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Using pedigrees to understand reproductive dynamics of imperiled populations of coho salmon (Oncorhynchus kisutch)

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SANTA CRUZ

USING PEDIGREES TO UNDERSTAND REPRODUCTIVE DYNAMICS OF IMPERILED POPULATIONS OF COHO SALMON (*ONCORHYNCHUS KISUTCH*)

A thesis submitted in partial satisfaction of the
requirements for the degree of

MASTER OF SCIENCE

in

OCEAN SCIENCES

by

Hayley M. Nuetzel

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Abstract

Using pedigrees to understand reproductive dynamics of imperiled populations

of coho salmon (*Oncorhynchus kisutch*)

by

Hayley M. Nuetzel

Populations of anadromous Pacific salmon and steelhead are increasingly threatened by degradation and loss of critical freshwater habitat, as well as poor survival at sea. Coho salmon (*Oncorhynchus kisutch*) populations in California, which represents the southern limit of the species' range, are particularly vulnerable to such environmental disturbances. Accordingly, all California populations are listed under the Endangered Species Act and have been placed into two Evolutionarily Significant Units (ESUs): the Southern Oregon/Northern California Coast (SONCC) ESU, which is listed as threatened; and the Central California Coast (CCC) ESU, which is listed as endangered. Despite these protections, many populations are threatened with extirpation and natural productivity is severely impacted by the lack of natural habitat. In order to facilitate recovery, several integrated recovery and conservation hatchery programs operate within these ESUs. However, how effectively these programs contribute to production in these high-risk populations has yet to be quantified, which prevents the development of evidence-based mitigation strategies that aim to incorporate artificial propagation.

In chapter one, I use pedigree reconstruction tools to understand current reproductive dynamics of coho salmon in the Shasta River, CA. I particularly focus on

evaluating the contribution of excess adults released from the nearby Iron Gate Hatchery (IGH) to production within the Shasta to determine the efficacy of directly supplementing the Shasta population with these hatchery releases. Less than 10% of the juveniles sampled in the Shasta each year were assigned to IGH releases, suggesting low reproductive success amongst these individuals within the Shasta River. In chapter two, I assess the efficacy of the current Captive Broodstock program at Kingfisher Flat Hatchery on Scott Creek, CA. I again use pedigree reconstruction methods to perform parentage-based assignment for juveniles collected in Scott Creek, CA against a parental pool of adults that spawned both within the hatchery and the creek. Few Captive Broodstock adults were recovered as parents, again suggesting low reproductive success amongst these individuals.

I ultimately discuss the implication of these results with respect to management, indicating measures that may optimize both ongoing and proposed conservation strategies to ensure long-term stability within these imperiled populations of coho salmon.

To everyone still fighting the good fight.

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Introduction

Coho salmon (*Oncorhynchus kisutch*), one of several Pacific salmonid species, is broadly distributed along the continental shelf throughout the North Pacific Ocean (Sandercock, 1991). In recent years, however, many populations of coho salmon have severely declined or been extirpated from historically productive watersheds. These range-wide declines can be attributed to a variety of disturbances, such as habitat modifications for agricultural and residential development, disruptions to flow regimes and migration patterns due to hydropower and water diversions, commercial and recreational harvest, and interactions with hatchery fish subject to domestication selection (Bradford & Irvine, 1999; Clemento *et al.,* 2009; Fritts *et al.,* 2007). Populations within California, the southern limit of the species range in North America, have experienced particularly dramatic declines in abundance and, as such, have been listed under the Endangered Species Act since the late-1990s (Brown, Moyle & Yoshiyama, 1994; Rogers *et al.,* 2016; Weeder *et al.,* 2016). California coho salmon populations are separated into two distinct Evolutionarily Significant Units (ESUs): 1) the Southern Oregon/Northern California Coast ESU (SONCC ESU), which is classified as threatened, and 2) the Central California Coast ESU (CCC ESU), which is classified as endangered. Several populations

within these respective ESUs suffer from unsustainable population sizes and low apparent rates of recovery. While several mitigation actions have been implemented to curtail dramatic declines in adult escapement to these vulnerable systems, assessments of productivity, such as quantification and identification of effective breeders, have yet to be undertaken.

Assessments of reproductive dynamics within natural populations can be achieved using pedigree reconstruction methods. Pedigree reconstruction methods use genetic data to infer genealogical relationships, such as parentage or sibling relationships, amongst sampled individuals (Blouin, 2003). Parentage-based inference has proven to be a strong tool for estimating reproductive success and the number of effective breeders within a population, as well as the inheritance of traits (Garant, Dodson & Bernatchez, 2001; Sheldon, Kruuk & Merila, 2003; Abadía-Carduso *et al.,* 2013). Additionally, pedigrees can provide information on existing mating systems and relatedness within populations that merit more intensive conservation efforts (Miller, Adams & Waits, 2003; DeSalle & Amato, 2004).

The preferred genetic markers used to construct these pedigrees are constantly developing, but with the goal always being to identify abundant, variable markers that provide sufficient resolving power to efficiently, and accurately, infer genealogical relationships. For many years, the highly polymorphic nature of microsatellites made them the marker of choice for pedigree-based analyses (Queller, Strassmann & Hughes, 1993; Jones & Ardren, 2003). However, microsatellites have several drawbacks, including relatively high incidences of homoplasy and genotyping error, relatively low genotyp-

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ing throughput and the difficultly of standardizing these markers across laboratories (Narum *et al.,* 2008). Single nucleotide polymorphisms (SNPs), or a variation in a single nucleotide at a specific position within the genome, have emerged as a low-cost, high-throughput alternative to microsatellites (Narum *et al.,* 2008; Hauser *et al.,* 2011). While SNPs are biallelic, and therefore do not provide as much information per locus as microsatellites, it has been demonstrated that as few as 60-100 SNPs may allow for accurate parentage inference, even within populations involving thousands of individuals (Anderson & Garza, 2006). Consequently, SNP-based pedigree reconstruction methods have become an increasingly powerful and efficient tool for informing ecological hypotheses within natural populations.

In both chapters one and two, I utilize SNP data to reconstruct pedigrees within populations of at-risk coho salmon. In chapter one, I apply pedigree reconstruction methods to a population of coho salmon in the Shasta River, California (SONCC ESU) to quantify the contribution of hatchery-released adults to productivity within the system. In chapter two, I apply pedigree reconstruction methods to a population of coho salmon in Scott Creek, California (CCC ESU) to assess the efficacy of ongoing hatchery and captive broodstock practices. Both studies represent the utility of pedigree analyses in describing reproductive dynamics within imperiled populations, and the importance of using this information to develop informed, effective management strategies.

Chapter 1

Using SNP-based parentage inference to estimate contribution of coho salmon (*Oncorhynchus kisutch*) from Iron Gate Hatchery to production in the Shasta River

1.1 Abstract

Coho salmon (*Oncorhynchus kisutch*) in the Shasta River (Klamath River basin) are considered a functionally independent population whose stability is essential to the recovery of the Southern Oregon/Northern California Coast Coho Salmon Evolutionarily Significant Unit. However, consistently low population size and negative

trends in productivity exacerbate the already high extinction risk of this critical population, demonstrating the need for an effective management plan. Supplementation of this population using excess adult coho salmon returning to the nearby Iron Gate Hatchery (IGH) has been suggested as a strategy to immediately increase effective population size. To determine whether the placement of excess broodstock from IGH would disrupt existing population dynamics within the Shasta River, we quantified the current contribution of IGH fish to productivity in the Shasta using SNP-based parentage analysis. Genotype data at 88 SNP loci were generated for both coho salmon juveniles collected in the Shasta River from 2013-2015, and adult fish that returned to IGH from 2010-2015. Identification of parent-offspring trios and single parent-offspring pairs via likelihood-based pedigree reconstruction methods indicated that only 8.67%, 1.72% and 1.29% of juveniles caught in 2013, 2014 and 2015, respectively, were offspring of IGH fish. This suggests the majority of the parents were unsampled. Given that sampling of returning adults at the IGH weir has been fairly complete since 2010, our results suggest that coho salmon production in the Shasta River may be predominately attributed to natural origin fish or, perhaps more likely, to a combination of natural origin and unsampled hatchery-produced fish that stray directly to the Shasta River to spawn. Given the low observed contribution by IGH fish, management efforts that directly place mature fish into appropriate spawning habitat, as well as habitat restoration, may be the best means of conserving this population.

1.2   Introduction

Coho salmon (*Oncorhynchus kisutch*) of the Klamath River basin fall within the Southern Oregon/Northern California Coast Coho Salmon Evolutionary Significant Unit (SONCC ESU), which is designated as threatened under the Endangered Species Act (ESA, Fed Reg 1997). Much of the decline in coho population density and diversity in the Klamath basin can be attributed to anthropogenic disturbances, such as agricultural development and dam construction, which have severely disrupted native salmonid habitat. The Shasta River, a tributary to the mainstem Klamath River, is no exception, with an estimated 22% of suitable fish habitat made inaccessible by the 1926 construction of the Dwinnell Dam and Parks Creek diversion (NMFS, 2004). In an attempt to mitigate some of the negative effects on fishes and habitat in the Klamath River basin, and to supplement natural yearling production, multiple hatchery programs have been established within the SONCC ESU.

The Iron Gate Hatchery (IGH) coho salmon program was instituted in the late 1960s, using Trinity River Hatchery coho as the founding stock. Since 1976, IGH broodstock has been composed exclusively of adults returning to IGH (California HSRG, 2012). Iron Gate Hatchery is located on the Klamath River approximately 13 miles upstream of the Shasta River confluence, and has a production goal of 75,000 coho salmon, in addition to 6 million Chinook salmon and 200,000 steelhead (NMFS, 2014). The location and productivity of IGH has impacted natural coho population dynamics in the Shasta, namely by perturbing intra- and interspecies interactions and altering

genetic diversity (NMFS, 2014).

Additionally, the number of adult coho salmon returning to spawn in the Shasta remains significantly lower than historical estimates, and the number of yearlings per adult has displayed a downward trend in recent years (NMFS, 2014). Approximately 163 adults were estimated to have returned to the Shasta in 2013, a high relative to recent years (Chesney and Knechtle, 2017); however, the depensation threshold is estimated at 531 spawning adults (Williams *et al.*, 2008). This disparity places coho salmon in the Shasta River at a high risk of depensatory effects and ultimately extirpation, necessitating a mitigation strategy that increases effective population size immediately, which increases spawning stock and potential for reproductive success. Given that natural habitat has been significantly reduced in the Shasta River basin, one may expect the potential for natural production to be similarly reduced, such that an immediate recovery strategy may ultimately require supplementation via artificial propagation as has been suggested for comparably depauperate and disturbed populations of Pacific salmon (Brannon *et al.*, 2004).

The existing Hatchery and Genetic Management Plan (HGMP) for IGH specifies the incorporation of wild fish into spawning matrices to reduce inbreeding and domestication selection of hatchery fish (CDFW, 2014). Additionally, current assessments of genetic divergence throughout the Klamath River Basin suggest the Shasta River is not significantly different from the Upper Klamath populations, including IGH (Gilbert-Horvath *et al.*, 2016), potentially due to introgression via ongoing straying of hatchery fish into the Shasta River (Garza, unpublished data). This hypothesis is sup-

ported by video evidence of adult IGH fish (marked by a left maxillary clip) naturally entering the Shasta River. Additionally, since 2011 excess broodstock from IGH have been marked with Passive Integrated Transponder (PIT) tags prior to release into the Klamath River, and a certain proportion of these PIT tagged fish have been detected in the Shasta River every year since (Chesney & Knechtle, 2012-2017). Consequently, one mitigation strategy that has been proposed is direct supplementation of Shasta River coho with these excess broodstock fish from IGH. These fish are individuals that return to the hatchery, are not selected for spawning, and instead are released back into the river as non-broodstock fish. This low-cost strategy would use readily available fish to immediately increase spawning stock in the Shasta River with little impact to IGH management. By using these non-broodstock, predominately hatchery-origin, fish for supplementation, rather than natural origin (NO) fish that return to the hatchery, IGH can continue to incorporate NO adults into spawning matrices per the standing HGMP. Hence, this will allow IGH to uphold operations that ultimately aim to reduce domestication selection amongst the hatchery fish that will continue to naturally stray into many areas of the Upper Klamath Basin (Ward & Kinziger, report from Upper Klamath Workshop, 2012).

