ENVIRONMENTAL, BIOLOGICAL, AND GENETIC FACTORS INFLUENCING LOCAL ADAPTATION OF PINK SALMON (*ONCORHYNCHUS GORBUSCHA*) IN AUKE CREEK, ALASKA

By

Christopher V. Manhard

RECOMMENDED:

Dr. Milo D. Adkison

Jeffrey J Hard

Dr. Jeffrey J. Hard

John E Joyce

Mr. John E. Joyce

W W Smoker

Dr. William W. Smoker

Dr. Anthony J. Gharrett, Advisory Committee Chair

Dr. Franz Mueter
Chair, Graduate Program in Fisheries

APPROVED:

Dr. S. Bradley Moran
Dean, School of Fisheries and Ocean Sciences

John C Eichelberger

Dr. John C. Eichelberger
Dean of the Graduate School

4/6/2016

Date

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A

DISSERTATION

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Christopher V. Manhard, M.S.

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Abstract

Pacific salmon form distinct, locally adapted populations because of the spatial and temporal precision with which they home to their natal streams. Local adaptation is recognized as an important component underlying the productivity and sustainability of salmonid populations, yet there remains uncertainty of the scale at which it occurs. This uncertainty was addressed by analysis of demographic, genetic, and experimental data collected from seasonally structured brood lines of Pink Salmon that spawn in Auke Creek, Alaska. An extensive background of research on this system has indicated that the timing of the adult and juvenile migrations is closely aligned with fitness and productivity in this stream; this background provided a framework for synthesizing the results of the analyses to address these questions: (1) What ecological factors influence productivity of the freshwater and marine life history stages; (2) Do these factors suggest a mechanism for evolution of migration time; (3) What are the consequences of disrupting fine-scale local adaptation of migration time? Freshwater productivity appeared to be influenced primarily by competition for spawning habitat, rather than variability in environmental conditions. Marine productivity, conversely, was associated with physical processes that influence survival of juveniles in the nearshore environment. Consistent with these findings, genetic evolution of earlier migration time, which was observed in both adults and juveniles over two generations, appeared to be driven by earlier vernal warming of the nearshore environment. Despite these environmental changes and resulting selection against late-migrating fish, recruitment to Auke Creek has remained stable, thereby indicating that seasonal structure of migration time has supported sustained productivity in a changing climate. Experimental relaxation of natural barriers to gene flow that maintain the seasonal structure resulted in intermediate adult migration times in two generations of hybrid fish. These patterns were consistent with an additive genetic basis for migration time and suggest that ecological outbreeding depression is a post-zygotic mechanism that maintains adaptive variation of migration time in Auke Creek. Collectively, these results provide evidence that fine-scale local adaptation can enhance productivity of salmonid populations while providing resilience to climate change.

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Introduction

The scale of local adaptation

Local adaptation is a process in which natural selection augments the intrapopulation frequency of traits that confer a survival or reproductive advantage in a local environment. An important consequence of local adaptation is that resident genotypes display, on average, higher Darwinian fitness in their local environment than genotypes originating from other habitats. Primary pre-requisites for local adaptation include sufficient genetic variation of fitness traits, restricted gene flow between diverging populations or demes, sufficient effective population size to mitigate stochastic genetic changes, and genotype-by-environment interactions of fitness traits (Kawecki and Ebert 2004). Genotype-by-environment interaction is a phenomenon in which different genotypes respond to environmental variation in different ways. When such an interaction is associated with fitness, it can prevent any single genotype from having the highest fitness across all environments. Spatial or temporal heterogeneity in environmental conditions promotes the maintenance of polymorphisms that show genotype-by-environment interaction, provided that frequency-dependent selection is operating within a population (Hedrick et al. 1976; Hedrick 1986). Frequency-dependent selection also increases the frequency of rare alleles that improve fitness in habitats in which most individuals perform poorly, thereby enabling variant strategies which capitalize on niches that are generally less favorable (Karlin and Campbell 1981). This is a mechanism by which adverse effects of high population density may be alleviated by the staggering of resource use in time or space.

Pacific salmon (*Oncorhynchus* spp.) are excellent subjects for the study of local adaptation because of their tendency to form local populations with distinct adaptive traits across diverse habitats ranging from temperate regions to the Arctic. In addition, Pacific salmon display high fidelity of homing and migration timing, which enables them to locate their ecological niche in a vast adaptive landscape and enhances reproductive isolation among populations (Quinn 2005). Pacific salmon exhibit substantial variation of fitness traits within and among populations including body morphology and meristics, life history characteristics, embryonic development rate and migration time, homing ability, and disease resistance (reviewed e.g. by Taylor 1991; Quinn 2005; Garcia de Leaniz et al. 2007; Fraser et al. 2011). Effective management of Pacific salmon stocks requires consideration of the adaptive variation that underlies population structure (Ricker 1972; Spangler et al. 1981). Given the great value of salmon in commercial fisheries (Woodby et al. 2005) and in subsistence fisheries essential to many indigenous people (O'Neil 2007), as well as their unique and vital ecological roles (e.g. Gende et al. 2002; Hocking and Reynolds 2011), it is important to understand the mechanisms that create and maintain population structure and the consequences of its deterioration.

Most empirical evidence of local adaptation in salmonids has been gathered from comparative studies that examined correlations between fitness traits and environmental characteristics. Other evidence of local adaptation has been gathered by examining differences in traits among populations or demes in a common environment (i.e. common garden experiments), observations of the performance of transplanted populations in non-native habitats and of domesticated stocks in the wild, and studies of genetic components of resistance to diseases and parasites (Garcia de Leaniz et al. 2007). Local adaptation in salmonids has been most commonly demonstrated by trait divergence of populations spawning in separate habitats (Woody et al. 2000; Hodgson and Quinn 2002; Meier et al. 2011). Most reports of sympatric temporal divergence are confounded by differences in spawning or rearing locations (Tallman 1986; Tallman and Healey 1991; Hendry et al. 2002; Fillatre et al. 2003), or do not demonstrate genetic differences between demes (Hendry et al. 1999).

Seasonal differences in life history schedules

In Pacific salmon, population-specific variation in migration and spawning time is presumed to be the consequence of adaptations by populations to local conditions and to have a basis in genetic variation (see reviews in Groot and Margolis 1991; Taylor 1991). This is supported by the high estimates of heritability of timing of adult migration and spawning that have been demonstrated in Pink (*O. gorbuscha*), Chinook (*O. tshawytscha*), and Sockeye (*O. nerka*) Salmon (Smoker et al. 1998; Quinn et al. 2000; Hard 2004; Dickerson et al. 2005; Crozier et al. 2011). Furthermore, salmon spawning streams and their associated nurseries often vary seasonally in environmental conditions critical to spawning success and juvenile survivorship, particularly in Pink Salmon. Critical environmental conditions that vary within spawning seasons include abiotic factors such as stream temperature and flow regimes (Mueter et al. 2005; Geiger et al. 2007) and biotic factors such as predation and the density of spawning adults within the stream (Fukushima et al. 1998; Mortensen et al. 2000). One important consequence of local adaptation of spawning segments to seasonal conditions is that it enables more niches to be filled within spawning habitats. In streams that exhibit high spawner densities during the adult migration period, partitioning of resource exploitation (e.g. by structuring of the timing of life history events) can reduce intraspecific competition, thereby elevating the carrying capacity of a population (Gharrett et al. 2013). In this manner, local adaptation may augment the average productivity of individual populations.

