 2012 (N = 128) and 2013 (N = 140). A small proportion of these fin clips originated from unmarked juveniles (no scute marks; N = 13), either from "river releases" by the KTOI or the product of natural reproduction. The juveniles were collected as part of the annual juvenile gill net monitoring program that takes place in both Idaho and BC (see Stephenson et al., 2014 for study design). Biologists preferentially collected tissue from non-PIT tagged juveniles from the 2004–2006 year classes across all gill-netting sites (Fig. 1) because the majority of fish from those year classes were released without a PIT tag. All tissue samples were sent to the lab for genotyping and parentage analysis.

2.3. Molecular methods

DNA was extracted from fin tissue using a PureGene DNA extraction kit (Promega). We quantified DNA using a FLA 5100 fluorimager (Fujifilm), normalized each sample to 20 ng DNA concentration, and genotyped each individual at 17 microsatellite loci previously developed for parentage assignment in the Kootenai River white

sturgeon population (AciG 2, AciG 35, AciG 46, AciG 51, AciG 52, AciG 53, AciG 61, AciG 110, AciG 177, AciG 203, As015, Atr 105, Atr 107, Atr 109, Atr 1101, Atr 1173) using previously published PCR protocols (Drauch Schreier et al., 2012). Genotype data was collected on a Life Technologies (LT) ABI 3730xl Genetic Analyzer and allele calling was conducted in GeneMapper v 4.0 (LT). As white sturgeon are ancestral octoploids with polysomic inheritance (Rodzen and May, 2002; Drauch Schreier et al., 2011), microsatellite alleles were treated as present/absent dominant loci for all genetic analyses (Israel et al., 2004; Rodzen et al., 2004; Drauch Schreier et al., 2012).

2.4. Data analysis

2.4.1. Parentage analysis

We used the Fortran program Parent.exe (Rodzen et al., 2004), which implements the log-likelihood method of Gerber et al. (2000), to make parentage assignments with dominant genetic datasets. The statistic delta (δ) was used to provide a confidence threshold for each assignment (Marshall et al., 1998). We applied a threshold δ of 2.5 (Drauch Schreier et al., 2012), which meant that assigned parents must have been $\geq 10^{2.5}$ (316) times more likely to be the true parent than the next best parent for an assignment to be accepted for further analysis.

Individuals were grouped for parentage analysis by year class inferred from scute removal patterns. To account for possible errors in scute pattern interpretation, the first parentage analysis for each year class included as potential parents that year's broodstock as well as those from the previous and following years. For example, an individual with a scute removal pattern indicating it belonged to the 2005 year class initially would be analyzed with broodstock from 2004, 2005, and 2006. Unmarked individuals were analyzed with all broodstock used from 2002–2012 for which we had genotypes. Due to the high genetic similarity between adult Kootenai River white sturgeon, we had many cases where one parent could be assigned confidently while the other parent did not have an adequate δ value for assignment. We used our confident parent assignment to "anchor" that juvenile in a particular year class. In other words, if a juvenile could be confidently assigned to a sire in 2006, we re-analyzed parentage using only parents from the 2006 year class. This "anchoring" procedure was only used when the parent assigned confidently had been spawned only once in the CAP's history. In nearly all cases, the same parent pair was identified in both analyses and often the second analysis using fewer possible parents allowed us to exceed the δ threshold of confidence for both parents to make an assignment.

2.4.2. Variance in recapture among families

The total number of individual recaptures from each family was counted. Families created and released into the river as larvae were excluded from this analysis, as were families not surviving to release from the hatchery. Of the remaining families (N = 209), those not represented by recaptured individuals were given a score of 0. We evaluated variability in recapture among families using the Shannon–Wiener diversity index. The Shannon–Wiener diversity index H' measures evenness of recaptures among families within each year class using log base 10, scaled by the evenness statistic J' (Zar, 1999). The homogeneity measure J' scales the diversity among categories (families in a year class; H') to the maximum diversity possible given the number of categories considered (H' max). J' ranges from 0–1, with values near 0 reflecting high variability among categories (families) and values near 1 reflecting similarity between categories.

Finally, we wished to determine whether our sample size was adequate to detect all hatchery-reared families at large in the Kootenai River. We modified the R program Rare-sampler (Schreier and May, 2012) to take subsamples of increasing size from our recapture dataset so we could calculate the number of unique families detected in each subsample of size N. Re-sampling was conducted without replacement (1000 iterations/N) to match our approach for assigning recaptures to each family, considering individuals only once no matter how many times they were recaptured. The number of unique detections was plotted as a function of increasing sample size to illustrate the adequacy of the sampling effort. If the function reached an asymptote, adding more samples to our data likely would not detect many additional families. Failure to reach an asymptote would suggest we would likely detect more families if we increased our sample size.

2.4.3. Genetic diversity

To determine whether variable post-release mortality among families might affect our estimate of genetic diversity conservation for the Kootenai River white sturgeon CAP, we identified which broodstock in the 2002 and 2004–2009 year classes were represented by surviving offspring that had been at large ≥3 years. We limited our analyses in this way because 1) we had genotype data for nearly all broodstock used in 2002 and 2004–2009 and 2). Dinsmore et al. (2015) found survival for individuals age-3 and older was ~93%. Individuals at large for ≥3 years post-release are likely to survive to sexual maturity and have the opportunity to pass their genetic material to the next generation. By excluding individuals and year classes with a lower probability of survival, we avoid overestimating genetic diversity conservation.

