STRAYING, STRESS, AND POTENTIAL FOR REPRODUCTIVE INTERACTIONS BETWEEN HATCHERY-PRODUCED AND WILD CHUM SALMON (*ONCORHYNCHUS KETA*) IN SOUTHEAST ALASKA

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Abstract

Approximately 1.5 billion juvenile hatchery-produced Pacific salmon (*Oncorhynchus* spp.) are currently released each year into Alaskan waters with goals of enhancing important fisheries and minimizing detrimental impacts on wild stocks. As the abundance of hatchery-produced salmon has increased, so have concerns about hatchery-origin strays entering wild systems and interactions with wild individuals on the spawning grounds. The influx of non-native strays and their associated fitness-related traits can reduce the resilience and productivity of recipient wild stocks, and is likely to be most deleterious when disparities in population sizes and heritable phenotypic characteristics between wild and hatchery fish exist. Thus, understanding the ecological and life-history mechanisms that regulate gene flow between hatchery and wild populations is crucial for conservation and management strategies in areas where hatchery enhancement is common. Currently, the ecology of strays on the spawning grounds and proximate physiological factors associated with straying (e.g., stress) are not well known. In this thesis I examine, 1) differences and similarities in several fitness-related phenotypic traits between naturally produced (presumably wild local individuals) and stray hatchery-produced chum salmon (*Oncorhynchus keta*) that died on the spawning grounds of Sawmill Creek, a small watershed near Juneau, Alaska, and 2) physiological differences in cortisol concentrations and the frequency of crystalline (vaterite) structure of otoliths between straying and correctly homing salmon. Hatchery-strays comprised 51.4% of the adult chum salmon that returned to Sawmill Creek during the 2015 spawning season. Hatchery males and females returned approximately seven days later, were consistently smaller (10% for males, 6% for females) in length, and younger on average than their naturally-produced counterparts. Additionally, hatchery-produced females lived fewer days on the spawning grounds during the

spawning season, and retained a higher proportion of their eggs than did naturally produced females.

To explore the potential role of stress on straying, I compared cortisol samples and frequency of vaterite formation in otoliths among groups of hatchery-produced fish that homed to the hatchery, hatchery-produced fish that strayed to Sawmill Creek, and naturally produced chum salmon that presumably homed to Sawmill Creek. No significant differences in cortisol concentration were found among any groups, though differences between the sexes were detected. Males of all groups had significantly lower cortisol concentrations than did females. No differences in frequency of vaterite occurrence were found between hatchery-stray and hatchery-home groups, though both hatchery groups were higher than naturally produced groups, which is consistent with findings of other studies. Thermal marking while at the hatchery during early development was not associated with vaterite formation, and no difference in frequency of vaterite formation was observed among groups of varying mark intensities.

Overall, these results revealed there was ample opportunity for reproductive interactions between stray hatchery-produced and naturally produced chum salmon in Sawmill Creek during the 2015 spawning season, and consistent differences in phenotypic traits suggests the potential for gene flow to alter population-level phenotypic variation. However, despite the potential for gene flow, these results also reveal potential barriers to introgression and indicate that at least some of the presumed locally adapted traits of the natural stock remain intact. It remains unknown what the characteristics of the wild stock were prior to regional hatchery production and the extent to which the traits of this population are reflections of genetic differences between the hatchery and wild groups or phenotypic plasticity. To the extent these results are generalizable, observed differences in fitness-related traits between naturally produced and stray

hatchery-produced fish may underlie the reduced reproductive success often reported in the literature. There were no differences in cortisol concentrations and frequency of vaterite occurrence between hatchery chum salmon that strayed and those that homed correctly, and the frequency of vaterite occurrence of hatchery chum salmon did not change as thermal mark intensity increased, which suggests that thermal marking may not directly alter homing ability of adults or development of juveniles, at least via otolith formation. Despite not having an effect on straying, the consistent findings of higher frequency of vaterite occurrence in hatchery-produced fish compared to naturally produced counterparts highlight the need for future work to uncover the causal underlying mechanisms and implications of vaterite on survival of the 1.5 billion salmon released each year in Alaskan waters.

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General Introduction

Natal site fidelity of Pacific salmon, termed "homing", facilitates reproductive isolation (Hasler and Wisby 1951; Quinn 1993; Hendry 2004), and when combined with site-specific selection pressures, promotes evolution of locally adapted populations (Taylor 1991; Peterson et al. 2014). The rich literature on the proximate and ultimate causes of homing has greatly increased our understanding of salmon ecology (Dittman and Quinn 1996; Quinn 2005; Quinn et al. 2006). Salmon that do not return to natal sites ("strays") can increase genetic diversity in the recipient population (Consuegra et al. 2005; McPhee et al. 2014), hedge against environmental disturbance (Leider 1989), and allow for colonization of newly suitable habitat (Milner et al. 2008; Pess et al. 2012). Straying rates vary among species and populations (Westley et al. 2013; Keefer and Caudill 2014) and are influenced by distances between sources and sinks (Piston and Heinl 2012), climatic factors (Westley et al. 2015), density dependence (Berdahl et al. 2016), or anthropogenic disturbance during juvenile imprinting (Keefer et al. 2008; Bond et al. 2017). Although extensive work has gone into quantifying rates of straying both from donor populations and into recipient populations (especially in the context of hatchery populations into wild populations), relatively little is known about the ecology of the strays themselves and how they interact with wild fish on the spawning grounds (see Lin et al. 2008 as rare exception).

Although straying is a fundamental aspect of salmon biology, the ecological and genetic interactions of straying hatchery-produced fish continue to be a conservation concern in North America (Waples 1991; Rand et al. 2012) and Europe due to escape of farm-raised Atlantic salmon (McGinnity et al. 2003). Prominent among these concerns are the erosion of locally adapted gene complexes resulting from the invasion of detrimental alleles from domesticated hatchery stocks (Waples 1991; Araki et al. 2007; Naish et al. 2007). Current estimates suggest

over 4.5 billion juveniles are released annually into the Pacific Ocean (Ruggerone et al. 2010; Larsen 2015), greatly increasing the likelihood of interaction between hatchery- and naturally produced populations on both common oceanic foraging and spawning grounds. This latter potential has prompted studies investigating recipient straying rates into natural populations around the Pacific Rim (Brenner et al. 2012; Piston and Heinl 2012; Zhivotovsky et al. 2012) as well as causes of straying, with the goal to mediate interactions and increase survival (and hence numerical returns) of hatchery-produced salmon (Keefer et al. 2008; Clarke et al. 2011; Westley et al. 2013, 2015; Bond et al. 2017). In contrast to the nomenclature of many previous salmon studies that use "Hatchery" or "Wild" to identify the origin of an individual fish, in this thesis I use the terms hatchery- and naturally produced instead. This is to account for the possibility of a non-marked fish being a hybrid between a hatchery-produced and a naturally produced mating pair, or the offspring of two hatchery-produced individuals that spawned naturally outside the hatchery system. For the remainder of this manuscript hatchery-produced and naturally produced will be abbreviated to H_p and N_p, respectively.

Research Objectives

Existing knowledge of interactions between natural and hatchery populations in Alaska has been gained primarily from numerical examination of spawning grounds (i.e., the percentage of carcasses of hatchery-origin; Josephson 2010; Brenner et al. 2012; Piston and Heinl 2012), although some evidence suggests varying levels of gene flow between N_p and H_p populations (Jasper et al. 2013). While these studies identified potential for interaction, and evidence of interaction, they are unable to identify the mechanisms mediating those interactions. The overarching goal of this thesis was to address gaps in knowledge regarding the potential for genetic interactions between H_p and N_p produced chum salmon (*Oncorhynchus keta*). I

approached this goal by quantifying phenotypic and life history traits known to influence fitness from N_p and stray H_p fish spawning in Sawmill Creek, north of Juneau, Alaska. I also investigated specific stress-related mechanisms that may influence rates of straying by H_p chum salmon, which ultimately mediate interaction potential.

The objective of my first chapter was to addresses the potential that ecological interaction H_p strays may have on a naturally occurring population by conducting a mark-recapture experiment, carcass surveys, analyses of body size and shape, and subsequent determination of origin through thermal mark identification in otoliths. By sampling chum salmon on average every other day throughout the spawning season, I examined the potential for ecological and reproductive interactions between H_p and N_p salmon through comparisons of a suite of fitness-related traits such as entrance timing onto the spawning grounds, body length and depth, snout size, age at maturity, length at age, instream lifespan, and egg retention in Sawmill Creek. The results of field efforts allowed me to 1) assess how each difference or similarity may affect reproductive success of the hatchery strays and 2) provide new evidence of the potential promoters and inhibitors of gene flow between hatchery-produced chum salmon and naturally produced conspecifics.

The objective of my second chapter was to assess the potential for stress to be a proximate cause of straying by examining differences in physiology of homing and straying chum salmon through comparisons of cortisol concentrations and rates of vaterite occurrence. Additionally, I quantified the effect that a stressful event early in development (i.e., thermal marking: intentional temperature fluctuations initiated by hatcheries to induce variation in growth rate) may cause developmental disruption during a critical imprinting period. Chapter 2 draws upon on the role cortisol (a stress hormone) plays in memory recall during olfactory

navigation by adult chum salmon (Carruth et al. 2000) and developmental disruption as indicators of chronic stress experienced during early life history (Palmer and Strobeck 1986). Through paired comparisons between H_p and N_p (presumed to have homed to natal areas), H_p chum salmon that are confirmed to have strayed onto natural spawning grounds (referred to hereafter as H_{ps}) and H_p chum salmon that returned home to the hatchery of rearing or release location (referred to hereafter as H_{ph}), I quantified stress through cortisol concentrations and correlated those concentrations to reproductive behavior and performance on the spawning grounds. As a complementary measure of stress, the frequency of vateritic otolith occurrence between H_{ps} and H_{ph} were compared, as well as the frequency of vaterite occurrence among hatchery groups that experienced a range of rapid stress-inducing thermal fluctuations, to determine if exposure to thermal stress early in development influences probability of straying later in life.

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Chapter 1: Do hatchery and naturally produced chum salmon (*Oncorhynchus keta*) differ in fitness-related traits in a small stream subject to chronic immigration of strays? [1]

Abstract

To assess the potential for introgression and competition between hatchery-produced (H_p) and naturally produced (N_p) chum salmon (*Oncorhynchus keta*), we quantified a suite of morphological and life-history traits on the spawning grounds of a small creek in southeast Alaska that has high rates of immigration from nearby hatchery programs. Using thermally marked otoliths to distinguish between H_p and N_p fish, we observed that 51.4% (n = 560) of chum salmon examined in 2015 were H_p (e.g. strays). Compared with their N_p counterparts, H_p males and females entered the creek significantly later, were younger at maturity, smaller in body length, and smaller for a given age. On average, H_p females lived two days less in freshwater during the spawning season than N_p females, and also had higher rates of egg retention. These differences in hatchery and naturally-produced ratios on the spawning grounds and differences in several heritable traits suggest that the naturally reproducing population may be resistant to the invasion of foreign traits through a combination of reduced reproductive success of strays or spatio-temporal segregation on the spawning grounds. At the same time, our finding of high proportions of hatchery-produced salmon on the spawning ground, combined with observed differences between hatchery- and naturally produced groups in Sawmill Creek also highlight the potential for introgression and outbreeding depression.

Introduction

It is well established that Atlantic and Pacific salmon (*Salmo* and *Oncorhynchus* spp., respectively) exhibit natal site fidelity for reproduction (Foerster 1936; Scheer 1939; Hasler and

[1]McConnell, C.M., Westley, P.A.H., and McPhee, M.V. 2017. Do hatchery produced and naturally occurring chum salmon (*Oncorhynchus keta*) differ in fitness-related traits in a small stream subject to chronic immigration of strays? Accepted in: *Aquaculture Environment Interactions*.

Wisby 1951). This homing behavior facilitates reproductive isolation (Quinn 1993; Hendry et al. 2000; Schtickzelle and Quinn 2007) which, in combination with natural selection, promotes the evolution of locally adapted populations (Taylor 1991; Hendry 2004; Fraser et al. 2011; Peterson et al. 2014). Individual salmon that return to non-natal freshwaters and attempt to spawn, termed "strays", are thought to reflect hedges against environmental disturbance (Leider 1989), provide a mechanism for colonization (Pess et al. 2012; Nielsen et al. 2013), and increase genetic diversity via gene flow (Consuegra et al. 2005; McPhee et al. 2014).

Although straying is a fundamental part of salmon biology, the ecological and genetic impacts of H_p strays on naturally occurring populations continue to be a concern in North America and Europe (McGinnity et al. 2003; Rand et al. 2012). Chief among these concerns are the domestication of hatchery stocks, reduction in genetic diversity, and the loss of local adaptation of native stocks through hybridization and introgression of maladaptive non-native alleles (Waples 1991; Naish et al. 2007). It is well known that introgression of non-native alleles from genetically distant populations of salmon can result in the loss of local adaptation and reductions in fitness due to outbreeding depression (Gharrett and Smoker 1991; McClelland et al. 2005; Muhlfeld et al. 2009). This sequence of events is exacerbated by hybridization with more distantly related populations or populations that have diverged through contrasting natural or artificial selection pressures (Gharrett et al. 1999; McClelland and Naish 2007).

Selection pressures experienced by N_p salmon differ from those reared in captivity. For example, the hatchery environment relaxes selection pressures from predators and can alter competitive interactions and selective pressures on body size and prominent secondary sexual characteristics (Fleming and Gross 1989; Hendry et al. 2003; Knudsen et al. 2006). Adaptation to the artificial environment of hatcheries increases reproductive success within the hatchery

system, but results in lowered reproductive success of hatchery salmon in the wild (Fleming and Gross 1994; Reisenbichler and Rubin 1999; Ford 2002; Araki et al. 2007; Frankham 2008). Genetic changes driven by spawning and rearing in the hatchery environment can occur over short timespans (Fleming and Einum 1997), even after a single generation in captivity (Christie et al. 2012; 2016).

Hatcheries employ a variety of protocols to promote healthy brood stocks, and regulate gene flow with surrounding salmon populations. Hatcheries that attempt to minimize or mitigate genetic changes of the H_p stock from their N_p ancestral genotype by continually incorporating gametes from N_p stocks into each generation are often referred to as "integrated" hatcheries (Mobrand et al. 2005). Conversely, "segregated" hatcheries create the each new generation by spawning only fish that return to the hatchery or hatchery release location. These segregated hatcheries are the primary producers of pacific salmon in the State of Alaska. Alaska's salmon hatchery program attempts to mitigate impacts on wild populations by regulating stock movements, establishing wild stock sanctuaries, maintaining genetic diversity within hatchery stocks, and specifying release locations (as detailed in the Alaska Department of Fish and Game (ADF&G) Genetic Policy) rather than through integrated brood stock programs because the goals of the hatcheries are to enhance harvest (rather than wild abundance) and protect wild stocks (Davis and Burkett 1989).