Genetic variation of migration and spawning time that is structured by seasonal environmental differences within a spawning habitat gives rise to distinct life history strategies, each of which is tailored to the environmental conditions that are typical of a particular time and location. The portfolio effect hypothesis, a concept that underscores the importance of adaptive variation, asserts that a rich diversity of life history traits confers increased resilience to climatic fluctuations by enhancing the likelihood that some individuals within a population will carry traits that are well-suited to future environmental regimes (Hilborn et al. 2003; Greene et al. 2010). This concept indicates that the capacity of populations to respond to inexorable climatic shifts is, in part, dependent upon genetic variation that is structured by local adaptation. Hence, an understanding of the mechanisms that create and maintain fine-scale differences in the timing of life history events (i.e. phenology) may yield insight into the means and extent to which salmonid populations will respond to climate changes.

In salmon populations for which migration time is temporally structured, local adaptation increases the allele frequencies of traits that optimize the likelihood of surviving and reproducing in a given environment, thereby enhancing average fitness within populations. Fine-scale temporal structure is only maintained when gene flow between seasonally distinct spawning segments is sufficiently constrained. A potential consequence of the deterioration of barriers to gene flow is reduced fitness of F_1 or later hybrids. This phenomenon is known as outbreeding depression. Outbreeding depression can manifest from both disruption of gene by environment interactions and deterioration of epistatic allele complexes (Lynch 1991; Edmands 2007). Experiments in which barriers to gene flow were artificially removed have demonstrated that interbreeding between genetically distinct populations can have detrimental fitness consequences (Gharrett et al. 1999; Gilk et al. 2004). These studies examined hybrids between populations separated by temporal or spatial barriers of sufficient size to preclude gene flow. The consequences of removing fine-scale barriers to gene flow, such as those that maintain adaptive differences between seasonally distinct spawning segments, have yet to be evaluated, but could yield insight into the scale at which local adaptation occurs.

Project objectives

The primary purpose of this study was to examine how local adaptation creates and maintains temporal structure in seasonally distinct spawning segments of Pink Salmon that home to Auke Creek, Alaska and to evaluate the consequences of such structure to the sustainability and productivity of salmonid populations. Specifically, my research objectives were (1) to identify biotic and abiotic environmental components of lifetime fitness that could drive local adaptation of phenology; (2) to describe how locally adaptive variation in phenology has enabled evolution of migration time in a changing climate; and (3) to evaluate whether the removal of a natural barrier to gene flow between early- and late-spawning fish would disrupt local adaptation and cause declines in fitness. These goals were achieved by analysis of multiple data sets that have been collected at or near Auke Creek Research Station, a permanent weir and salmon hatchery that is operated by the U.S. National Marine Fisheries Service. These data sets included measurements of environmental variables, complete daily counts of migrating adult and juvenile Pink Salmon, observations of a genetic marker, and experimental data. An extensive background of research on Auke Creek Pink Salmon provided the basis for the hypotheses and conclusions in this paper.

Chapter 1 explores abiotic and biotic determinants of reproductive success and survival at various life stages of Pink Salmon that spawn in Auke Creek. A substantial body of theory and empirical evidence pertaining to this and other salmonid populations suggests that abiotic environmental characteristics, such as stream temperature and flows, along with biotic factors, such as intraspecific competition and predation, influence fitness of salmon at specific life history stages. These variables, which exhibit interannual variation in Auke Creek, would potentially explain a significant portion of the variation in recruitment among brood years. Annual counts of migrating juveniles and adults conveniently divide the life cycle of Auke Creek Pink Salmon into freshwater and marine components. These annual counts were used to describe the population dynamics of the freshwater and marine environments. Deviations of observed abundances of spawners and juveniles from those estimated by models of the population dynamics provided two indices of fitness: freshwater productivity and marine survival. These indices were likely to reflect interannual variation in environmental conditions. Environmental covariates were constructed by estimating indices of habitat quality during the appropriate life history periods. Available indices of habitat quality were based on direct measurements of temperatures of Auke Creek and Auke Bay and estimates of stream flows of Auke Creek derived from measurements of precipitation and air temperature. Furthermore, daily counts of Coho Salmon smolts and Dolly Varden charr outmigrating from Auke Creek in the spring provided indices of predator density encountered by juvenile Pink Salmon in the nearshore marine environment. A generalized additive modeling (GAM) framework was used to elucidate functional relationships between the indices of fitness and the environmental covariates. Multivariate parametric models, based upon the functional relationships expressed by the GAMs, were compared with one another through an information theoretic approach.

Chapter 2 evaluates mechanisms underlying recent changes in the seasonal structure of Pink Salmon that home to Auke Creek. The adult migration is divided into two sympatric spawning segments whose median spawning dates have been historically offset by approximately two weeks, largely reflected in differences in genetic architecture (Smoker et al. 1998, Gharrett et al. 2013). New evidence indicates that, in recent years, there has been a reduction in the size of the late-spawning segment and an overall truncation of the adult migration period, which potentially reflects a response to a warming trend (0.34 °C per decade) that has been observed in Auke Creek over the past 40 years (Taylor 2008). Furthermore, observations of a genetic marker, which was experimentally elevated in late-run fish from the odd-year brood line, suggest that these changes reflect contemporary evolution (Kovach et al. 2012). While it is evident that the demography of this population shifted dramatically between the adult migrations of 1989 and 1991 as a consequence of anomalously poor fitness of late-run fish, the specific life history stage in which this fitness decline first occurred remains unknown. The purpose of this chapter was to use census and genetic data collected from outmigrating fry during each odd-broodyear outmigration from 1984-1992 to determine the life history stage at which the genetic changes occurred. Furthermore, these data were synthesized with environmental data collected at Auke Creek in order to determine whether environmental factors in the freshwater or marine environment have contributed to these changes. Finally, daily counts of adult Pink Salmon collected at the Hugh Smith Lake weir in Southeast Alaska were examined to look for shifts in adult migration time parallel to those at Auke Creek. Congruent migratory shifts between Pink Salmon spawning in the Hugh Smith Lake system and Auke Creek would support the hypothesis that environmental processes operating at large spatial scales have caused the recent demographic changes in Auke Creek. Importantly, because Hugh Smith Lake lies more than 250 miles southeast of Auke Creek, the absence of a parallel response between these two systems would not necessarily confirm that large-scale climatic forcing does not underlie recent demographic changes; the local environmental signatures of large-scale climate patterns may simply differ among habitats separated by substantial distances.

Chapter 3 evaluated the consequences of removing a partial barrier to gene flow between the early- and late-spawning segments. Two generations of experimental hybrids between these spawning segments were reared at the Auke Creek hatchery and released to sea to complete their life cycle. Observations of fitness traits in hybrids relative to experimental controls can yield information regarding the genetic structuring of Auke Creek Pink Salmon and the extent to which these spawning segments are locally adapted to distinct environmental regimes. Depending on the nature of differences between subpopulations, signatures of outbreeding may not manifest until the second generation of hybridization (Lynch 1991; Edmands 1999). Hence, Chapter 3 evaluated the consequences of hybridization over two generations. Specifically, marine survival was evaluated by using log-linear models to look for difference among experimental lines. Parentage analysis was used to determine the number of returning adult Pink Salmon per full-sib family, and these data were used to construct Bayesian hierarchical models that compared family-specific marine survival among hybrid and control lines. Bayesian mixed effects models were used to quantify components of variation of adult migration time and to demonstrate phenotypic shifts resulting from hybridization. Finally, a line-cross analysis was used to evaluate the genetic architecture of adult migration time, thereby providing a complement to the mixed effects models.

