bioRxiv preprint doi: https://doi.org/10.1101/087973; this version posted November 16, 2016. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

What can genomics tell us about the success of enhancement programs in anadromous Chinook salmon? A comparative analysis across four generations

Charles D. Waters[a], Jeffrey J. Hard[b], Marine S.O. Brieuc[a], David E. Fast[c], Kenneth I. Warheit[d], Robin S. Waples[b], Curtis M. Knudsen[e], William J. Bosch[c], and Kerry A. Naish[a]

[a]School of Aquatic and Fishery Sciences, University of Washington, 1122 NE Boat St., Seattle, WA 98105, USA
[b]National Oceanic and Atmospheric Administration, Northwest Fisheries Science Center, 2725 Montlake Blvd. East, Seattle, WA 98112, USA
[c]Yakama Nation Fisheries, P.O. Box 151, Toppenish, WA 98948, USA
[d]Washington Department of Fish and Wildlife, 600 Capitol Way North, Olympia, WA 98501, USA
[e]Oncorh Consulting, 2623 Galloway SE, Olympia, WA 98501, USA

Author of correspondence: Charles D. Waters cwaters8@uw.edu

This paper was presented in an invited session entitled; Genomics for improved fisheries management and conservation: have the promises been fulfilled? at the 7th World Fisheries Congress which was sponsored by the OECD Co-operative Research Programme on Biological Resource Management for Sustainable Agricultural Systems, whose financial support made it possible for the invited speakers to participate in the Special Session.

The opinions expressed and arguments employed in this publication are the sole responsibility of the authors and do not necessarily reflect those of the OECD or of the governments of its Member countries.

bioRxiv preprint doi: https://doi.org/10.1101/087973; this version posted November 16, 2016. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Abstract

Population enhancement through the release of cultured organisms can be an important tool for marine restoration. However, there has been considerable debate about whether releases effectively contribute to conservation and harvest objectives, and whether cultured organisms impact the fitness of wild populations. Pacific salmonid hatcheries on the West Coast of North America represent one of the largest enhancement programs in the world. Molecular-based pedigree studies on one or two generations have contributed to our understanding of the fitness of hatchery-reared individuals relative to wild individuals, and tend to show that hatchery fish have lower reproductive success. However, interpreting the significance of these results can be challenging because the long-term genetic and ecological effects of releases on supplemented populations are unknown. Further, pedigree studies have been opportunistic, rather than hypothesis driven, and have not provided information on "best case" management scenarios. Here, we present a comparative, experimental approach based on genome-wide surveys of changes in diversity in two hatchery lines founded from the same population. We demonstrate that gene flow with wild individuals can reduce divergence from the wild source population over four generations. We also report evidence for consistent genetic changes in a closed hatchery population that can be explained by both genetic drift and domestication selection. The results of this study suggest that genetic risks can be minimized over at least four generations with appropriate actions, and provide empirical support for a decision-making framework that is relevant to the management of hatchery populations.

Introduction

Enhancement, the release of cultured organisms to increase population abundance, is an important fishery management tool (Lorenzen *et al.* 2010). But genetic risks associated with artificial propagation are well known and may compromise the wild populations that enhancement is intended to support (Naish *et al.* 2008; Laikre *et al.* 2010). Supportive breeding programs are a form of enhancement used extensively in the management of Pacific salmon in North America. Such programs aim to increase population sizes by rearing a fraction of juveniles in captivity and then releasing them into the natural environment along with their wild-born conspecifics (Ryman & Laikre 1991). Concerted efforts have been directed at mitigating the effects of domestication selection, genetic drift and inbreeding (Mobrand *et al.* 2005) associated with these programs, because in many cases populations have not recovered and cannot support sustainable fisheries (Naish *et al.* 2008; Beamish *et al.* 2010; Scheuerell *et al.* 2015). Practical recommendations to mitigate genetic risks have focused on theoretical models that examine the influence of gene flow in reducing divergence between cultured and wild populations (Duschene & Bernatchez 2002; Ford 2002; Baskett & Waples 2013). Specifically, the intentional use of natural-origin broodstock in the creation of the hatchery population in each generation may reduce risks, especially when gene flow from the hatchery to the wild population is limited (Mobrand *et al.* 2005). Such "managed gene flow" has seen widespread adoption in the Pacific Northwest of the USA (Paquet *et al.* 2011), but few practical examples on their efficacy exist.