Given the large numbers of fish currently being produced within hatcheries for enhancement or other purposes, and the natural propensity to stray, instances arise where H_p strays can overwhelm numerically small naturally occurring populations (Waples 1999; Bett et al. 2017). Since 1980, N_p pink salmon returns to Prince William Sound have ranged from 1.2 to 24.6 million, whereas H_p pink salmon returns have ranged from 1.7 million to 52.7 million

(Ruggerone et al. 2010). Chum salmon returns to Southeast Alaska since 1980 have ranged from 1.8 to 10 million N_p adults and 100,000 to 14 million H_p adults (Ruggerone et al. 2010). Recent studies in Alaska report that H_p chum salmon can comprise up to 78% of the returning adults in some natural systems, though in other streams no H_{ps} salmon were detected (Piston and Heinl 2012). This disparity in recipient stray rates is largely reflective of distance recipient streams were from hatchery-release locations and size of wild populations (Brenner et al. 2012; Bett et al. 2017). Although our understanding of the geographic and numeric extent of H_p strays in natural systems is expanding, we know little about the mechanisms facilitating interaction with naturally occurring populations or if precautions (genetic or otherwise) taken to mitigate interactions are adequate. To date, it is not known whether Alaskan H_p and N_p adult chum salmon differ in phenotypic traits such as run timing, body size, or body shape which may influence reproductive success in the wild. Understanding the intricacies of factors determining relative reproductive success may help identify mechanisms facilitating, or barriers to, genetic interactions.

We addressed this unknown by comparing H_p and N_p adult chum salmon spawning in a small representative watershed in Southeast Alaska. Given the close proximity to release locations, small size of naturally occurring population (Piston and Heinl 2014), high recipient stray rates, and short stream length, Sawmill Creek is a prime location for exploring interactions between H_p and N_p on the spawning grounds. Prior straying studies estimated recipient stray rates in Sawmill Creek, near Juneau Alaska, as 8% in 1995, 78% in 1996 and 2009 and 47% in 2010 (Josephson 2010; Piston and Heinl 2012). By sampling chum salmon throughout the spawning season, we examined the potential for ecological and reproductive interactions between H_p and N_p salmon while controlling for potentially confounding influences of migration distance, observer bias, and ancestral genetic origins. Specifically, our objectives were to

quantify differences between hatchery and wild chum salmon in the following traits: i) freshwater entrance timing, ii) body size and shape, iii) age at maturity, iv) instream lifespan, and v) egg retention rates. These traits were chosen based on a combination of their established genetic basis (Dickerson et al. 2005; Carlson and Seamons 2008), widespread divergence among locally adapted populations (reviewed in Quinn 2005), and known effects on fitness (Taylor 1991; Fleming and Gross 1994).

Methods

Site Description

Sawmill Creek (58.715°N, -134.944° W) is located approximately 40 km north of Juneau, Alaska (Fig. 1.1). It is a short stream (7 km), with approximately 500 m of available spawning habitat accessible to anadromous fish due to an impassable (ca. 15 m high) waterfall. The average width of Sawmill Creek is approximately 15 m, and estimated discharge ranges from 0.5 m^3/s to 1.12 m^3/s during summer base flow (i.e., not influenced by heavy rains or significant snow melt). The number of chum salmon entering Sawmill Creek is highly variable among years, with observations of 100 to 4,500 made during previous surveys (Piston and Heinl 2014).

Hatchery Description

Douglas Island Pink and Chum hatchery which releases chum salmon nearest Sawmill Creek originally developed its brood stock from five streams in the region (including Sawmill Creek) as well as a supplement from Hidden Falls Hatchery (which itself is comprised of three stocks from southern southeast Alaska (Stopha 2014; Fig 1.1). Currently, the chum salmon at DIPAC are a segregated stock, as only fish that return to the hatchery are used for egg takes. Egg takes occur throughout the season, and artificial selection for spawner attributes (e.g., body size, size-matched mating) is unlikely as entire batches of fish are processed at once regardless of size, though in years of high returns late returning adults are not spawned for brood stock

purposes if egg quotas had previously been reached (C. McConnell, personal observation). Two sites for remote release of hatchery-reared chum salmon are located within 30 km of Sawmill Creek (Amalga Harbor, 58.49°N, 134.79°W; Boat Harbor, 58.63°N, 135.16°W; Fig. 1.1). Combined releases of chum salmon fry at these two locations averaged 61 million annually from 2010 to 2015 (White 2011; Vercessi 2012; 2013; 2014; 2015; Stopha 2016).

Data Collection

Sampling of Sawmill Creek took place throughout the 2015 spawning season between July 7 (the day after chum salmon were first observed entering the creek) and August 21 (no live chum salmon were observed), though the creek was visited several times just prior to the run and just after to confirm exact start and end dates. Most stream visits consisted of an exhaustive carcass survey throughout the entire area available to spawning fish, starting at the tide line and ending at the barrier falls, and ranging into the forest approximately 10 m on each side of the creek to account for bear-kills. Every carcass encountered was measured for length (mm; mid-eye to end of hypural plate, hereafter referred to as MEHL). Carcasses of un-scavenged females were dissected and remaining eggs were counted to estimate egg retention. Sagittal otoliths were removed from all carcasses found in Sawmill Creek and stored until origin could be determined by examining otoliths for the absence or presence of a thermal mark indicating hatchery origin (Volk et al. 1999). Once the barrier falls was reached during a survey, the crew worked downstream, enumerating live chum salmon in the entire creek down to the high tide line (except on 7 occasions when poor water clarity obscured identification of fish in deep pools).

A mark-recapture experiment was conducted to estimate instream lifespan and allow for identification of origin of fish whose blood was drawn for a secondary study (see Chapter 2), and also allowed an estimate of the escapement population size. Tagging commenced upon completion of the carcass and enumeration surveys if untagged chum salmon were present. Fish

were captured by dip net and hook and line. To obtain accurate estimates of entrance date and lifespan, only chum salmon that had recently entered Sawmill Creek were targeted for tagging. Upon capture fish were distinguished as newly entered based on vibrant coloration, lack of fin erosion, presence of sea lice, and visual assessment of females being fully gravid (full, round, and dimpled showing egg pressure from inside body cavity). After capture, fish were anesthetized with Aqui-S 20E® (35 ppm), measured for MEHL, body depth (vertically from insertion of dorsal fin), and snout length (tip of snout to mid-eye). Fish were subsequently marked with individually labeled 1" Peterson disc tags and their sex determined. After a tagged specimen had fully recovered (as determined by regained equilibrium and normal response to handling, i.e., attempting avoidance/escape) it was released back into Sawmill Creek near the location it was captured.

To estimate age of N_p chum salmon, the whole right otolith was soaked in deionized water and examined under a dissecting microscope to count annuli following methods described by Bilton and Jenkinson (1968). When otoliths were difficult to age, a second or third trained reader was consulted. If consensus could not be reached, no age was assigned to that individual (n=17). Thermal mark patterns, unique to a year of release and hatchery of origin, were used to assign origin location and age to H_p fish. Verification of presence/absence and thermal code identifications were made by professional readers at Alaska Department of Fish and Game Mark, Tag, and Age Lab in Juneau, Alaska following standardized quality control protocol procedures described by Fernandez and Moffitt (2016). Each otolith was assessed for thermal marks doubly blind to the other reader's classification and conflicts were resolved by consultation with third read, also done blindly to previous reads. If consensus on mark identification could not be reached, no origin was assigned and the individual was not used for analysis.

Statistical Analysis

Entrance Timing

Non-parametric Kruskal-Wallis tests were used to determine whether H_p and N_p groups (separated by sex) significantly differed with regards to entrance timing distributions since distributions were found to be non-normal.

Body Length, Body Depth, and Snout Length

Analysis of covariance (ANCOVA) was used to compare morphological traits between H_p and N_p chum salmon within Sawmill Creek while controlling for seasonal trends:

$$y_{ik} = \mu_k + \beta_k x + \varepsilon_{ik}$$

where y is the generic response for fish *i* of origin *k* (body length, depth, or snout length of N_p or H_p), μ is the mean response for N_p or H_p, β is the effect of date (x) on the response, which may differ between hatchery and wild fish, and ε is the residual error term. Different slopes for hatchery and wild fish were estimated by including an interaction term in the model. If the interaction term was insignificant ($p > 0.05$), it was removed to formulate a simplified model:

$$y_{ik} = \mu_k + \beta x + \varepsilon_{ik}$$

Seasonality was considered because larger, older individuals typically enter streams first (Clark and Weller 1986; Morbey 2000), and comparing fish not present at similar times is not as informative as comparisons between potential competitors. If a seasonal trend was not identified as significant it was removed from the model and a standard ANOVA was used. Because secondary sexual characteristics, such as body depth and jaw length, shrink after spawning and are thus not necessarily representative of an individual's spawning morphology (Quinn and Blair 1992; Hendry and Berg 1999), untagged carcasses recovered after senescence were measured only for MEHL. Body depth and snout length were standardized to a common body length for

each sex (males = 537 mm, females = 510 mm) following Hendry and Quinn (1997) prior to comparison by ANCOVA. Comparisons were only made from measurements taken from fish with a confirmed origin (i.e., readable otoliths collected from carcasses).

Age at Maturity

Kruskal-Wallis tests were performed to account for non-parametric age-at-maturity distributions and compare ages of H_p and N_p chum salmon by sex recovered during the 2015 spawning season. Only groups of fish age-4 or age-5 were used to assess differences in age at maturity because sample sizes for age-3 and age-6 fish were inadequate.

Length at Age

Body length (MEHL) measurements of age-4 and -5 H_p and N_p chum salmon were analyzed using ANCOVA tests while controlling for death date as a covariate to assess length at age differences. If the covariate was not significant it was removed and ANOVA tests were used for the comparisons.

Instream Lifespan

Instream lifespan was calculated as the number of days between estimated entrance date and observed death date of recovered tagged salmon that died of natural causes. An ANCOVA was used to compare instream lifespan of H_p and N_p fish of both sexes while controlling for seasonal trends by including entry date as a covariate (similarly to ANCOVA tests for length, etc). If entry date was not significant it was removed and ANOVA tests were used to determine differences in lifespan between H_p and N_p groups.

Egg Retention

Egg retention was expressed as the number of eggs retained relative to total fecundity as predicted from the fish's MEHL. We constructed a length-fecundity relationship by sampling ten fully mature females from the fish ladder at DIPAC and 25 ripe females from Salmon Creek

directly adjacent to the hatchery. For each female, MEHL was recorded and twenty eggs were measured through volumetric displacement in a 10-ml graduated cylinder to determine the pooled average volume of an individual egg (0.25 ml ± 0.04 ml). The remaining eggs were then displaced in 1000-ml graduated cylinders to determine total gonad volume. The gonad volume-to-length relationship was estimated with a general linear model ($r^2 = 0.38$, $p < 0.001$) and expected fecundities (in number of eggs) were calculated by using model coefficients and the observed length to calculated expected egg volume, then dividing the expected volume by the pooled average egg volume to get expected fecundity as number of eggs. Egg retention percentages were then calculated by dividing number of counted eggs that remained in un-scavenged body cavities by the expected fecundity (similar to Quinn et al. 2007). While the fecundity to length relationship was not tight, it was expected as fecundity associations are typically variable within salmon (Beacham and Murray 1993; Fleming and Gross 1994).

A generalized linear model (GLM) was used to compare the proportion of eggs retained in the body cavity of dead H_p and N_p females while controlling for the effect of death date. A binomial distribution and logit link function were specified and interaction terms were tested. If interaction terms were insignificant they were removed from the model.

All statistical tests and data manipulations were conducted using R version 3.2.2 (2015) and statistical significance was based on $\alpha = 0.05$.

Results

Sawmill Creek was visited on 26 occasions during the 46-day chum salmon spawning season in 2015 which spanned July 6th to August 21st. Though tagging effort was not specifically standardized proportionally to the observed number of live fish, effort bracketed the entire run and sampling was most intensive during the peak of the run; thus, tagged fish were likely

representative of the 2015 spawning population as a whole. Additionally, all data used for hatchery/wild comparisons came from confirmed-origin carcasses. All carcasses found were sampled thereby reducing potential for subsampling bias (see Table 1.1). A total of 184 chum salmon entering the spawning grounds of Sawmill Creek were tagged and released. A total of 121 (67%) of tagged individuals were recovered: 18 were killed by bears (*Ursus* spp.) and the remaining 103 died either naturally (n = 99) or of an undetermined cause (n = 4).

Otoliths from 560 of 561 chum salmon were readable for the presence/absence of a thermal mark, of which 51.4% were of hatchery origin. Using a Chapman estimator to provide a conservative estimate of the number of spawners (Seber 1982), the estimated returns of chum salmon to Sawmill Creek in 2015 was 854±40. According to this estimate, and assuming a closed population (i.e., no tagged fish left Sawmill Creek during the tag recovery period), approximately 62 - 68% of the population was sampled.

Entrance Timing

N_p chum entered the creek earlier than H_p strays (Fig. 1.2). Median entry for N_p males was July 17, significantly earlier (10 days) than that of H_p males, whose median entry day was July 27 (Kruskal-Wallis; df = 1; $\chi^2 = 10.12$; $p = 0.001$). Similar to males, N_p females arrived earlier than their H_p counterparts, with median entry being 8 days apart, July 24th and August 2nd, respectively (Kruskal-Wallis; df = 1; $\chi^2 = 10.65$; $p = 0.001$). Despite the difference in median entry date, H_p chum salmon of both sexes were present on the spawning grounds during 92% of the surveys spanning the entire duration of the spawning season, though H_p to N_p rates varied widely near either end of the season. For example, during the first 10 days carcasses were found 26.5% were H_p, whereas during the last 10 days 80.7% of carcasses found were H_p.

Body Length, Body Depth, and Snout Length

The body length of individuals entering the creek declined over the course of the spawning season, but even so, H_p males were on average 10% smaller than N_p males after controlling for effects of date (Table 1.2; Fig. 1.3a; ANCOVA; $F = 56.52$; $p < 0.001$). Similarly, the body length of H_p females was 6% smaller than the average N_p female after controlling for date of recovery (Fig. 1.3b; ANCOVA; $F = 69.49$; $p < 0.001$).