While IGH fish do appear to be increasing adult escapement estimates in the Shasta River, how effectively these non-broodstock IGH adults contribute to coho salmon productivity in the Shasta River needs to be quantified. This can be achieved by performing parentage-based inference on coho juveniles collected in the Shasta River. Parentage based tagging techniques utilize genetic data to accomplish large-scale pedi-

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gree reconstruction (Anderson & Garza, 2006). Single nucleotide polymorphisms (SNPs) have become widely accepted as cost-effective, unambiguous genetic markers, and are increasingly utilized to reliably genotype thousands of individuals at numerous target loci to inform parentage inference analyses (Morin *et al.* 2004; Abadía-Cardoso *et al.,* 2013; Hauser *et al.,* 2011; Weinman *et al.,* 2014).

In the present study, we utilized parentage-based tagging techniques to assign juveniles caught in the Shasta River from 2013-2015, to a potential parent pool of fish that passed through IGH both broodstock and non-broodstock fish from 2010-2015. The resulting proportion of juveniles confidently deemed to be offspring of IGH returning adults was then used to assess the degree of successful reproduction by IGH non-broodstock fish currently occurring within the Shasta River. Given the documented presence of IGH adults in the Shasta River, we expect a fair proportion of juveniles to be assigned to one or two IGH non-broodstock parents. This finding would suggest that excess fish from IGH migrate downstream to the Shasta upon release to spawn and are able to successfully reproduce within the Shasta River. We use this application of parentage-based tagging techniques to advance our understanding of reproductive dynamics within a particularly vulnerable natural system, and to ultimately inform conservation practices.

Table 1.1: Number of adult coho sampled each year at Iron Gate Hatchery (IGH). An individual was removed from analysis if it was missing data at more than 10 loci (78 loci minimum) or if it was determined to be a non-coho species.

Spawn Year	Total no. samples	No. of samples analyzed	No. of females analyzed	No. of males analyzed
W2010/2011	513	441	218	223
W2011/2012	553	533	188	345
W2012/2013	601	576	170	406
W2013/2014	1350	1300	661	639
W2014/2015	395	386	89	297
Total	*3412*	*3236*	*1326*	*1910*

1.3 Methods

1.3.1 Tissue collection and DNA extraction

Tissue samples were collected from 3412 adult coho returning to IGH from 2010-2015 (Table 1.1), and from 2185 juveniles passing through the downstream rotary trap on the Shasta River from 2013-2015 (Table 1.2). The weir-trapped, sampled adults were predominately IGH-origin fish (marked by a left maxillary clip) and included both adults taken to be spawned at the hatchery ("broodstock"), and adults sampled and released ("non-broodstock") during those years. Adults that returned to the Shasta were not sampled. Tissue samples were digested in Proteinase K lysis buffer and extracted on a QIAGEN BioRobot 3000, following the DNeasy 96 Tissue Kit protocol (QIAGEN Inc., Hilden, Germany).

Table 1.2: Number of juvenile coho sampled each year in the Shasta River. An individual was removed from analysis if it was missing data at more than 10 loci (78 loci minimum) or if it was determined to be a non-coho species.

Collection Year	Total no. of samples	No. of samples analyzed
2013	162	150
2014	429	408
2015	1594	1555
Total	*2185*	*2113*

1.3.2 SNP loci and genotyping

A panel of 95 SNP loci was selected from predesigned assays known to target SNPs with minor allele frequencies conducive to parentage-based tagging inference (Anderson and Garza, 2006; Starks, Clemento & Garza, 2016; Smith *et al.,* 2006; Campbell & Narum, 2011). One additional species-specific locus was utilized to distinguish coho from their sister species Chinook (*Oncorhynchus tshawytscha*) salmon. All individuals were genotyped with predesigned TaqMan (Applied Biosystems Corporation, Foster City, U.S.A.) or SNP Type (Fluidigm Corporation, San Francisco, CA, U.S.A.) assays, using 96.96 Dynamic Genotyping Arrays on the Fluidigm EP1 Genotyping system. All genotypes were called using SNP Genotyping Analysis Software v 3.1.3 (Fluidigm Corporation, San Francisco, U.S.A.). Because genotyping began using a panel of 95 loci originally optimized for TaqMan chemistry and later transitioned to SNPtype, the downstream analyses utilized a subset of 88 loci that were shared across all genotyping panels (Table S1 in Supporting Information). Any individuals missing data at more than 10 loci were not considered in downstream analyses (Table 1.1; Table 1.2).

1.3.3 Parentage Analysis

Using known age-length relationships (Chesney *et al.,* 2007), the majority of the juveniles collected in the Shasta River were estimated to be age 1, with a small number of age 0 or age 2 individuals. These age data were then used to construct a pool of potential parents, consisting of all adults returning to IGH during biologically plausible spawn years for each Shasta juvenile collection year (Table 1.3). Parentage analysis was performed using three separate pedigree reconstruction methods: SNPPIT (Anderson, 2010), COLONY2 (version 2.0.6.1) (Jones & Wang, 2009; Wang, 2016), and FRANz (version 2.0) (Riester, Stadler & Klemm, 2009). We compared the results of all three analyses, filtering by confidence thresholds and concordance to generate a robust estimate of parentage between Shasta River juveniles and IGH adults.

Utilizing multiple inference methods was particularly important for this dataset given that non-broodstock fish released from IGH are likely to mate with wild fish as well as other IGH strays in the Shasta River, creating a potentially common situation in which only one parental genotype may be sampled. Determining parentage when the parental population is not completely sampled is one of the primary limitations to recovering accurate pedigrees amongst natural populations, as identification of parents may be impossible for some juveniles or have a higher likelihood of erroneous assignment (Jones & Arden, 2003; Pemberton, 2008). Additionally, given that SNPPIT was optimized for hatchery-produced Pacific salmonids, assignments are restricted to parent-offspring trios (Anderson, 2012), requiring the application of additional pedigree

Table 1.3: Potential parent pool for each Shasta River juvenile collection. The potential parents include all adults that returned to IGH in the listed spawn years and passed quality filtering criteria.

Juvenile collection year	Total no. of potential offspring	Spawn years of potential parents	Total no. of potential parents
2013	150	W2010/2011, W2011/2012, W2012/2013	1550
2014	408	W2011/2012, W2012/2013, W2013/2014	2409
2015	1555	W2012/W2013, W2013/2014, W2014/2015	2262

reconstruction methods (COLONY2 and FRANz) to identify single parent-offspring pairs.

1.3.3.1 Accuracy assessment of single parent-offspring inference using COLONY2 and FRANz

Prior to performing parentage analysis on the Shasta River empirical datasets we conducted an accuracy assessment on a dataset for which 24.8% of the parental population was removed. This was accomplished by identifying parent-offspring trios in SNPPIT, using the adult IGH coho returning in winters 2012/2013 and 2013/2014 as offspring, and the 2010/2011 and 2011/2012 returning adults as potential parents. Resulting trios were first filtered by FDR ≤ 0.01. SNPPIT reports statistical confidence in any given trio via the false discovery rate (FDR) parameter, which is the rate of false assignments that can be anticipated should that trio, and any trios with lower FDR values, be accepted (Anderson, 2012). By only accepting trio assignments with a FDR ≤ 0.01, one can expect every 1 in 100 assignments to be inaccurate.

The parents in these filtered trios were then compared to spawning lot informa-

tion from IGH to determine if the mother and father selected by SNPPIT represented a known spawning pair. For each trio meeting these criteria, a single parent was randomly removed. The genotype data from the remaining parents and all of the offspring were then supplied to COLONY and FRANz using input parameters described below. The random removal of one parent on a trio-by-trio basis allowed for the recovery of single parent-offspring pairs and parent-offspring trios, due to the practice of mating a female with multiple males in the hatchery, as well as the possibility for a male-female pair to have multiple offspring. The resulting assignments were then compared to the list of confirmed spawn pairs to assess the accuracy of the resulting trios and single parent-offspring pairs.

1.3.3.2 Parentage analysis parameters for empirical data

Parent-offspring trios were first assigned using SNPPIT, assuming a genotyping error rate of 0.005. Each juvenile sampling year was analyzed separately, and SNPPIT was informed of the sex and spawning year of all potential parents. Because only trios concordant between several parentage inference methods were accepted (see following section), resulting trios were filtered by a slightly less stringent FDR (FDR ≤ 0.05).

Each FRANz and COLONY2 run was supplied with the same offspring-parent pool supplied to SNPPIT (Table 3). The FRANz analysis utilized prior information, including the sex and birth year of individuals, as well as a list of accepted pedigrees created from SNPPIT trios with a FDR ≤ 0.05. It is important to note that the accuracy assessment run using FRANz was not supplied with a list of accepted pedigrees as

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the accuracy assessment input dataset was created only from individuals assigned to trios by SNPPIT at FDR $\leq$ 0.01. Consequently, supplying such a list of pedigrees would simply reproduce the results of the SNPPIT run. The input parameters for each FRANz run were as follows: reproductive age of 2-4 years for both sexes, 300 estimated candidate mothers and fathers, and no simulated annealing optimization. All single parent pair and trio assignments recovered by FRANz were filtered by posterior probability $\geq$ 0.95. For the COLONY2 analyses, the input parameters for each run were as follows: both sexes polygamous and dioecious, no sibship size prior or full sibship scaling, full likelihood estimation with medium run length and precision, and no updating of allele frequencies. The allelic dropout rate and genotyping error rate were estimated at 0.0025 each. Resulting parentage assignments were then filtered by posterior probability $\geq$ 0.95.

1.3.3.3 Consensus method for accepting parentage assignments

We examined all parent-offspring trios and single parent-offspring pairs passing confidence thresholds for concordance between at least two of the pedigree reconstruction methods to maximize the recovery of well-supported pedigrees. For parent-offspring trios, we accepted assignments generated by SNPPIT with FDR $\leq$ 0.05, and which were also recovered by COLONY2 at a posterior probability $\geq$ 0.95. Concordance between SNPPIT and FRANz is uninformative as FRANz was supplied with a list of SNPPIT-derived trios.

Additionally, we accepted any trios that were recovered by both COLONY2

and FRANz at posterior probabilities ≥ 0.95. Similarly, for the single parent pairs we accepted any assignments that were recovered by both COLONY2 and FRANz at posterior probabilities ≥ 0.95.

1.3.4   Sibship analysis

To analyze the family structure and estimate the number of adults successfully reproducing in the Shasta River each year, we performed sibship analysis in COLONY2. COLONY2 produces two estimates of support for each Full Sibling (FullSib) family: inclusive (Prob(Inc.)) and exclusive (Prob(Exc.)) probability. The inclusive probability indicates to what extent a given family can be split into two or more additional families, while the exclusive probability indicates the likelihood that the given family is missing full siblings (Wang, 2016). For example, a high inclusive but low exclusive probability suggests the proposed full sibship is well supported but may have been split, and is therefore incomplete. However, all FullSib families including only one member will automatically be assigned P(Inc.) $= 1$ because the family cannot be split. Consequently, we filtered the resulting FullSib families by Prob(Inc.) ≥ 0.95 and Prob(Exc.) ≥ 0.95, to prevent biasing our family structure estimates towards likely incomplete single-member families.

In order to estimate the proportion of adults that produced offspring during the sample years, we first combined the filtered FullSib and parentage results to identify any full sibling families attributed to two IGH broodstock fish. These FullSib families were then removed, given that such families do not represent production within the

Shasta River. Additionally, we removed any FullSib families that appeared questionable given the metadata associated with the assigned parent(s) (i.e., when only one parent was found and it was a confirmed broodstock fish). We then tallied the number of unique maternal and paternal genotypes. The total number of unique parental genotypes was then compared to escapement estimates during the parental spawn seasons for each juvenile collection to infer the proportion of adults successfully contributing to production within the Shasta.