We calculated the number of microsatellite alleles possessed by broodstock used in 2002 and 2004–2009 that had contributed offspring surviving ≥3 years post-release and compared this number to the total amount of genetic diversity represented by all broodstock used within and among those year classes. We used Wilcoxon signed rank tests (VassarStats) to compare numbers of alleles per locus between broodstock with surviving offspring and all broodstock, to test whether loss of families reduced genetic diversity conservation within and among years of the CAP. To examine reductions in N_e in each year class due to variance in apparent survival, we compared demographic estimates of inbreeding N_e between all broodstock and those broodstock with progeny surviving ≥3 years post release using the following equation:

$$N_e = \frac{4NfNm}{N_f + N_m}.$$

We used a demographic estimate because our dominant genetic data precluded use of genetic estimators of N_e. Year classes including families with unresolved paternity were excluded from the N_e comparison due uncertainty about the total number of males contributing progeny.

We wanted to determine whether the N_e of the CAP estimated from broodstock with surviving offspring was significantly lower than N_e estimated from all broodstock used from 2002–2009. However, estimating N_e across years of the CAP violates the assumptions of N_e estimation as mating between parents in different year classes is not possible (not a true population). Instead, we ranked differences in the two N_e estimates for each year class and used a Wilcoxon signed rank test (VassarStats) to evaluate the significance of N_e reduction among years of the CAP.

2.4.4. Relatedness as a predictor of recapture

Offspring of highly related parents may suffer from inbreeding depression and experience lower survival than outbred progeny of unrelated parents. We used generalized linear modeling to determine whether relatedness between parents could explain the variability in recapture among families. We used the program Relate (Rodzen et al., 2004), which implements the Lynch and Milligan (1994) approach for estimating relatedness from dominant data, to calculate pairwise relatedness values between parent pairs from year classes 2002 and 2004–2012. We only considered crosses of known parentage where parents had no or little (one microsatellite) missing data (N = 107 families). The number of years at large was

included as a predictor because progeny released in earlier years of the program have a longer time in which to be recaptured than recently released progeny.

Recapture number is a count variable so we used packages glm, glm.nb and pscl (R Core Team; Venables and Ripley, 2002; Zeileis et al., 2008) in R to explore whether a Poisson, quasipoisson, negative binomial, zero-inflated Poisson, or zero-inflated negative binomial model best fit the data. Data were overdispersed and we selected a zero-inflated negative binomial model because it was nearly equidispersed, had a high log-likelihood, and low AIC value.

3. Results

3.1. Variance in recapture among families

We were able to assign 76 of 268 unmarked juveniles to family with parentage analysis bringing the total number of recaptures to 4508. Recaptures determined from parentage assignment were consistent with recapture patterns observed in the database. Families with many recaptures of tagged juveniles also had many untagged juvenile recaptures. Of the 209 families released as juveniles by the CAP since 1995, 116 were represented by at least one recaptured individual. Between 18–100% of families in each year class were recaptured between 1995 and 2013 (Table 1; Fig. 2). A higher proportion of families released from the KTH were represented by recapture (79%) relative to families released from the KTOI hatchery (43%). Although families at large for a longer time period tended to have more recaptures, high numbers of recaptures in several recent years (2008, 2011; Table 1) suggested that variables other than time at large contributed to interannual variability.

The J' evenness statistic indicated that in many years, there was high variability among families in the number of recaptures. Three different patterns emerged. Some year classes were characterized by both a high proportion of recaptures and high J' value (e.g. 1995, 1998, 2002; Fig. 2). These year classes had many recaptures that were distributed fairly evenly among families. Other year classes had a high proportion of families with recaptures but a moderate J' value, indicating greater variability among families in number of recaptures (e.g. 2001; Fig. 2). Finally, some year classes had few families represented by recaptures and a low J' value (e.g. 2007, 2010; Fig. 2). Those years tended to be dominated by one or two families with a majority of families having few or no recaptures.

Table 1
The total number of progeny released for each year class from 1995–2012 (Tot rel), the total number of recaptures (Tot recap), number of families comprising each year class (No. fam), number of families with recaptures (Fam recap), range in number of recaptures per family (Recap/fam), mean number of recaptures per family, and standard deviation in number of recaptures among families.

Year	Tot rel	Tot recap	No. fam	Fam recap	Recap/fam	Mean	Std dev
1995	2,030	716	4	4	85–300	179	89.2
1998	308	56	6	6	1–23	9.33	8.29
1999	4,259	1093	12	11	0–304	91.1	117
2000	7,301	539	16	14	0–164	33.7	41.9
2001	8,856	126	10	7	0–70	12.6	21.4
2002	14,234	84	10	9	0–19	8.40	7.09
2003	12,531	863	13	7	0–275	66.4	94.8
2004	30,174	56	15	7	0–22	3.50	5.99
2005	16,598	86	10	8	0–29	8.60	9.69
2006	31,433	9	11	4	0–5	0.81	1.54
2007	3,253*	9	17	3	0–7	0.53	1.70
2008	14,739	464	17	8	0–102	27.3	42.7
2009	15,633	80	18	11	0–38	4.44	8.79
2010	16,290	69	17	3	0–66	4.06	16.0
2011	22,409	170	17	11	0–40	10.0	15.1
2012	10,704	88	15	4	0–32	5.87	11.3

* This value is an underestimate as release records were missing for three families.