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Chapter 1 - Ecological factors influencing lifetime fitness of Pink Salmon (*Oncorhynchus gorbuscha*) in an Alaskan stream[1]

Abstract

An understanding of lifetime fitness can provide insight into ecological processes that influence productivity in populations of anadromous Pacific salmon (*Oncorhynchus* spp.). We used a 29-year time series of local environmental data and complete censuses of migrating adult and juvenile Pink Salmon (*O. gorbuscha*) collected at Auke Creek, Alaska to examine ecological factors that influence reproductive performance in fresh water and survival in the nearshore marine environment. The importance of ecological factors to each life history stage was quantified by using an information-theoretic approach to select linear models based on parsimony. Freshwater productivity appeared to be influenced primarily by competition for limited spawning habitat in this small stream (323 m long) and less so by indices of water quality (e.g. stream temperature and flow) that were of putative importance to success of spawning and embryo development. Furthermore, we observed a temporal trend of declining freshwater productivity, which may be related to observed declines in spawning substrate quality and in the duration of the adult migration. Marine survival was highly variable among brood years and appeared to be influenced by densities of nearshore predators and by physical conditions in the nearshore marine environment; warm sea-surface temperatures during nearshore residency were associated with higher marine survival rates, whereas high stream flows late in the fry outmigration period were associated with reduced marine survival. Collectively, these observations confirm that predation, physical factors that contribute to early marine growth of fry, and dynamic, hydrological characteristics of the estuary are important to lifetime fitness of salmon.

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[1] Manhard, C.V., J.E. Joyce, W.W. Smoker, and A.J. Gharrett. Ecological factors influencing lifetime fitness of Pink Salmon (*Oncorhynchus gorbuscha*) in an Alaskan stream. Formatted for submission to the *Canadian Journal of Fisheries and Aquatic Sciences*

Introduction

The life history of anadromous Pacific salmon (*Oncorhynchus* spp.) involves movements within and between marine and freshwater habitats, and the ability of these habitats to support reproduction or survival is a composite of ecological factors that vary seasonally and interannually. Successful deposition of fertilized embryos and survival of those embryos during the freshwater stage determines the production of fry, and survival during the marine stage determines the number of adults that return as spawners. Hence, the ecological factors that comprise lifetime fitness in a salmonid population may be partitioned by dividing its life history into freshwater and marine stages, and examining each of these stages separately.

Freshwater productivity is probably influenced by the stream conditions encountered by spawning adults in many salmonid populations. Inadequate stream flows and high stream temperatures can impose high levels of stress on migrating salmon, thereby causing pre-spawning mortality (Fukushima and Smoker 1997). Additionally, depending on the location and hydrology of the stream, low flows and high temperatures may delay stream entry of early-migrating spawners (Shapovalov and Taft 1954; Sheridan 1962; Jonsson 1991). One potential consequence of delayed stream entry is temporal compression of the migration and consequent increases in spawner density. High densities of spawning adults may exacerbate the effects of low flows and high temperatures by causing rapid depletion of dissolved oxygen within the stream and corresponding increases in mortality among unspawned adults (Murphy 1985). Furthermore, in streams with limited spawning substrate, high spawner densities may be associated with increased intraspecific competition and reduced reproductive success (Essington et al. 2000; Gharrett et al. 2013).

Embryos that are successfully deposited and fertilized are subjected to both abiotic and biotic sources of mortality, which, along with genetic factors, determine the number of juveniles that emerge from the gravel in spring. Mortality during embryonic development is often very high, and survival from deposition to emergence is thought to commonly range from 10-20% in salmon, but may be as low as 1% in some years (reviewed by Heard 1991). A potential abiotic determinant of embryonic survival is stream flow. Although moderate levels of stream flow are necessary for developing salmonid embryos (Gibson and Myers 1988; Cowan 1991), high stream flows can scour the streambed and sweep embryos out of the gravel (Montgomery et al. 1996;

but see Fukushima et al. 1998). Several studies have demonstrated a negative correlation between embryonic survival and high stream flows (Thorne and Ames 1987; Scrivener and Brownlee 1989). Density-dependent effects associated with high spawner densities may also manifest during the embryonic development period. A primary biotic source of embryonic mortality is disturbance of redds of early-spawning salmon by the activity (e.g. redd digging, superimposition) of subsequent spawners (McNeil 1964; Fukushima et al. 1998). Consequently, factors that contribute to high spawner densities should increase embryonic mortality.

For juvenile salmon that successfully outmigrate from their natal stream, rapid growth probably maximizes survival during early marine residency (e.g. Mortensen et al. 2000; Beamish et al. 2004). Growth opportunities ultimately determine the size that salmon attain after the early marine period, and there are two stages in which size may influence marine survival. The first stage occurs during the early marine period when larger juvenile salmon may be less vulnerable to predation (Parker 1971; Hargreaves and LeBrasseur 1986), presumably because larger fish exceed the size range of many gape-limited predators and have faster swimming speeds. The second stage occurs in late fall and winter of the first marine year, when increased energy reserves of larger fish likely enhance their probability of surviving the fall and winter (Beamish and Mahnken 2001). The importance of early growth, when coupled with the strong positive correlation between water temperature and growth rate of juvenile salmonids, Pink Salmon in particular (Mortensen et al. 1991), suggests that juveniles that have access to warm water should benefit from increased marine survival. However, because high predator densities often coincide with environmental conditions that are favorable for growth, the relationship between thermal conditions and juvenile survival may involve trade-offs between predator avoidance and enhanced growth (Mortensen et al. 2000).

Stream discharge can influence primary productivity by stratifying the water column in the nearshore marine environment. Although strong stratification reduces fluxes of nutrients to the surface waters by inhibiting vertical mixing of the water column, it also constrains phytoplankton to the photic zone (Royer et al. 2001). In high-latitude estuaries, stratification may increase primary productivity by facilitating optimal light exposure in advance of nutrient depletion (Gargett 1997). Consequently, enhanced stream flows during early spring may provide favorable mixed layer conditions, thereby fueling primary production in the nearshore marine environment that is critical to early growth of juvenile salmon.

Because of their simple life history, Pink Salmon (*O. gorbuscha*) are an excellent species for modeling the factors that underlie fitness of anadromous salmonids. Because adults nearly always reach maturity in their second year of life throughout their natural range (Anas 1959; Bilton and Ricker 1965), Pink Salmon populations have non-overlapping generations. Adults in coastal populations typically enter their natal stream in late summer and spawn in or just above the intertidal zone near the stream mouth. Embryos develop and hatch within the gravel beds of the stream during the fall and winter months and fry emerge during early spring. Juvenile Pink Salmon lack a protracted freshwater period; fry outmigrate to the nearshore marine environment shortly after emerging from the gravel. Juveniles form schools in the nearshore marine environment and live there for several weeks before moving further offshore in late spring. During this early life-history phase, growth is fueled by predation on epibenthic organisms and zooplankton (Mortensen et al. 2000). Pink Salmon usually range in the North Pacific Ocean for 15 to 18 months prior to maturation and their anadromous migration to their natal stream to spawn (reviewed by Heard 1991).