1.4 Results

1.4.1 Parentage

1.4.1.1 COLONY2 and FRANz accuracy assessment

After filtering all SNPPIT trios for the 2012/2013 and 2013/2014 IGH adult coho as offspring by FDR $\leq$ 0.01 and concordance with known IGH spawning information, 1254 parentage assignments remained. From these 1254 trios, either a father or mother was randomly removed, resulting in 59 unique maternal genotypes and 61 unique paternal genotypes. This random subsample of parental genotypes was then supplied to COLONY2 and FRANz, along with all 1254 offspring genotypes.

After filtering the COLONY2 output by posterior probability $\geq$ 0.95, 1173 family groups remained, of which 659 were parent-offspring trios and 514 were single parent-offspring pairs. No errors were found amongst these 1173 assignments (Table S2). For the FRANz analysis, after filtering by posterior probability $\geq$ 0.95, 1063 assignments

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remained, of which 709 were parent-offspring trios, 353 were single parent-offspring pairs, and 1 recovered no parents. Of the 1063 assignments, 15 were deemed inaccurate (1.41% inaccuracy rate). FRANz appeared to be more biased to Type Ib errors, or false positives when the putative parent was not supplied (12 of the 15 erroneous assignments) (Harrison *et al.,* 2012) (Table S3).

1.4.1.2   Parent-offspring trios

Applying the confidence thresholds described above, 11 parent-offspring trios for the 2013 Shasta River juveniles, four trios for the 2014 juveniles and 11 trios for the 2015 juveniles were deemed concordant between at least two inference methods. Of the 11 trios including 2013 Shasta juveniles, two juveniles were assigned to a pair of non-broodstock releases, while nine juveniles were assigned to confirmed IGH broodstock spawn pairs. Of the four trios recovered for the 2014 juveniles, all parent pairs were denoted as IGH broodstock and three were confirmed spawn pairs. Of the 11 trios including 2015 Shasta juveniles, five juveniles were assigned to a pair of non-broodstock releases, while six were assigned to confirmed IGH broodstock spawn pairs (Table 1.4).

1.4.1.3   Single parent-offspring pairs

Given the confidence thresholds and consensus limitations described above, a small number of single-parent offspring pairs were recovered in all three juvenile cohorts (Table 1.5). All single parents were identified as non-broodstock releases, and within the 2015 juvenile cohort, all single-parent offspring pair assignments were attributed to

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Table 1.4: Concordant parent-offspring trio assignments (SNPPIT filtered by FDR $\leq$ 0.05; COLONY2 by P $\geq$ 0.95; FRANz by P $\geq$ 0.95). The percentage in parentheses represents the proportion of juveniles assigned to two parents of that hatchery status for each collection year. The "unassigned" category includes juveniles for which no parent could be found, as well as assignments that did not pass the filtering criteria.

	No. juveniles assigned broodstock parents	No. juveniles assigned non-broodstock parents	No. of unassigned juveniles
2013 Juveniles	9 (6.0%)	2 (1.3%)	139 (92.7%)
2014 Juveniles	4 (0.98%)	0 (0.0%)	404 (99.02%)
2015 Juveniles	6 (0.39%)	5 (0.32%)	1544 (99.29%)

Table 1.5: Concordant single parent-offspring pairs (COLONY2, P $\geq$ 0.95; FRANz, P $\geq$ 0.95)

	No. juveniles assigned a single parent	No. of unique parental genotypes	Mean Posterior Probability (FRANz)	Mean Posterior Probability (COLONY2)
2013 Juveniles	2	2	0.9999 (+/- 0.0001)	0.9987 (+/- 0.0017)
2014 Juveniles	3	2	0.9993 (+/- 0.0007)	1.0000 (+/- 0.0000)
2015 Juveniles	9	1	0.9915 (+/- 0.0142)	1.0000 (+/- 0.0000)

the same putative mother.

1.4.1.4   Total contribution of IGH fish to Shasta River productivity

In order to estimate the contribution of IGH fish to productivity within the Shasta River, all trios involving two broodstock parents were removed, as these trios likely represent hatchery-produced smolts entering the Shasta River during their migration to sea, rather than reproduction that occurred within the Shasta. We also removed these assigned offspring from the total counts of juveniles each year to calculate the proportion of IGH non-broodstock adults contributing to production each sampling year.
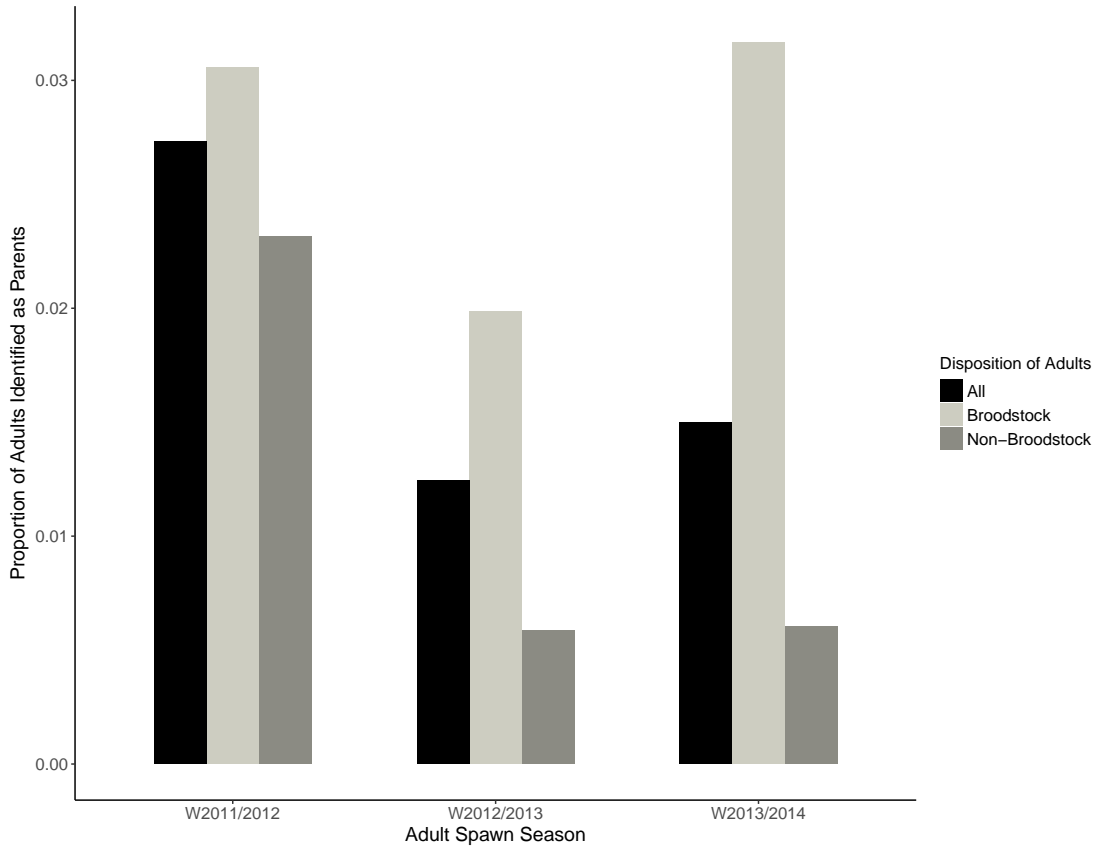
Figure 1.1: Proportion of adults identified as parents for spawn years in which assignments were recovered. Adults are categorized by disposition: all Iron Gate Hatchery (IGH) adults, all broodstock IGH adults, and all non-broodstock IGH adults. Includes all trio and single-parent assignments passing filtration criteria and concordance limitations.

So by summing the remaining non-broodstock parent-offspring trios and single parent-offspring pairs, the total estimated contribution of IGH non-broodstock adults to Shasta productivity was 2.84% (4 of 141 juveniles) in 2013, 0.74% (3 of 404 juveniles) in 2014 and 0.90% (14 of 1549 juveniles) in 2015. Ultimately, across the three relevant parental spawn years a maximum of 2.32% of all non-broodstock adults (in Winter 2011/2012) successfully contributed to production in the Shasta River (Figure 1.1). Even when all trio and single-parent assignments passing filtration criteria for at least one inference method are summed, only 21.5% of production can be attributed to non-broodstock IGH adults in 2013, 9.44% in 2014 and 7.38% in 2015 (data not shown).

1.4.2 Sibship

After filtering by confidence thresholds and removing families with metadata issues or which were attributed to two broodstock adults, we recovered 21 FullSib families amongst the 2013 juveniles, 22 FullSib families amongst the 2014 juveniles, and 64 FullSib families amongst the 2015 juveniles. The most common family size across all juvenile cohort years was a single-offspring family (Figure 1.2). Additionally, in each cohort year the largest FullSib family (23 in 2013, 26 in 2014, and 43 in 2015) was assigned to parents whose genotypes were not found amongst any of the genotyped IGH adults.

The inferred FullSib families amongst the 2013 juveniles were attributed to 40 unique parental genotypes, the FullSib families amongst the 2014 juveniles were attributed to 35 unique parental genotypes, and the FullSib families amongst the 2015
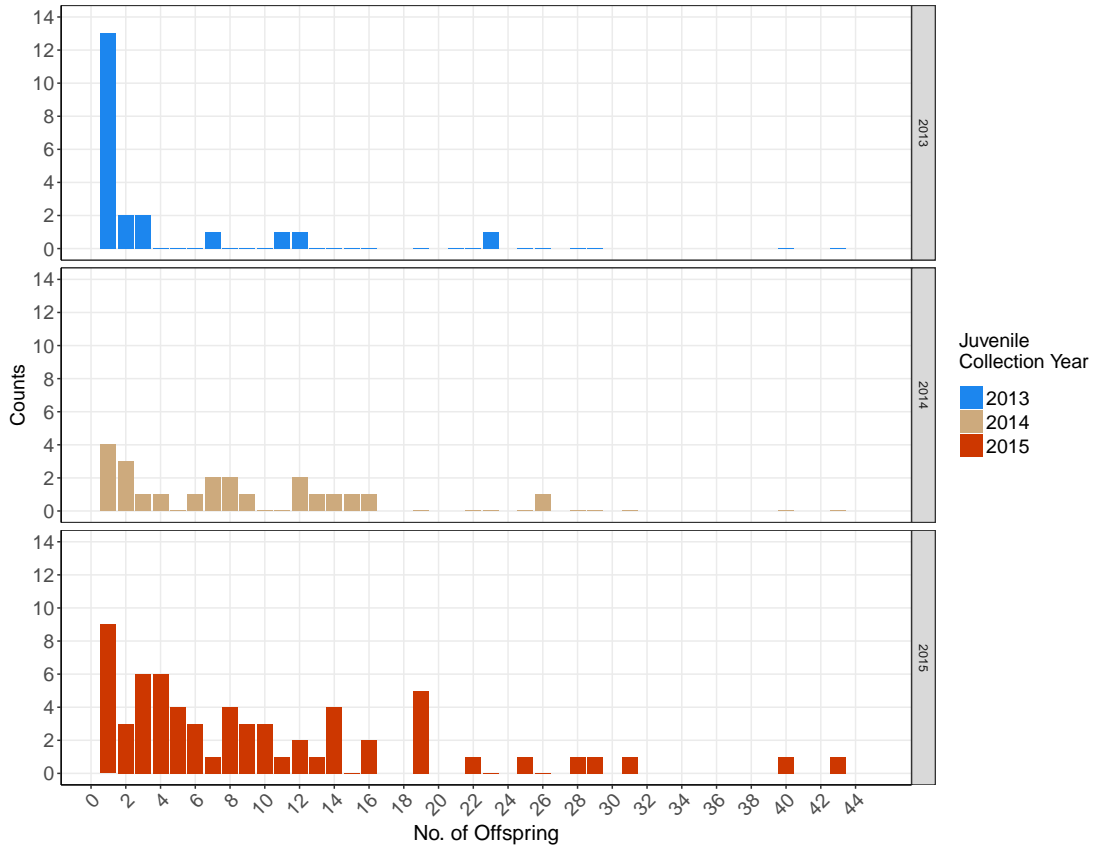
Figure 1.2: Distribution of full sibling (FullSib) families by number of offspring amongst the 2013, 2014, and 2015 Shasta River juvenile sampling years. The most common family size was single-offspring families across all sample years.

juveniles were attributed to 104 unique parental genotypes. In all three juvenile collections, the number of unique parental genotypes recovered was not simply twice the number of FullSib families, indicating polygamous matings. Amongst the 2013 and 2015 collection, the number of males and females with multiple mates were equivalent: in the 2013 collection, one male and female were found to have two mates; in the 2015 collection, 11 males and females were identified in multiple matings, with a maximum of three mates per any individual. In the 2014 collection, four males were found to have multiple mates, while two females were found to have multiple mates. The maximum number of mates per male or female individual was three.