An ideal way to test whether managed gene flow is effective at reducing genetic divergence between hatchery and wild populations is to empirically compare cultured populations with and without gene flow. Such a comparison would provide results on the range of possible outcomes of these management approaches, and would be especially informative if conducted longitudinally. The use of population genomic approaches provides a way to survey temporal changes in genetic divergence, to measure the rate of change with each generation since founding, and to identify factors driving divergence. We conducted such a study in two populations of Chinook salmon (*Oncorhynchus tshawytscha*) released from a hatchery on a tributary of the Columbia River (Waters *et al.* 2015). Both hatchery populations were founded from the same source population; however, one population remained integrated with the wild and used only wild-born broodstock in each generation, while the second hatchery population was maintained separately and received no gene flow from the wild. Our results over three generations revealed little change in the integrated line compared to the founding population. Most of the genetic divergence in the segregated line could be attributed to genetic drift, but there was also evidence for directional selection at specific locations in the genome. However, it is unclear over how many generations managed gene flow may be effective at mitigating genetic risks, because processes occurring in the wild could mitigate or exacerbate the effects over time (Ford 2002; Baskett & Waples 2013). Here we aimed to test whether the use of natural-origin broodstock was effective at reducing divergence over several generations by extending our earlier study for an additional fourth generation.

Materials and Methods

A spring Chinook salmon hatchery program was initiated in 1997 at the Cle Elum Supplementation and Research Facility (CESRF, Fig. 1) to supplement the declining upper Yakima River Chinook salmon population while minimizing possible genetic and ecological risks associated with supportive breeding. Local, wild adults were collected for broodstock from 1997 to 2002 as they passed the Roza Dam Adult Monitoring Facility (Roza Dam, Fig. 1). Adults were then transferred to CESRF and held until spawning. Eggs and juveniles were reared in the hatchery for approximately 18 months before they began their migration to the ocean. Adult hatchery fish first returned to the Yakima River in 2001 and were allowed to spawn naturally. In 2002, both wild and hatchery-origin adults were spawned at CESRF to create two contrasting hatchery lines. The integrated (INT) line is derived only from wild or natural-origin adults, and all fish from this line are

bioRxiv preprint doi: https://doi.org/10.1101/087973; this version posted November 16, 2016. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

allowed to spawn naturally. Here, natural-origin fish are those that were born in the river but may have some hatchery ancestry. The segregated (SEG) line, however, uses only hatchery-origin broodstock, and no fish can spawn in the river.

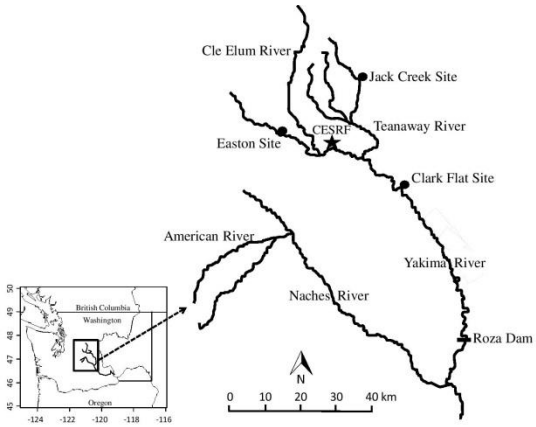

Figure 1. From Waters *et al*. (2015). Map of the Yakima River system. The upper Yakima Chinook salmon population is the target of the Cle Elum Supplementation and Research Facility (CESRF). All returning adults are sampled at Roza Dam and allowed to spawn naturally (natural origin and integrated line fish) or are removed from the system (all segregated line fish). Spawning and rearing for the hatchery lines occurs at CESRF. Prior to outmigration in spring, juveniles are transferred to the Easton, Jack Creek, and Clark Flat acclimation sites, where they are held for approximately two months before volitional release.