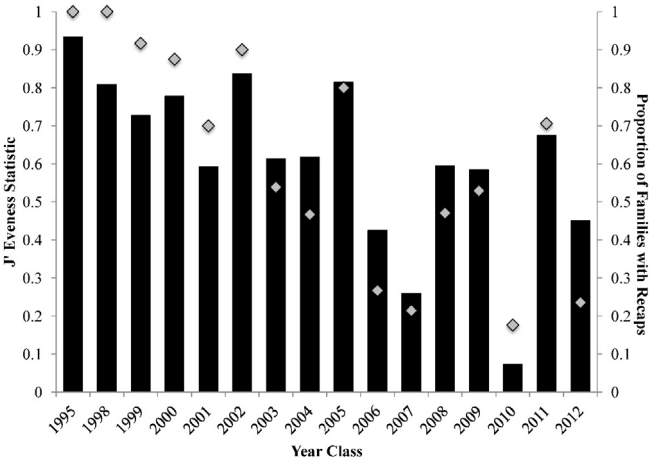

Fig. 2. The J' evenness statistic (bars) measures the similarity or dissimilarity in number of recaptures among families. J' values near 1 indicate a more even distribution of recaptures among families while J' values closer to 0 indicate dissimilarity in recapture number among families. The secondary axis (diamonds) shows the proportion of families for which progeny have been recaptured.

Re-sampling analysis simulated the number of unique families that would be detected in samples ranging in size from 40–4500 individuals. A plot of the mean number of families detected for each N began to asymptote around N = 4000 (Fig. 3). Over 90% of detected families were represented in samples containing ≥2320 individuals. Therefore our sample size of N = 4508 likely captured the majority of white sturgeon families at large in the Kootenai River.

3.2. Genetic diversity

The number of microsatellite alleles possessed by broodstock represented by recaptured juveniles at large for ≥3 years was nearly always significantly lower than the number of alleles represented by all broodstock for a particular year class (Fig. 4). The only year in which this was not the case was 2002, where all but one family were represented by recaptures. However, when we calculated the number of alleles across years in broodstock with recaptured offspring at large for ≥3 years, there was no significant difference from the number of alleles detected in all broodstock used in the CAP during this time period (Fig. 4).

Similarly, the N_e calculated from broodstock with recaptured juveniles at large for ≥3 years was less than the N_e calculated from all broodstock in nearly all years (Fig. 5). Although it was inappropriate to calculate cumulative N_e of the CAP from 1995–2009, a Wilcoxon signed rank test indicated a significant reduction in N_e when broodstock with progeny surviving for ≥3 years post-release were compared to all broodstock spawned in each of those years ($W = 55$, $n_s/r = 10$; $P = 0.005$). Further examination revealed that this reduction was driven by a smaller total number of parents represented by surviving progeny rather than increased sex ratio disparity. In fact, sex ratio disparity decreased when considering only broodstock with progeny surviving for ≥3 years post-release in nine of the 12 year classes examined.

3.3. Relatedness as a predictor of recapture

Pairwise relatedness between parents ranged from 0.02–0.54 (mean = 0.20, variance = 0.02). Although not statistically significant, there was a positive and marginally significant relationship between the number of recaptures and relatedness between parents (Table 2). Number of years at large did not predict likelihood of recapture (Table 2). Neither relatedness nor time at large predicted the incidence of zeros in the recapture dataset (P = 0.464 and 0.270, respectively).

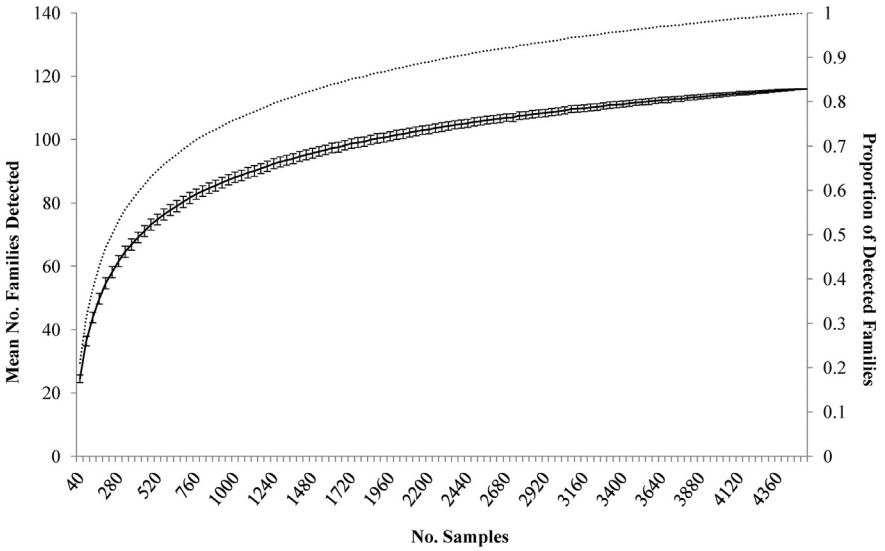

Fig. 3. A re-sampling analysis indicates our sample size is sufficient to detect the majority of hatchery released families at large in the Kootenai River. The mean number of families detected is plotted in a solid line and the proportion of detected families in each sample is plotted with a dotted line. Error bars indicate standard deviation in mean number of families detected.