From time series of census data collected at the permanent salmon weir at the National Oceanographic and Atmospheric Administration (NOAA) Auke Creek Research Station and of environmental data that reflect habitat quality at critical life history periods, we modeled the importance of biotic and abiotic factors to lifetime fitness of Pink Salmon. These census data included daily counts of both immigrant adults in summer and emigrant juveniles in spring, which conveniently divide the life history into freshwater and marine components. We quantitatively modeled relationships between abundances of adults and juveniles throughout the time series. Residuals from abundance-based models were in turn modeled as functions of indices of habitat quality in order to determine environmental components of lifetime fitness. The primary questions that these analyses address are (1) Are compensatory or depensatory processes evident in the population dynamics at different life history stages? and (2) Which environmental factors are most important to lifetime fitness?

Methods

Census data source

Study site - Auke Creek, a short (323 m), steep outlet stream that flows from Auke Lake to the estuary of Auke Bay (Figure 1.1), is a migratory corridor and spawning ground for Pink Salmon. In accordance with the strict 2-year life cycle of Pink Salmon, genetically distinct even- and odd-year brood lines inhabit Auke Creek. Within each brood line, time of return of spawning adults has followed a bimodal distribution; migration into Auke Creek of 'early' spawners typically occurs between mid and late August, whereas migration of 'late' spawners typically occurs between early and mid-September (Taylor 1980; Figure 1.2). Although a few of the Pink Salmon homing to the Auke Lake system spawn in the intertidal area and in Lake Creek, a tributary of Auke Lake, most spawn in the spatially limited (< 600 m^2) habitat of Auke Creek. Located at the demarcation line separating the intertidal and upstream habitats is Auke Creek Research Station, a permanent weir and salmon hatchery that is operated by the U.S. National Marine Fisheries Service. The weir allows for censuses of upstream-spawning adults and outmigrating fry. Censuses of outmigrating fry have been made at Auke Creek since 1972, but censuses prior to 1980 were made with less accurate fyke nets rather than the permanent counting structure used in later years. In addition to uncertainty about the quality of early juvenile censuses made with nets, there is uncertainty about censuses of wild adults made prior to 1983 because of substantial releases of unmarked experimental fish from the Auke Creek hatchery in the 1970s and early 1980s. Consequently, only censuses of naturally-spawning adult salmon made after the fall of 1983 were included in this study. The time series of adult census data includes each adult migration period from 1984-2012, and the juvenile census data include each fry outmigration period from 1983-2013. Together, these census data were used to construct separate models of the population dynamics of Auke Creek Pink Salmon in the freshwater and marine environments over 29 brood years.

Abundance-based models of productivity

Freshwater productivity - The relationship between the number of fry that outmigrated in spring and the number of adults that returned to Auke Creek during the previous summer was analyzed in order to explore the freshwater population dynamics. Studies of Auke Creek Pink Salmon have suggested that resource limitation may manifest in the form of competition for limited spawning substrate within the stream and juvenile mortality that is directly imposed by spawning adults (Fukushima and Smoker 1997; Fukushima et al. 1998; Gharrett et al. 2013). The Ricker model (Ricker 1954) captures such a relationship. It produces a dome-shaped relationship between recruits and spawning stock, which results from the compensatory effect that spawners exert on juvenile production as the spawning stock increases. The form of the Ricker model is given by:

$$J_t = S_{t-1}\exp\left(\alpha - {S_{t-1}}/{K}\right)\exp(\varepsilon_t)$$

where the number of outmigrating fry in the spring of the t^{th} year (J_t) is related to the number of wild adult spawners from the previous summer (S_{t-1}) by parameters that are intrinsic to the population and describe its productivity (α) and carrying capacity (K). This model can be rewritten by dividing both sides by the number of adult spawners and taking the natural logarithm of both sides:

$$log\left({J_t}/{S_{t-1}}\right) = \alpha - {S_{t-1}}/{K} + \varepsilon_t$$

$$\varepsilon_t \sim N(0, \sigma^2)$$

The residual errors (ε), which are assumed to be log-normally distributed (Hilborn and Walters 1992), provide an index of interannual variability in freshwater productivity that is likely due to environmental factors.

Marine productivity - We explored marine-phase population dynamics with models of the relationship between number of adults that returned to Auke Creek in summer and the number of fry that outmigrated in the spring of the previous year. In contrast to the freshwater phase, in which a density-dependent relationship between fry and spawners is supported by theory and empirical observations, the population dynamics of the marine phase are less well understood. Because we did not have strong a priori expectations about this relationship, we used a linear model in which the response was log transformed to normalize the residuals:

$$\log(S_t) = b + \beta * J_{t-1} + \varepsilon_t$$

$$\varepsilon_t \sim N(0, \sigma^2)$$

where the abundance of wild adult spawners in the summer of the t^{th} year is S_t, the model intercept is b, and the abundance of wild outmigrating fry from the spring of the previous year, J_{t-1}, is related to the abundance of adults by the parameter β. The residual errors (ε) provided an index of interannual variability in marine productivity that likely had an environmental basis.

Environmentally-based models of productivity

The residuals from the abundance-based models provided an opportunity to evaluate how environmental factors (e.g. stream conditions, predator abundance) influence freshwater and marine productivity of Auke Creek Pink Salmon. The residuals from each abundance-based model were treated as separate response variables, which were modeled as functions of covariates that were of hypothetical ecological importance to relevant life history stages. The goal of this modeling approach was to determine the most parsimonious model that could be constructed from the available environmental covariates. Given that we did not have a priori expectations about the relationships between the environmental covariates and residuals, a Generalized Additive Modeling (GAM) framework was used to explore these relationships. The GAM framework allows for response variables to be modeled as smooth functions of parameters, thereby accommodating a wide variety of response distributions and allowing the data to dictate an appropriate functional relationship. To accomplish this, the 'mgcv' package in R (R Core Team 2015) was used to determine whether any non-linear relationships existed. The 'gam' function estimates the degrees of freedom for each term as part of the model-fitting algorithm by minimizing the Generalized Cross Validation (GCV) of the entire model, which is represented as the effective degrees of freedom (*edf*) in the model output. An *edf* for a parameter that is close to 1 suggests that a non-linear relationship does not exist and that the smooth term can be replaced by a parametric linear term (Wood 2001). Based on the functional form that was specified by the GAM, a non-linear or linear parametric model was fit for each covariate and subsequently tested for significance with parametric tests (F-tests). Finally, an information theoretic approach based on Akaike's Information Criterion was used to select the suite of covariates and interactions that minimized the information loss and, therefore, comprised the most parsimonious model. The relative probability P_R that the i^{th} model minimized the information loss was estimated as:

$$P_R = exp\left(\frac{AIC_{min} - AIC_i}{2}\right)$$

where AIC_{min} corresponded to the model with the lowest AIC score (Burnham and Anderson 2002).

Covariates of freshwater productivity

Median adult migration date - Daily counts of migrating wild adults were used to quantify interannual variability in adult migration time (i.e. stream entry date). Delays in stream entry may lead to density-related factors constraining freshwater productivity, such as egg retention and redd superimposition (Fukushima et al. 1998). To examine this, the median migration date was calculated each year in order to provide an index of the annual timing of the adult salmon migration. This index was expected to covary negatively with freshwater productivity due to increased spawner densities in years in which migration was delayed.