Tissues for DNA were sampled from adults of both hatchery lines in 2014 during spawning at CESRF and stored in 100% ethanol. These adults represent the fourth (F_4) generation of each line. DNA was extracted using DNeasy Blood & Tissue kits (Qiagen, Valencia, CA, USA) following the animal tissue protocol. Restriction site-associated (RAD) libraries (Baird *et al.* 2008) were prepared using the restriction enzyme *SbfI* and sequenced on the Illumina HiSeq 2000 platform with 36 individuals per lane. All raw RAD sequences from the F_4 generation were combined with raw data from our previous comparative analysis (P_1 founders and F_1-F_3 generations, Waters *et al.* 2015). Filtering and genotyping were performed following Waters *et al.* (2015), with two additional steps to improve data quality. First, loci were removed if more than 50% of individuals in any population were not genotyped. Then, loci were removed if they were out of Hardy-Weinberg equilibrium (*q*-value < 0.05) in more than one population, as determined by the Monte Carlo procedure with $1x10^5$ permutations in the R-package *adegenet* (v. 1.3-9, Jombart 2008). *Q*-values were computed using the R-package *qvalue* (v. 1.28.0, Storey 2002).

As the aim of the present study was to extend previous comparisons between the integrated and segregated hatchery lines by another generation, genetic change was evaluated using the same methods described in Waters *et al*. (2015). Population-level genetic change between each generation of the hatchery lines was evaluated using measures of F_{ST}, computed in *Genepop* (v. 4.1, Raymond & Rousset 1995), and a discriminant analysis of principal components (DAPC), conducted in the R-package *adegenet*. The relative effect of genetic drift within each hatchery line was determined using estimates of effective numbers of breeders, N_b. Temporal and linkage disequilibrium (LD) estimates of N_b were computed with N_E Estimator (v. 2.01, Do *et al.* 2014) using only four-year-old adults, which represented a single cohort of individuals. Steps taken to reduce potential bias in N_b estimates due to selection, overlapping generations, and fluctuating population size were identical to those of Waters *et al.* (2015). Lastly, loci and genomic regions exhibiting signals of diversifying selection in the hatchery lines were identified using three independent tests: F_{TEMP} (Therkildsen *et al.* 2013), *Bayescan* (Foll & Gaggiotti 2008), and a sliding-window approach (Brieuc *et al.* 2015; Waters *et al.* 2015). We focused on loci and regions that were identified by multiple tests and were divergent across multiple generations.

Results

Tissues from 72 individuals (36 from each line) were sequenced from the F_4 generation. The raw data was combined with RAD sequences from the previous generations and filtered, yielding 9266 bi-allelic RAD loci with minor allele frequencies >0.05 in at least one population and less than 50% missing genotypes within each population. A total of 465 individuals from the five generations were genotyped at >50% of these loci and retained for analyses (Tables S1, S2). Tests of HWE identified 158 loci that significantly deviated from expectations in more than one population. Following removal of these loci, the final data set comprised 9108 loci (Table S1), including 4214 loci that aligned to the Chinook salmon linkage map (Brieuc *et al.* 2014). Population-level divergence of the two hatchery lines followed previously documented trends (Waters *et al.* 2015). Values of pairwise F_{ST} between the lines and the P_1 founders was approximately four times higher in the F_4 SEG population (F_{ST} =0.0125, P < 0.001, Table S3) than in the F_4 INT population (F_{ST} =0.0033, P < 0.001). Divergence between the two hatchery lines in the F_4 generation also continued to increase (F_{ST} =0.0126, P < 0.001). Patterns of genetic change were further supported by a discriminant analysis of principal components, conducted on the first 63 PCs as recommended by the *optim.a.score* function in *adegenet*. The segregated line diverged from the P_1 founders and integrated line over time along the first discriminant function; this axis explained 59.2% of the retained variation (Fig. 2). Genetic change between the later generations of the integrated line and the P_1 founders was evident along the second discriminant function, which explained 16.6% of the retained variation.