Body depth of live male chum salmon entering the creek did not change throughout the season (ANCOVA; $F = 0.45$; $p = 0.50$) and did not differ between groups (Table 1.2, ANOVA; $F = 0.071$; $p = 0.79$). Similarly, female body depths did not vary through the spawning season (ANCOVA; $F = 2.001$; $p = 0.16$) and there were no differences of N_p or H_p females (Table 1.2; ANOVA; $F = 0.98$; $p = 0.32$).

Snout length did not vary by entry date for males (ANCOVA; $F < 0.01$; $p = 0.98$) or females (ANCOVA; $F = 1.33$; $p = 0.25$). Snout lengths of males did not differ between H_p and N_p groups (Table 1.2; ANOVA; $F = 0.04$; $p = 0.84$), however, snout lengths did differ significantly between females, with snouts of N_p females being approximately 4% smaller than those of H_p females (Table 1.2; ANOVA; $F = 4.87$; $p = 0.03$).

Age at Maturity

Age at maturity was significantly younger for H_p males (Kruskal-Wallis; df = 1; $\chi^2 = 10.39$; $p = 0.001$) and females (Kruskal-Wallis; df = 1; $\chi^2 = 10.51$; $p < 0.001$) than their naturally occurring counterparts. H_p males primarily matured at 4 years of age (79%) whereas age at maturity of N_p males was evenly split between age-4 and age-5 (Table 1.3). The proportion of N_p females that matured at age-4 was greater than the proportion that matured at age-5 (63% and

37%, respectively), however a markedly higher proportion of H_p females matured at age-4 than at age-5 (82% and 18%, respectively; Table 1.3).

Length at Age

N_p individuals were significantly longer than their hatchery-produced counterparts after controlling for date of recovery (Table 1.3), in both age-4 males (approx. 9.4%; ANCOVA; $F = 29.89$; $p < 0.001$) and age-5 males (approx. 8.9%; ANCOVA; $F = 6.84$; $p = 0.01$). Age-4 N_p females were longer than H_p females after controlling for date of recovery (approx. 5.9% larger ; ANCOVA; $F = 48.23$; $p < 0.001$), as were age 5 N_p females (approx. 4.4% larger ; ANCOVA; $F = 8.88$; $p = 0.003$).

Instream Lifespan

The instream lifespan of males did not differ significantly between H_p (7.5 ± 2.3 days) and N_p (8.3 ± 1.9 days) chum salmon (Table 1.2; ANCOVA; $F = 1.29$; $p = 0.26$). Conversely, N_p females lived approximately 28% longer (8.1± 2.1 days compared to 5.8 ± 1.9 days) than H_p females (Table 1.2; ANCOVA; $F = 6.94$; $p = 0.01$).

Egg Retention

Mean egg retention rates increased as the spawning season progressed (Fig. 1.4). Rates did not differ significantly between N_p or H_p females when controlling for the effect of death date (GLM; df = 168; $Z = -1.32$; $p = 0.18$). Overall, however, N_p females retained an average of 18.7% of their eggs whereas H_p females averaged 47% egg retention (Table 1.2) and retention rates of both groups ranged from 0.0% to 100%.

Discussion

The interactions between N_p and H_p fish on spawning grounds have been a long-standing focus of conservation efforts (Waples 1991; McGinnity et al. 2003; Rand et al. 2012), and the magnitude of potential genetic impacts is expected to grow with increasing phenotypic

divergence between strays and the recipient populations (Hendry 2004; Peterson et al. 2014). Given that stray rates in Sawmill Creek have been consistently high (Josephson 2010; Piston and Heinl 2012), and that nearby releases of similar magnitude have occurred for nearly 28 years (roughly 5 to 8 generations), there has been ample opportunity for interactions between N_p and H_p salmon to occur. Despite the potential for genetic introgression, we found consistent differences between N_p and H_p chum salmon in heritable phenotypic and life-history traits.

The present study is the first to document phenotypic and life-history differences between N_p and H_p produced chum salmon on spawning grounds, which have underpinned previous concerns regarding hatchery and wild interactions (Fleming and Gross 1992; Rand et al. 2012; Zaporozhets and Zaporozhets 2012). Furthermore, the data collected here provides more fine-scale information on run timing and interaction potential than previous carcass surveys on Sawmill Creek (Josephson 2010; Piston and Heinl 2012), which were conducted periodically or once per spawning season. Another unique aspect of this project is the genetic makeup of the H_p salmon in that 1) their ancestry was (partially) derived from N_p chum salmon in Sawmill Creek 2) have essentially been a segregated hatchery population since then, and 3) that any interactions on spawning grounds is unwanted by state salmon management making interpretation of results more complex, albeit interesting. Differences observed in Sawmill Creek during 2015 could result from one or more of the following causes: maintenance of pre-zygotic reproductive isolation between groups; potential differences in reproductive success between origins; reduced survival of hybrid offspring; and/or a considerable role for environment to shape these traits.

Entrance Timing

Typical of salmon migrations (Morbey 2000), males arrived first to Sawmill Creek, followed by females. The timing of arrival, however, was staggered such that N_p males arrived first, followed by N_p females and H_p males (whose median arrival day was only three days after

the median naturally-produced female arrival day). On average, H_p females arrived last on the spawning grounds. This contrasts with Prince William Sound (PWS), Alaska, where H_p chum salmon arrived at spawning grounds prior to N_p chum salmon, possibly due to differences in brood stock used in hatchery production (early runs were selected for hatchery brood stocks in PWS, Brenner et al. 2012). These results highlight that differences in run timing are likely site- and region-specific (and dependent on brood stock of the hatchery) making generalizations difficult. In our study, the situation may be further complicated by initial brood collection requirements that necessitated early-returning salmon to successfully escape before surplus could be collected for brood stock development (Brock Meredith, DIPAC, Juneau, Alaska. personal communication, May 2017). Risk of interaction between N_p spawners and H_p strays may be mitigated within some systems by artificial selection for run timing within hatcheries or spatial segregation (Mackey et al. 2001; Williamson et al. 2010). Spatial isolation of spawning areas within Sawmill Creek is unlikely, given that all chum salmon spawning took place within a 300m section of the 500m of accessible habitat. Complete temporal isolation of N_p Sawmill Creek chum salmon is similarly unlikely as H_p strays were present on the spawning grounds during 92% of the surveys and male salmon remain reproductively active during their entire stream life.

Despite overlaps in time and space, there remain distinct entrance timing differences between N_p and H_p strays. Unfortunately, the historic entrance timing of Sawmill Creek chum salmon prior to start of nearby hatchery releases is unknown. It is also unknown if H_p strays found in Sawmill Creek first arrive at their release locations (likely Amalga or Boat harbors), fail to find suitable spawning habitat, and then disperse to nearby creeks, which would mask their true arrival timing. Regardless of mechanism, later arrival and spawning of H_p strays likely

affects their reproductive success and could influence the reproductive success of N_p salmon. Male N_p salmon may be at a competitive disadvantage when examining entrance timing alone, as we documented that late arriving H_p males would have access to many of the remaining females of both origins at the end of the spawning season with little competition from N_p males. For example, after August 4th (latest confirmed entry date by naturally produced male) there were only 16 N_p male carcasses collected, whereas 44 H_p males were collected, as well as 124 H_p and 36 N_p females.

In order to successfully spawn, a late arriving female must attempt to displace females already in prime locations, wait for nest guarding females to die, or choose a less desirable location (reviewed in Fleming and Reynolds 2003). Prior residency is a key factor in the successful defense of a redd (nest) location from invasion, even from larger females (Foote 1990). In Sawmill Creek, H_p females generally had later arrival dates (and smaller body size), which would put them at a competitive disadvantage when attempting to secure a spawning location.

Body Length, Body Depth, and Snout Length

Salmon can experience divergent regimes of natural and sexual selection based on the environmental pressures of a discrete stock (Quinn et al. 2001). For example, male sockeye salmon spawning in the relative absence of bird or bear predators and no stranding risk in shallow water (e.g., lake spawners) tend to be deep-bodied (Quinn and Foote 1994), whereas shallow body shapes are favored in creek-spawning populations where risk of predation and stranding is high (Quinn and Buck 2001; Carlson et al. 2009). Predation on chum salmon in Sawmill Creek was low in 2015 (58 chum salmon deaths attributed to bear predation out of 558 positively identified causes of death), likely due to the high abundance of pink salmon, which were approximately two orders of magnitude more abundant than chum salmon. Additionally,

the creek was deep enough such that stranding was not an appreciable risk (only one salmon died of stranding as tide receded, C. McConnell, pers. obs.). Therefore, we expect that large and deep body-size might be favored through sexual selection in Sawmill Creek. However, we observed no significant differences between N_p or H_p males or females for either trait. We found a small difference in snout length between females, where H_p females had larger size-adjusted snout lengths compared to N_p counterparts. Although size of secondary sexual characteristics such as jaw size can relate to migration distance (Kinnison et al. 2003; Crossin et al. 2004) and all sources of DIPAC brood are from relatively short streams we believe this result to be spurious.

We found that N_p chum salmon of both sexes were significantly longer than H_p chum salmon throughout the spawning season, which might benefit N_p individuals in competitive interactions. Male dominance is strongly correlated with size and prior access to females, and dominant male chum salmon usually sire a higher proportion of offspring than satellite males (Schroder 1982; Foote 1990; Fleming and Gross 1994). Therefore, established and larger N_p males should have higher reproductive success than late-coming and smaller H_p males.

Being large bodied (and thus more conspicuous) likely had little negative impact on the reproductive success of female chum salmon, because most females preyed upon by bears were killed after spawning had occurred (Gende et al. 2004). Additionally, pink salmon are much more abundant in Sawmill Creek; therefore, predation is unlikely to be a major agent of selection in this system and relative reproductive success of larger females should be higher than smaller females through acquisition of preferential access to spawning sites, ease of redd defense, ability to attain greater egg burial depth, and greater fecundity (van den Berghe and Gross 1984; Steen and Quinn 1999). The relationship between body size and reproductive success is not rigid, however, as small females are occasionally able to utilize spawning areas not available or

optimal for large females (van den Berghe and Gross 1984), and in some circumstances large body size alone conveys no reproductive advantage (Holtby and Healey 1986). Additionally, if redd superimposition caused by late returning H_p females was common and occurred prior to epiboly of N_p embryos, the relative reproductive success of the early spawning N_p females could be reduced (Gharrett et al. 2013).

Age at Maturity

H_p males and females matured younger and were less variable in age than N_p chum salmon. Similar differences in age at maturity and age structure between natural and hatchery populations have been found in Atlantic salmon and Pacific salmon populations and have mainly been attributed to fishery selection, different growth trajectories, and within-hatchery effects (Knudsen et al. 2006; Imai et al. 2007; Zaporozhets and Zaporozhets 2012). Fishery selection that causes age-at-maturity declines is more likely to occur in fisheries that target multiple maturity stages (Healey 1986; Kuparinen and Merilä 2007). Chum salmon in northern southeast Alaska are commercially harvested with gill and seine net (Gray et al. 2016) and on smaller scale by trolling (Skannes et al. 2016), all of which have size selective potential (Milne; 1955; Kendall and Quinn 2012) but only target maturing individuals. It is possible, but unknown given the available data, that the largest H_p fish were selectively caught, leaving the smaller, younger fish to potentially stray and skew observed age-at-maturity observations made in Sawmill Creek. However, N_p salmon returning to Sawmill Creek and nearby streams are also subject to commercial harvest and would also experience similar (though likely lower intensity) fishery selection than H_p chum salmon, which are targeted first in common property fisheries and again in cost-recovery fisheries to cover hatchery operation costs (Macaulay Salmon Hatchery 2016).

In chum salmon, like other fishes, rapid growth tends to be associated with younger age at maturation (Myers et al. 1986; Vollestad et al. 2004; Morita et al. 2005; Claiborne et al. 2011) and accelerated growth in the hatchery and release at a large size may underpin the pattern of maturing younger by H_p chum salmon. H_p chum salmon are fed for several months following emergence to increase their size prior to release with the goal of increasing survival rates (A. Zaleski, DIPAC, Juneau, Alaska. personal communication, December 2016). Hatchery chum salmon released in northern southeast Alaska are larger and more energy dense than newly emigrated wild chum salmon (Reese et al. 2008), but interestingly, when sampled several weeks later in areas along their shared migratory pathway towards the Gulf of Alaska, their size and energy densities were similar (Sturdevant et al. 2012). Several weeks later in the eastern Gulf of Alaska N_p chum salmon had a larger mean length (Kohan et al. 2013) suggesting either compensatory growth by the N_p fish, selective mortality against the fastest growing H_p fish, or a combination of the two.

The different patterns of maturation between H_p and N_p chum salmon found in Sawmill Creek may alternatively reflect altered trade-offs between size and maturation. Attaining a large size that would be favored during natural spawning comes at the cost of longer growth period or heightened feeding rates, both of which incur additional mortality risks (Walters and Juanes 1993; Tillotson and Quinn 2016). In absence of the fitness benefits associated with large body size experienced in natural populations, less time at sea may be the favored life history strategy of H_p salmon (Gross 1985), which could explain the lower age-at-maturity observed of H_p chum salmon found in Sawmill Creek.

Furthermore, H_p males and females were less variable in age at maturity, a heritable trait (Carlson and Seamons 2008), and returns were dominated by age-4 fish. Complex age structures

act as a buffer against environmental variability and act as a stabilizer of population size (Moore et al. 2014) and positively influences population productivity (Greene et al. 2010). However, in this instance, being less variable in age structure may make hatchery populations more prone to "boom and bust" cycles which could increase and decrease numerical interaction potential in some years.

Length at Maturity

In Sawmill Creek we found that for the most common ages (age- 4 and age-5) H_p strays were significantly shorter than their N_p counterparts (Table 1.3). Smaller size at age of H_p fish suggests slower growth in the marine environment, yet a younger age at maturation usually correlates with a period of rapid growth (Morita and Fukuwaka 2006). Given the relatedness of growth and age at maturity, these results appear somewhat paradoxical. A possible scenario that explains our findings is that rapid growth within the hatchery environment puts H_p chum salmon on a trajectory to mature young but leads to domestication effects that either 1) may expose the fastest growing and more aggressive juveniles to higher predation related mortality once released (Yamamoto and Reinhardt 2003), or 2) causes a lag in transition to wild food types which slows growth potential (Sturdevant et al. 2012). Dietary differences as juveniles may temporarily interrupt growth trajectories; however, Morita and Fukuwaka (2006) found that chum salmon maturity schedules were more closely linked to growth rates during later growth stages (specifically, the growth season prior to maturity and spawning), so differences early in life may not adequately explain different body lengths at maturity.