1.5 Discussion

1.5.1 COLONY2 and FRANz accuracy assessment

Hence, the simultaneous parentage and sibship reconstruction approach (group method) utilized by COLONY2 appears to perform well when the parents are not completely sampled. However, the accuracy assessment dataset may have been particularly well suited to the computational algorithms employed by COLONY2. For example, the full-pedigree likelihood method benefits from datasets with large families, as all potentially related individuals may be simultaneously considered and contribute to pedigree inference, increasing the accuracy of any given assignment (Jones and Wang, 2009). Accordingly, despite our expectations that family size would be reduced amongst the accuracy assessment data set given that the offspring were returning adults, which are

subject to increased mortality, the mean full sibling (FullSib) family size was higher than all three empirical sample collections. The mean FullSib family size amongst the individuals included in the accuracy assessment was 16.3 offspring, versus 3.6 for the 2013 juveniles, 7.7 for the 2014 juveniles and 10 for the 2015 juveniles. Conversely, FRANz utilizes a pairwise approach, inferring pedigrees from parent-offspring relationships, and therefore does not incorporate all sibship relationships when assessing parentage. Ultimately, the proportion of inaccurate assignments generated by FRANz is fairly low at 1.41%, but these inaccuracies remain concerning for their apparent bias towards misleading Type I (false positive) errors. Thus, while the proportion of erroneous assignments recovered by both inference methods is fairly low, employing a consensus approach produces more robust and confident estimates of parentage (Herbinger, O'Reilly & Verspoor, 2006; Walling *et al.,* 2010; Harrison *et al.,* 2012). The results of these assessments suggest that applying these methods to natural systems in which the parental generation is often not completely sampled is a reasonable approach to understanding family structure.

1.5.2 Parentage

Despite utilizing multiple inference methods, the vast majority of Shasta River juveniles could not be assigned to any IGH adult from the corresponding potential parent pool, implying that the true parents were unsampled. Given that sampling of returning adults at the IGH weir has been fairly complete since 2010, this suggests the majority of juvenile coho salmon produced in the Shasta River during the study years were not

progeny of adult fish that entered IGH (either broodstock or non-broodstock). It is possible spawning between natural-origin and non-broodstock releases may occur more frequently than is represented in this analysis due to the strict confidence thresholds and consensus methods utilized for single parentage inference. However, even when all assignments passing confidence thresholds regardless of concordance were considered, a maximum of 21.5% of juveniles were attributed to IGH non-broodstock fish in any sampling year. Nonetheless, our sampling method did not include returning IGH adults that stray directly to the Shasta, and may therefore underestimate the contribution of IGH fish to within river productivity.

Of the juveniles that were assigned to trios, the majority were identified as offspring of IGH broodstock spawn pairs in all sample years. This suggests the Shasta River may actually be utilized as a temporary rearing habitat by hatchery-origin coho salmon prior to ocean entry (Gorman, 2016; Witmore, 2014). This could perhaps be explained by the unique location of the Shasta River basin. Glacial melt from nearby Mt. Shasta results in several spring-fed tributaries, particularly in and downstream of Big Springs Creek, resulting in relatively cool temperatures and consistent water levels year-round (Jeffres, Dahlgren, Deas, *et al.,* 2009; NMFS, 2014). Certain reaches of the Shasta River may therefore act as thermal refugia for hatchery-raised smolts, which are typically released in late March to early April when water temperatures may already be rising to above-optimal levels (California HSRG, 2012).

1.5.3 Sibship

Sibship inference for the 2013 juveniles identified 21 full sibling families, and 40 unique parental genotypes. Given that the majority of juveniles were estimated, or inferred by parentage assignment to be age 0 or age 1, the predominant parental spawn seasons would include winters 2011/2012 and 2012/2013. Escapement of coho salmon to the Shasta River in the parental years of 2011 & 2012 was estimated as 177 adults. However, the Shasta River Fish Counting Facility weir is not operational the entirety of the coho spawn season primarily due to prohibitively high flows as early as mid-December. Therefore, we calculated estimates of total escapement for each of the parental years by tabulating the mean fraction of the run that was captured per year, for years in which the weir was in place beyond early to mid-December. We used escapement data from 2001-2014 to estimate these numbers. We then used the estimated totals to calculate the proportion of the total run that successfully reproduced. Hence, with 21 FullSib families amongst the 2013 juveniles, 19.42% of adults that entered the Shasta during the 2011/2012 and 2012/2013 spawn seasons successfully contributed to production. Similarly, the Fullsib family and parentage assignment amongst the 2014 juvenile cohort suggests 11.71% of entering adults successfully reproduced, while the Fullsib family and parentage assignment amongst the 2015 juveniles suggests 49.76% of entering adults successfully reproduced (Table 1.6).

The finding that the largest family groups in each year were assigned to unsampled parents may suggest higher productivity per spawning effort among natural

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Table 1.6: Estimated proportion of adults returning to the Shasta River that contributed to production. Number of unique parental genotypes derived from full-sibling sibship results. The estimated total escapement accounts for the early removal of the weir and the resulting fraction of the run that may not have been captured each year. Both the recorded and estimated escapements reflect the sum of the given parental spawn years. The proportion of the run contributing to production is calculated from the estimated total escapement.

Juvenile collection	No. unique parental genotypes	Parental spawn years	Recorded escapement	Estimated total escapement	Estimated proportion of adults contributing to production
2013	40	2011 & 2012	177	206	19.42%
2014	35	2012 & 2013	249	299	11.71%
2015	104	2013 & 2014	180	209	49.76%

origin fish. This observation would support the paradigm that natural origin fish are better adapted to the conditions that uniquely characterize a given river system, and aligns with previous findings of differential reproductive success between salmonid populations with little to substantial hatchery influence (Chilcote, 2003; McLean, Bentzen & Quinn, 2003; Chilcote, Goodson & Falcy, 2011). However, we cannot conclusively attribute these large sibling families exclusively to natural-origin fish, as the parents could include unsampled hatchery fish straying into the Shasta River instead of returning to IGH.

1.5.4 Application to Management

Ultimately these results suggest that a substantial proportion of production within the Shasta River can be attributed to natural origin, or some combination of natural origin and straying hatchery-produced fish. These findings are somewhat un-

expected given our previous predictions of hatchery influence on coho salmon in the Shasta River. For example, the average percentage of hatchery coho salmon observed in the Shasta River from 2007-2014 was 51%, peaking at 80% in 2014, and we therefore expected to recover more parentage assignments involving IGH non-broodstock fish simply due to the prevalence of hatchery fish within the system (Chesney & Knechtle, 2017). However, low reproductive success amongst non-broodstock releases has also been observed in Bogus Creek, a Klamath tributary less than one mile from IGH, where 66% of non-broodstock females died without spawning (Chesney & Knechtle, 2013). This aligns with our findings of relatively few well supported, and even fewer large, families linking Shasta juveniles and IGH parents in more recent years. Hence, while IGH, and specifically IGH non-broodstock, fish represent a substantial number of the adults that escape to the Shasta River each year (Table 1.7), these fish do not appear to significantly contribute to production (Table 1.8). However, we must acknowledge that these estimates only reflect the contribution to production amongst the sampled juveniles each year, which is a small proportion of the total estimated juvenile population. It is therefore possible that non-broodstock fish produced more offspring that were simply not sampled for genetic analysis. Nonetheless, the overall contribution to production is difficult to predict without making several assumptions to estimate the fraction of families actually included in the juvenile sample.

The observed lack of reproductive success may be due to various confounding factors. For example, IGH non-broodstock fish were detected in the Shasta an average of 11 and 17 days after release from IGH in 2012 and 2013, respectively (Chesney &

Table 1.7: Estimated proportion of Iron Gate Hatchery (IGH) fish entering the Shasta River during spawn years relevant to this study. Total number coho reflects number of fish recorded entering the Shasta while the weir was operational during the given year. Since 2011, all non-broodstock (NB) fish from IGH have been PIT tagged prior to release, and therefore can be detected and recorded upon entry and movement within the Shasta River. Adapted from (Chesney and Knechtle, 2016).

Year	Total no. coho	Estimated no. IGH fish	No. IGH NB detected	Estimated contribution of IGH fish to escapement	Contribution of IGH NB to escapement
2010	44	11	N/A	25%	N/A
2011	62	44	17	71%	27.42%
2012	115	81	50	70%	43.48%
2013	163	101	85	62%	52.15%
2014	46	37	31	80%	67.39%

Knechtle, 2013; 2014). This gap between arrival at IGH and entry in the Shasta could lead to overripening, which has been associated with egg mortality and malformation within as few as eight days (Gaudemar & Beall, 1998). Additionally, the majority of IGH broodstock fish are often detected at the PIT tag antenna nearest the Shasta-Klamath River confluence, and detection events decrease as one moves further upstream into the Shasta River (Chesney & Knechtle, 2014). Therefore, these IGH non-broodstock fish may not simply have time to identify suitable spawning and rearing habitat, but rather attempt to spawn immediately upon entering the Shasta.

Given that both conservation and hatchery management objectives for coho salmon in California aim to preserve the genetic integrity and associated phenotypes of natural-origin fish, direct supplementation with excess hatchery fish would not necessarily be the most obvious or ideal mitigation strategy in the Shasta River. However, the extremely low population size in the Shasta River ultimately merits a fast-acting mitiga-

Table 1.8: Estimated proportion of IGH non-broodstock (NB) fish that contributed to production amongst the sampled juveniles each year. Number of detected NB adults is derived from PIT tag antenna data. We again used the mean fraction of the run that is not sampled per year post weir removal to estimate how many additional NB releases may have entered the Shasta River undetected. Adapted from (Chesney and Knechtle, 2016).

Juvenile collection	Parent spawn years	No. NB adults detected	Estimated no. of NB present	No. NB adults assigned offspring	Estimated proportion of NB adults contributing to production
2013	2011 & 2012	67	79	8	10.1%
2014	2012 & 2013	135	161	7	4.3%
2015	2013 & 2014	116	135	5	3.7%

tion strategy to avoid depensatory effects and decrease extinction risk in the near future. If the lack of observed reproductive success amongst IGH non-broodstock fish is, in fact, largely due to excessive time between maturation and spawning, direct supplementation to the Shasta may effectively combat this issue. Nonetheless, habitat restoration efforts may ultimately be the most effectual means to promoting coho salmon longevity in the Shasta River. Additionally, because much of the Shasta River basin has been dramatically altered for agricultural development and hydropower, supplementation without restoration could sustain the population in the short-term, but would likely have little impact on long-term recovery. Therefore, continued habitat restoration and direct supplementation may be the best approach to combatting low effective population size and stimulating productivity in this critically threatened population.