Bias-adjusted LD and temporal estimates of effective number of breeders, N_b, supported earlier results and suggested that the relative effect of genetic drift was much greater in the segregated line than in the integrated line. The LD estimate of N_b in the F_4 INT population was nearly eight times higher than that obtained in the F_4 SEG population (Fig. 3;

bioRxiv preprint doi: https://doi.org/10.1101/087973; this version posted November 16, 2016. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Tables S4a, S5). The temporal N_b estimates, which applied to the entire sampling period of 1998-2014, were also markedly different between the two lines (Fig. 3; Table S4b). While the average broodstock size of the integrated line (363 ± 15) exceeded that of the segregated line (85 ± 15), the difference was not sufficient to explain the higher N_b of the integrated line (Table S6).

In addition, the N_b estimates for the F_4 generation revealed a result that was not apparent from the census data alone. The ratio of effective number of breeders to census size (N_b/ N_{census}) declined from 0.21 (95% CI: 0.20-0.23) in the F_3 INT sample to 0.04 (95% CI: 0.03-0.04) in the F_4 INT sample, despite the fact that the census sizes increased from 3364 to 8374 adults. This result is important because the N_b/N_{census} ratio provides a metric for understanding factors which cause deviations from N_b=N_{census} (e.g. variance in reproductive success) and affect genetic variation over time.

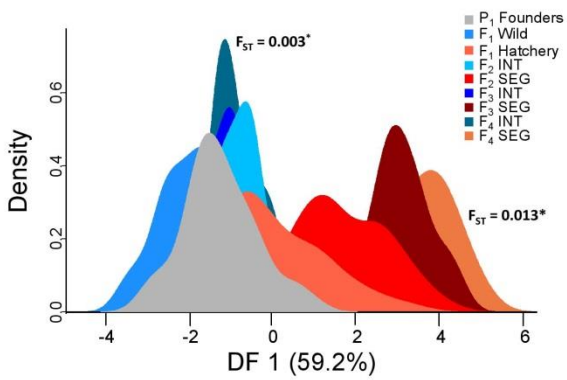

Figure 2. Density plot of individuals along the first discriminant function from the discriminant analysis of principal components (DAPC) for the wild founders (P_1 Founders, black) and four generations of the integrated (INT, blue colors) and segregated (SEG, red colors) hatchery lines. Pairwise F_{ST} values for the F_4 generation compared to the P_1 founders are shown for each hatchery line.

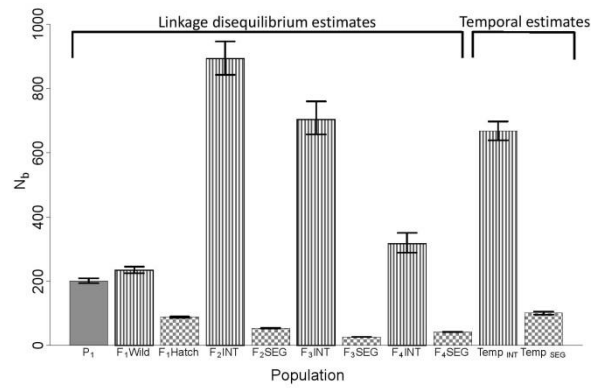

Figure 3. Estimates of effective number of breeders, N_b, and 95% confidence intervals produced by the linkage disequilibrium (LD) and temporal methods. The LD method enables estimation of N_b for every generation, while a single estimate for the sampling period is produced by the temporal method. LD estimates are adjusted for physical linkage and other potential biases as described in Waters *et al*. (2015).

Three independent tests identified loci and genomic regions that exhibited signals of diversifying selection. The F_{TEMP} method identified 78 loci that exceeded neutral expectations in the integrated line and 198 in the segregated line (Table S7). Thirty-five loci were outliers in both hatchery lines. *Bayescan*, conducted using all populations combined, identified 120 loci putatively under diversifying selection (Table S7). There was considerable overlap between *Bayescan* and F_{TEMP}, as 48 and 72 *Bayescan* outliers were also identified by F_{TEMP} in the integrated and segregated lines, respectively. Genomic regions that exhibited significantly elevated levels of divergence compared to the P_1 founders were identified in both hatchery lines by sliding window analyses (Table S8). However, divergence in the segregated line was more consistent across the F_1, F_2, F_3, and F_4 generations than in the integrated line. For example, seven regions were significantly elevated in at least three generations of the segregated line while none were observed in the integrated line (e.g. Fig. 4). Five of these regions also contained outlier loci identified by F_{TEMP} and *Bayescan* (Fig. 4), providing further support that selection – likely due to continued exposure to the hatchery environment – has also contributed to the higher levels of divergence observed in the segregated line. Previous work has identified genes in such regions of overlap that may be targeted by selection in captivity (Waters *et al.* 2015).