Stream temperature - Daily measurements of stream temperature that have been collected at the Auke Creek weir were available for the time series. The first half of the adult migration, which enters the stream in August, frequently experiences high stream temperatures (Figure 1.2). The potentially deleterious influence of high stream temperatures on early-migrating fish was evaluated by estimating an index of the mean stream temperature experienced by the first 50% of adults to pass Auke Creek weir. Each early-migrating fish was assumed to have a stream life of seven days, based on observations of stream life in Auke Creek (Fukushima and Smoker 1997). The mean stream temperature $\bar{T}_{ij}$ experienced by the i^{th} fish within the j^{th} spawning season was estimated by averaging stream temperatures over a period spanning from the date the fish passed the weir to seven days after that date:

$$\bar{T}_{ij} = \frac{T_t + T_{t+1} + T_{t+2} + T_{t+3} + T_{t+4} + T_{t+5} + T_{t+6} + T_{t+7}}{8}$$

An index of the average stream temperature experienced by early-migrating adults in the j^{th} spawning season was then estimated by averaging $\bar{T}_{ij}$ across n_j returned adults:

$$\bar{T}_j = \frac{\sum_{i=1}^{n_j} \bar{T}_{ij}}{n_j}$$

This index was expected to covary negatively with freshwater productivity because reduced spawning success has been observed during years of high stream temperatures in Auke Creek.

Stream flow - Although measurements of stream flow rates in Auke Creek were not available during the span of the salmon abundance time series, daily measurements of precipitation at Auke Bay were available from the Auke Bay Laboratories Climatological Series (ABLCS). Daily stream flow measurements that were collected at Auke Creek from 1962-75

(U.S. Geological Survey) were used to model stream flows in late summer and early fall (July-October) as a linear function of precipitation over the previous 16 days (Supplementary Materials; Table S1). The model was then used to reconstruct stream flows during autumn from 1984-2012. Low stream flows, which are commonly encountered by the first half of the adult migration (Figure 1.2), may be associated with declines in spawning habitat quality (Murphy 1985; Fukushima et al. 1998). In order to evaluate this, an index of the average stream flow rate experienced by early-migrating salmon during each spawning season was estimated with the approach that was detailed for stream temperature. This index was expected to covary positively with freshwater productivity due to enhanced spawning success in years of high stream flow.

The embryo incubation period, which typically begins in late August or September, is marked by heightened stream flows and frequent freshets (Figure 1.2). An index of stream flows during the initial incubation period was estimated as the mean stream flow rate between 1 September and 31 October each year. This index was expected to covary negatively with freshwater productivity due to scouring of embryos during years of high stream flow.

Covariates of marine productivity

Juvenile outmigration date - Daily counts of migrating Pink Salmon fry were used to quantify interannual variability in the date at which fry entered Auke Bay. The date of their peak outmigration may influence the quality of habitat encountered by fry (Mortensen et al. 2000). The median outmigration date was calculated each year in order to provide an index of the annual timing of the fry outmigration. This index was included in the environmentally-based models of marine productivity.

Predator abundance - Daily counts of two other salmonid species that migrate from Auke Creek in spring, Coho Salmon (*O. kisutch*) smolts and Dolly Varden (*Salvelinus malma*) charr, were available for the time series. These species prey upon Pink Salmon fry (Mortensen et al. 2000), and they typically migrate into Auke Bay shortly after the peak of the fry outmigration (Supplementary Materials; Figure S1). The timing and magnitude of these predator migrations may influence survival of Pink Salmon fry. This possibility was examined by estimating separate indices of abundance for each predatory species. An index of predator abundance was estimated as the number of predators that had passed the Auke Creek weir prior to the end of the fry outmigration, which was defined as the first date on which 95% of outmigrating fry had left the stream. Counts of predators were extended two weeks past this date to include predators entering Auke Bay during the initial marine residency period of the latest outmigrating fry. Indices of predator abundance were expected to covary negatively with marine productivity.

Nearshore sea-surface temperature - Daily measurements of sea-surface temperature of Auke Bay, which were available over the time series from the ABLCS (Figure 1.3), were used to estimate an index of growth opportunity related to the temperature of Auke Bay. An annual index of the temperature of Auke Bay during the typical fry outmigration period (Supplementary Materials; Figure S1) was estimated by averaging sea-surface temperatures between 15 March and 15 May. This index was expected to covary positively with marine productivity due to more favorable growth conditions in years of warmer nearshore temperatures.

Stream flow - Measurements of air temperature and precipitation, which were available over the time series from the ABLCS, were used to reconstruct daily stream flows of Auke Creek during spring (March-May) under a modeling framework similar to that described for autumn stream flows (Supplementary Materials; Table S2). The hydrology of the nearshore environment of Auke Bay (Figure 1.1) depends on freshwater input from the Auke Lake system, as well as its other major tributaries, which include Waydelich Creek and Auke Nu Creek. Additional freshwater input from the Mendenhall River and Fish Creek, which both discharge into marine waters in nearby Fritz Cove, also influences the nearshore environment of Auke Bay. Since stream flow in these other systems is likely determined by similar environmental factors, Auke Creek was assumed to be a proxy for the aggregate freshwater influx into Auke Bay. An index of freshwater influx in advance of and during the typical extent of the primary plankton bloom (Bienfang and Ziemann 1995) was estimated by averaging stream flows between 1 March and 30 April. This index was expected to covary positively with marine productivity due to more favorable mixed layer conditions in years of high freshwater influx and corresponding high food availability. Another index, reflecting the period of high and variable stream flows that occur late in the nearshore residency period (Figure 1.3), was estimated by averaging stream flows between 1 May and 31 May. This index was expected to covary negatively with marine productivity due to offshore displacement of fry or their planktonic prey during years of high stream flows.

Results

Abundance-based model of freshwater productivity - Census data from wild Pink Salmon were used to examine the relationship between the abundances of outmigrating fry and spawning adults across 29 brood years. Annual censuses varied substantially over the time series; the abundance of spawning adults ranged between 1,500 and 28,000, while the abundance of outmigrating fry ranged between 12,000 and 243,000. The abundance-based model of freshwater productivity (Figure 1.4) revealed that there was a positive, density-dependent relationship (R^2 = 0.542, $P = 5.371*10^{-6}$) between the abundances of spawning adults and outmigrating fry and estimated that the carrying capacity (K) of Auke Creek was 16,224 adults during the time series. Individual analyses of each brood line had similar results, so the data were combined to increase power. Temporal plots of residuals from the abundance-based model did not reveal any trends of first- or second-order autocorrelation, and statistical tests of autocorrelation, performed with the 'acf' function in R, did not reveal any significant autoregressive coefficients through five orders.

Environmentally-based model of freshwater productivity - Relationships between environmental conditions in Auke Creek and freshwater productivity were examined by modeling the residual errors from the abundance-based model as a function of hypothetical indices of habitat quality. The indices included the median adult migration date, mean stream temperature and flow experienced by spawners, and mean stream flow during early embryo incubation. Adult migration year was included as a covariate to account for temporal trends. None of the five covariates exhibited a non-linear relationship with the residual errors ($edf \sim 1$), so parametric linear models were used. Computation of Pearson's correlation coefficients revealed moderately significant correlation ($P < 0.10$) between some pairs of covariates (Supplementary Materials; Table S3). General linear models were constructed with all possible combinations of non-correlated covariates, and interactions were included where theory supported them (Supplementary Materials; Table S5). The model with the lowest AIC value included the year of adult migration (Figure 1.5), which exhibited a significant, negative linear relationship with the residual errors ($R^2 = 0.148$; $P = 0.039$). Comparisons of this model with the other models indicated that there were three bivariate models that had AIC values that were nearly as low. These models, which separately included discharge during the incubation period, discharge during the adult migration period, and temperature during the adult migration period, ranged between 0.37 and 0.43 times as likely to minimize the information loss.

