NMFS FELLOW - YEAR 1 REPORT

NMFS Fellows - 2016 - Year 1
Charles Waters
NMFS/Sea Grant Population and Ecosystem Dynamics Graduate Fellowship (Charles D. Waters): Modeling the effects of inbreeding in salmon hatcheries on the eco-evolutionary dynamics of supplemented wild populations
R/E/I-26
Submitted On: 03/16/2017 04:27:36 AM

METRICS & MEASURES

Metric/Measure	Value	Note
Acres of coastal habitat	0	This project is still on-going. As such, final results have not been generated and disseminated to management agencies, stake-holders, and user groups. The project will assist the enhancement and restoration of coastal ecosystems after its completion and dissemination.
Fishermen and seafood industry personnel	0	This project is still on-going. As such, final results have not been generated and disseminated to management agencies, stake-holders, and user groups. The project will assist fishermen, the seafood industry, and aquaculture projects after its completion and dissemination.
Communities - economic and environmental development	0	This project is still on-going. As such, final results have not been generated and disseminated to management agencies, stake-holders, and user groups. The project will (hopefully) improve the economic and environmental development of communities after its completion and dissemination.
Stakeholders - sustainable approaches	0	This project is still on-going. As such, final results have not been generated and disseminated to management agencies, stake-holders, and user groups. The project will provide information to stakeholders that will improve the sustainability of aquaculture practices in the future.
Informal education programs	2	Contributed to ocean science education of high school students by volunteering at Orca Bowl, the Washington State competition for the National Ocean Science Bowl. Another lab member also volunteered on my behalf to give tours of our laboratory to visiting high school students.
Stakeholders who receive information	0	
Volunteer hours	16	These represent hours spent volunteering for Orca Bowl.
P-12 students reached	100	This is a conservative estimate of the number of high school students that participated in Orca Bowl.
P-12 educators	0	

REQUESTED INFORMATION

Publications

What can genomics tell us about the success of enhancement programs in anadromous Chinook salmon? A comparative analysis across
Publication Type: Non-peer-reviewed: Complete Issues of Journals, Periodicals; Magazines, Miscellaneous Reports, Papers, Special Collections
Publication Year: 2016
Publication Authors:
Publisher Info: bioRxiv (pronounced "bio-archive") is a free online archive and distribution service for unpublished preprints in the life sciences. It is operated by Cold Spring Harbor Laboratory, a not-for-profit research and educational institution.
Notes: In addition to this work being published online to bioRxiv, the paper is being included as part of a larger review article, currently in press, that will be published in the peer-reviewed journal "Trends in Ecology and Evolution."
Related URLs:
Keywords: genomics, enhancement, conservation,
Publication URLs: http://biorxiv.org/content/early/2016/11/16/087973.article-metrics
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-released individuals relative to wild individuals, and ten 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 o 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 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 fou generations with appropriate actions, and provide empirical support for a decision-making framework that is relevant to the management of hatchery populations.
Citation: Waters CD, Hard JJ, Brieuc MSO, et al. (2016) What can genomics tell us about the success of enhancement programs in anadromous Chinook salmon? A comparative analysis across four generations. bioRxiv DOI: 10.1101/087973.
Citation for Coverpage:
SG can post PDF online?: Yes
Uploaded File: Waters_et_al_2016_What_can_genomics_tell_us.pdf

Students Supported

Charles Waters (Continuing Student)
cwaters8@uw.edu
University of Washington, School of Aquatic and Fishery Sciences

Field of Study: Genetics and Evolution
Advisor: Dr. Kerry Naish
Degree Type: PhD
Degree Year: 2018

Student Project Title: Modeling the effects of inbreeding in salmon hatcheries on the eco-

evolutionary dynamics of supplemented wild populations

Involvement With Sea Grant This Period (capstone, fellow, intern, etc.): NMFS-Sea Gran Fellow

Post-Graduation Plans (employer, grad school, etc.):

Was this thesis/dissertation supported by Sea Grant?: Yes

Thesis / Dissertation: Effectiveness of managed gene flow to reduce genetic and phenotypic change associated with captive breeding of Chinook salmon

New or Continuing?: continuing

Degree awarded this reporting period?: No

Financially supported?: Yes

Narratives

Modeling the effects of inbreeding in salmon hatcheries on the eco-evolutionary dynamics of supplemented wild populations
Uploaded File: REI-26_narrative2017.pdf