4. Discussion

Here we synthesize data from multiyear genetic monitoring of the captive component and abundance monitoring of the in situ component of a supportive breeding program to evaluate how post-release mortality affects genetic diversity conservation. We assume that recapture data are an appropriate proxy of family representation and that all families have an equal probability of detection in the study area. We believe that these assumptions are justified because juvenile monitoring occurs over a wide range of the Kootenai River system that is inhabited by white sturgeon (rkm 18–244) and multiple mesh sizes were used to sample juveniles, reducing the likelihood that inadequate sampling and/or size selectivity for certain age classes drives the patterns we observed. Although there are fewer sampling locations in Kootenay Lake than in the Kootenai River proper, family composition of recaptures in Kootenay Lake is similar to that of the Kootenai River recaptures, suggesting probability of recapture is not biased by family specific habitat partitioning. We also assume that our sample size is adequate to detect the majority of surviving families in the Kootenai system, a conclusion supported by our re-sampling analysis.

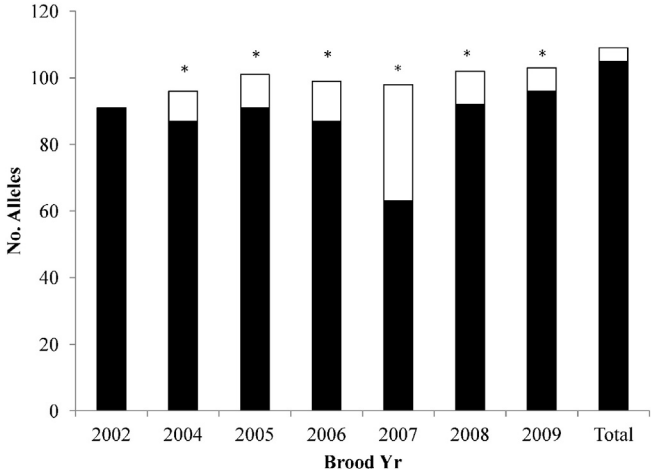

Fig. 4. Significant reductions in genetic diversity were observed when broodstock represented by recaptured progeny at large for ≥3 years (black) in a given year were compared to all broodstock used in that year (white). Significance ($P \leq 0.05$; one-tailed test) is indicated by asterisks. No significant difference was observed when years 2002–2009 were pooled (Total).

Mark-recapture data collected during juvenile abundance monitoring indicates that white sturgeon families exhibit marked differences in recapture after release into the wild from a captive environment. Despite extensive abundance monitoring efforts for over 20 years, surviving progeny were encountered for only 55% of families released from the KTOI white sturgeon CAP and there was high variability in the number of unique progeny recaptured within and across years for remaining families. Even year classes with fairly high J' values and a high proportion of detected families had per family recapture numbers that spanned one to two orders of magnitude (Table 1). Our findings suggest families experience different rates of post-release mortality and often families are extirpated from the river altogether.

The biotic and abiotic factors driving the observed variability in recapture among families is unknown. Our model including parental relatedness and time at large as predictors of recapture did not suggest that either variable significantly explained variability in the data. Although the positive relationship between parental relatedness and recapture was marginally significant (Table 2), the nature of the relationship did not make biological sense. Other studies in a variety of taxa report no relationship between relatedness and survival (Lind et al., 2009) or a negative relationships between inbreeding and survival (Ryman, 1970; Saccheri et al., 1998; Arkush et al., 2002). High numbers of zero counts in our data (nearly 50% of observations) may confound

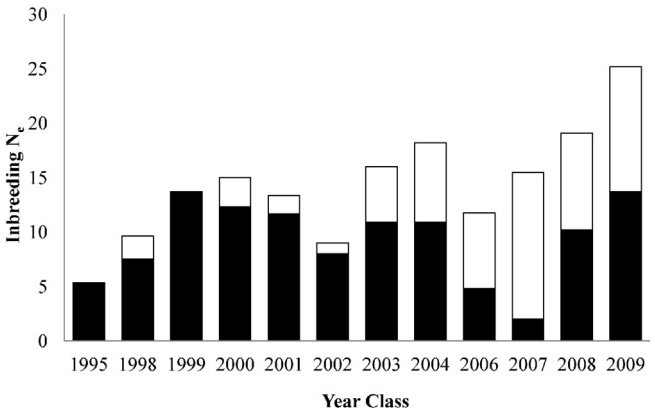

Fig. 5. Demographic estimates of inbreeding N_e for all broodstock used to produce a given year class (white) and only those broodstock in that year that were represented by recaptured offspring ≥3 years of age (black). Year class 2005 is excluded due to uncertain paternity in several families.