Abundance-based model of marine productivity - Census data from wild Pink Salmon were used to examine the relationship between the abundances of returned adults and outmigrating fry across 29 brood years. The abundance of outmigrating fry exhibited a significant, positive relationship with the abundance of returned adults and explained a moderate portion of its variability ($R^2 = 0.317$, $P = 0.002$). Individual analyses of each brood line had similar results, so the data were combined to increase power. A plot of the observed data and predicted values from the model indicated that the relationship between abundances of adults and fry was curvilinear, with some evidence of depensation over the time series (Figure 1.6). There were no patterns of first- or second-order autocorrelation evident in plots of residuals from this model against brood years. This observation was supported by statistical tests of autocorrelation, which did not produce any significant autoregressive coefficients across five orders.

Environmentally-based model of marine productivity - Relationships between environmental conditions encountered by fry and subsequent abundances of returned adults were examined by modeling the residual errors from the abundance-based model of marine productivity as a function of indices of habitat quality in the nearshore marine environment. The indices included median outmigration date, Coho Salmon smolt and Dolly Varden charr abundances, mean sea-surface temperature of Auke Bay during the spring algal bloom, and stream flows of Auke Creek during the spring bloom and late-nearshore residency period. Year of fry outmigration was included as a covariate to account for temporal trends. Generalized additive models did not reveal a significant non-linear relationship between the residual errors and any of the covariates ($edf \sim 1$), so parametric linear models were used. Moderately significant correlations ($P < 0.10$) existed between some pairs of covariates (Supplementary Materials; Table S4). General linear models of marine productivity were constructed with each possible combination of non-correlated covariates, and interactions were included where they were supported by theory (Supplementary Materials; Table S6). The model with the lowest AIC value included sea-surface temperature during the spring bloom, which exhibited a significant, positive linear relationship with the residual errors, and stream flow during the late-nearshore residence period, which exhibited a significant, negative linear relationship with the residual errors. However, there were two other models that had AIC values that were nearly as low. Those models, which separately included Dolly Varden and Coho Salmon abundance as covariates in addition to stream flow and sea-surface temperature, were 0.99 and 0.41 times as probable to minimize the information loss, respectively. In those models (Figure 1.7), the abundance of Dolly Varden and Coho Salmon each exhibited a weak, negative association with the residuals. Together, these indices of habitat quality and predator abundance explained a large portion of the environmentally-based variation in marine productivity ($R^2 > 0.500$; $P = < 0.001$). The inclusion of these environmental covariates in the abundance-based model of marine productivity improved the fit of the model; together, the abundance of wild fry and the selected environmental covariates accounted for more than two thirds of the interannual variability in the abundance of returned adults.

Discussion

Recruitment of juvenile Pink Salmon to Auke Bay is determined by the abundance of spawners during the previous fall, the fecundity and reproductive success of those spawners, and the survival of embryos and alevin. The Ricker model provided a good approximation of the relationship between abundances of outmigrating fry and adult spawners. The model accounted for more than half of the variation in juvenile recruitment and estimated the carrying capacity as 16,244 spawners in Auke Creek (Figure 1.4). This suggests that density limitation controls fry production in Auke Creek. Throughout this 29-year time series, runs of spawning adults have averaged nearly 10,000 fish and have exceeded the estimated carrying capacity in more than 20% of years. Furthermore, the majority of these fish spawn within an expanse of habitat between the weir and Auke Lake that is less than 300 meters long. Consequently, large numbers of fish engaged in spawning within a limited space probably hinders successful deposition and survival of embryos in some years. Indeed, high levels of egg retention and redd superimposition have been observed in association with high spawner densities in Auke Creek (Fukushima and Smoker 1997; Fukushima et al. 1998).

Based on observations of this and other salmon populations, the two environmental variables that were hypothesized to have the greatest influence on freshwater productivity were stream discharge and temperature during the spawning season. Among the models of freshwater productivity with the lowest AIC values were two models that separately included stream discharge and temperature during the spawning season. However, the confidence intervals of the coefficient estimates for discharge and temperature were very wide in those models, and the signs of the coefficients were inconsistent with expectations. Collectively, there was minimal support for an influence of either of those environmental covariates on freshwater productivity.

The inability of our study to detect a significant, deleterious effect of high stream temperature or low discharge was unanticipated, given that poor stream conditions in Auke Creek have been linked to low spawning success in some brood years (Taylor 2008). In a study of Pink Salmon spawning in Auke Creek during a single season, sustained stream temperatures in excess of 15 °C were associated with egg retention levels in early-migrating fish that were abnormally high relative to those reported in other Pink Salmon populations. Furthermore, low stream flows were associated with shortened stream life of females, presumably because of delayed stream entry and reduced oxygen supply (Fukushima and Smoker 1997). While stream conditions described seasonal variation in freshwater productivity in those studies, they failed to capture interannual variation in this trait in our study. One possible explanation for this is that pre-spawning mortality of early-migrating adults in years of poor stream conditions may leave more unseeded spawning habitat for late-spawning adults. Under this hypothesis, gains in freshwater productivity later in the season would partially offset losses in productivity earlier in the season, thereby stabilizing the aggregate productivity among years.

While this study detected no evidence of an association between freshwater productivity and interannually variable environmental conditions in Auke Creek, a significant, negative temporal trend in freshwater productivity was detected (Figure 1.5). None of the available indices of water quality exhibited a significant temporal trend over the 29-year time series (Supplementary Materials; Table S3), which suggests that the decline in fry production may be related to the amount of spawning habitat in Auke Creek. The recent history of Auke Creek provides a possible explanation for this trend. Improvement of spawning beds in this stream in 1963 transformed much of the streambed upstream of the weir from primarily large cobble, boulder, and bedrock to gravel substrate. These modifications likely created about 1,000 m^2 of new spawning habitat in Auke Creek. However, since the improvement of the spawning beds, periodic floods have washed away substantial amounts of gravel, causing the streambed to gradually revert toward its natural state (Taylor 2008). These changes in streambed composition have probably reduced the amount of available spawning habitat over the past 40 years. Alternatively, the trend in fry productivity may be more related to significant declines in the duration of the adult migration, which have been observed in both brood lines over a 40-year span (Kovach et al. 2012). Staggering of the use of limited habitat over the spawning season is thought to enhance the carrying capacity of this population (Gharrett et al. 2013) and, consequently, temporal compression of the adult migration may have contributed to declines in fry production.

Recruitment of spawning adults to Auke Creek is determined by the abundance of outmigrating fry during the previous spring and their marine survival rates. Our hypothesis was that, because spawning habitat limits the number of fry that migrate into the marine environment each spring, competition for resources in the nearshore environment is probably minimal, and a linear model should capture the marine population dynamics effectively. While a linear model provided a reasonable fit to the data (Figure 1.6), juvenile abundance accounted for less than a third of the variability in adult recruitment ($R^2 = 0.317$). This suggests that there is a substantial amount of interannual variability in marine survival arising from environmental factors. It is possible that some of this variability may be attributable to the harvest of returning Auke Creek Pink Salmon in the commercial fishery. The influence of the commercial fishery in Southeast Alaska on individual stocks is difficult to determine because the harvest is not terminal and includes mixtures of Pink Salmon stocks that are biologically and geographically diverse (Piston and Heinl 2011). Furthermore, the fishery is primarily conducted by spatially dispersed purse seiners. Although harvest rates of Auke Creek Pink Salmon are unknown in this fishery, the total annual catch of Pink Salmon in Northern Southeast Alaska has commonly exceeded 10 million fish and has varied substantially over the past 30 years (Supplementary Materials; Figure S2). Collectively, these characteristics suggest that, while substantial commercial harvest probably occurs, its effects are more likely to be stochastic than deterministic. Hence, the ecological effects that we report here have been resolved in spite of background noise originating from the commercial fishery.