Partners This Period

Yakama Nation, Cle Elum Supplementation and Research Facility
Types: Other
Scale: Tribal
Notes:

Washington Department of Fish and Wildlife
Types: Government
Scale: STATE
Notes:

Northwest Fisheries Science Center, National Marine Fisheries Service
Types: Government
Scale: FEDERAL or NATIONAL
Notes:

University of Washington, School of Aquatic and Fishery Sciences, College of the Environment (UW)
Types: Academic Institution
Scale: STATE
Notes:

STANDARD QUESTIONS

Community Hazard Resilience

No **Community Hazard Resilience** information reported

Economic Impacts

No **Economic Impacts** information reported

Impacts and Accomplishments

(1)

Type	accomplishment
Title	Quantify levels of inbreeding and its effects on fitness in two salmon hatchery populations
Relevance	Captive breeding programs can rebuild depleted populations and aid in the recovery of threatened or endangered species. However, numerous factors within captive breeding programs can increase the risk of inbreeding, or mating between relatives. Inbreeding can decrease the fitness of individuals (called inbreeding depression), which may then negatively affect the adaptive potential of the entire population and increase the risk of extinction. Despite the possible negative consequences of inbreeding and inbreeding depression, research efforts have focused on other risks associated with captive breeding programs (e.g. adaptation to captivity). Thus, there is a need to develop a comprehensive understanding of the impacts of inbreeding and inbreeding depression in captive and supplemented wild populations, so that the success of management and recovery efforts can be maximized. Bridging this gap between research and conservation can have impacts on local, state, and regional scales.
Response	This project is part of a larger research collaboration between the University of Washington, the Yakama Nation, the Washington Department of Fish and Wildlife, and NOAA's Northwest Fisheries Science Center. Specifically, a long-term data set from the Cle Elum Supplementation and Research Facility has been used to address other fundamental and applied research questions related to captive breeding (e.g. Washington Sea Grant project number R/HCE-4). This project builds upon previous efforts and used existing data to quantify multi-generational impacts of inbreeding and inbreeding depression in two hatchery populations of Chinook salmon. The two hatchery populations were derived from the same source but are now managed as separate lines, one integrated with and one segregated from the wild population. By comparing these two populations, the effectiveness of different management practices on captive populations can be assessed. First, inbreeding coefficients were estimated across four generations of each hatchery line using genomic data generated by previous

	WSG-funded research. Then, the effects of inbreeding on five fitness-related traits in adult salmon were quantified using linear mixed-effects models.
Results	Preliminary results show that integrated hatchery management, or deliberate breeding between hatchery and wild fish, reduces the risk of inbreeding over four generations when compared to segregated hatchery management. Small but statistically significant correlations between inbreeding coefficient and fitness were observed for some of the fitness-related traits. The next phase of this project is to incorporate these results into a model that quantifies the effects of inbreeding depression in hatchery salmon on the eco-evolutionary dynamics of supplemented wild populations. This work is still on-going and thus has not had any direct impacts on hatchery management and policy. However, this work is extremely important, because wild and hatchery-reared salmon provide millions of dollars to local economies and are inherently important to the culture of the Pacific Northwest. Unfortunately, many wild salmon populations in the region are declining. Numerous hatchery supplementation programs exist to bolster these populations, but their long-term effectiveness has been questioned due to associated genetic and phenotypic change that may occur due to captive rearing. This project is one of the first to explicitly link inbreeding with population dynamics, identify critical levels of inbreeding for fisheries management, and examine the effectiveness of integrated management to reduce potential risks. The findings will inform management practices that maximize the fitness of captive-reared fish and aid the restoration of salmon populations across the West Coast. The multi-generational results of two different management approaches will also provide managers with a range of outcomes for hatcheries, which will be highly relevant to future risk assessments of such programs.
Recap	Inbreeding is likely an unrecognized cause of reduced fitness of hatchery-reared salmon, and this project explicitly quantified its impacts to the fitness of hatchery fish, and will then examine its ecological and evolutionary effects on supplemented wild populations.
Comments	
Primary Focus Area	Sustainable Fisheries and Aquaculture
Secondary Focus Areas	