Table 2
Model coefficients, standard error (SE) and P values for zero inflated negative binomial model of relatedness and time at large as predictors of recapture.

	Coefficient	SE	P
Relatedness	4.635	2.493	0.063
Time at large	−0.145	0.088	0.102

the model. Alternatively, non-genetic variables may have a greater influence on individual survival in the Kootenai River system. Dinsmore et al. (2015) examined the effect of fork length, weight, age, season of release, and hatchery of release on survival of Kootenai River white sturgeon year classes and found in general that larger fish and those released in spring had a greater likelihood of survival. This corroborates our finding of higher recapture proportions in the KTH relative to the KTOI hatchery as the KTH has consistently released juveniles in the spring at larger sizes while release times and sizes in the KTOI have varied over time.

Regardless of the cause, family loss due to post-release mortality has significant implications for genetic diversity conservation in captive breeding programs. Previous conclusions about genetic diversity conservation in the Kootenai River white sturgeon CAP were based upon genetic diversity levels in broodstock that produced offspring surviving to release from captivity (Drauch Schreier et al., 2012). However, this study reveals that genetic diversity represented by captive-reared juveniles in the wild is significantly less than broodstock genetic diversity in seven of the eight year classes examined. Similarly, a significant reduction in N_e was detected across years when comparing values calculated from all broodstock and estimates from only those broodstock contributing offspring likely to survive to adulthood in the wild. This means that the rate of genetic diversity loss in the supplemented white sturgeon population is more rapid that originally predicted. Although high proportions of genetic diversity have been preserved by the Kootenai River white sturgeon CAP due to stocking over multiple years and including 209 unique parents, captive or supportive breeding programs for critically endangered populations with a smaller parent pool, fewer progeny to release, or fewer release events would be more susceptible to significant genetic diversity losses due to stochastic (drift) or non-random (selection) post-release mortality.

Genetic diversity loss and low N_e in captive breeding programs are undesirable because these programs typically supplement wild populations already suffering from low genetic diversity and/or low N_e. Therefore, it is critical that managers are able to accurately characterize transmission of genetic diversity to reintroduced and supplemented populations. In the case of the Kootenai River white sturgeon, captive breeding will continue until natural recruitment is restored; therefore future spawning of unique wild adults and improved stocking practices will make up for genetic diversity losses due to variability in survival among families. However, programs that have specific genetic diversity or N_e "targets" may be negatively impacted if post-release mortality is not considered when captive or supportive breeding programs are designed. For example, managers may aim to include a particular number of parents in captive breeding and equalize of family sizes to reach a target N_e in the released population. Unequal mortality or even extirpation of families occurring after release would lower the actual N_e below the desired value predicted from genetic or demographic data taken before release. Post-release genetic monitoring can characterize the magnitude of genetic diversity loss and help managers mitigate for post-release mortality when developing breeding and release strategies.

Unfortunately, post-release genetic monitoring is rarely done (Schwartz et al., 2007). We found few examples of studies examining the genetics of released cohorts (Drauch and Rhodes, 2007; Gonzalez et al., 2008; Karlsson et al., 2008; Alcaide et al., 2010; this study) and these examined only a single year class or provided a single "snapshot" of the genetic composition of multiple year classes rather than examining trends in genetic composition over time. Likely constraints on post-release monitoring include time/labor investment, financial limitations, low recapture probability, and desire to avoid imposing handling stress on valuable captive-released individuals. However, advances in genetic technology can alleviate several of these concerns. New techniques allow for genotyping individuals from trace samples such as hair, feathers, feces, urine or even footprints (Waits and Paetkau, 2005; Dalén et al., 2007). Therefore, biologists can virtually "recapture" individuals without the need for direct encounter or handling.

Coupling genetic monitoring and post-release monitoring over time not only will characterize genetic diversity trends in released cohorts but also will allow for adaptive management of captive and supportive breeding programs to maximize survival and N_e in wild populations into which releases occur. The ultimate goal of captive and supportive breeding programs is to produce viable, self-sustaining populations. Post-release monitoring can provide a direct link between program practices and indicators of genetic and demographic health in wild populations. For example, stocking practices in the KTOI CAP have evolved as post-release monitoring has revealed relationships between programmatic practices, environmental variables, and post-release survival. Juveniles are now released exclusively in the spring and at larger sizes to maximize survival in the first year at large. In light of advances in genetic technology and the findings of this case study, we encourage conservation biologists to consider ways in which post-release monitoring may be incorporated into their captive and supportive breeding programs to improve genetic diversity conservation of endangered populations.