The early marine period is a critical stage for Pacific salmon. This stage is characterized by rapid growth (LeBrasseur and Parker 1964; Healey 1979) and high mortality (Parker 1968;

Hartt 1980) relative to the aggregate marine period. A potentially important determinant of early marine survival is the degree of synchrony between the emergence of fry and the rapid increase in zooplankton abundance that follows the primary plankton bloom (Pitcher and Hart 1982). In Auke Bay, the dates of initiation and peak biomass of the estuarine primary plankton bloom are relatively invariant (Bienfang and Ziemann 1995); consequently, the degree of synchrony between fry emergence and food availability is probably determined by the timing of peak fry outmigration. In spite of this, we failed to detect an association between median migration time and marine survival. While the median migration date ranged between 1 April and 1 May over the time series, its coefficient of variation was only 0.07, suggesting that, aside from a few anomalous years, there was minimal interannual variability in the median date of fry outmigration. For example, 80% of the median migration dates occurred within a two week window. This could reflect the action of strong stabilizing selection, which has enabled fry to consistently time their migrations within a narrow range that typically coincides with optimal productivity.

Because predation is likely a large component of mortality of fry during their initial marine residency, we hypothesized that abundance indices of two migratory predators (Dolly Varden charr and Coho Salmon smolts) would covary negatively with early marine survival. Some support for this was provided by the fact that two of the three most parsimonious models of marine productivity separately included Dolly Varden and Coho Salmon abundance, which each exhibited a negative association with productivity. Between the two models that included predator abundance, the model with Dolly Varden abundance was better supported. This observation may be explained by body size differences. Because multiple size classes of Dolly Varden migrate from Auke Creek after overwintering in Auke Lake, whereas Coho Salmon outmigrate exclusively as smolts, Dolly Varden inhabiting Auke Bay in spring are generally larger than Coho Salmon smolts and may have a higher predatory capacity. Although Dolly Varden and Coho Salmon abundance were included in two of the most parsimonious models, the relationships between these predator indices and marine productivity were weak, and a much longer time series would likely be required to observe significant associations. An important consideration is that the presence of other predators in Auke Bay may diminish the influence of individual species. A survey of several fish species present in Auke Bay revealed that, in addition to Coho Salmon smolts and Dolly Varden charr, three sculpin species (Great Sculpin, *Myoxocephalus polyacanthocephalus*; Pacific Staghorn Sculpin, *Leptocottus armatus*; and Buffalo Sculpin, *Enophrys bison*) frequently had remains of Pink Salmon fry in their stomachs (Mortensen et al. 2000). The influence of individual species may be further reduced if fry abundance buffers against predation rates (i.e. depensation). This idea is supported by the fit of the abundance-based model, which provided some evidence of depensatory mortality. Furthermore, the coefficient of variation of the annual number of outmigrating Pink Salmon (0.57) was approximately twice as large as that of Coho Salmon (0.28) over this time series, suggesting that interannual fluctuations in Pink Salmon abundance may overwhelm Coho Salmon abundance.

As we predicted, sea-surface temperatures in Auke Bay during early marine residency exhibited a significant, positive association with marine survival (Figure 1.7). In addition to its direct influence on growth of fry (Mortensen et al. 2000), sea-surface temperature may also indirectly influence growth of fry by modulating the dynamics of their nearshore prey. For example, the abundance and size of calanoid copepods, an energetically dense food source for fry, is related to water temperatures in Auke Bay. Warmer waters in Auke Bay during spring are associated with increased growth rates and egg production of copepods when primary production is high (Bienfang and Ziemman 1995), thereby providing another mechanism by which warm temperatures may enhance growth of fry. Studies of tagged juvenile Pink Salmon from Auke Creek have demonstrated that marine survival is correlated with growth rate during the nearshore residency period (Mortensen et al. 1991), and a mechanism linking early marine growth to survival is provided by the previously detailed critical-size hypothesis (Parker 1971; Hargreaves and LeBrasseur 1986).

Research has demonstrated that marine survival of Alaskan salmon stocks tends to covary positively with freshwater discharge, suggesting that the interaction between mixed layer conditions and plankton dynamics is an important determinant of early marine survival (Mueter et al. 2005). Based on this, we hypothesized that high stream flows of Auke Creek during the typical extent of the primary plankton bloom would favor increased plankton production in Auke Bay and that marine survival would therefore covary positively with stream flow. However, our analysis failed to detect a significant association between stream flow and marine survival during this time period. In Auke Bay, the peak biomass of phytoplankton during the bloom invariably exceeds that which would be required to saturate grazing zooplankton (Bienfang and Ziemann 1995). Consequently, environmental factors that contribute to interannual variability of the spring plankton bloom might be uncoupled from zooplankton abundance in the nearshore environment. This could explain our inability to detect an association between stream flows in early spring and marine survival.

In contrast to early spring, we observed that stream flows late in the fry outmigration period exhibited a significant, negative association with marine survival (Figure 1.7). The late outmigration period is characterized by steadily increasing stream flows. Furthermore, stream flows in late spring are highly variable among years (Figure 1.3). Riverine freshwater input can have a dramatic impact upon dynamic hydrological features in estuaries such as river plume fronts, which may be important to juvenile salmon (De Robertis et al. 2005; Burla et al. 2010). In addition to providing an interface between fresh and saline waters for newly emergent salmon acclimating to the marine environment, river plume fronts can concentrate zooplankton and potentially provide feeding opportunities for fry (Morgan et al. 2005). It follows that high stream flows and corresponding offshore displacement of the plume could cause a shift in the estuarine distribution of juvenile salmon. A study in Prince William Sound reported that dispersal of Pink Salmon fry from the shallow nearshore habitat to offshore waters occurred in association with nearshore declines in zooplankton abundance and resulted in a five-fold increase in predatory mortality (Willette 2001). The distribution of salmon fry in estuaries likely reflects a trade-off between the protected, shallow nearshore habitat and the energetically rich offshore waters, and the presence of nearshore fronts may present an opportunity to realize the benefits of both habitats. Tagging studies conducted in Auke Bay, which demonstrated that nearshore residence time of juvenile Pink Salmon from Auke Creek varied both seasonally and among brood years (Mortensen 2000), provide some support for this hypothesis. Unfortunately, the determinants, characteristics, and variability of freshwater fronts in Auke Bay have not been explored in detail, and without information of this nature, this hypothesis remains largely speculative.

Conclusions

Our results indicate that freshwater productivity of Auke Creek Pink Salmon is primarily influenced by spawning habitat availability rather than interannual fluctuations in water quality. Auke Creek supports large numbers of migrating adults (mean ~ 10,000) that spawn primarily in a short expanse of habitat just above the intertidal zone. This creates opportunity for competition for limited spawning habitat and negative interactions between spawning adults and developing eggs (i.e. intraspecific mortality). An additional facet of the characteristic pattern of this spawning migration is that, since spawning occurs within several hundred meters of the stream mouth, limited in-stream migration time probably reduces exposure to stressful stream conditions. The life history characteristics of Auke Creek Pink Salmon are archetypical of this species, which suggests that productivity of other populations may be limited primarily by the area and composition of the streambed near the tidal interface and less so by water quality. However, it is less sensible to generalize our results to other Pacific salmon species, which often exhibit life characteristics that differ dramatically from Pink Salmon. Another important factor to consider is that our study of freshwater productivity examined only freshwater conditions. Pacific salmon grow rapidly in the marine environment prior to returning to fresh water, and this accumulation of body mass ultimately contributes to the amount of energy that is channeled into reproductive output (e.g. Bagenal 1969; Tamate and Maekawa 2000). Consequently, growth conditions in the marine environment may be an important component of salmon fecundity, and there is a possibility that some of the environmentally-based variation in freshwater productivity that our models failed to account for is attributable to interannual variation in oceanic conditions.