Secondary Focus Areas	Healthy Coastal Ecosystems
Goals	Ocean and coastal habitats are protected, enhanced and restored.|Aquaculture operations and shellfish harvests are safe, environmentally sustainable and support economically prosperous businesses.|Fisheries are safe, responsibly managed and economically and culturally vibrant.
Partners	University of Washington, Yakama Nation, Washington Department of Fish and Wildlife, NOAA's Northwest Fisheries Science Center
PI Draft	

Leveraged Funds

No **Leveraged Funds** information reported

Meetings, Workshops, Presentations

(1)

Type of Event	Public or professional presentation
Description	I presented my preliminary results at the "Yakima Basin Spring Chinook Internal Project Review" meeting. The meeting is held every year and serves as a venue for everyone involved in Yakima Basin Spring Chinook activities to give updates on their research. The meeting attendees comprise hatchery managers and researchers from the Yakama Tribe, the Washington Department of Fish and Wildlife, the National Marine Fisheries Service, and the Columbia River Inter-tribal Fish Commission
Event Date	02-16-2017
Number of Attendees	20

(2)

Type of Event	Public or professional presentation
Description	I presented preliminary results to members of the Conservation Biology Division at NOAA's Northwest Fisheries Science Center in Seattle, Washington at one of their lab meetings.

Event Date	12-06-2016
Number of Attendees	12

Tools, Technologies, Information Services / Sea Grant Products

(1)

Description	This project will develop tools and information that hatchery managers and state/federal government agencies can adopt to reduce the risk of inbreeding in hatchery and wild populations. Specifically, I will build an Integral Projection Model (IPM) to determine how inbreeding affects phenotypic variability and population size in supplemented wild populations over time. This model will be well documented and well annotated, so that other people can use it and, if necessary, modify it to investigate questions in other systems and organisms. Additionally, I will conduct sensitivity analyses to identify levels of inbreeding to avoid in hatchery management. The "critical levels" of inbreeding that I identify can be incorporated in hatchery programs throughout the Pacific Northwest, thereby improving the conservation of salmon populations and the resilience of populations to future stressors. It is important to note that this project is still underway. As such, these tools and guidelines have not yet been developed and have not yet influenced management.
Developed (in the reporting period)?	No
Used (in the reporting period)?	No
Used for EBM?	No
ELWD product?	No
Number of managers	0
Description/Names of managers	The tools are not yet developed
Reported in previous year?	

Progress Report 8/1/2016-2/28/2017

Modeling the effects of inbreeding in salmon hatcheries on the eco-evolutionary dynamics of supplemented wild populations, R/E/I-26

Charles D. Waters, University of Washington, NMFS-Sea Grant Fellow
Kerry A. Naish, University of Washington, Faculty Advisor
Jeff Hard, NOAA Northwest Fisheries Science Center, NMFS Mentor

Activities Carried Out:

This project aims to quantify the genetic and demographic risks of inbreeding in hatchery populations of Chinook salmon. Inbreeding coefficients will first be estimated from genomic data in two closely related hatchery populations, and the effects of inbreeding on fitness-related traits will be quantified. Empirical results will then be incorporated into an Integral Projection Model (IPM) to quantify the effects of inbreeding depression in hatchery salmon on the eco-evolutionary dynamics of supplemented wild populations. Sensitivity analyses of the IPM will also help to identify critical levels of inbreeding to avoid in hatchery propagation. The specific project objectives are:

1. To estimate inbreeding coefficients from genomic data within two hatchery populations of Chinook salmon with contrasting levels of gene flow with the natural population.
2. To assess the effect of inbreeding on fitness traits in each hatchery population by determining if level of inbreeding is correlated with changes in fitness traits.
3. To build an Integral Projection Model (IPM) to determine how inbreeding affects phenotypic variability and population size in supplemented wild populations over time.
4. To identify levels of inbreeding to avoid in hatchery management and to determine if managed gene flow mitigates potential risks of inbreeding.

Our activities to date for each of these tasks are as follows:

1) We used existing genomic data, generated by previous WSG-funded research (Project R/HCE-4), to generate preliminary estimates of inbreeding coefficients for 465 adult Chinook salmon from four generations (1998, 2002, 2006, and 2010). We also estimated levels of relatedness between pairs of individuals within each generation of each hatchery line, as this measure can influence future levels of inbreeding.
2) Potential negative effects of inbreeding on fitness (i.e. inbreeding depression) were then assessed using linear mixed effects models and five fitness-related traits – fork length, weight, return time, maturation time, and daily growth coefficient. These traits are of particular interest, as previous studies have documented significant inbreeding depression in similar traits in a related species, steelhead trout.