Acknowledgments

Funding for this study was provided by the Kootenai Tribe of Idaho (award #198806400 Contracts #57791 and #63815) and the United States Fish and Wildlife Service Recovery Initiative (awards #F12AP00934 and #F14AP00612). SY is employed by the Kootenai Tribe of Idaho and SS is under subcontract to the KTOI and they assisted with data collection (SS), interpretation (SS, SY) and manuscript writing (SS, SY). The US Fish and Wildlife Service (USFWS) provided no input in study design, analysis, or publication. Research was carried out under a Section 10 permit from the United States Fish and Wildlife Service (no. TE798744-7). We would like to thank Chris Lewandowski and staff of the KTOI White Sturgeon Conservation Hatchery and Ron Ek of the Kootenay Trout Hatchery for recording data and providing access to records. Thanks also to Diane Wakkinen of the Idaho Department of Fish and Game for help with recapture database access. Ed Heist and Kim Scribner made helpful suggestions that improved data analysis. We appreciate the assistance of Alison Muller and Alisha Goodbla who provided technical support in the laboratory. Finally, this manuscript was improved by the thoughtful comments external reviewers.

References

Alcaide, M., Negro, J.J., Serrano, D., Antolín, J.L., Casado, S., Pomarol, M., 2010. Captive breeding and reintroduction of the lesser kestrel Falco naumanni: a genetic analysis using microsatellites. Conserv. Genet. 11, 331–338. http://dx.doi.org/10.1007/s10592-009-9810-7 (accessed August 26, 2014)).

Araki, H., Berejikian, B.A., Ford, M.J., Blouin, M.S., 2008. SYNTHESIS: fitness of hatchery-reared salmonids in the wild. Evol. Appl. 1, 342–355. http://dx.doi.org/10.1111/j.1752-4571.2008.00026.x (accessed May 28, 2013).

Araki, H., Cooper, B., Blouin, M.S., 2007. Genetic effects of captive breeding cause a rapid, cumulative fitness decline in the wild. Science 318, 100–103 (Available from http://www.ncbi.nlm.nih.gov/pubmed/17916734 (accessed May 22, 2013)).

Arkush, K.D., Giese, A.R., Mendonca, H.L., McBride, A.M., Marty, G.D., Hedrick, P.W., 2002. Effects of inbreeding and major histocompatibility complex genotypes. Can. J. Fish. Aquat. Sci. 59, 966–975.

Bailey, J.K., Schweitzer, J.A., Rehill, B.J., Lindroth, R.L., Martinsen, G.D., Whitham, T.G., 2004. Beavers as molecular geneticists: a genetic basis to the foraging of an ecosystem engineer. Ecology 85, 603–608.

Beamesderfer, R., Garrison, T., Anders, P., 2014. Abundance and Survival of the Remnant Kootenai River White Sturgeon Population. Bonners Ferry, ID.