Regardless of causality, disparities in size and age at maturity between chum salmon of different origins may have consequences for long-term reproductive success of the N_p population if genetic introgression or size-mediated competition occurs. H_p females on average were smaller

for their age than N_p chum, which could negatively affect the overall fecundity of hybrids, as well as impact their ability to build and protect redds (van den Berghe and Gross 1989).

Instream Lifespan

Instream lifespans of both sexes decreased as the spawning season progressed, consistent with other observations in semelparous salmon breeding systems (McPhee and Quinn 1998; Hendry et al. 2003; Dickerson et al. 2005; Doctor and Quinn 2009). For males, the difference in lifespan of one day was not statistically significant, though it could be biologically significant given that males can continue to spawn throughout their freshwater lifespans and that additional breeding opportunities decline non-linearly given changes in the operational sex ratio (Dickerson et al. 2005). In contrast, N_p females lived significantly longer in freshwater than did H_p females, which would provide a competitive advantage when vying for quality spawning locations or guarding redds from other females (McPhee and Quinn 1998; Hendry et al. 2003). In fact, our observations of the entirety of Sawmill Creek revealed several preferred locations that female chum continually spawned in throughout the season, reinforcing the importance of female longevity and entrance timing when maximizing relative reproductive success.

Egg Retention

Hatchery-produced females retained roughly the same proportion of eggs when compared to N_p females spawning at the same time, though over the course of the spawning season hatchery females retained over twice the expected number of eggs than did naturally produced females. This may be due to N_p chum salmon arriving and spawning prior to the peak pink salmon spawning activity, whereas the mean entry time of H_p chum salmon was later, coinciding with peak pink salmon spawning activities and hence greatly increased competition for space (Tillotson and Quinn 2017). Once pink salmon spawning activities commenced in late July, the variability in egg retention increased in both H_p and N_p chum salmon. The spawning

aggregations of pink salmon in Sawmill Creek during late July and early August of 2015 were dense (minimum density of 1 pink salmon/m^2) and significantly overlapped the period of chum salmon spawning dates, which reinforces and emphasizes the potentially stark consequences of run timing and prior residency on reproductive success. Ultimately, these results suggest that egg retention (shaped by sex-specific run timing) may be a key factor limiting reproductive success of H_p fish on the spawning grounds and minimizing genetic interaction, despite chronic opportunity for gene flow.

Conclusions

Proposed thresholds at which a population can maintain integrity of locally adapted traits and thereby resist outbreeding depression assume a single fixed proportion of H_p to N_p spawners during a spawning season, and do not account for unequal reproductive success. In Sawmill Creek, the threshold proportions of 5% H_p within a stream suggested by Mobrand et al. (2005) and 10% suggested by Ford (2002) bracket an instream estimate of 8% H_p spawners in 1995, and were greatly exceeded in 1996, 2009, 2010, and 2015 with estimates of 78%, 78%, 47%, and 51%, respectively (Josephson 2010; Piston and Heinl 2012). The 1995, 1996, and 2009 estimates were generated after only a single day of sampling whereas samples from 2010 were collected during three separate visits. During the intensive sampling that occurred during 2015, it was evident that the proportion of H_p chum salmon in Sawmill Creek changed through time, and that N_p fish entering early were somewhat protected from introgression based on low H_p proportions present at that time. However, near the later portion of the run very few naturally produced males were being collected as carcasses whereas numerous H_p males and N_p females were being recovered, indicating a higher potential for hybridization in later stages of the spawning season. Furthermore, differences in traits such as body size, lifespan, and egg retention further undermine the assumption of equal reproductive success in proposed thresholds. In Sawmill

Creek during 2015 these differences generally favored the N_p over H_p chum salmon in terms of relative reproductive success, indicating that this population may be able to withstand higher proportions of H_p strays than proposed. However, it is conceivable that in other N_p populations relative reproductive success may favor H_p strays and proportions of 5% of 10% may be sufficient to allow for substantial genetic introgression. Ultimately, it is becoming readily apparent that acceptable rates of straying are fluid, subject to caveats, and that introgression concerns should not be founded quantitatively, but qualitatively as well.

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Table 1.1. Summary of run timing, tagging effort, and carcass recoveries during 2015 spawning season. Live Count indicates the number of live chum salmon observed within Sawmill Creek on that date. Tagged is the number of fish tagged on that date, and Recovered is number of deployed tags from that sample day that were eventually recovered later in the season. Carcasses Recovered is the number of both tagged and untagged carcasses (for which origin was positively identified) sampled on date of visit. Dashes (-) indicate dates Sawmill Creek was visited but counts or tagging did not occur, and non-sequential dates indicate which days Sawmill Creek was not visited.

Date	Live Count	Tagged/Recovered	Carcasses Recovered
7/6/2015	10	-	0
7/7/2015	20	-	0
7/8/2015	-	3/0	
7/10/2015	-	2/0	0
7/12/2015	-	9/7	1
7/14/2015	72	10/6	0
7/15/2015	108	18/13	4
7/16/2015	103	17/16	0
7/19/2015	134	12/5	11
7/20/2015	137	2/2	16
7/22/2015	132	5/5	48
7/23/2015	-	-	19
7/24/2015	-	17/12	44
7/26/2015	124	14/12	24
7/29/2015	-	-	42
7/30/2015	97	9/6	32
7/31/2015	113	10/4	22

Table 1.1 continued

8/2/2015	95	12/7	32
8/4/2015	90	16/8	41
8/6/2015	88	10/5	19
8/8/2015	102	7/3	26
8/10/2015	95	15/10	24
8/12/2015	-	-	37
8/13/2015	58	-	36
8/17/2015	25	-	65
8/20/2015	10	-	14
8/21/2015	0	-	0

Table 1.2. Mean trait values for groups of hatchery- produced (H_p) and naturally-produced (N_p) chum salmon of both sexes, with standard deviation in parentheses and sample sizes italicized. Units for entry date is day of year (where day 200 corresponds to July 19); mid-eye to hypural length (MEHL), body depth, and snout length are millimeters; instream lifespan is days spent alive in freshwater; egg retention is proportion of eggs remaining in body cavity after natural death (range for both wild and female egg retention was 0.0 to 1.0).

Trait	N_p Male	H_p Male	N_p Female	H_p Female
Entry Date	199.9 (6.3) *45*	207.2 (10.7) *19*	205 (7.6) *25*	213.2 (9.2) *32*
MEHL	559 (41) *138*	502 (35) *86*	529 (32) *128*	497 (25) *199*
Body Depth	152 (9) *45*	151 (6) *19*	130 (7) *25*	132 (8) *32*
Snout Length	84 (6) *45*	83 (7) *19*	54 (4) *25*	56 (4) *32*
Instream Lifespan	8.3 (1.9) *45*	7.5 (2.3) *19*	8.1 (2.1) *21*	5.8 (1.9) *30*
Egg Retention			0.18 *128*	0.43 *199*

Table 1.3. Mean mid-eye to hypural length (MEHL, mm; with standard deviation in parentheses) by age and sample sizes (N) for groups of hatchery-produced (H_p) and naturally-produced (N_p) chum salmon of both sexes in dominant age classes.

Age	Sex	Origin	MEHL (SD)	N
4	M	N_p	548 (43)	58
4	M	H_p	496 (29)	63
5	M	N_p	568 (37)	61
5	M	H_p	517 (45)	17
4	F	N_p	523 (32)	72
4	F	H_p	492 (21)	157
5	F	N_p	539 (26)	42
5	F	H_p	515 (31)	32

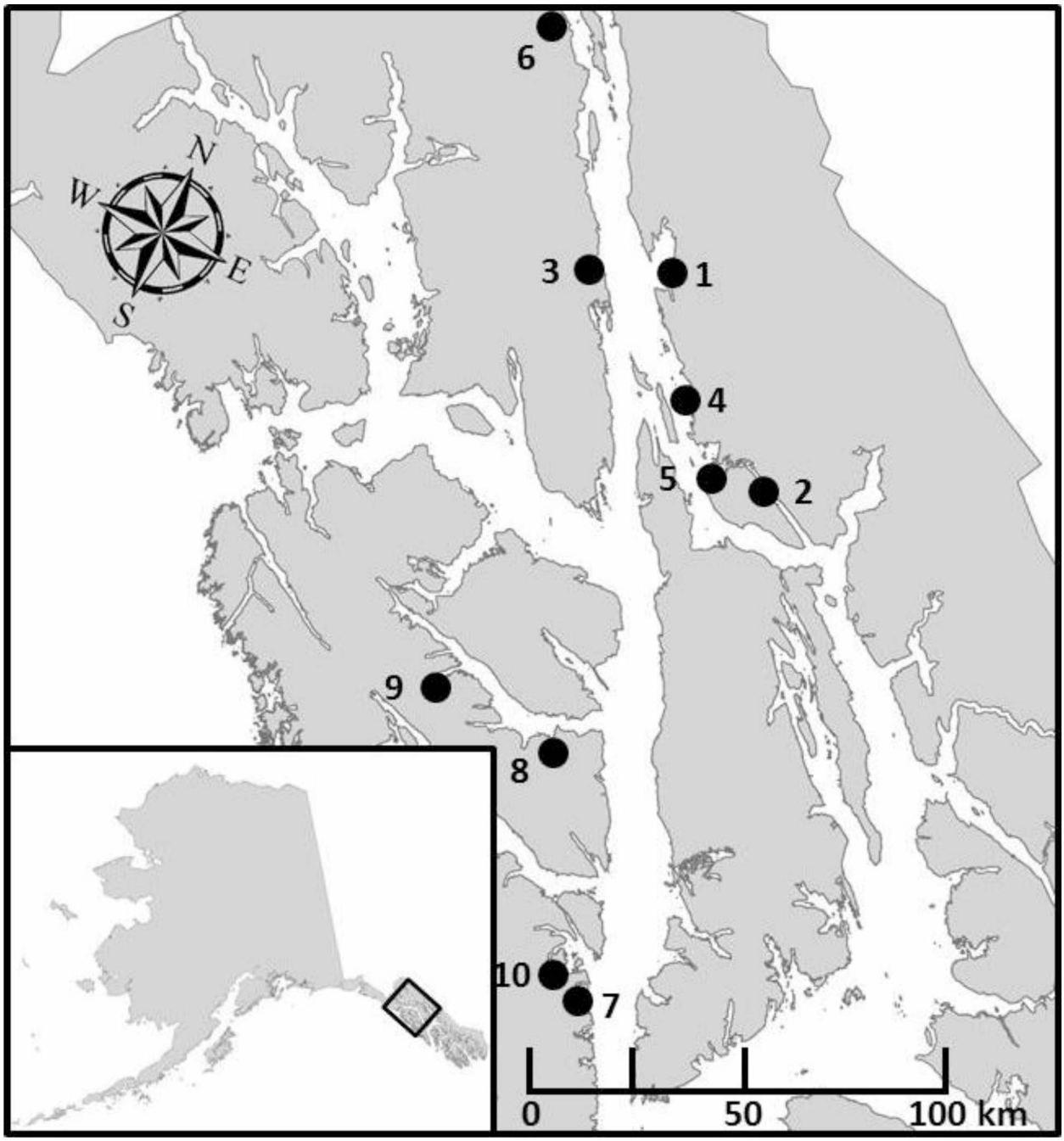

Fig. 1.1. Map indicating broodstock collection sources for chum salmon released in close proximity to Sawmill Creek (1) and DIPAC hatchery (2); remote release locations of Boat Harbor (3) and Amalga Harbor (4); and source locations for DIPAC broodstock as defined in Stopha (2014) as Sawmill Creek (1), Fish Creek (5), and Klehini River (6), as well as Salmon Creek and Kowee Creek (not shown; each within 3km of DIPAC). Hidden Falls Hatchery (7) stocks were developed from Kadashan River (8), Seal Bay Cove (9), and Clear River (10).

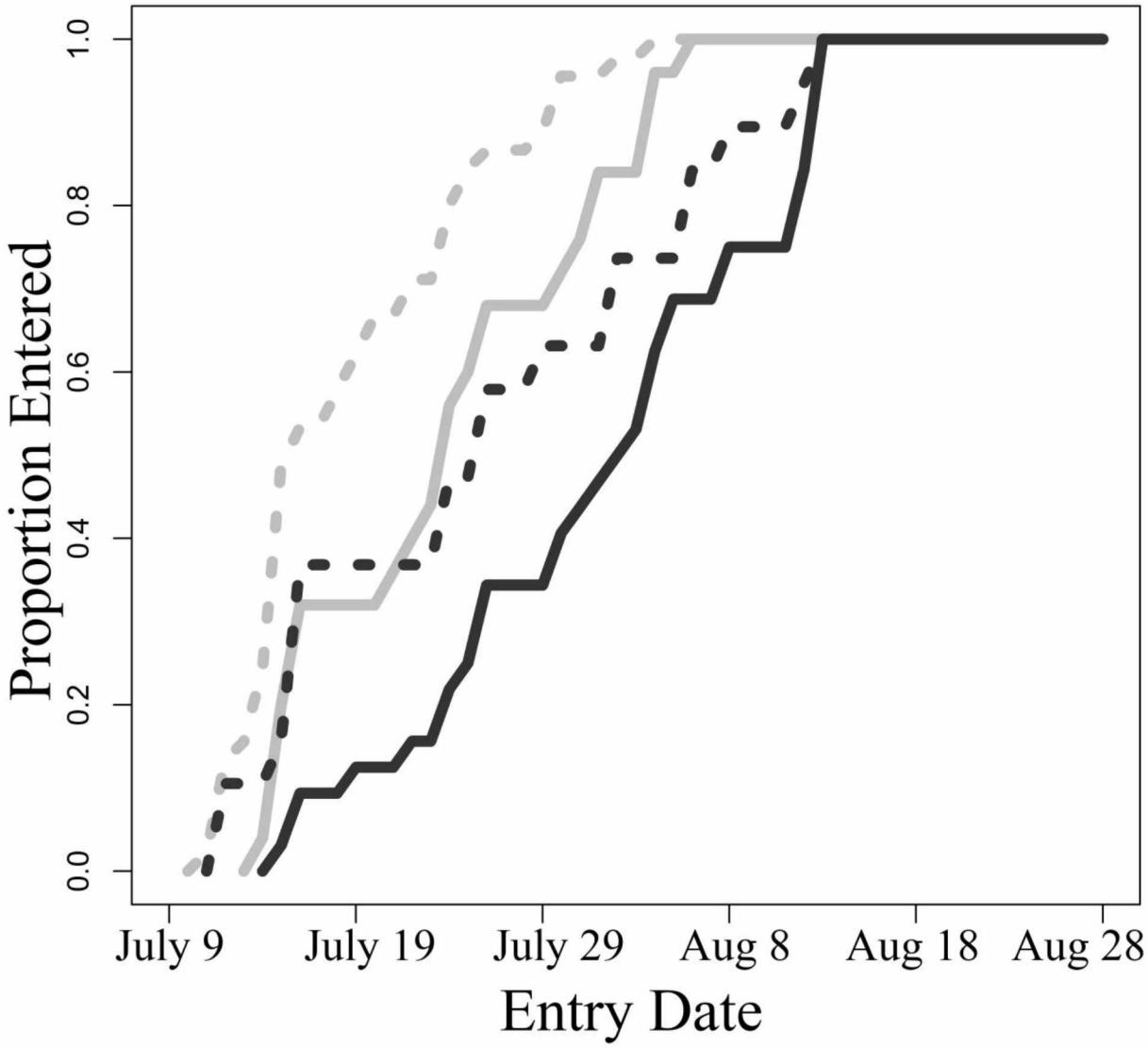

Fig. 1.2. The cumulative proportion of chum salmon entering Sawmill Creek across the spawning season of 2017. Males are represented by dashed lines while females are represented by solid lines. Origin is denoted by shade, with naturally produced salmon being represented by grey and hatchery-produced salmon by black.