Participants:

This project is part of a larger research collaboration between the University of Washington, the Yakama Nation, the Washington Department of Fish and Wildlife, and NOAA's Northwest Fisheries Science Center. Specifically, a long-term data set from the Cle Elum Supplementation and Research Facility has been used to address other fundamental and applied research questions related to captive breeding (e.g. Washington Sea Grant project number R/HCE-

4). This project builds upon previous efforts and used existing data to accomplish the two activities described in the previous section.

Results:

Average levels of inbreeding and pairwise relatedness were not significantly different between the integrated and segregated hatchery lines (Figs. 1-2). However, the variances of inbreeding coefficients and estimates of pairwise relatedness were larger in the segregated line. In particular, the variance of pairwise relatedness increased over time in the segregated line. These results suggest that integrated management successfully reduced the genetic and phenotypic risks of inbreeding over three hatchery generations. In contrast, the risks associated with inbreeding will only increase within the segregated line in the future.

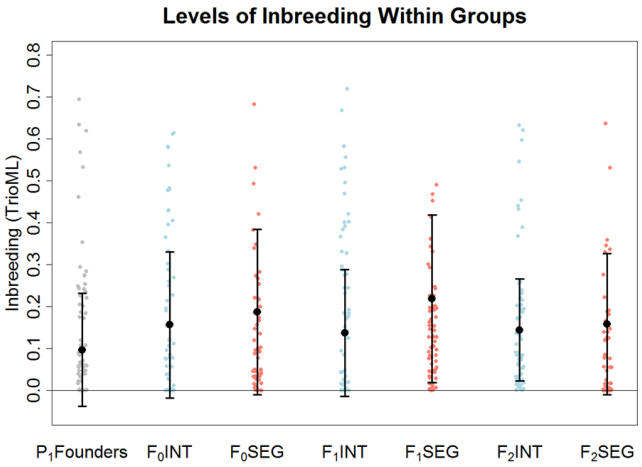

Figure 1. Estimates of inbreeding coefficients for individuals within each generation of each hatchery line. Estimates for the 1998 wild founders (P_1) are in gray while the three generations of the integrated and segregated hatchery lines are in blue and red, respectively.

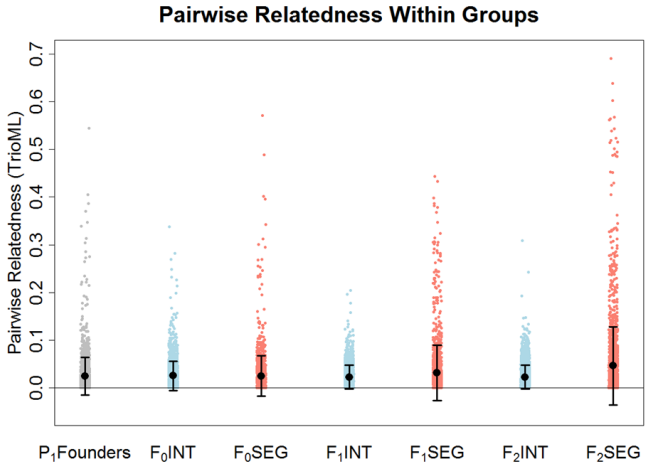

Figure 2. Estimates of pairwise relatedness for individuals within each generation of each hatchery line. Estimates for the 1998 wild founders (P_1) are in gray while the three generations of the integrated and segregated hatchery lines are in blue and red, respectively.

Linear mixed effects models revealed small but statistically significant negative correlations between inbreeding coefficient and phenotype for both fork length and weight. No association between inbreeding coefficient and phenotype was observed for return time, maturation time, and daily growth coefficient. However, these model results are preliminary and require further exploration before any conclusions can be made.

Challenges Encountered:

No challenges were encountered for the project during this time period.

Changes in Project Direction:

None

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.

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

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.