Chapter 2

Parentage-based analysis of an endangered coho salmon (*Oncorhynchus kisutch*) population to assess and inform hatchery practices

2.1 Abstract

Populations of anadromous Pacific salmon and steelhead are increasingly threatened by degradation and loss of critical freshwater habitat, as well as poor survival at sea. Consequently, coho salmon in the Central California Coast Evolutionarily Significant Unit (CCC ESU) are listed as endangered, and production is supplemented by two conservation hatchery programs. Scott Creek in the Santa Cruz Mountains is the southernmost persistent population of coho salmon, and is supported by the Kingfisher Flat

Conservation Hatchery and Captive Broodstock Program. While Scott Creek is one of the few streams within the CCC ESU to display increases in adult abundances in recent years, how effectively the hatchery programs contribute to production remains to be quantified. We performed parentage-based analysis on three years of juveniles sampled in the creek with a parent pool of captive broodstock fish, and ocean returning fish of both hatchery- and natural-origin. Approximately 34.2%, 33.4% and 5.48% of the juveniles sampled in 2015, 2016 and 2017, respectively, were assigned into parent-offspring trios. Across all juvenile collection years, 111 unique parent pairs were recovered, of which 63 were captive broodstock spawn pairs and 48 were pairs that spawned in the stream. The in-stream spawners produced 82.7% (459 of 555) of the assigned offspring. Amongst the in-stream spawners, only one trio and one (of three) single parent-offspring pair involved a captive broodstock fish released from the hatchery as an adult. Additionally, we used these pedigree reconstruction data to support the positive relationship between female length and reproductive success, and the lack of such relationship for males. Finally, we also found no significant difference in reproductive success between age-2 and age-3 males, although this comparison is hindered by low sample size for age-2 males. Ultimately, we use these results to inform hatchery practices and offer recommendations for future modifications that maximize efficacy and long-term population stability.

2.2 Introduction

In recent years, many stocks of anadromous Pacific salmon and steelhead (*Oncorhynchus* spp.) have experienced declines in abundances throughout their native ranges, with numerous populations now extirpated from historically productive watersheds. Coho salmon (*O. kisutch*) within the Central California Coast Evolutionarily Significant Unit (CCC ESU) are one particularly poignant example. The CCC ESU extends from Punta Gorda in Humboldt County to Aptos Creek in Santa Cruz County, and represents the southernmost extent of the species range in North America (Williams *et al.,* 2016). Theory predicts individuals at the edge of a species distribution often possess reduced diversity and therefore suppressed adaptive potential to environmental fluctuations (Pearson *et al.,* 2009). This makes the indiscriminate, range-wide disturbances to Pacific salmon habitat, such as damming for hydropower or the removal of critical estuarine habitat for human development, even more impactful for populations at the edge of the species range. Accordingly, coho salmon within the CCC ESU have been listed as endangered since 2005, and all populations south of the Golden Gate (entry to San Francisco Bay) remain at a high risk of extinction (Spence & Williams, 2011; Williams *et al.,* 2016).

The Scott Creek watershed in Santa Cruz County supports the southernmost persistent population of coho salmon (Rogers *et al.,* 2016). Despite some recent improvement in abundance, Scott Creek and all additional populations within the Santa Cruz Mountains remain well below recovery targets and are threatened with extirpa-

tion (Rogers *et al.*, 2016). In an effort to curtail negative trends in abundance, many artificial propagation (i.e., hatchery) programs have been instituted throughout the range of ESA-listed salmonids. Two conservation hatchery programs propagate CCC ESU fish: the Don Clausen/Warm Springs Fish Hatchery, which supports populations within the Russian River watershed and nearby basins, and the Kingfisher Flat Hatchery and Conservation Program (KFH), which supports populations within the Scott Creek watershed and nearby basins. As conservation hatcheries, both programs exist to rebuild natural-origin stocks by producing fish with genetic and ecological characteristics representative of natural-origin fish within their respective regions (Flagg & Nash, 1999).

In addition to the standard artificial propagation of fish for eventual release from the hatchery, both hatcheries also operate captive broodstock programs, in which fish are raised in captivity throughout their life cycle. Ensuring survival to reproductive maturity is particularly important in Scott Creek, where population abundance has displayed an overall downward trend, often to precipitously low and unsustainable levels (Williams *et al.*, 2016). Individuals selected for the captive broodstock program at KFH are ultimately spawned following matrices that seek to optimize genetic diversity and reduce inbreeding depression. The resulting offspring are reared in the hatchery until age-1 (smolt stage), whereupon they are released during the spring (March - May) using a staggered protocol, first implemented in 2013 (Williams *et al.*, 2016). A certain proportion of these captively-raised adults, however, will be selected for release into the stream without being spawned in the hatchery each spawn season.

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The Kingfisher Flat Hatchery and Captive Broodstock program play a significant role in sustaining the highly imperiled population of coho salmon that returns to Scott Creek. In fact, Scott Creek recently experienced its largest run of coho salmon in ten years with approximately 163 ocean returns during the 2014/2015 season (Williams *et al.,* 2016). This surge in escapement has been largely attributed to modified hatchery practices, including the inclusion of broodstock from Warm Springs Hatchery for outbreeding, and the apparent success of the staggered juvenile release strategy (Williams *et al.,* 2016). Additionally, the low incidence of natural-origin fish within this system suggests hatchery-origin fish must play a significant role in sustaining productivity; however, we have yet to tease apart contributions to productivity by hatchery-origin ocean-returning adults versus captive broodstock adult releases. To this end, we performed parentage analysis on juveniles sampled in Scott Creek during three consecutive years to estimate reproductive success amongst 1) captive broodstock adults, 2) hatchery-origin ocean returns, and 3) natural-origin ocean returns. The captive broodstock adults ultimately have three fates upon reaching maturity within the hatchery: 1) an individual may be spawned in the hatchery and then culled, 2) an individual may be spawned in the hatchery and then released, or 3) an individual may be released into the stream to spawn naturally (i.e. the captive broodstock release). The hatchery-origin ocean returns refer to adults that were produced in the hatchery, released as age-1 smolts and returned to their natal stream to spawn. The natural-origin ocean returns refer to individuals that hatched and reared within the stream, emigrated to the ocean as smolts, and escaped to Scott Creek as adults to spawn. Both hatchery-origin and natural-origin

ocean returns may be returned to the stream to spawn after sampling, or brought to the hatchery to spawn with captive broodstock depending on spawning matrix priorities during a given season.

This pedigree reconstruction analysis will inform our understanding of current biological and reproductive dynamics within the stream, and identify hatchery practices that could be modified to maximize productivity and preservation. Given that the recovery of coho salmon in Scott Creek likely requires continued supplementation via artificial propagation, it is especially important to optimize hatchery practices that maximize production while maintaining adaptability to wild conditions and facilitating long-term population stability.

2.3 Methods

2.3.1 Tissue collection and DNA extraction

Tissue samples were collected from a total of 1924 juvenile coho salmon collected throughout the Scott Creek watershed in 2015, 2016 and 2017 (Table 2.1). Juvenile sampling was fairly extensive, using e-fishing, downstream migrant trapping and seining throughout the watershed to sample individuals from early Spring to late Summer. Therefore, the juvenile collections are expected to be a representative sample of the individuals residing within the stream each year.

Additionally, tissue samples were collected from adults potentially contributing to production during the winter 2013/2014 (W1314), 2014/2015 (W1415) and 2015/2016

Table 2.1: Number of juvenile coho sampled from Scott Creek, CA. Individuals were sampled using e-fishing, downstream migrant trapping and seining. Collections therefore contain an assortment of age-0 and age-1 individuals. An individual was removed from analysis if it was missing data at more than 10 loci (76 loci minimum) or if it was determined to be a non-coho species.

Collection year	Total no. of samples	Total no. of samples analyzed
2015	1181	1091
2016	597	572
2017	146	146
Total	*1924*	*1809*

Table 2.2: Number of adult coho sampled per spawn season. Adults include captive broodstock fish, hatchery-origin ocean returns, and natural-origin ocean returns. An individual was removed from analysis if it was missing data at more than 10 loci (76 loci minimum) or if it was determined to be a non-coho species.

Spawn Season	Total no. of samples	Total no. of samples analyzed	No. of ocean returns	No. of Captive Broodstock
2013/2014	413	405	19	386
2014/2015	523	499	106	393
2015/2016	319	317	3	314
Total	*1255*	*1221*	*128*	*1093*

(W1516) spawn seasons, totaling 1255 individuals (Table 2.2). The adults included captive broodstock fish spawned and/or released from the hatchery, as well as ocean returns of hatchery and presumably natural-origin. Tissue samples were digested in proteinase k and extracted on a QIAGEN BioRobot 3000, following the DNeasy 96 Tissue Kit protocol (QIAGEN Inc., Hilden, Germany).

2.3.2 SNP loci and genotyping

All individuals were genotyped using a panel of 95 SNP loci with minor allele frequencies conducive to parentage-based tagging inference (Anderson and Garza, 2006; Starks, Clemento & Garza, 2016; Smith *et al.,* 2006; Campbell & Narum, 2011). Additionally, one species specific marker was utilized to distinguish coho salmon from its sister species Chinook (*Oncorhynchus tshawytscha*) salmon. All individuals were genotyped with predesigned Taqman (Applied Biosystems Corporation, Foster City, U.S.A.) or SNPtype (Fluidigm Corporation, San Francisco, CA, U.S.A.) assays, using 96.96 Dynamic Genotyping Arrays on the Fluidigm EP1 Genotyping system. All genotypes were called using SNP Genotyping Analysis Software v 3.1.3 (Fluidigm Corporation, San Francisco, U.S.A.). Because genotyping began using a panel of 95 loci optimized for Taqman chemistry and then transitioned to SNPtype, a total of 88 loci were shared across all genotyping assays. Additionally, excessive Mendelian incompatibilities were associated with two markers across many potential families, likely due to high genotyping error rates at these loci. Consequently, the final panel used for all downstream analyses included 86 loci (Table S1 in Supporting Information). Individuals missing genotype data at more than 10 loci were dropped from the analysis.

2.3.3 Parentage analysis

Since juveniles were sampled using a variety of collection methods (i.e. smolt trapping, seining and electrofishing), we obtained an assortment of age-0 and age-1 fish in 2015 (sampled March-November) and 2016 (sampled March-August). Consequently,

parentage assignment for juveniles captured in 2015 was performed using a pool of potential parents from the W1314 and W1415 spawn seasons. We did not include W1213 due to inadequate metadata accounting in years previous to the W1314 spawn season. Assignment for the juveniles captured in 2016 was performed using a pool of potential parents from the W1314, W1415 and W1516 spawn seasons. Assignment for the juveniles collected in 2017 was performed using a pool of potential parents from the W1415 and W1516 spawn seasons. We did not include W1617 in the potential parent pool for the 2017 juveniles, as the sampling dates and length data for these fish suggested they were all age-1. Prior to performing parentage inference, however, all juvenile samples within a single collection year were compared to account for potential repeat sampling. Individuals compared at ≥ 76 of 86 loci and differing at no more two alleles were accepted as duplicate samples and only one representative from the matching samples was subsequently used in the parentage-based tagging analysis.

Parent-offspring trio assignments were identified using SNPPIT (Anderson, 2010), assuming a genotyping error rate of 0.005. After removing duplicate individuals, each juvenile cohort was analyzed separately, and each run was constrained by parent sex and spawn season, such that only adults of opposite sex and the same spawn season could be identified as a parent pair. The resulting trios were filtered using a false discovery rate (FDR) of ≤ 0.01. The FDR parameter indicates the rate at which one may expect any assignment to be erroneous given the data (Anderson, 2012). For example, a FDR cutoff of 0.01 suggests 1 in every 100 assignments may be inaccurate.

Single parent-offspring pairs were identified using COLONY2 (version 2.0.6.1)

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(Jones & Wang, 2009) and FRANz (version 2.0) (Riester, Stadler & Klem, 2009). Each COLONY2 and FRANz run was supplied with the same offspring-parent pool supplied to SNPPIT after filtering for missing data and duplicate samples. The input parameters for each run in COLONY2 were as follows: both sexes polygamous and dioecious; no sibship size prior or full sibship scaling; full likelihood estimation with medium run length and precision; and no updating of allele frequencies. The allelic dropout rate and genotyping error rate were estimated at 0.0025 each. Each FRANz run was informed of sex and birth year of all individuals, and supplied with an accepted pedigree created from SNPPIT trios with a FDR $\leq$ 0.01. Given the difficulty of accurately constructing pedigrees when the parental population is incompletely sampled, as in most natural populations, only single parent - offspring pairs identified by both COLONY2 and FRANz at a posterior probability $\geq$ 0.95 were accepted (Jones & Arden, 2003; Pemberton, 2008).