Discussion

Here, we have demonstrated the utility of genomic-based methods to test alternative management approaches for population enhancement and to monitor fine scale genetic changes in populations over several generations. Many theoretical studies have indicated that ongoing gene flow between hatchery and wild fish may ultimately compromise the fitness of the natural population (Ford 2002; Baskett & Waples 2013). However, the degree to which the natural population is affected depends on many factors that likely fluctuate over time, such as selection intensity, the proportion of wild-origin individuals on the spawning grounds, reproductive rate in the hatchery and wild, and carrying capacity of the natural system. Thus, our multigenerational findings extend complementary studies that evaluate reproductive success in single populations over one or two generations (Christie *et al.* 2014). The results from the fourth hatchery generation largely supported observations

bioRxiv preprint doi: https://doi.org/10.1101/087973; this version posted November 16, 2016. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

from a previously published longitudinal study (Waters *et al.* 2015). Little genetic change occurred in the integrated hatchery line, which frequently exchanged migrants with the founding wild population. In contrast, consistent temporal trends in divergence were documented in the segregated hatchery line, which is maintained as a closed population. Such consistency was observed on the population-level and at specific genomic regions, despite the fact that environmental change likely occurred during the sixteen years over which this study was conducted. This result might be explained by domestication selection imposed by the relatively uniform hatchery environment on the segregated hatchery population. Genomic regions exhibiting potential signals of domestication selection can be further examined to identify candidate genes (e.g. Waters *et al.* 2015) and mechanisms underlying genetic adaptation to captivity, and to inform management practices to possibly reduce this risk.

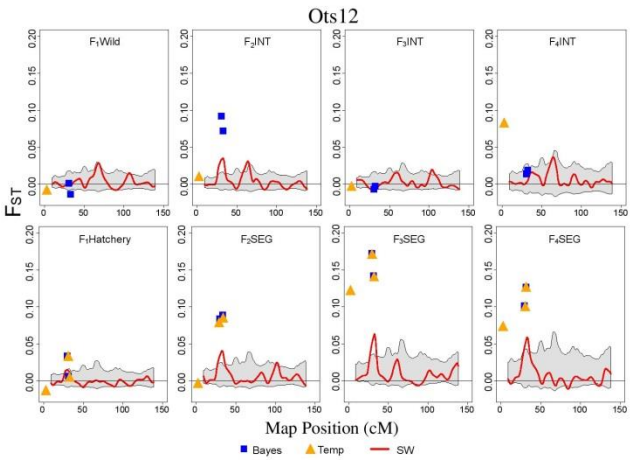

Figure 4. Loci and regions of the genome showing signatures of adaptive divergence, based on pairwise F_{ST} compared to the P_1 founders, on chromosome Ots12 for the integrated (top panel) and segregated (bottom panel) hatchery lines through the F_1, F_2, F_3, and F_4 generations. Blue squares are loci that were identified as outliers with *Bayescan* and orange triangles are outliers identified by F_{TEMP}. The red line represents the kernel smoothed moving average of F_{ST} and the grey shaded area is the 95% confidence interval.