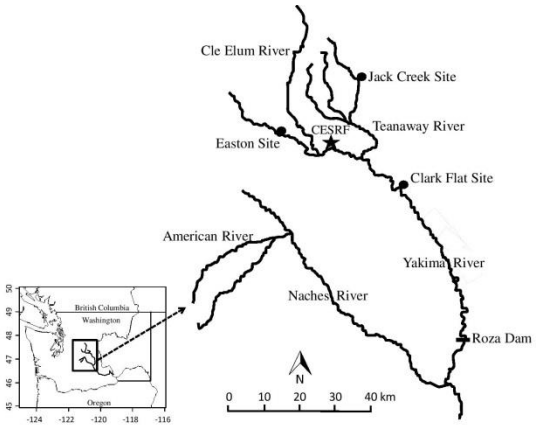

Figure 1. From Waters *et al*. (2015). Map of the Yakima River system. The upper Yakima Chinook salmon population is the target of the Cle Elum Supplementation and Research Facility (CESRF). All returning adults are sampled at Roza Dam and allowed to spawn naturally (natural origin and integrated line 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;

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.

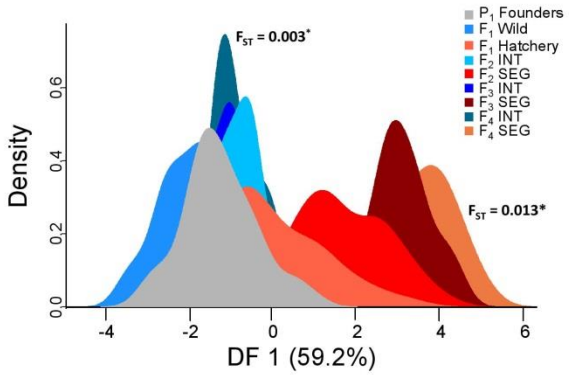

Figure 2. Density plot of individuals along the first discriminant function from the discriminant analysis of principal components (DAPC) for the wild founders (P_1 Founders, black) and four generations of the integrated (INT, blue colors) and segregated (SEG, red colors) hatchery lines. Pairwise F_{ST}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

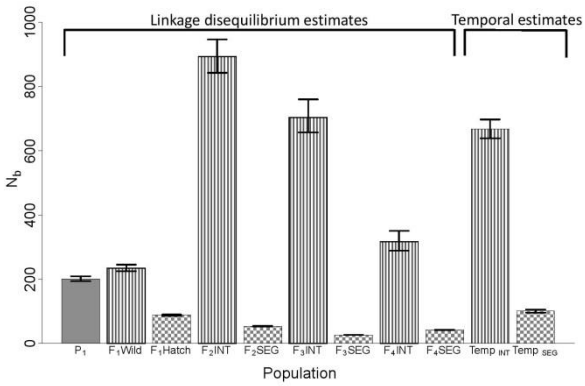

Figure 3. Estimates of effective number of breeders, N_b, and 95% confidence intervals produced by the linkage disequilibrium (LD) and temporal methods. The LD method enables estimation of N_b for every generation, while a single estimate for the sampling period is produced by the temporal method. LD 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

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.

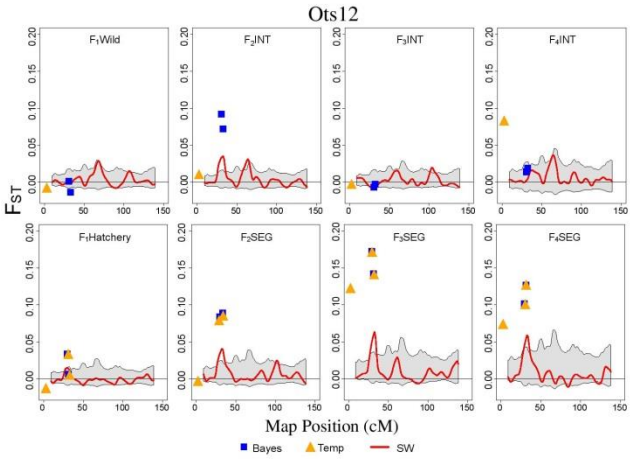

Figure 4. Loci and regions of the genome showing signatures of adaptive divergence, based on pairwise F_{ST} compared to the P_1 founders, on chromosome Ots12 for the integrated (top panel) and segregated (bottom panel) hatchery lines through the F_1, F_2, F_3, and F_4 generations. Blue squares are loci 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.

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