2.3.4   Assessments of reproductive success

We utilized the resulting SNPPIT trios to determine the number of offspring assigned to each male and female parent individually, as well as to infer family size per unique parent pair. We collected disposition information for all identified parents to compare reproductive success (i.e. number of assigned offspring) amongst captive broodstock, hatchery-origin and natural-origin adults identified as parents. By collating the disposition information, we were also able to infer spawning location, and thereby the initial rearing habitat of the juveniles as either within the hatchery or

stream. However, given that collection efforts only sample juveniles in the stream and hatchery produced juveniles are not released until age-1, age-0 offspring could only be attributed to in-stream spawners. Therefore, we only included age-1 offspring in the total count of offspring attributed to each unique parent pair and individual adult to avoid upwardly biasing reproductive success for individuals that happened to spawn in the stream. Further, due to the practice of spawning adults with several partners in the hatchery, we summed the total offspring assigned to each mother or father individually for comparisons of reproductive success with respect to spawning location. We performed a Mann-Whitney-Wilcoxon test to compare the mean number of offspring attributed to 1) adults spawned in the stream versus the hatchery, 2) adults originating in the hatchery and released as smolts versus adults born and raised in the hatchery as captive broodstock, and 3) hatchery-origin adults spawning in the stream versus natural-origin adults spawning in the stream.

Additionally, we utilized our parentage assignment methods to generate estimates of relative reproductive success per individual adult with respect to length at spawning for males and females, as well as age at spawning for males (i.e. age-2 versus age-3 spawners). Again, to avoid biasing estimates of reproductive success simply due to juvenile collection methods, we only counted the number of age-1 offspring per male or female. Additionally, as length is a characteristic by which males may be selected for spawning in natural environments, we analyzed reproductive success amongst all males, as well as only amongst males spawning in the stream. A correlation coefficient (Pearson) was calculated for comparisons of number of assigned offspring versus length

at spawning for both sexes. A Mann-Whitney test was used to determine whether the mean number of offspring assigned to age-2 (i.e. jacks) and age-3 males was significantly different.

2.4 Results

2.4.1 Parentage analysis

Approximately 34.2%, 33.4% and 5.5% of the 2015, 2016 and 2017 juveniles, respectively, were assigned a parent pair (Table 2.3), after filtering all SNPPIT trios as previously described. When the parent pairs recovered for each juvenile collection year separately were combined, we identified 21 parent pairs that apparently produced offspring from multiple sample years: 18 parent pairs were assigned to juveniles sampled in 2015 and 2016; and three parent pairs were assigned to juveniles sampled in 2015, 2016 and 2017. These 21 parent pairs produced the majority of assigned juveniles (398 of 572; 69.6%) across all years. We then compared the genotypes of these 398 juveniles against each other to identify potential re-sampling across years. We found 15 occurrences of duplicate individuals, all including juveniles presumably sampled as age-0 in 2015 and again as smolts (age-1+) in 2016. These duplicates were accounted for when totaling the number of offspring assigned to each parent pair across years.

Ultimately, a total of 555 unique offspring were assigned to 111 distinct parent pairs when the trios across all juvenile sampling years were combined. The trios included parent pairs spawned in the hatchery and the stream. The disposition of parent fish

Table 2.3: Parent-offspring trio assignment results for each juvenile collection separately (SNPPIT, FDR ≤ 0.01).

Juvenile collection year	Total no. assigned	No. unique parent pairs	No. of families with age-0 offspring	No. of families with age-1 offspring	No. of families with age-1+ offspring
2015	373 (34.2%)	89	45	44	0
2016	191 (33.4%)	43	0	43	0
2017	8 (5.5%)	5	0	2	3

included captive broodstock fish, ocean-returning fish of hatchery-origin, and ocean-returning fish of natural-origin. Across all three sample years, 96 juveniles (17.3%) were assigned to 63 parent pairs that were spawned in the hatchery and involved at least one KFH captive broodstock adult. Sixty of these 63 pairs were confirmed spawning partners from hatchery records. The remaining 459 assigned juveniles (82.7%) were attributed to 48 parent pairs that spawned in the stream. The majority of these in-stream spawning parent pairs were of hatchery-origin (31 of 48), while 15 pairs included one natural-origin parent, and one pair had two natural-origin parents. Only one of the in-stream spawn pairs included a female captive broodstock adult release mating with a male hatchery-origin ocean return in W1415 to produce one age-0 juvenile.

Three single parent-offspring pairs were concordant between COLONY2 and FRANz. These three offspring were attributed to three unique parents: 1) a 2016 juvenile assigned to a male, captive broodstock fish spawned in the hatchery and then released in W1516; 2) a 2016 juvenile assigned to a male, returning from the ocean in W1415; and 3) a 2017 juvenile assigned to a female, returning from the ocean in W1516.

2.4.2 Assessments of reproductive success

When the number of offspring attributed to each mother or father was conditioned upon spawning location, the average number of offspring attributed to individuals spawned in the hatchery versus the stream was significantly different at 2.33 versus 9.88 offspring, respectively (W = 814, p-value = 0.0002; Table 2.4). Additionally, the maximum number of offspring assigned to an individual that spawned in the hatchery was 11, versus 61 for an in-stream spawning adult (Figure 2.1). Further, a significant difference was found when comparing the mean number of offspring assigned to hatchery-origin fish that were released as smolts and returned to spawn in the stream versus those retained as captive broodstock and spawned in the hatchery (W = 705, p-value = 0.0009; Table 2.5). However, when comparing the mean number of offspring assigned to individuals that spawned within the stream on the basis of parent origin, there was no significant difference in the mean number of offspring assigned to hatchery-origin versus natural-origin fish (W = 61.5, p-value = 0.605; Table 2.5).

A comparison of total assigned offspring and known length at spawning for all identified fathers produced a non-significant correlation coefficient (r = -0.032; p-value = 0.84) (Figure 2.2). The comparison between length and the total number of offspring attributed to only in-stream spawning males was also non-significant (r = -0.32, p-value = 0.54) (Figure 2.3). Conversely, when the reproductive success of all female adults was compared against known length at spawning, the correlation coefficient was positive and significant (r = 0.36, p-value = 0.033) (Figure 2.4).

Table 2.4: Number of age-1 offspring assigned to each single adult across all juvenile sampling years. The recovered mothers and fathers are sorted by spawn location. The sample size (n) reflects the number of adults that fall within the respective spawn location category. Number of offspring per single adult was computed from filtered parent-offspring trios (FDR $\leq$ 0.01).