Notably, extending the earlier study by another generation also revealed fluctuations in N_b/ N_{census} that would otherwise have been missed. We previously reported N_b/N_{census} ratios of 0.11 (95% CI: 0.10-0.12) and 0.21 (95% CI: 0.20-0.23) for the F_2 and F_3 INT samples, respectively, which reflected the first two generations of naturally-spawning adults that included hatchery fish from the integrated line. These estimates show a positive trend in N_b/N_{census}, which, if taken alone, could possibly be attributed to successful supplementation efforts. However, the decline of N_b/N_{census} to 0.04 in the F_4 INT sample may be indicative of the Ryman-Laikre effect (Ryman & Laikre 1991), where supportive breeding reduces the effective size of a wild population. Alternatively, ratios of 0.04-0.21 are within the range of those documented in natural populations of many species (including salmonids; Frankham 1995; Naish *et al.* 2013), and temporal fluctuations could simply reflect changes in the natural environment. It is impossible to identify the true source(s) of the observed fluctuations, particularly since there is no wild control population for comparison. Rather, our results emphasize the importance of continued monitoring and the need integrate processes affecting the productivity of natural systems with enhancement efforts. Finally, while this study does not evaluate fitness directly and lacks an unsupplemented control population, rates of genetic divergence measured here provide a range of multigenerational outcomes for contrasting management regimes. These findings, in turn, can assist managers and policy-makers when assessing the relative benefits and risks of conservation decisions, particularly in cases where population recovery may depend on supportive breeding.

Country-specific information (United States)

Fisheries Genomics Work Funding Sources

NOAA – National Sea Grant Program, Saltonstall-Kennedy, National Science Foundation, Bonneville Power / Federal Columbia River Power System (FCRPS) Biological Opinion Remand Funds, US Department of Agriculture

Funding sources accessed to support Genomics research

NOAA – National Sea Grant Program, Washington Sea Grant Program, National Science Foundation, Federal Columbia River Power System (FCRPS) Biological Opinion Remand Funds, US Department of Agriculture

Examples of genetic/genomic information to inform fisheries management and/or policy decisions in USA

Many of the listings under the Endangered Species Act rely on genetic information. Such data has been used to delineate "Distinct Population Segments" (Conservation Units) that are the subject of management actions. There are many publications associated with this activity, including reports. NOAA maintains a list of technical reports on this website: https://www.nwfsc.noaa.gov/publications/scipubs/displayinclude.cfm?incfile=technicalmemorandum2016.inc

The publications of interest are the "status reviews". The Hatchery Scientific Review Group on the West Coast of the USA has been extensively involved in developing "best practices" for the recovery and enhancement of salmon populations. http://www.hatcheryreform.us/hrp/welcome_show.action. Many of the reforms are based on a wide number of papers that have been published on the impacts of hatchery fish on wild fish. Work in this area is also influencing the management of other fish species, as well as molluscan population management.

bioRxiv preprint doi: https://doi.org/10.1101/087973; this version posted November 16, 2016. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Mobrand, L. E., J. Barr, L. Blankenship, D. E. Campton, T. T. P. Evelyn, T. A. Flagg, C. V. W. Mahnken, L. W. Seeb, P. R. Seidel, and W. W. Smoker. 2005. Hatchery reform in Washington State: Principles and emerging issues. *Fisheries* **30**:11-23.

Araki, H., B. Cooper, and M. S. Blouin. 2007. Genetic effects of captive breeding cause a rapid, cumulative fitness decline in the wild. *Science* **318**:100-103.

Paquet, P. J., T. Flagg, A. Appleby, J. Barr, L. Blankenship, D. Campton, M. Delarm, T. Evelyn, D. Fast, J. Gislason, P. Kline, D. Maynard, L. Mobrand, G. Nandor, P. Seidel, and S. Smith. 2011. Hatcheries, Conservation, and Sustainable Fisheries-Achieving Multiple Goals: Results of the Hatchery Scientific Review Group's Columbia River Basin Review. *Fisheries* **36**:547-561.

Seamons, T. R., L. Hauser, K. A. Naish, and T. P. Quinn. 2012. Can interbreeding of wild and artificially propagated animals be prevented by using broodstock selected for a divergent life history? *Evolutionary Applications* **5**:705-719.

Hess, M. A., C. D. Rabe, J. L. Vogel, J. J. Stephenson, D. D. Nelson, and S. R. Narum. 2012. Supportive breeding boosts natural population abundance with minimal negative impacts on fitness of a wild population of Chinook salmon. *Molecular Ecology* **21**:5236-5250.