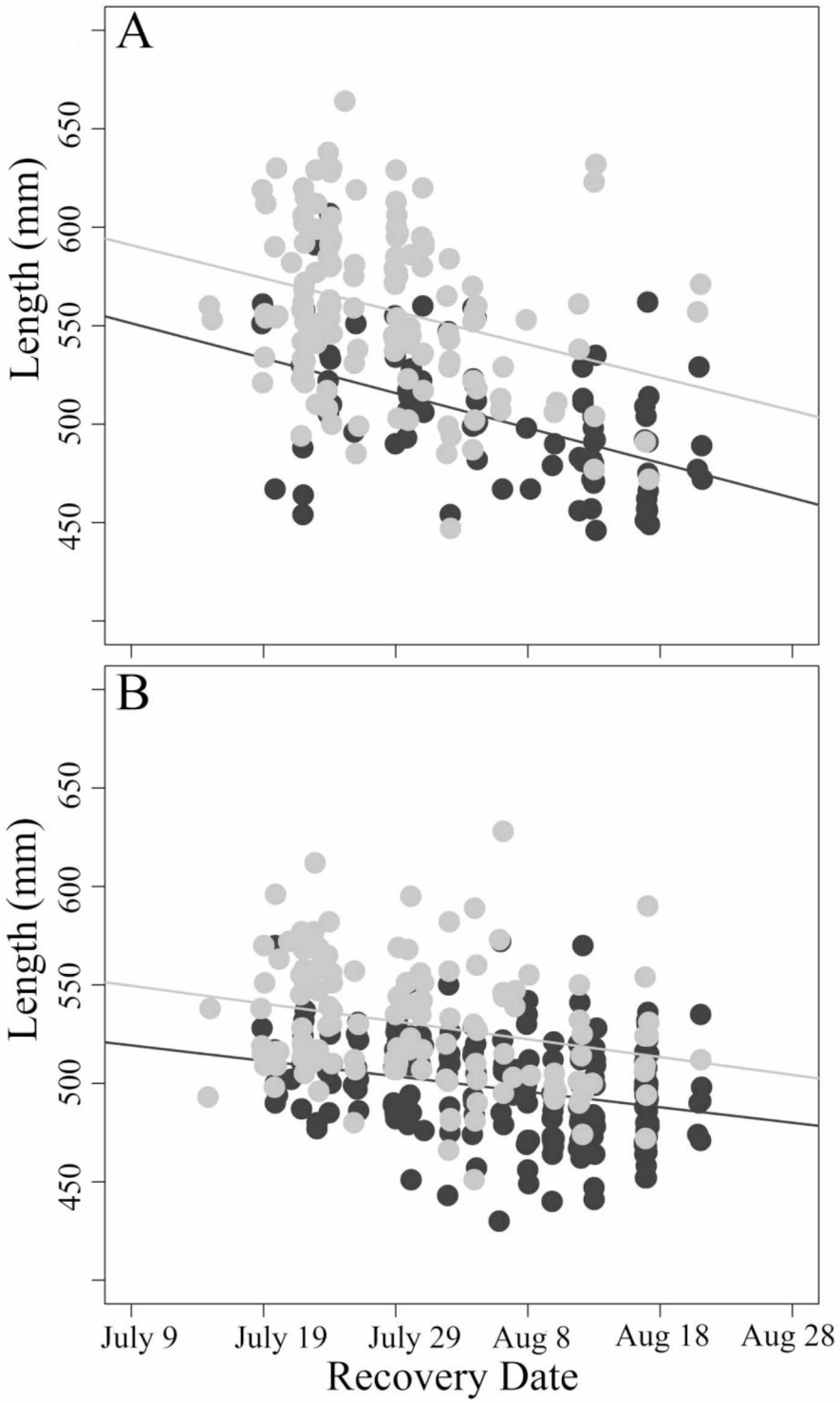

Fig. 1.3. Body lengths (measured from mid-eye to end of hypural plate, in mm) of male (Panel A) and female (Panel B) chum salmon by recovery date. Origin is denoted by shade, with naturally produced salmon being represented by grey and hatchery-produced salmon by black. Solid lines are simple linear regressions through group.

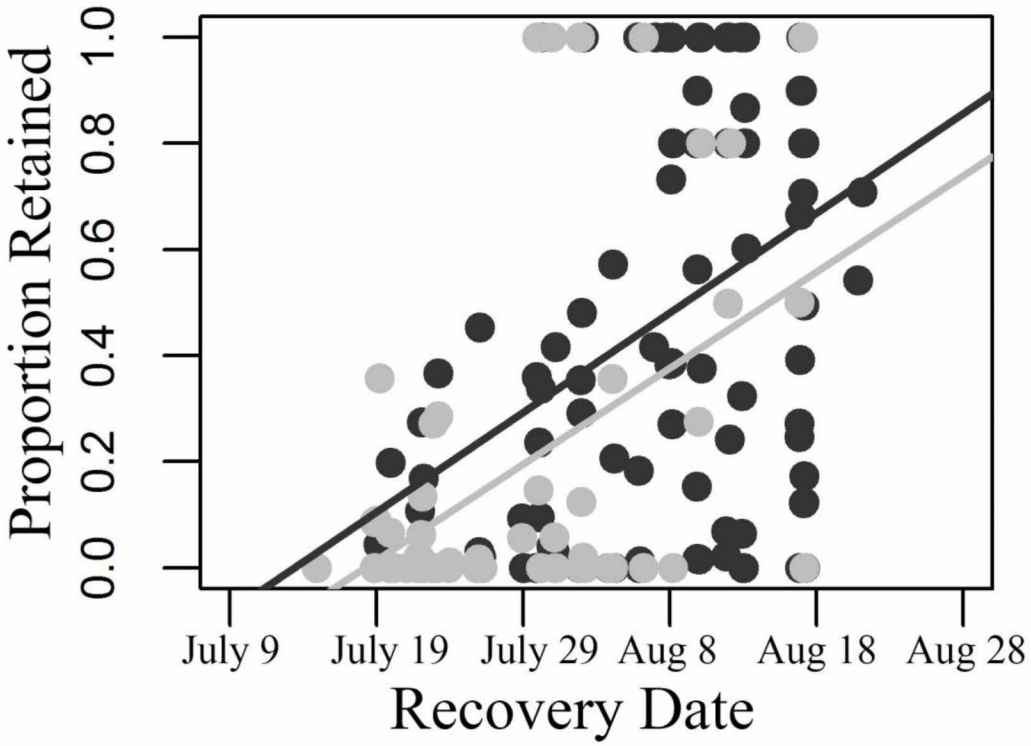

Fig. 1.4. Proportion of eggs retained by naturally produced (grey) and hatchery-produced (black) female chum salmon across breeding season.

Chapter 2: Is stress associated with straying of hatchery-produced chum salmon (*Oncorhynchus keta*)? Cortisol, vateritic otoliths, and potential consequences for enhancement programs.[2]

Abstract

Hatchery programs around the Pacific Rim release approximately five billion Pacific salmon each year into the North Pacific Ocean, prompting concerns about interactions with wild counterparts. Straying of hatchery-produced individuals mediates interactions with wild spawning populations, though relatively little is known about the proximate factors shaping straying. In this paper we assess the potential influence of stress on homing and straying rates of hatchery-produced salmon by comparing cortisol concentrations between hatchery-produced salmon that have homed (correctly returning to imprinting location) to those that strayed (returned to location other than release site). Cortisol levels were associated with fitness-proxies such as the number of days a fish lived on the spawning grounds and extent of egg retention in females. As an additional proxy for stress, we quantified the frequency of abnormal vateritic otolith development between homing and straying hatchery-produced salmon. Finally, we assessed the potential for otolith thermal marking, a widely used approach that exposes developing individuals to abrupt temperature fluctuations to induce a visible mark within the otolith, to influence rates of vaterite occurrence. No differences were found between hatchery-produced chum that had homed or strayed in cortisol concentrations of either males (stray = 113.4 ± 99.7 ng/ml; home = 124.66 ± 113.81 ng/ml) or females (stray = 329 ± 208.9 ng/ml; home = 294.12 ± 134.8 ng/ml) or rates of vaterite occurrence (stray = 40% vaterite; home = 45% vaterite). Instream lifespan was negatively correlated with cortisol concentrations, though egg retention rates were not related to cortisol concentration. There was a slight, though not

[2] McConnell, C.J., Westley, PAH., McPhee, M.V., Atkinson, S., and Oxman, D. 2017. Is stress associated with straying of hatchery-produced chum salmon (*Oncorhynchus keta*)? Cortisol, vateritic otoliths, and potential consequences for enhancement programs. Formatted for the *Canadian Journal of Fisheries and Aquatic Sciences*.

statistically significant, increase in vaterite occurrence in individuals with high thermal mark intensities (low = 32%, medium = 32%, high = 39%). The lack of differences in cortisol concentrations and rates of vaterite occurrence between correctly homing and straying groups suggest that straying is not significantly linked to these physiological measures, at least on the spawning grounds. Additionally, increasing mark complexity did not result in an increase in frequency in vaterite occurrence.

Introduction

The homing of Pacific salmon (*Oncorhynchus* spp.) is intimately tied to the ecology, evolution, and management of the species (Myers et al.1998; Dittman and Quinn 1996). The alternative to homing, termed 'straying', has garnered much attention given it mediates interactions between hatchery and wild fish on the spawning grounds (Quinn 1993; Rand et al. 2012). Although it is clear that straying is influenced by phenotypic attributes of dispersers such as size or sex (Lin et al. 2008) and is plastic in response to climatic factors (Westley et al. 2015), density dependence (Berdahl et al. 2016), and anthropogenic disturbance during juvenile imprinting (Keefer et al. 2008; Bond et al. 2017), the proximate physiological factors associated with straying are not well known (reviewed in Dittman and Quinn 1996).

Advances in genetic and mass marking techniques have facilitated the rapid growth of literature regarding salmonid straying (Keefer and Caudill 2014). Also, increased efforts to conserve and assess responses to changing environmental and anthropogenic forces have prompted similar increases in literature regarding stress physiology of fish (Baker et al. 2013). Although there is considerable justification for expecting relationships between stress physiology and straying, studies linking proximate physiological mechanisms to migration decisions are limited (Carruth et al. 2002; Cooke et al. 2008).

The hypothalamopituitary-adrenal/interrenal axis plays a large role in facilitating life-history transitions across a wide range of vertebrate taxa, including fishes (Crespi et al. 2013). Corticosteroids, and the hormone cortisol in particular, serve a fundamental role in the physiological processes of salmon undergoing the parr-smolt transformation (Langhorne and Simpson 1986; Iwata 1995) and the strenuous migration to spawning grounds (McBride et al. 1986). Circulating cortisol (hereafter referred to simply as "cortisol") may mediate less obvious stressful life-history transitions; for example, homeward migrating kokanee (*O. nerka*) had elevated cortisol concentrations even though their migration was not especially rigorous and lacked salinity changes associated with stress responses in salmon migrating from a marine environment (Carruth et al. 2000a). It is unclear how the potential benefits of elevated cortisol involved with homing may be balanced with the detrimental impacts of chronically high cortisol levels which alter maturation rates, reduce immune capacity, accelerate senescence, and contribute to pre-spawn mortality (Schreck et al. 2001; Hruska et al. 2010; Cook et al. 2011; McConnachie, et al. 2012).

In addition to helping mitigate the effects of stress, hormones such as cortisol may be involved in the recall of odor memories, as regions of the salmon brain associated with memory and olfaction are sensitive to corticosteroids (Carruth et al. 2000b). The elevation of cortisol concentrations during the homeward migration phase could be an adaptive mechanism to enhance recall of learned natal odors, which assists with successful navigation to home streams (Hasler and Scholz 1983; Dickhoff 1989; Dittman 1994; Carruth et al. 2002). Cortisol concentrations may vary among individuals in response to homing/straying, predator avoidance, or thermal stress, and may play a part in controlling or influencing other aspects of a fish's life (Wendelaar-Bonga 1997; McConnachie et al. 2012). Thus, elevated concentrations of cortisol

may be expected in homing salmonids, and may differ between groups that successfully home and those that fail to navigate to their imprinted locations. If the physiological response that leads to correct identification of natal source is significantly different from the response that causes homing failure, there may then be cascading effects of physiological differences between homing and straying salmon. A difference in levels of a stress response may further help to explain why strays typically display lower reproductive success than correctly homing salmon, beyond typical local adaptations and phenotypic differences (Peterson et al. 2014).