Chargé, R., Sorci, G., Saint Jalme, M., Lesobre, L., Hingrat, Y., Lacroix, F., Teplitsky, C., 2014. Does recognized genetic management in supportive breeding prevent genetic changes in life-history traits? Evol. Appl. 7, 521–532.
Cooper, A.M., Miller, L.M., Kapuscinski, A.R., 2009. Conservation of population structure and genetic diversity under captive breeding of remnant coaster brook trout (*Salvelinus fontinalis*) populations. Conserv. Genet. 11, 1087–1093. http://dx.doi.org/10.1007/s10592-009-9841-0 (accessed September 3, 2014).
Crossman, J.A., Scribner, K.T., Yen, D.T., Davis, C.A., Forsythe, P.S., Baker, E.A., 2011. Gamete and larval collection methods and hatchery rearing environments affect levels of genetic diversity in early life stages of lake sturgeon (*Acipenser fulvescens*). Aquaculture 310, 312–324 (Elsevier B.V. Available from http://linkinghub.elsevier.com/retrieve/pii/S0044848610007404 (accessed August 8, 2013)).
Crutsinger, G.M., Collins, M.D., Fordyce, J.A., Gompert, Z., Nice, C.C., Sanders, N.J., 2006. Plant genotypic diversity predicts community structure and governs an ecosystem process. Science 313, 966–968 (Available from http://www.ncbi.nlm.nih.gov/pubmed/16917062 (accessed July 31, 2014)).
Dalén, L., Götherström, A., Meijer, T., Shapiro, B., 2007. Recovery of DNA from footprints in the snow. Can. Field Nat. 121, 321–324.
DFO, 2007. Recovery potential assessment for white sturgeon. DFO Can. Sci. Advis. Sec. Sci. Advis. Rep. 2007/014.
Dinsmore, S.J., Rust, P.J., Hardy, R.S., Ross, T.J., Stephenson, S.M., Young, S.P., 2015. Kootenai River Juvenile White Sturgeon Population Analyses.
Drauch, A.M., Rhodes, O.E., 2007. Genetic evaluation of the lake sturgeon reintroduction program in the Mississippi and Missouri Rivers. N. Am. J. Fish Manag. 27, 434–442. http://dx.doi.org/10.1577/M06-024.1 (accessed October 21, 2011).
Drauch Schreier, A., Gille, D., Mahardja, B., May, B., 2011. Neutral markers confirm the octoploid origin and reveal spontaneous autopolyploidy in white sturgeon, *Acipenser transmontanus*. J. Appl. Ichthyol. 27, 24–33. http://dx.doi.org/10.1111/j.1439-0426.2011.01873.x (accessed January 31, 2012).
Drauch Schreier, A., Rodzen, J., Ireland, S., May, B., 2012. Genetic techniques inform conservation aquaculture of the endangered Kootenai River white sturgeon *Acipenser transmontanus*. Endanger. Species Res. 16, 65–75 (Available from http://www.int-res.com/abstracts/esr/v16/n1/p65-75/ (accessed January 26, 2012)).
Duke, S., et al., 1999. Recovery plan for Kootenai River white sturgeon (*Acipenser transmontanus*). J. Appl. Ichthyol. 15, 157–163.
Fiumera, A.C., Porter, B.A., Looney, G., Asmussen, M.A., Avise, J.C., 2004. Maximizing offspring production while maintaining genetic diversity in supplemental breeding programs of highly fecund managed species. Conserv. Biol. 18, 94–101.
Frankham, R., Manning, H., Margan, S.H., Briscoe, D.A., 2000. Does equalization of family sizes reduce genetic adaptation to captivity? Anim. Conserv. 4, 357–363.
Gerber, S., Mariette, S., Streiff, R., Bodénès, C., Kremer, A., 2000. Comparison of microsatellites and amplified fragment length polymorphism markers for parentage analysis. Mol. Ecol. 9, 1037–1048 (Available from http://www.ncbi.nlm.nih.gov/pubmed/10964223).
Gonzalez, E.B., Nagasawa, K., Umino, T., 2008. Stock enhancement program for black sea bream (*Acanthopagrus schlegelii*) in Hiroshima Bay: monitoring the genetic effects. Aquaculture 276, 36–43 (Available from http://linkinghub.elsevier.com/retrieve/pii/S0044848608000835 (accessed August 25, 2014)).
Hedrick, P.W., Kalinowski, S.T., 2000. Inbreeding depression in conservation biology. Annu. Rev. Ecol. Syst. 31, 139–162.
Israel, J.A., Cordes, J.F., Blumberg, M.A., May, B., 2004. Geographic patterns of genetic differentiation among collections of Green Sturgeon. N. Am. J. Fish Manag. 24, 922–931.
IUCN/SSC, 2013. Guidelines for Reintroductions and Other Conservation Translocations. Version 1.0. Gland, Switzerland.
Ivy, J.A., Miller, A., Lacy, R.C., Dewoody, J.A., 2009. Methods and prospects for using molecular data in captive breeding programs: an empirical example using parma wallabies (*Macropus parma*). J. Hered. 100, 441–454 (Available from http://www.ncbi.nlm.nih.gov/pubmed/19423700 (accessed October 24, 2014)).
Justice, C., Pyper, B.J., Beamesderfer, R.C.P., Paragamian, V.L., Rust, P.J., Neufeld, M.D., Ireland, S.C., 2009. Evidence of density- and size-dependent mortality in hatchery-reared juvenile white sturgeon (*Acipenser transmontanus*) in the Kootenai River. Can. J. Fish. Aquat. Sci. 66, 802–815.
Karlsson, S., Saillant, E., Bumguardner, B.W., Vega, R.R., Gold, J.R., 2008. Genetic identification of hatchery-released red drum in Texas bays and estuaries. N. Am. J. Fish Manag. 28, 1294–1304. http://dx.doi.org/10.1577/M07-181.1 (accessed September 11, 2014).
Kock, T.J., Congleton, J.L., Anders, P.J., 2006. Effects of sediment cover on survival and development of white sturgeon embryos. N. Am. J. Fish Manag. 26, 134–141. http://dx.doi.org/10.1577/M05-073.1.
Laikre, L., Schwartz, M.K., Waples, R.S., Ryman, N., 2010. Compromising genetic diversity in the wild: unmonitored large-scale release of plants and animals. Trends Ecol. Evol. 25, 520–529 (Available from http://www.ncbi.nlm.nih.gov/pubmed/20688414 (accessed July 15, 2014)).