Waters, C. D., J. J. Hard, M. S. O. Brieuc, D. E. Fast, K. I. Warheit, R. S. Waples, C. M. Knudsen, W. J. Bosch, and K. A. Naish. 2015. Effectiveness of managed gene flow in reducing genetic divergence associated with captive breeding. *Evolutionary Applications* **8**:956-971.

There is a growing interest in "parentage based tagging" (PBT) for tagging fish populations, which is viewed as an alternative for the coded wire tag program. PBT has significant potential to contribute to fisheries management.

Anderson, E. C., and J. C. Garza. 2006. The power of single-nucleotide polymorphisms for large-scale parentage inference. Genetics **172**:2567-2582.

Campbell, N. R., S. A. Harmon, and S. R. Narum. 2015. Genotyping-in-Thousands by sequencing (GT-seq): A cost effective SNP genotyping method based on custom amplicon sequencing. *Molecular Ecology Resources* **15**:855-867.

Steele, C. A., E. C. Anderson, M. W. Ackerman, M. A. Hess, N. R. Campbell, S. R. Narum, and M. R. Campbell. 2013. A validation of parentage-based tagging using hatchery steelhead in the Snake River basin. *Canadian Journal of Fisheries and Aquatic Sciences* **70**:1046-1054.

Molecular-based methods are used extensively for stock identification, mixed stock analysis and measures of abundance

Dann, T. H., C. Habicht, T. T. Baker, and J. E. Seeb. 2013. Exploiting genetic diversity to balance conservation and harvest of migratory salmon. *Canadian Journal of Fisheries and Aquatic Sciences* **70**:785-793.

Hess, J. E., J. M. Whiteaker, J. K. Fryer, and S. R. Narum. 2014. Monitoring Stock-Specific Abundance, Run Timing, and Straying of Chinook Salmon in the Columbia River Using Genetic Stock Identification (GSI). *North American Journal of Fisheries Management* **34**:184-201.

Satterthwaite, W. H., M. S. Mohr, M. R. O'Farrell, E. C. Anderson, M. A. Banks, S. J. Bates, M. R. Bellinger, L. A. Borgerson, E. D. Crandall, J. C. Garza, B. J. Kormos, P. W. Lawson, and M. L. Palmer-Zwahlen. 2014. Use of Genetic Stock Identification Data for Comparison of the Ocean Spatial Distribution, Size at Age, and Fishery Exposure of an Untagged Stock and Its Indicator: California Coastal versus Klamath River Chinook Salmon. *Transactions of the American Fisheries Society* **143**:117-133.

Acknowledgements

We thank everyone who was involved in establishing CESRF, shaping its research direction, and sampling broodstock, including Levi George, Melvin Sampson, Steve Schroder, Craig Busack, past and present members of the Independent Scientific Review Panel, and the Yakama Nation Tribal Council. We are grateful to Maren Wellenreuther and Louis Bernatchez, and the OECD Co-operative Research Programme for the opportunity to present this research at the World Fisheries Congress in Korea. Funding for this study was provided by NOAA Fisheries/Federal Columbia River Power System (FCRPS) Biological Opinion Remand Funds (to K.A.N. and J.J.H.), Washington Sea Grant (Award NA14OAR4170078 to K.A.N), and the Hall Conservation Genetics Research Award from the University of Washington (to C.D.W.).

Literature Cited

Baird NA, Etter PD, Atwood TS, *et al.* (2008) Rapid SNP discovery and genetic mapping using sequenced RAD markers. *Plos One* **3**, 7.

Baskett ML, Waples RS (2013) Evaluating alternative strategies for minimizing unintended fitness consequences of cultured individuals on wild populations. *Conservation Biology* **27**, 83-94.

bioRxiv preprint doi: https://doi.org/10.1101/087973; this version posted November 16, 2016. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Beamish RJ, Sweeting RM, Lange KL, *et al.* (2010) Early Marine Survival of Coho Salmon in the Strait of Georgia Declines to Very Low Levels. *Marine and Coastal Fisheries* **2**, 424-439.

Brieuc MSO, Ono K, Drinan DP, Naish KA (2015) Integration of Random Forest with population-based outlier analyses provides insight on the genomic basis and evolution of run timing in Chinook salmon (*Oncorhynchus tshawytscha*). *Molecular Ecology* **24**, 2729-2746.