As in other taxa (reviewed by Palmer 1994), chronic stress during development of salmonids can be manifested in easily identifiable bioindicators of otherwise difficult to measure developmental abnormalities (Palmer and Strobeck 1986; Cambell 2003). Specifically, evaluating environmental or genetic stress (*sensu* Oxman et al. 2013) experienced by fish is commonly quantified by comparing degree of difference in counts of paired fin rays, branchiostegal rays, maxillary length, gill rakers (Bryden and Heath 2000; Dann et al. 2010) and differences in otolith size, shape, and composition (Oxman et al. 2005; Díaz-Gil et al. 2015). Otoliths are typically formed of aragonite, though they may occasionally be composed of vaterite, a polymorph of calcium carbonate that appears clear and is commonly described as crystalline (Campana 1999). The mechanistic cause of aragonite vs. vaterite deposition is unknown, though aquaculture-produced salmon consistently show higher rates of vaterite compared to wild counterparts (Sweeting et al. 2004; Bowen et al. 2011; Reimer et al. 2016). The factors inducing potential stress responses may include crowding, feeding, noise, or mechanical shock that occurs within hatcheries (Strong et al. 1986; Sweeting et al. 2004; Fagerlund et al. 2011). Temperature fluctuation is another within-hatchery stressor hypothesized to be associated with the occurrence of vaterite (Oxman et al. 2005). During the embryonic and

larval stage of the life history, virtually all chum salmon released each year into Alaska's waters are intentionally exposed to abrupt water temperature changes (typically 3 to 4°C) that disrupt natural growth leaving distinct visible marks within the otolith (Volk et al. 1999, Vercessi 2014). This process of 'thermal marking' serves to mass mark individuals with unique codes that allow for identification of origin, release location, and age at a later date. The thermal marking events can last days or weeks and vary considerably in complexity based on the coding requirements necessary for sufficiently distinguishing among codes and on the ambient water temperature in which embryos or alevin are reared (Volk et al. 1999). If stress-induced developmental disruptions occur early in life during the olfactory imprinting process, then the ability to return to precise natal locations as adults may be compromised. It is necessary for imprinting processes of many populations of chum and pink salmon (*O. gorbuscha*) to begin just following emergence given many spawn and emerge from very short natal streams or intertidal areas (Thendinga et al. 2000; Bett et al. 2016). Additionally, fine-scale homing accuracy to specific stream segments has been shown in sockeye salmon (*O. nerka*), consistent with the presence of olfactory imprinting years prior to the parr-smolt transformation (Tilson et al. 1995; Quinn et al. 2006). Furthermore, population-specific rates of straying are hypothesized to correlate with environmental stability during development, with populations of salmon that experience more stable developmental conditions thought to stray less frequently than those experiencing unstable conditions (Thendinga et al. 2000; Keefer and Caudill 2014).

In this paper, we report on a series of tests for associations between stress and straying in hatchery-produced chum salmon. Through paired comparisons between naturally produced (N_p) fish presumed to have homed to natal areas, hatchery-produced (H_p) fish that were known to have strayed (H_{ps}) onto wild spawning grounds, and H_p fish that returned home successfully

(H_{ph}), we quantified a stress response through cortisol concentrations and evaluated correlations between cortisol concentration and two traits associated with reproductive success: instream lifespan and egg retention on the spawning grounds. Additionally, we compared rates of vateritic otolith crystallization between straying and homing salmon to test the hypothesis that thermal fluctuations experienced during early development influences probability of straying later in life. To the extent that stress may influence homing and straying, we expected that 1) H_{ps} would have higher cortisol concentrations compared to either N_p or H_{ph} chum salmon, individuals with higher cortisol concentrations would have shorter instream lifespans, and display higher rates of egg retention, 3) that H_{ps} strays would differ in incidence of vaterite occurrence compared to H_{ph} groups, and 4) that frequency of vaterite occurrence would increase as thermal mark intensity and complexity increases.

Methods

Sawmill Creek (Fig. 2.1) is located 15 km and 30 km from the two closest remote release locations of the Douglas Island Pink and Chum hatchery (DIPAC) and is known to routinely attract a substantial number of H_{ps} that are not harvested in common property commercial fisheries (Piston and Heinl 2012; McConnell et al. in review). Because of its high rates of recipient straying, small size (<1 km in length), and relatively remote location, Sawmill Creek offers a rare opportunity to test hypotheses related to straying and homing, and stress physiology of those groups. Additionally, because 100% of chum salmon released from DIPAC and Southern Southeast Regional Aquaculture Association (SSRAA) hatcheries during the study period were thermally marked and a standardized protocol for identifying marks exists, the misidentification of origin of chum salmon used in this study is highly unlikely (Scott et al. 2001). For the purposes of this study, fish classified as H_{ph} were mature adults that were

collected at their appropriate release location, either the hatchery facility or a remote-release location, and are confirmed "home" by possessing the correct thermal mark. However, salmon that lacked thermal marks which were captured in freshwater were assumed to have correctly homed to their natal stream, though it is not possible to precisely quantify the rate of straying among natural populations. Individuals representative of N_p and H_{ps} groups were collected in Sawmill Creek, while H_{ph} samples came from either Salmon Creek (water source of DIPAC), the raceway at DIPAC itself, or from SSRAA release sites (Fig. 2.1).

Sample Collection

To compare cortisol concentrations between N_p, H_{ps}, and H_{ph} individuals, we targeted chum salmon that had recently entered freshwater for capture in Sawmill Creek and Salmon Creek and captured them using dip nets. Anesthesia was applied quickly using a 35ppm dose of Aqui-S 20E (Aqui-S, New Zealand Ltd). We aimed to collect blood in under five minutes from onset of capture process to avoid bias caused by chasing/handling stress (Kobokawa et al. 1999) and the fish was uniquely tagged with a 1" disc tag, then revived and released back into Sawmill Creek. Instream lifespan and egg retention estimates are detailed in McConnell et al., in review, though briefly lifespan was calculated by counting the days between tagging and recovery, and egg retention was calculated by enumerating remaining eggs within an un-scavanged body cavity and converting to a percentage based on a fecundity/length relationship. Otoliths removed from tagged carcasses from Sawmill Creek were used to link cortisol concentration to origin through presence or absence of thermal marks. Otoliths from tagged and untagged carcasses were also used to determine the frequency of vaterite occurrence for N_p and H_{ps} groups. The H_{ph} individuals were sampled for cortisol concentrations in Salmon Creek using identical methods to those in Sawmill Creek. H_{ph} cortisol samples were collected from Salmon Creek rather than the fish ladder returning directly to the hatchery because Salmon Creek more accurately represents

natural stream conditions and is closer to conditions experienced by fish captured in Sawmill Creek. Moreover, densities of chum salmon in the fish ladder are frequently very high, and it is possible that by sampling here could have introduced bias that could mask the cortisol stress response associated with homing or straying.

Otoliths for vaterite comparisons between H_{ph} and H_{ps} were collected directly at the hatchery during brood stock egg collection. Otoliths used to examine relationships between thermal marking and vaterite occurrence were provided by Southern Southeast Alaska Regional Aquaculture Association (SSRAA), and represent the best available samples to compare rates of vaterite occurrence across a gradient of thermal mark intensities. The samples used represent the greatest degree of difference between low and high complexity and duration of thermal marks applied to chum salmon while simultaneously controlling for confounding effects related to straying. Specifically, these samples all come from individuals collected from release locations where they are presumed to have homed correctly, and they all come from the same brood stock and brood year, thus controlling for potential stock-specific or interannual environmental influences on rates of vaterite occurrence.

Analysis of Cortisol

Plasma cortisol concentrations (circulating cortisol) were assayed using enzyme immunoassays (EIA) obtained from Enzo Life Sciences (Farmingdale, NY 11735) and processed following Atkinson (2015). Detailed assay validation results and other EIA accuracy information can be found in Appendix 2.A. Distributions of cortisol concentrations were found to be non-normal using Shapiro-Wilk tests and remained non-normal after log-transformation, thus comparisons between H_{ph}, H_{ps}, and N_p groups of both sexes were made using non-parametric Kruskal-Wallis tests. Comparisons of instream lifespan and egg retention rates to cortisol concentrations upon freshwater entry were made using generalized linear models (specified

Gaussian distribution for lifespan GLMs and a binomial distribution for GLM of egg retention rates) on untransformed data.

Concentrations of plasma cortisol of salmon are continuously changing as osmoregulatory shifts occur, maturation continues, spawning activities commence, environmental conditions shift, and senescence approaches (summarized in McConnachie et al. 2012). In order for comparisons between groups in Sawmill Creek and Salmon Creek to be as unbiased as possible, we collected samples of salmon at the same life stage and sexual maturity level. Additional exploratory analyses were conducted to identify outliers (if any) and verify that other confounding effects were not biasing data. These included checking for differences in cortisol concentrations between up- and downstream groups (Kruskal-Wallis tests) in Sawmill Creek and testing (using generalized linear models with Gaussian distributions) for relationships between cortisol concentration and duration of sample collection and freshwater entrance timing (i.e., through time by day of year), as well as temperature, dissolved oxygen, and salmon density, which could elicit acute stress responses, masking basal cortisol concentrations (Table 2.2). The highlighted cell in Table 2.2, indicating a significant relationship between male chum cortisol concentration and temperature based on p-value, is not concerning given the low R^2 value and directional difference displayed by females.

Analysis of Vaterite and Associations with Thermal Mark Intensity

To quantify the occurrence of vaterite, otoliths were soaked in deionized water for 5-10 minutes then placed in a black petri dish under a dissecting microscope for examination. Projected overhead light and double-polarizing filters were used to maximize contrast between vaterite and aragonite. Normal aragonite appeared opaque and white, whereas vaterite was semi-transparent and was more jagged around edges (Fig. 2.3). In contrast to previous studies (Sweeting et al. 2004), we dichotomously categorized otoliths as either aragonitic (normal) or

vateritic (crystalline), given that the majority of otoliths were either purely aragonite or mostly vaterite (Table 2.3). Because no directional difference in vaterite was observed, the fish's right otolith was used unless it was lost or damaged and in such instances the left otolith was used (N = 50).

To determine if vaterite occurrence was associated with straying, we compared H_{ps} otoliths collected from DIPAC-produced chum salmon in Sawmill Creek to H_{ph} DIPAC-produced chum. Quantifying the extent of thermal marking's influence on developmental disruption was conducted by comparing vaterite rates between 1) differing mark intensities, 2) mark complexity, and 3) duration each mark took to apply. Mark intensity was defined as the number of thermal cycles induced during the thermal marking process. Mark complexity was a categorical variable based on the number of thermal marking sequences. For example, a thermal mark designation of 4H would have undergone four thermal cycles prior to hatching (denoted by "H"), but only one sequence of thermal cycles, and would be identified as a mark intensity of four, and a complexity of one. A fish with a thermal mark designation of 1,6H would have undergone one sequence of one cycle, then another sequence of six cycles prior to hatching, giving it an intensity of seven and a complexity of two. Mark duration was a continuous variable defined by the number of hours that the fish was subjected to temperature fluctuations from initial temperature spike until the final return to ambient temperature signaling the end of the thermal marking process.

Differences in frequency of vaterite occurrence between H_{ph} (collected at DIPAC) and H_s (collected at Sawmill Creek) groups, as well as between H_{ps} and N_p (collected from Sawmill Creek) groups were tested using two-tailed Z-Score tests for two population proportions. To test if frequency of vaterite occurrence increased with increasing thermal mark intensity, complexity,

or duration (SSRAA otoliths) logistic regressions were used with a specified binomial distribution. All statistical analyses were conducted in R (version 3.2.2) and significance was determined at the $\alpha = 0.05$.

Results

Cortisol, Straying, and Performance on the Spawning Grounds

Males had significantly lower cortisol concentrations (104.9 ± 92.9 ng/ml) than female (319.9 ± 176.9 ng/ml) chum salmon (Kruskal-Wallis $\chi^2 = 77.7$, $p < 0.001$), so comparisons were conducted within sexes. We found no evidence that cortisol was associated with straying. Cortisol samples collected from 15 H_{ph} males in Salmon Creek (mean: 124.6 ±113.8, range: 32.7 to 516 ng/ml) were not significantly different (Kruskal-Wallis; $\chi^2 = 0.88$, p=0.347) from the 18 H_{ps} males collected in Sawmill Creek (mean: 113.4 ±99.7, range: 23.0 to 412 ng/ml). Twenty-five H_{ph} females were sampled and their cortisol concentrations (mean: 294.1 ±134.8, range: 109.6 to 586 ng/ml) were also not significantly different (Kruskal-Wallis; $\chi^2 = 0.12$, $p = 0.729$) than the 31 H_{ps} females (mean: 329.0 ±208.9, range: 55 to 988 ng/ml). Similarly, no significant differences were found between pooled H_{ph} and H_{ps} males and 43 N_p males (mean: 95 ±83.7, range: 7.2 to 412 ng/ml; Kruskal-Wallis; $\chi^2 = 0.35$, p=0.55), or between pooled H_{ph} and H_{ps} females and 24 N_p females (mean: 307.6 ±175.7, range: 25.6 to 820 ng/ml; Kruskal-Wallis; $\chi^2 = 0.02$, $p = 0.871$; Fig. 2.2).

While there were no differences between N_p and H_{ps}, higher cortisol concentrations were associated with shorter instream lifespan in both males and females (Table 2.1). Egg retention was not significantly related to cortisol concentrations (Table 2.1). No other biological or ecological measures were significantly correlated with cortisol concentrations, except for a positive relationship with temperature among male chum salmon (Table 2.2). Female chum

salmon cortisol levels were non-significantly correlated and the direction of relationship was opposite.

Frequency of Vaterite Occurrence, Straying, and Thermal Mark Intensity

Vaterite occurrence was not associated with straying. Rates of vaterite occurrence did not differ between H_{ps} (40% vaterite) and H_{ph} (45% vaterite) chum salmon (Z-test; Z score = 1.22, $p = 0.222$) despite controlling for brood-year effects and comparing between groups with large sample sizes (Table 2.3). Similarly, thermal marking was not associated with observed rates of vaterite occurrence regardless of thermal marking intensity ($z = 1.27$, $p = 0.203$), complexity ($z = 1.27$, $p = 0.259$), or duration ($z = 1.36$, $p = 0.172$) in hatchery groups from SSRAA. The frequency of vaterite occurrence in N_p chum salmon in Sawmill Creek was 24%, lower than any of the H_p groups (Table 2.3).

Discussion

The straying of salmon from their natal stream into different areas and attempting to spawn can lead to numerous outcomes. It can facilitate geneflow between wild populations or expand the range of the species, or in the case of hatchery-produced populations, their straying can impact native populations (Keefer and Caudill 2014). In situations where interaction between hatchery and wild populations is of concern or unwanted altogether, understanding drivers and physiology related straying would be beneficial to fisheries managers seeking to mitigate interactive potential. We found no evidence that stress, as measured by cortisol concentrations and frequency of vaterite occurrence in otoliths, was associated with straying in hatchery-produced chum salmon. Moreover, we detected no effect of increasing aggressiveness of thermal marking (intensity, complexity, or duration of marks) on likelihood of straying. We did, however, observe that stream life declined with cortisol concentrations but that the likelihood of prespawning mortality did not. Consistent with the literature, we showed that

hatchery produced fish had higher incidence of vaterite than naturally produced fish, though the consequences for survival are unclear. Taken as a whole, these results suggest that the propensity to stray may not be determined by a single life-history event, and that straying is not the direct result of a hormonal difference influencing olfactory recall.