Lind, C.E., Evans, B.S., Taylor, J.J.U., Jerry, D.R., 2009. The consequences of differential family survival rates and equalizing maternal contributions on the effective population size (Ne) of cultured silver-lipped pearl oysters, *Pinctada maxima*. Aquac. Res. 41, 1229–1242. http://dx.doi.org/10.1111/j.1365-2109.2009.02410.x (accessed December 20, 2013).
Lynch, M., Milligan, B.G., 1994. Analysis of population genetic structure with RAPD markers. Mol. Ecol. 3, 91–99.
Marshall, T.C., Slate, J., Kruuk, L.E., Pemberton, J.M., 1998. Statistical confidence for likelihood-based paternity inference in natural populations. Mol. Ecol. 7, 639–655.
McLean, J.E., Seamons, T.R., Dauer, M.B., Bentzen, P., Quinn, T.P., 2007. Variation in reproductive success and effective number of breeders in a hatchery population of steelhead trout (*Oncorhynchus mykiss*): examination by microsatellite-based parentage analysis. Conserv. Genet. 9, 295–304. http://dx.doi.org/10.1007/s10592-007-9340-0 (accessed May 21, 2013).
Paragamian, V.L., Beamesderfer, R.C.P., Ireland, S.C., 2005. Status, population dynamics, and future prospects of the endangered Kootenai River white sturgeon population with and without hatchery intervention. Trans. Am. Fish. Soc. 134, 518–532. http://dx.doi.org/10.1577/T03-011.1 (accessed December 23, 2013).
Partridge, F., 1983. Kootenai River fisheries investigations. Idaho Department of Fish and Game, Federal Aid to Fish and Wildlife Restoration, Project F-73-R-5. Boise, ID.
Reusch, T.B.H., Ehlers, A., Hämmerli, A., Worm, B., 2005. Ecosystem recovery after climatic extremes enhanced by genotypic diversity. Proc. Natl. Acad. Sci. U. S. A. 102, 2826–2831 (Available from http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=549506&tool=pmcentrez&rendertype=abstract).
Rodzen, J.A., Famula, T.R., May, B., 2004. Estimation of parentage and relatedness in the polyploid white sturgeon (*Acipenser transmontanus*) using a dominant marker approach for duplicated microsatellite loci. Aquaculture 232, 165–182 (Available from http://linkinghub.elsevier.com/retrieve/pii/S0044848603004502 (accessed May 25, 2013)).
Rodzen, J.A., May, B., 2002. Inheritance of microsatellite loci in the white sturgeon (*Acipenser transmontanus*). Genome 45, 1064–1076.
Ryman, N., 1970. N: a genetic analysis of recapture frequencies of released young of salmon (*Sulmo sulur* L.). Hereditas 65, 159–160.
Ryman, N., Laikre, L., 1991. Effects of supportive breeding on the genetically effective population size. Conserv. Biol. 5, 325–329.
Saccheri, I., Kuussaari, M., Kankare, M., Vikman, P., Hanski, I., 1998. Inbreeding and extinction in a butterfly metapopulation. Nature 392, 491–494.
Saltzgiver, M.J., Heist, E.J., Hedrick, P.W., 2012. Genetic evaluation of the initiation of a captive population: the general approach and a case study in the endangered pallid sturgeon (*Scaphirhynchus albus*). Conserv. Genet. 13, 1381–1391. http://dx.doi.org/10.1007/s10592-012-0381-7 (accessed March 28, 2013).
Schreier, A., May, B., 2012. Comparison of Two Conservation Aquaculture Techniques for Capturing Upper Columbia White Sturgeon Genetic Diversity — Final Report. Wellpinit, WA.
Schwartz, M.K., Luikart, G., Waples, R.S., 2007. Genetic monitoring as a promising tool for conservation and management. Trends Ecol. Evol. 22, 25–33 (Available from http://www.ncbi.nlm.nih.gov/pubmed/16962204 (accessed May 27, 2013)).
Seddon, P.J., Griffiths, C.J., Soorae, P.S., Armstrong, D.P., 2014a. Reversing defaunation: restoring species in a changing world. Science 345, 406–412. http://dx.doi.org/10.1126/science.1251818 (accessed July 24, 2014).
Seddon, J.M., Lee, K.E., Johnston, S.D., Nicolson, V.N., Pyne, M., Carrick, F.N., Ellis, W.A.H., 2014b. Testing the regional genetic representativeness of captive koala populations in South-East Queensland. Wildl. Res. 41, 277–286.
Stephenson, S., Evans, V., Neufeld, M., 2014. Kootenay White Sturgeon: Juvenile and Adult Sampling in British Columbia 2013–14. Ministry of Forest, Lands and Natural Resource Operations, Nelson, BC.
Theodorou, K., Couvet, D., 2004. Introduction of captive breeders to the wild: harmful or beneficial? Conserv. Genet. 5, 1–12.
Tzika, A.C., Remy, C., Gibson, R., Milinkovitch, M.C., 2008. Molecular genetic analysis of a captive-breeding program: the vulnerable endemic Jamaican yellow boa. Conserv. Genet. 10, 69–77. http://dx.doi.org/10.1007/s10592-008-9519-z (accessed September 3, 2014).
USFWS, 1994. Determination of endangered status for the Kootenai River white sturgeon population. Fed. Regist. 59, 45989.
Venables, W.N., Ripley, B.D., 2002. Modern Applied Statistics with S. 4. Springer, New York.
Waits, L.P., Paetkau, D., 2005. Noninvasive genetic sampling tools for wildlife biologists: a review of applications and recommendations for accurate data collection. J. Wildl. Manag. 69, 1419–1433.
Waples, R.S., 1999. Dispelling some myths about hatcheries. Fisheries 24, 12–21.
Woodworth, L.M., Montgomery, M.E., Briscoe, D.A., Frankham, R., 2002. Rapid genetic deterioration in captive populations: causes and conservation implications. Conserv. Genet. 3, 277–288.
Zar, J.H., 1999. Biostatistical AnalysisFourth Edi. Prentice Hall Inc., Upper Saddle River, NJ.
Zeileis, A., Kleiber, C., Jackman, S., 2008. Regression models for count data in R. J. Stat. Softw. 27 (Available from < Go to ISI>://000258207100001).
Zeisset, I., Beebee, T.J.C., 2012. Donor population size rather than local adaptation can be a key determinant of amphibian translocation success. Anim. Conserv. 1–8. http://dx.doi.org/10.1111/acv.12003 (accessed February 15, 2013).