Brieuc MSO, Waters CD, Seeb JE, Naish KA (2014) A dense linkage map for Chinook salmon (*Oncorhynchus tshawytscha*) reveals variable chromosomal divergence after an ancestral whole genome duplication event. *G3-Genes Genomes Genetics* **4**, 447-460.

Christie MR, Ford MJ, Blouin MS (2014) On the reproductive success of early-generation hatchery fish in the wild. *Evolutionary Applications* **7**, 883-896.

Do C, Waples RS, Peel D, *et al.* (2014) N_EESTIMATOR v2: re-implementation of software for the estimation of contemporary effective population size (N_e) from genetic data. *Molecular Ecology Resources* **14**, 209-214.

Duschene P, Bernatchez L (2002) An analytical investigation of the dynamics of inbreeding in multi-generation supportive breeding. *Conservation Genetics* **3**, 45-58.

Foll M, Gaggiotti O (2008) A genome-scan method to identify selected loci appropriate for both dominant and codominant markers: a Bayesian perspective. *Genetics* **180**, 977-993.

Ford MJ (2002) Selection in captivity during supportive breeding may reduce fitness in the wild. *Conservation Biology* **16**, 815-825.

Frankham R (1995) Effective population size/adult population size ratios in wildlife: a review. *Genetical Research* **66**, 95-107.

Jombart T (2008) *adegenet:* a R package for the multivariate analysis of genetic markers. *Bioinformatics* **24**, 1403-1405.

Laikre L, Schwartz MK, Waples RS, Ryman N, GeM_Working_Group (2010) Compromising genetic diversity in the wild: unmonitored large-scale release of plants and animals. *Trends in Ecology & Evolution* **25**, 520-529.

Lorenzen K, Leber KM, Blankenship HL (2010) Responsible Approach to Marine Stock Enhancement: An Update. *Reviews in Fisheries Science* **18**, 189-210.

Mobrand LE, Barr J, Blankenship L, *et al.* (2005) Hatchery reform in Washington state: Principles and emerging issues. *Fisheries* **30**, 11-23.

Naish KA, Seamons TR, Dauer MB, Hauser L, Quinn TP (2013) Relationship between effective population size, inbreeding and adult fitness-related traits in a steelhead (Oncorhynchus mykiss) population released in the wild. *Molecular Ecology* **22**, 1295-1309.

Naish KA, Taylor JE, Levin PS, *et al.* (2008) An evaluation of the effects of conservation and fishery enhancement hatcheries on wild populations of salmon. *Advances in Marine Biology* **53**, 61-194.

Paquet PJ, Flagg T, Appleby A, *et al.* (2011) Hatcheries, Conservation, and Sustainable Fisheries-Achieving Multiple Goals: Results of the Hatchery Scientific Review Group's Columbia River Basin Review *Fisheries* **36**, 547-561.

Raymond M, Rousset F (1995) Genepop (version 1.2) - Population-genetics software for exact tests and ecumenicism. *Journal of Heredity* **86**, 248-249.

Ryman N, Laikre L (1991) Effects of supportive breeding on the genetically effective population size. *Conservation Biology* **5**, 325-329.

Scheuerell MD, Buhle ER, Semmens BX, *et al.* (2015) Analyzing large-scale conservation interventions with Bayesian hierarchical models: a case study of supplementing threatened Pacific salmon. *Ecology and Evolution* **5**, 2115-2125.

Storey JD (2002) A direct approach to false discovery rates. *Journal of the Royal Statistical Society Series B-Statistical Methodology* **64**, 479-498.

Therkildsen NO, Hemmer-Hansen J, Als TD, *et al.* (2013) Microevolution in time and space: SNP analysis of historical DNA reveals dynamic signatures of selection in Atlantic cod. *Molecular Ecology* **22**, 2424-2440.

Waters CD, Hard JJ, Brieuc MSO, *et al.* (2015) Effectiveness of managed gene flow in reducing genetic divergence associated with captive breeding. *Evolutionary Applications* **8**, 956-971.