Parent spawn location	n	Mean no. assigned offspring	Max no. assigned offspring
Hatchery	83	2.33	11
Stream	34	9.88	61
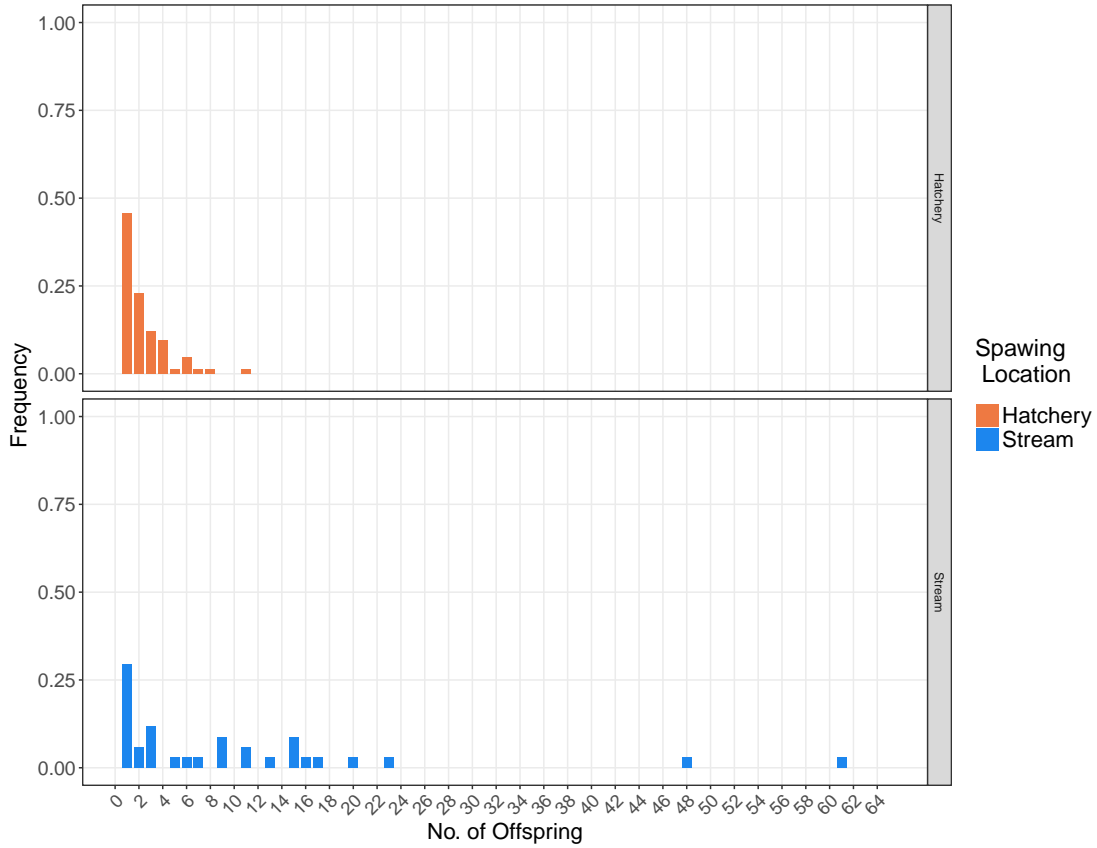
Figure 2.1: Distribution of the number of age-1 offspring assigned to individual adults according to spawning location of the single parent across all juvenile sampling years.
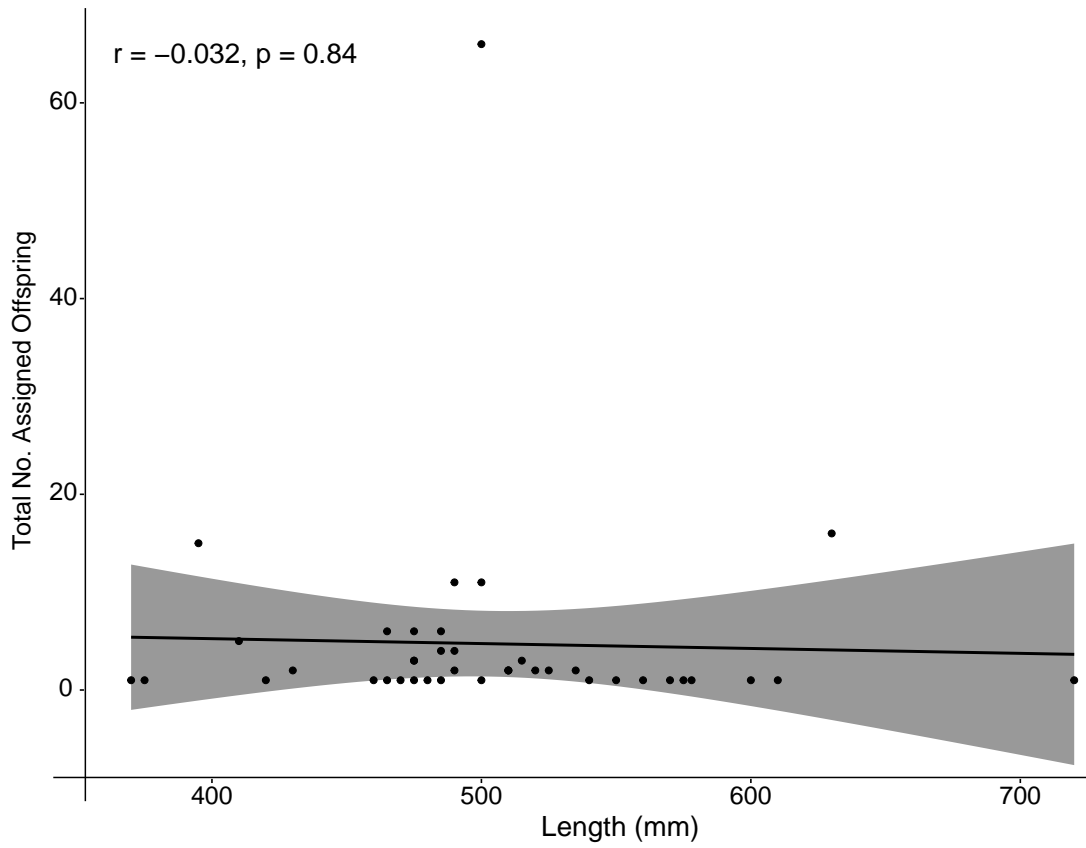
Figure 2.2: Relationship between length at spawning and reproductive success per adult male. This included all males assigned age-1 offspring, irrespective of spawn location. A Pearson test produced a non-significant correlation coefficient (r = -0.032, p = 0.84).
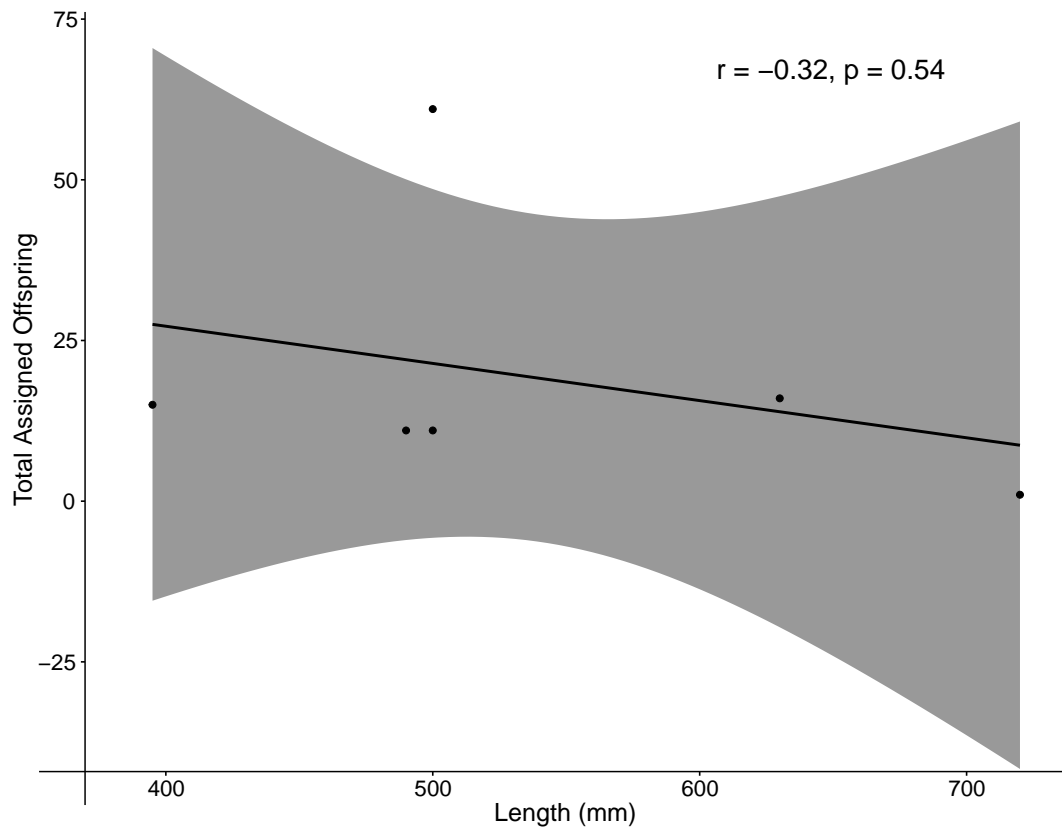
Figure 2.3: Relationship between length at spawning and reproductive success per adult male, known to have spawned in Scott Creek. The number of offspring per male only includes offspring determined to be age-1. A Pearson test produced a non-significant correlation coefficient (r = -0.32, p = 0.54).
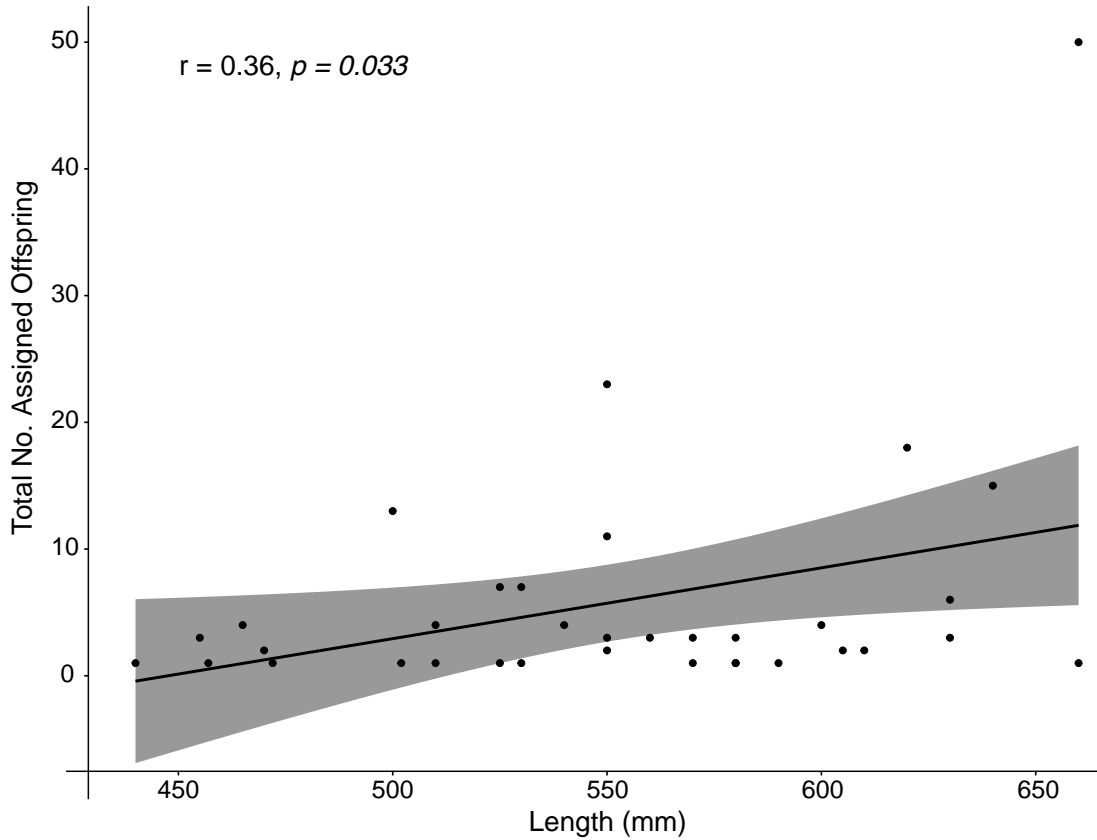
Figure 2.4: Relationship between length at spawning and reproductive success per adult female. This included all females assigned age-1 offspring, irrespective of spawn location. A Pearson test produced a significant correlation coefficient (r = 0.36, p = 0.033).

Table 2.5: Number of age-1 offspring assigned to each single adult across all juvenile sampling years. The recovered mothers or fathers are categorized by spawning location and origin. The sample size (n) reflects the number of adults that fall within the respective spawn location and origin category. Number of offspring per single adult was computed from filtered parent-offspring trios (FDR $\leq$ 0.01).

| Parent spawn location	Parent origin	n	Mean no. assigned offspring	Max no. assigned offspring
Hatchery	Captive Broodstock	81	2.32[*]	11
Hatchery	Natural	2	2.5	3
Stream	Hatchery	29	8.66[*^]	48
Stream	Natural	5	17[^]	61

[*]Indicates comparison performed between these two parent dispositions was significant

[^]Indicates comparison performed between these two parent dispositions was non-significant

Table 2.6: Number of age-1 offspring assigned to each single adult across all juvenile sampling years. The recovered fathers are sorted by spawn location. The sample size (n) reflects the number of adults that fall within the respective spawn location category. Number of offspring per single adult was computed from filtered parent-offspring trios (FDR $\leq$ 0.01).

Age of male at spawning	n	Mean no. of assigned offspring per male	Max no. of assigned offspring per male
Age 2	3	4.67	10
Age 3	60	3.95	66

Amongst the identified fathers with known age at spawning, three were estimated to be age-2 spawners, whereas 60 were age-3 spawners. The distribution of total offspring by age group is slightly overlapping, but with more occurrences of large family sizes per male for age-3 spawners (Figure 2.5). The Mann-Whitney test for comparison of mean offspring per male between age-2 and age-3 spawners was not significant with the mean number amongst age-2 spawners being 4.67, and the mean amongst age-3 spawners being 3.95 (W = 114, p-value = 0.4214; Table 2.6).
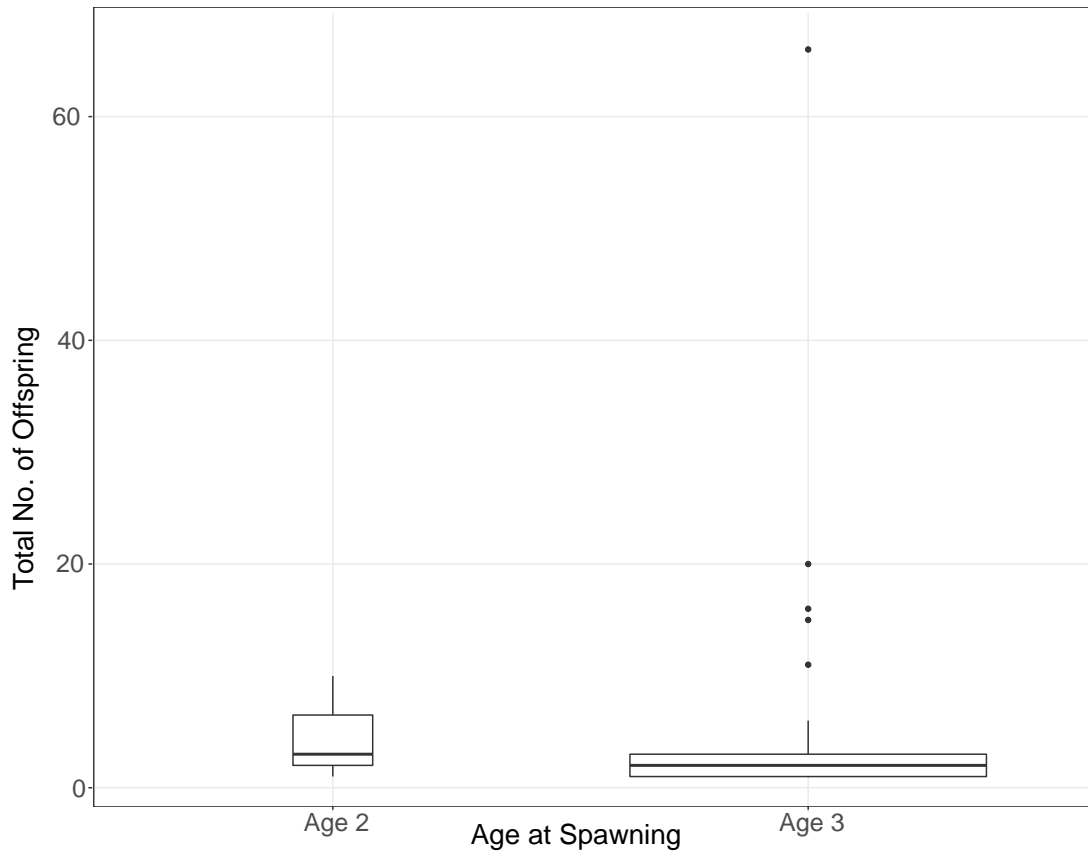
Figure 2.5: Number of offspring assigned to age-2 (i.e. jacks) and age-3 males. Number of offspring only includes offspring determined to be age-1. The mean number of offspring amongst age-2 spawners was 4.67, and the mean amongst age-3 males was 3.95. A Mann-Whitney test for comparison of mean offspring attributed to each male age group was not significant (W = 114, p-value = 0.4214).