Cortisol

The majority of cortisol concentrations we measured in homing and straying chum salmon fell within the range of concentrations for sexually maturing salmonids (Pottinger et al. 1995; Carruth et al. 2000*a*). However, we note wide variation in our samples with some concentrations being higher than those measured following acute stress in juvenile Chinook salmon (*O. tshawytscha*) (Maule et al. 1988). The high variances in cortisol concentrations (exceeded variances found by Hruska 2010; Cook et al. 2011) may have been due to ongoing osmoregulatory shifts related to acclimation to freshwater (Wendelaar-Bonga 1997) as the Hruska et al. (2010) and Cook et al. (2011) studies took place far from the marine environment and Sawmill Creek is less than 1 km in length, where fish were sampled shortly after entering the stream.

Despite this large variation, male chum salmon in Sawmill and Salmon Creek exhibited significantly lower cortisol concentrations than did females, contrary to Fagerlund (1967) and Cook et al. (2014) who found no difference between sexes in sockeye salmon, but similar to Kobokawa et al. (1999) and Cook et al. (2011) who found both male sockeye salmon and male pink salmon had significantly lower basal cortisol concentrations than conspecific females. The lower concentration of cortisol of males may be in part explained by the timing of our sampling, which purposefully took place prior to competitive interactions with other males for access to spawning females. These spawning activities could increase basal cortisol levels, confounding our ability to test hypotheses regarding links between cortisol and behaviors that happened prior

to spawning. Relatively high female cortisol concentrations may reflect the final processes of ova development (Pickering and Pottinger 1987), but are unlikely to reflect sex-specific run timing as cortisol concentrations did not vary systematically throughout the season.

Previous evidence suggests that elevated cortisol concentrations might be associated with home stream odor recall during migration (Carruth 2000*b*). If this is true, one might expect that fish that have higher cortisol concentrations to be more likely to remember stream odors and correctly home. Alternatively, elevated cortisol levels could result from the inability to distinguish a natal stream odor if cortisol is continually produced in an attempt to correctly stimulate areas of the brain responsible for memory recall, or simply an individual 'knowing' it is lost may be sufficient to induce a stress response. In contrast to our expectations, we found no evidence that cortisol concentrations differed between fish that homed or strayed, nor between H_p and N_p individuals. A possible explanation for our findings could be that H_{ps} entering Sawmill Creek could have first successfully homed to their imprinted remote release location, found no suitable spawning habitat, and left the area in search of suitable streams exhibiting a physiological response which mirrors that of fish that homed correctly. This interpretation has some merit, as nearby release location Boat Harbor contains only one small creek that is not identified as chum salmon spawning habitat (Alaska Department of Fish and Game anadromous waters catalog: https://www.adfg.alaska.gov/sf/SARR/AWC/, accessed June 2016) and for the majority of the summer a weir is constructed on the freshwater source of Amalga Harbor (second nearest release location to Sawmill Creek; Fig. 2.1), preventing returning hatchery fish from entering (A. Zaleski, Douglas Island Pink and Chum, Inc. Juneau, Alaska, personal communication, December 2016). Alternatively, if cortisol levels declined as the need for olfactory recall decreases, then that signal could have been lost as the individual's physiology

responded to other physiological and environmental stressors such that it may not be possible to attribute a hormonal change to a single factor in such an uncontrolled circumstance.

While our findings of no discernable differences between homing and straying individuals did not support our hypothesis, it did allow examination of cortisol's role on instream lifespan and egg retention. Results from these opportunistic comparisons are consistent with existing literature regarding cortisol concentrations and salmon lifespans on the spawning grounds (Cook et al. 2011; McConnachie et al. 2012) in that increased cortisol concentrations of Sawmill Creek chum salmon correlated with decreased lifespans of both sexes, although observed rates of egg retention did not change significantly with increasing cortisol concentrations. Previous studies that have linked reductions in relative reproductive success of migrants and native populations have focused on phenotypic or genetic differences (McGinnity et al. 2003; Peterson et al. 2014). If cortisol concentrations had differed among straying and homing groups it may have further explained why migrants (either wild or hatchery) tend to display lower reproductive success (Chilcote et al. 1986; McGinnity et al. 2003; Hendry 2004).

Vaterite

Contrary to our hypothesis, H_{ph} chum salmon did not differ in rates of vaterite occurrence than did the H_{ps} group. This result combines to suggests that 1) the mechanism, stress related or not, that initiated the switch from deposition of aragonite to vaterite did not correlate with increased proclivity to stray later in life and 2) the functional impact of an abnormally formed otolith may have had no influence on homing ability.

Given this result, it suggests that thermal marking may have little impact on the processes of imprinting in salmon; however, we were not able to compare straying rates by individuals that were not thermally marked (as the mark provided the tag to assess straying). It is possible that the thermal marking procedure does not take place during the critical period when chum salmon

imprint and thus avoids disrupting the development processes surrounding olfactory imprinting (Dittman and Quinn 1996; Ueda et al. 2015). This is consistent with the observation that many otoliths transitioned from aragonite to vaterite prior to their first winter (see Fig. 2.3, panels B and C) rather than near or at the primordia (very early age) when chum may be imprinting on freshwater olfactory cues. We initially hypothesized that the frequently observed switching of aragonite to vaterite near (but not at) the core of H_p salmon might result from the forced transition to marine conditions and disruption of growth. Forced transitions would theoretically introduce changes in osmoregulation, temperature, and foraging more abruptly than if transitions were volitional, though wild salmon can also display smoltification checks in their otoliths and scales that correspond to disrupted growth during the transition to saline environment (Vega et al. 2017), suggesting that even volitional transitions can be stressful.

However, a study by Reimer et al. (2017) linked higher prevalence of vaterite in groups of salmon that were reared at higher temperatures, suggesting changes of growth rates may be a cause of vaterite formation. However, those results showed increased growth rates were responsible for increased frequency of vaterite occurrence (Reimer et al. 2017) so the cause of switching from aragonite to vaterite around the commonly observed transition area may be attributable to accelerated growth potential after release into a marine environment rather than a disruption in growth.

We found no evidence linking vaterite occurrence to different levels of intensity, complexity, or duration of the thermal marking procedure. Due to our reliance on archived otolith samples, we were limited to measuring vaterite presence as a potential indicator of developmental instability, whereas comparison of a suite of responses may have yielded different interpretations (Palmer and Strobeck 2001; Campbell 2003). Curiously, post-hatch chum salmon

that had been thermally marked during hatching had higher cortisol concentration levels than control groups (Sanders 2012). While the results of Sanders (2012) support the inferred mechanism for thermal marking (i.e., minute growth disruptions caused by short-lived thermal stress), it remains to be demonstrated that this stress influences the development of other sensory organs or has other lasting effects on the physiology of thermally marked fish.

Wild chum salmon had consistently lower rates of vaterite occurrence (24% vateritic) than hatchery origin individuals that had strayed into Sawmill Creek (40% vateritic), consistent with previous literature on hatchery and wild salmon otolith composition (Sweeting et al. 2004; Reimer et al. 2016). While some aspect(s) of rearing within the hatchery environment clearly influences increases the formation of vaterite (Reimer et al. 2017), our results indicate that it is not directly related to the widely used thermal marking procedure. Also, H_{ps} and H_{ph} had similar rates of vaterite occurrence, indicating that any possible link (if any) between vaterite occurrence within a population and that population's propensity to stray is not a direct one. Having the ability to discriminate stock of origin, and to ensure high return rates of hatchery-produced salmon are both key management goals of fishery and hatchery managers, and our results suggest that thermal marking does not confound these management objectives. The lack of difference in cortisol concentrations among N_p, H_{ps}, and H_{ph} salmon suggests other aspects of salmon development and physiology may dictate the homing abilities more directly. To better understand proximate mechanisms regulating straying for managerial purposes, future research may be focused on aspects of remote-releases or volitional transition from freshwater to marine systems. Also, given the high levels of variation and the number of external factors which may have affected cortisol levels, controlled experiments should be considered in future investigations of straying physiology.

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Table 2.1. Generalized linear model results of cortisol instream lifespan and egg retention.

	Estimate	St. Error	*t*	*p*	*df*
Male Instream Lifespan	-0.007	0.002	-2.366	0.021	60
Female Instream Lifespan	-0.004	0.001	-3.068	0.003	48
Female Egg Retention	<0.001	0.001	0.341	0.733	31

Table 2.2. Biological and ecological correlates that could bias plasma cortisol concentration observations. Temperature and dissolved oxygen values used correspond to mean daily value for the day cortisol was sampled. Entrance Timing is a seasonality correlate showing how cortisol concentrations may have changed as the run progressed. Draw Time was the duration of chase, capture, and blood draw. Pink Density is an estimated proportion of the density of pink salmon as determined by visual counts of live pink salmon in an index section on the day cortisol samples were taken. Similarly, chum density is a proportion of chum present divided by the maximum number of chum observed in Sawmill Creek. Body length and age are biological correlates that match to an individual salmon's cortisol concentrations.

	Males		
	Slope	R^2	p-value
Temperature	-60.090	0.065	**0.027**
Dissolved Oxygen	0.488	<0.001	0.932
Entrance Timing	-2.235	0.047	0.093
Draw Time	-0.236	0.014	0.362
Chum Density	-102.600	0.043	0.108
Pink Density	-129.860	0.046	0.104
Body Length	0.213	0.012	0.387
Age	21.050	0.019	0.330
	Females		
	Slope	R^2	p-value
Temperature	8.796	<0.001	0.895
Dissolved Oxygen	-18.510	0.030	0.210
Entrance Timing	-0.915	0.002	0.751
Draw Time	-0.677	0.018	0.331
Chum Density	160.400	0.018	0.332
Pink Density	-24.300	<0.001	0.894
Body Length	-0.237	0.001	0.768
Age	-4.196	<0.001	0.927

Table 2.3. Summary of otolith collections location, group designation and associated mark and vaterite findings. Mark codes are read by number of temperature fluctuations per sequence and pauses between sequences are indicated by a comma. "H" symbol refers to the hatching event. Intensity is the total number of temperature fluctuations and complexity is the number of fluctuation sequences needed to induce the thermal mark code. Duration is expressed as hours that temperature manipulations were occurring during thermal marking, not including pauses between pre-and post-hatch sequences. Number of otoliths within the total sample size (per designation by collection location) that were found to contain vaterite are noted in vateritic column, and the proportion of vateritic otoliths by location in designated groups is listed in Proportion column.

Collection Location	Designation	Mark Code	Intensity	Complexity	Duration	Sample Size	Vateritic	Proportion
Sawmill Cr.	Np	-	-	-	-	220	52	0.24
	Hps	4H	4	1	83.75	191	77	0.40
	Hps	1,6 H	7	2	122.5	46	17	0.37
DIPAC	Hph	4H	4	1	83.75	181	85	0.45
	Hph	1,6H	7	2	122.5	39	15	0.38
SSRAA Term. Harvest	Hph	5H	5	1	176	97	31	0.32
	Hph	3,4,2H	9	3	184	84	27	0.32
	Hph	4,4H4n,2	14	4	478	153	60	0.39

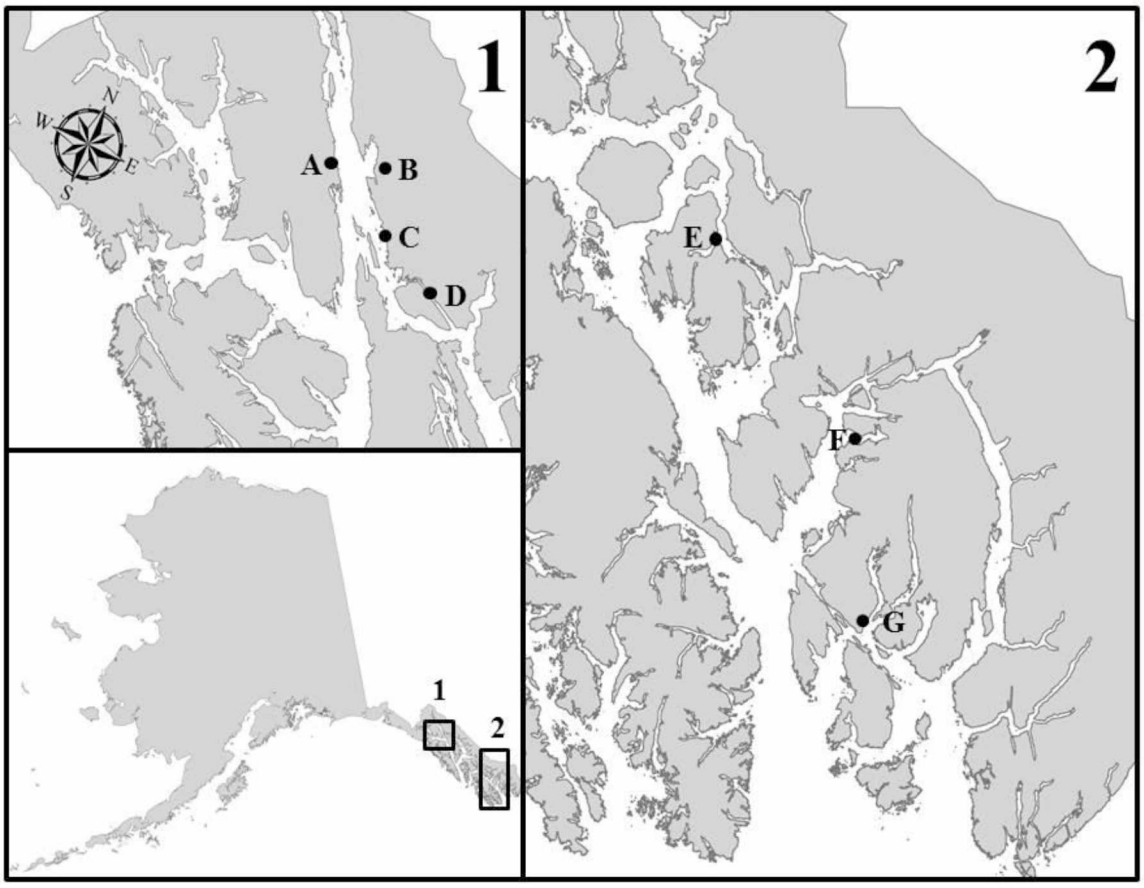

Fig. 2.1. Map of study area, including northern southeast Alaska (inset 1) and southern southeast Alaska (inset 2). Filled circles indicate positions of sample collection locations or points of interest. A) Douglas Island Pink and Chum Hatchery (DIPAC) remote release location at Boat Harbor, B) Sawmill Creek, C) DIPAC remote release location at Amalga Harbor, D) Salmon Creek and DIPAC facility where egg collection and rearing occurs, E) Anita Bay Terminal Harvest Area, F) Southern Southeast Regional Aquaculture Association (SSRAA) rearing facility and release location in Neets Bay, G) SSRAA Whitman Lake rearing facility.