2.5 Discussion

2.5.1 Parentage

This parentage-based tagging analysis of coho salmon in Scott Creek informed our understanding of hatchery influence and efficacy within the watershed, and identified potential practice modifications, particularly regarding release protocols, that may facilitate population stability. For example, one of our initial motivating questions was to quantify the contribution of released captive broodstock adults to juvenile production within the watershed. Released captive broodstock fish are one mechanism for genetic integration between the captively raised fish and fish returning to Scott Creek to spawn naturally, and are therefore quite important in preventing significant genetic and ultimately ecological divergence between captive and any remaining wild fish. Amongst the recovered parent-offspring trios, only one included a released captive broodstock adult and this pair was assigned a single age-0 juvenile. One additional captive broodstock fish that was released after spawning in the hatchery was recovered in a single-parent offspring pair and therefore presumably mated with an unsampled, ocean return. While the estimated number of adult releases during the spawn seasons included in this study is fairly low (Table 2.7), a general lack of reproductive success amongst these individuals suggests the adult release strategy, as practiced, for the captive broodstock program is not effectively contributing to production. This may be due to a propensity to release smaller or even immature adults that are less desirable or unsuitable mates for ocean returning fish that spawn season. The relative reproductive failure of these captive re-

Table 2.7: Estimated number of Captive Broodstock adults released with and without spawning in the hatchery each season. The practice of spawning adults in the hatchery and then releasing in the stream is exclusive to males. The number in parentheses represents these spawned and released males, which were identified as parents.

Spawn season	No. of released adults	No. of spawned and released adults	No. of released adults assigned offspring
W1314	11	7	0 (0)
W1415	58	11	1 (0)
W1516	43	32	0 (1)

leases suggests a necessary re-evaluation of the criteria utilized to determine whether a fish is appropriate for release, in order to uphold the operational goals of a conservation hatchery, such as Kingfisher Flat.

2.5.2 Assessments of reproductive success

The comparisons of mean number of offspring assigned to each individual parent according to origin and/or spawning location found significant differences when comparing all in-stream versus in-hatchery spawners, as well as when comparing only hatchery-origin fish that spawned in the stream versus the hatchery. In contrast, the mean number of offspring attributed to a hatchery-origin fish spawning in the stream versus a natural-origin fish spawning in the stream were not significantly different. Hence, significant differences in this measure of relative reproductive success were apparent when comparing on the basis of spawn location, but not parental origin. However, it is important to note the small sample size of natural-origin fish, which may make the identification of significant differences according to parental origin difficult. Nonetheless, these findings may suggest maturation in the ocean, or alternatively a lifetime

in a hatchery environment, may be very critical in influencing reproductive success. However, previously observed differences in emigration behavior between hatchery and naturally-produced smolts may have also biased sampling towards juveniles produced by in-stream spawning fish. Hatchery produced steelhead (*O. mykiss*) and coho salmon in Scott Creek have been observed to emigrate from the system faster and in a more concentrated time period than naturally produced smolts (Hayes *et al.,* 2004), which may have resulted in the sampling of more naturally produced smolts, thereby leading to an artefactual increase in the number of assigned offspring for adults that spawned in the stream.

This may also explain the significantly lower assignment rate amongst 2017 juveniles. Given that all of the juveniles sampled in 2017 were estimated to be age-1, we would expect the parents to have spawned in winter 2015/2016. However, escapement to Scott Creek was particularly low during the W1516 season, with only 13 ocean-returning adults observed and three captured (Joseph Kiernan, personal communication, 10 Jan 2018; Table 2.2). Therefore, if juvenile sampling is in fact biased towards naturally produced fish due to differences in smolt emigration behavior, we may simply not have genetic tags for the adult fish that produced the majority of the 2017 juveniles. This seems even more plausible given that the majority of assigned age-1 offspring in 2015 and 2016 were attributed to in-stream spawn pairs.

Assessments of reproductive success according to length and age generally followed previously held understandings. For example, as expected, female length appears to be significantly and positively correlated with reproductive success (Van den Berghe

& Gross, 1989). This relationship was not seen amongst males, regardless of whether individuals spawned in the hatchery were included. While the mean number of off-spring assigned to age-2 males was not significantly different than the number assigned to age-3 males, age-3 males had more family sizes that exceeded the upper limit of the inter-quartile range (Figure 2.5). However, we are reluctant to suggest any biologically significant difference between age-2 and age-3 males in regards to reproductive success, given the low sample size of age-2 males.

2.5.3   Conclusions

While potential differences in smolt emigration behavior due to juvenile rearing habitat may confound comparisons of relative reproductive success between hatchery and natural spawners, it does not dismiss the finding that captive broodstock releases appear to have low success. Juveniles produced by these fish should rear in the stream and therefore be more prone to sampling. Therefore, the low contribution to production amongst these fish presents an existing practice that can be re-assessed to facilitate productivity and genetic exchange between captively reared fish and ocean returns each year. Ultimately, the Kingfisher Flat Hatchery plays a critical role in maintaining the population of coho salmon returning to Scott Creek, and this study suggests hatchery-origin ocean returns are particularly successful and effectively contribute to production. Consequently, slight modifications to the Captive Broodstock program, with particular focus on releasing fish with a higher likelihood of successful reproduction, are likely the best means of adding to existing measures that aim to rebuild this highly imperiled

population.

Conclusions and Future Directions

This study demonstrates the utility of parentage-based tagging inference in understanding reproductive dynamics within natural populations of coho salmon (*Oncorhynchus kisutch*), in order to create more informed management strategies. In particular, this study evaluates the efficacy of current conservation hatchery practices and identifies practices that can be modified to more effectively facilitate recovery. As we become increasingly reliant on artificial propagation to supplement natural productivity, it becomes absolutely critical that we identify the best operating procedures that will allow for long-term adaptive potential and survival of these high-risk populations.

Within each system, I have identified future actions or practice modifications that may augment productivity. Additionally, continued monitoring and parentage inference of these populations will supplement the findings of this study, and allow for the evaluation of the proposed strategies.

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Supplementary Information

Table S1: List of loci used in parentage analysis for both chapters with corresponding dbSNP IDs. The two bold-face markers were the loci not included in analyses for chapter 2. The species identification marker is italicized. The reference refers to the study that identified each marker (1 = Starks, Clemento & Garza, 2016; 2 = Campbell & Narum, 2011; 3 = Smith *et al.*, 2006).

Locus	dbSNP	Reference	Locus	dbSNP	Reference
Oki_afp4-10	ss263196994	3	Oki_arp-105	ss263196997	3
Oki_gdh-189	ss263197003	3	Oki_gshpx-152	ss263197005	3
Oki_HGFA-311	ss49845917	2	Oki_hsc713-56	ss263197006	3
Oki_ins-167	ss49845899	2	Oki_itpa-85	ss263197010	3
Oki_LWSop-554	ss49845907	2	Oki_nips-159	ss263197013	3
Oki_p53-20	ss263197014	3	Oki_pigh-33	ss263197016	3
Oki_rpo2j-235	ss263197019	3	Oki_SClkF2R2-120	ss49845937	2
Oki_txnip-35	ss263197027	3	Oki100771-83	ss974293200	1
Oki100974-293	ss974293202	1	Oki101119-1006	ss974293203	1
Oki101419-103	ss974293204	1	Oki101554-359	ss974293205	1
Oki101770-525	ss974293206	1	Oki102213-604	ss974293207	1
Oki102414-499	ss974293209	1	Oki102457-67	ss974293210	1
Oki102801-511	ss974293211	1	Oki102867-667	ss974293212	1
Oki103271-161	ss974293213	1	Oki103577-70	ss974293214	1
Oki103713-182	ss974293215	1	Oki104515-99	ss974293216	1
Oki104519-45	ss974293217	1	Oki104569-261	ss974293218	1

Continued on next page

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Locus	dbSNP	Reference	Locus	dbSNP	Reference
Oki105105-245	ss974293219	1	Oki105115-49	ss974293220	1
Oki105132-169	ss974293221	1	Oki105235-460	ss974293222	1
Oki105385-521	ss974293223	1	Oki105407-161	ss974293224	1
Oki105897-298	ss974293225	1	Oki106172-60	ss974293226	1
Oki106313-353	ss974293227	1	Oki106419-292	ss974293228	1
Oki106479-278	ss974293229	1	Oki107336-45	ss974293230	1
Oki107607-213	ss974293231	1	Oki107974-46	ss974293232	1
Oki108505-331	ss974293233	1	Oki109243-480	ss974293234	1
Oki109874-122	ss974293237	1	Oki109894-418	ss974293238	1
Oki110064-418	ss974293239	1	**Oki110078-191**	**ss974293240**	**1**
Oki111681-407	ss974293243	1	Oki113457-324	ss974293244	1
Oki114315-360	ss974293246	1	Oki114448-101	ss974293247	1
Oki114587-309	ss974293248	1	Oki116362-411	ss974293249	1
Oki116865-244	ss974293250	1	Oki117043-374	ss974293251	1
Oki117144-64	ss974293252	1	Oki117286-291	ss974293253	1
Oki117742-259	ss974293254	1	Oki117815-369	ss974293255	1
Oki118152-314	ss974293256	1	Oki118175-264	ss974293257	1
Oki118654-330	ss974293258	1	Oki120024-226	ss974293259	1

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Locus	dbSNP	Reference	Locus	dbSNP	Reference
Oki122593-430	ss974293264	1	Oki123205-88	ss974293266	1
Oki123921-90	ss974293268	1	Oki124162-62	ss974293269	1
Oki125998-340	ss974293270	1	Oki127236-383	ss974293272	1
Oki127760-301	ss974293273	1	Oki128302-547	ss974293274	1
Oki128757-232	ss974293276	1	**Oki128851-185**	**ss974293277**	**1**
Oki129870-552	ss974293278	1	Oki130295-48	ss974293279	1
Oki130524-184	ss974293280	1	Oki131460-243	ss974293282	1
Oki131802-368	ss974293283	1	Oki94903-192	ss974293192	1
Oki96127-66	ss974293194	1	Oki96158-278	ss974293195	1
Oki96376-63	ss974293197	1	Oki97954-228	ss974293199	1
Oki120255-113-sppID	*ss974293262*	*1*			

Table S2: Results of COLONY2 accuracy assessment for incompletely sampled parent population, after filtering by Posterior Prob. $\geq$ 0.95. Type Ia error = false positive when true parent is present; Type Ib = false positive when true parent is absent; Type II error = false negative when true parent is present.

	No. of assignments	No. of Type Ia errors	No. of Type Ib errors	No. of Type II errors
Both parents supplied	659	0	0	0
Putative mother supplied	153	0	0	0
Putative father supplied	361	0	0	0

Table S3: Results of FRANz accuracy assessment for incompletely sampled parent population, after filtering by Posterior Prob. $\geq$ 0.95. Type Ia error = false positive when true parent is present; Type Ib = false positive when true parent is absent; Type II error = false negative when true parent is present. Assignment in parentheses, marked by [*] had two different error types, as indicated, for each parent.

	No. of assignments	No. of Type Ia errors	No. of Type Ib errors	No. of Type II errors
Both parents supplied	696	0	0	0
Putative mother supplied	111	1	1	0
Putative father supplied	256	0	11 (1[*])	1 (1[*])