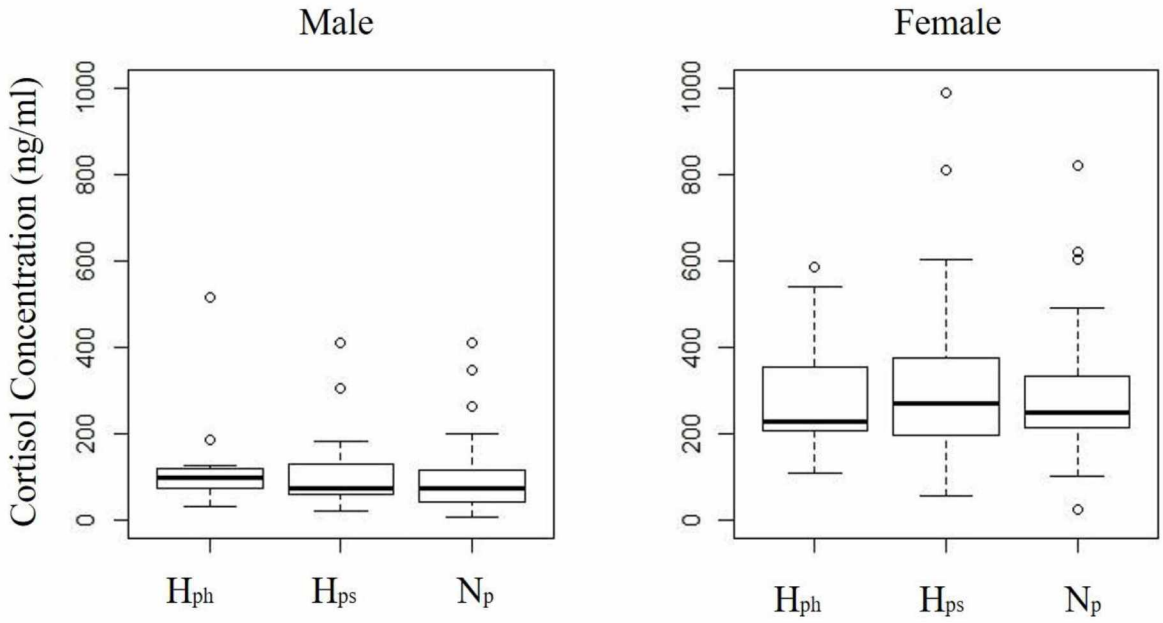

Fig. 2.2. Cortisol concentrations (ng/ml) of hatchery-home (H_{ph}), hatchery-stray (H_{ps}), and naturally produced (N_p) groups separated by sex.

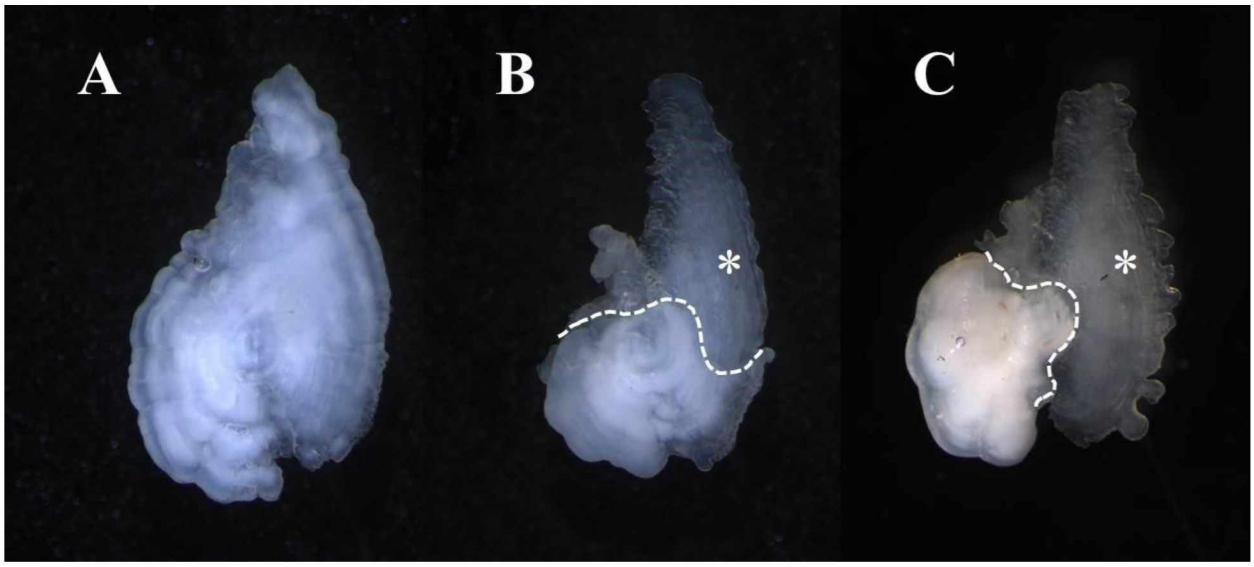

Fig. 2.3. Normal otolith comprised of aragonite (A), and otoliths with aragonite core that partially transitioned to vaterite (B and C). Dashed lines in panels B and C detail transition zone and the vateritic portion is denoted by the * symbol. Otoliths were photographed sulcus side down under objective microscope using double polarizing filters and projected light.

Appendix 2.A. Analytical Validation of Cortisol Samples
Testing validity of plasma cortisol concentrations

All EIA's were analyzed using a Chromate microplate reader (Awareness Technologies, Palm City, FL 34990) utilizing a 405nm filter and mass was calculated using a 4-parameter logistic curve. For validations, pools for each sex were serially diluted (range: undiluted to 1: 128) in the appropriate assay buffer supplied by the manufacturer and dilutions were run as samples according to manufacturer instructions. Accuracy of each EIA for both sexes was determined by addition of a standard material to the pools of each sex and comparison through regression of the mass added to the pool versus the mass measured for each assay. Serial dilutions for both sexes exhibited displacement parallel to that of the standard curve. The accuracy check resulted in slopes of $y = -12.11 + 1.00x$; $r^2 = 0.99$ and $y = -5.20 + 0.99x$; $r^2 = 0.99$ for males and females, respectively. Percent recovery of added standard was 98.72% (± 5.43 S.D; 5.51 % CV) for females and 98.33% (± 1.93 S.D; 1.97 % CV) for females. EIA's were prepared by diluting neat serum in the appropriate assay buffer to 1:200 and to avoid inter-assay variability all samples were run in duplicate within an individual tray of assays.

General Conclusions

This thesis sought to contribute to our understanding of some of the potential causes and consequences of straying hatchery-produced on wild Pacific salmon. Based on work conducted in Sawmill Creek and the surrounding areas of southeast Alaska, I explored variation in traits associated with fitness of hatchery-produced (H_p) strays and naturally produced (N_p) chum salmon in a natural system, and investigated physiological differences between H_p chum salmon that strayed (H_{ps}) or homed (H_{ph}), and N_p salmon. The salient findings of this thesis are as follows:

- H_{ps} differed in a suite of phenotypic and life history traits compared to at least some natural populations with which they interact; H_p male salmon arrived later, were significantly shorter in length, younger at age of return, smaller for their age, but were not different in body depth, snout size, and instream lifespan, when compared to N_p male chum salmon in Sawmill Creek. The H_{ps} female chum salmon were significantly shorter in length, younger at age of return, smaller for their age, entered freshwater later, and survived for less time in freshwater, though were similar in body depth, and had longer snouts than did N_p females found in Sawmill Creek.
- Markedly higher egg retention rates of H_p fish on the spawning grounds may be a factor in possibly lower reproductive success of H_p fish in Sawmill Creek and serve as a barrier to introgression of H_p fish in the wild. Egg retention increased as spawning season progressed for both H_p and N_p females though because of the difference in run timing, where the H_p strays primarily returned later and N_p chum returned earlier, the proportion of eggs retained by each group was very different, with H_p females retaining higher proportion of their eggs than N_p females.

- Cortisol concentrations did not differ between N_p, H_{ps}, and H_{ph} chum salmon. Instream lifespan was negatively correlated with cortisol concentrations, but egg retention did not change with increasing cortisol concentrations.
- Stress induced by thermal marking during early development was not associated with straying or rates of vaterite deposition. The rates of vaterite occurrence in N_p populations was less than that of H_p populations, though no differences were found between H_{ps} or H_{ph} groups.
- The rate of vaterite occurrence did not increase significantly as the intensity, complexity, or duration of thermal mark application increased.

Observed rates of straying and implications for conservation

The proportion of H_p strays observed on the spawning grounds of Sawmill Creek in 2015 was much greater than proposed stray rate thresholds of 5%, or 10% (Ford 2002; Mobrand et al. 2005), and exceeded a 2% guideline set in Prince William Sound for pink salmon by the Prince William Sound Comprehensive Salmon Management Plan (PWS CRRPT 1994). Despite this, and that hatchery straying has occurred at consistently high rates over a duration spanning several chum salmon generations (Piston and Heinl 2012), it appears that at least currently some of the presumed locally adapted traits of the naturally produced stock remain intact. Thus, while H_p and N_p groups were nearly equal numerically, their relative reproductive success was likely not. In most circumstances the differences found in Sawmill Creek (with the exception of H_p female snout size, and H_p male entry timing) point to theoretic disadvantages for H_p chum salmon attempting to spawning in this natural system (Fleming and Gross 1989). However, the entrance timing of H_p male chums, which was matched very closely to that of N_p females, may counteract the slightly shorter lifespans of H_p males and their smaller on average body size,

because prior residency of males (in this instance generally naturally-produced males) does not increase relative reproductive success as clearly as in female salmon (Schroder 1981). Furthermore, the proposed thresholds at which existing stocks can resist gene flow and fitness loss (Ford 2002; Mobrand et al. 2005) assume constant proportions of strays, identical run timing, and equal relative fitness of strays, all of which are likely untrue. Within the 2015 spawning season I found a wide range of H_p to N_p ratios in Sawmill Creek due to the differences in run timing. Given that mismatched run timing by H_p and N_p stocks in Sawmill Creek was also found by Brenner et al. (2012) in Prince William Sound, violations of proposed stray rate thresholds may be the rule rather than the exception in natural systems located close to hatchery release sites. My findings of mismatched run timing, and likely probability of uneven relative reproductive success between H_p and N_p groups suggest that the proportion of strays in proposed thresholds would be conservative in this circumstance, as differences between groups point to limiting introgression between H_p and the N_p stock, however, the observed H_p composition in Sawmill Creek during 2015 was well above any proposed thresholds.

A goal of Alaskan hatcheries is to enhance fisheries value by increasing harvest yield while protecting wild stocks; thus it is desirable to fishermen and managers that H_p salmon have high rates of survival and high homing precision. It has been shown that presence of vaterite is associated with hearing loss over portions of auditory range (Oxman et al. 2007), a potential detriment to survival in the wild (Reimer et al. 2016). If thermal marking influenced otolith development or the imprinting process, survival and straying rates of H_p salmon could be affected. Despite their widespread use, surprisingly little is known about the impacts that thermal marking events can have on the development and physiology of salmon (Sanders 2012; Sturdevant et al. 2012; Kohan et al. 2013). In a key finding of this thesis, I detected no evidence

to suggest that the frequency of vaterite occurrence (indicative of developmental stress) was related to thermal marking procedures. Additionally, H_{ps} and H_{ph} had no significant differences in the frequency of vaterite occurrence, suggesting that it is unlikely that homing abilities are directly impacted by vaterite formation, though specific tests may be necessary to determine this with ultimate certainty.

Understanding the physiological responses to homing or straying allows for a better understanding of the ecological consequences of straying. Hormones such as cortisol are linked to a variety of physiological functions including osmoregulation and olfactory recall (Carruth et al. 2002), and that cortisol levels may be a factor indicative of reproductive potential (Cook et al. 2011; McConnachie et al. 2012). Given that straying salmon routinely exhibit lower reproductive success than individuals that correctly home (Fleming and Gross 1992; McGinnity et al. 2003; Hendry 2004; Peterson et al. 2014) it is possible that an initial difference in cortisol concentrations as salmon arrive at the spawning grounds contributes to their success or failure. Although I detected no difference in cortisol concentrations between straying and homing salmon in this uncontrolled natural system, differences may be observable if measured in a more controlled experimental setting.

Ultimately, this work highlighted differences between H_{ps} and N_p chum salmon in Alaska, contributing to our broader understanding of the interactions between hatchery and wild populations on spawning grounds. The numerous differences between H_{ps} and N_p salmon, particularly entrance timing, body size, and egg retention, likely serve as barriers to introgression and may mitigate the genetic exchange in small creeks such as Sawmill Creek. However, given this study was conducted on one location, in one year, it must be acknowledged that results are difficult to generalize and may be different in other species, years, and locations in Alaska. A

continuation of this study and incorporation of parentage analysis as part of a long-term monitoring of Sawmill Creek (over the course of perhaps two full generations) or other similar sites represents the obvious next step to link the ecological observations made during the research to the long-term consequences for the maintenance of locally adapted populations, which is known to contribute to fishery sustainability.

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Appendix A: IACUC Approval Form

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Institutional Animal Care and Use Committee
909 N Koyukuk Dr. Suite 212, P.O. Box 757270, Fairbanks, Alaska 99775-7270

April 6, 2016

To:	Megan McPhee Principal Investigator
From:	University of Alaska Fairbanks IACUC
Re:	[726223-3] Ecological causes and consequences of straying: stress anad competition on the spawning grounds between wild and hatchery produced chum salmon (Oncorhynchus keta).

The IACUC has reviewed the Progress Report by Designated Member Review and the Protocol has been approved for an additional year.

Received:	April 6, 2016
Initial Approval Date:	April 20, 2015
Effective Date:	April 6, 2016
Expiration Date:	April 20, 2017

This action is included on the April 14, 2016 IACUC Agenda.

PI responsibilities:

- *Acquire and maintain all necessary permits and permissions prior to beginning work on this protocol. Failure to obtain or maintain valid permits is considered a violation of an IACUC protocol and could result in revocation of IACUC approval.*
- *Ensure the protocol is up-to-date and submit modifications to the IACUC when necessary (see form 006 "Significant changes requiring IACUC review" in the IRBNet Forms and Templates)*
- *Inform research personnel that only activities described in the approved IACUC protocol can be performed. Ensure personnel have been appropriately trained to perform their duties.*
- *Be aware of status of other packages in IRBNet; this approval only applies to this package and the documents it contains; it does not imply approval for other revisions or renewals you may have submitted to the IACUC previously.*
- *Ensure animal research personnel are aware of the reporting procedures detailed in the form 005 "Reporting Concerns".*

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