In contrast to the freshwater phase, our results suggest that marine productivity of Auke Creek Pink Salmon is more related to local environmental conditions in the nearshore environment than habitat availability. This is consistent with an increasingly well supported hypothesis, which asserts that adult recruitment is determined largely by early marine survival (e.g. Karpenko et al. 1998; Beamish et al. 2004; Farley et al. 2007). One of the underpinnings of this hypothesis is that predation on fry, which is thought to be size-selective, is a critical component of early marine survival. Our results supported that idea and indicated that local environmental factors that condition the vulnerability of fry to predators had a greater influence on early marine survival than predator abundance itself. Pink Salmon fry migrate into Auke Bay immediately after emerging from the gravel, which is a ubiquitous trait of both Pink and many Chum Salmon populations. The absence of a protracted feeding stage prior to marine entry may render fry from these species particularly vulnerable to predation compared to other salmonid species, such as Sockeye and Coho Salmon, which generally rear in fresh water over one or more summers prior to outmigrating. Additional support for the importance of the nearshore stage was provided by observations from the Southeast Coastal Monitoring project, which demonstrated that annual catch per unit effort (CPUE) of juvenile Pink Salmon was significantly correlated with the annual commercial harvest of Pink Salmon in Southeast Alaska (a proxy for adult recruitment) over a 13-year monitoring period (Orsi et al. 2012). The CPUE data were obtained from offshore surveys conducted in the seaward migratory corridor of Icy Strait, which indicated that much of the marine mortality of juvenile Pink Salmon was sustained during the nearshore residence period.

An important implication of our results is that ecological factors, such as competition for limited spawning substrate and estuarine conditions during the fry outmigration period, have shaped the characteristic bimodal spawning migration of Auke Creek Pink Salmon and conferred distinct life history adaptations in early- and late-spawning salmon. Adaptation of embryonic development trajectory is evident in embryos of early spawners, which face higher levels of mortality due to redd superimposition by late spawners (Fukushima et al. 1998). In order to increase their survival probabilities, early-spawned embryos have evolved to complete epiboly, a shock-resistant developmental stage that confers resistance to redd superimposition, faster than their late-spawned counterparts (Joyce 1986). Indeed, the bimodal spawning migration may be maintained largely by dispersive selection against eggs that are deposited in advance of the late-spawning segment, but without sufficient time to reach epiboly (Smoker et al. 1998; Gharrett et al. 2013). Furthermore, early-spawned embryos compensate for warmer stream temperatures during incubation, thereby increasing the likelihood that fry emerge concordantly with the vernal warming of Auke Bay (Hebert et al. 1998). The adaptive significance of developmental compensation is supported by the relationship between sea-surface temperature and early marine survival that we demonstrated. Given the dynamic nature of Auke Creek and Auke Bay, it is likely that neither spawning segment possesses a consistent lifetime fitness advantage. Instead, the existence of temporal structure and diverse life history traits likely buffers against interannual fluctuations in environmental conditions, thereby providing some stability to the productivity of Auke Creek Pink Salmon.

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Figures

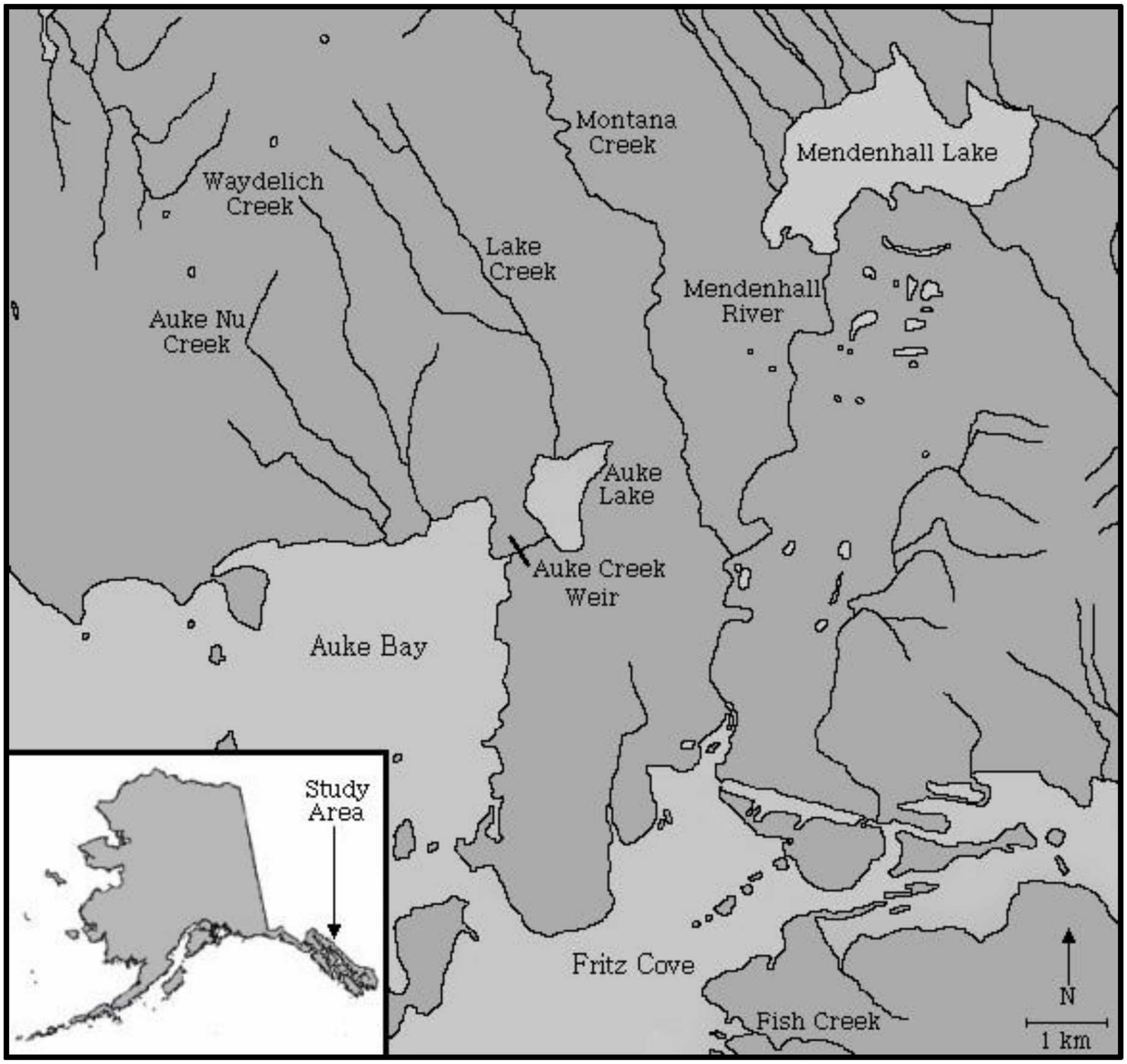

Figure 1.1 - Map of Auke Bay, Alaska. The position of the Auke Creek weir is depicted by a bold line.

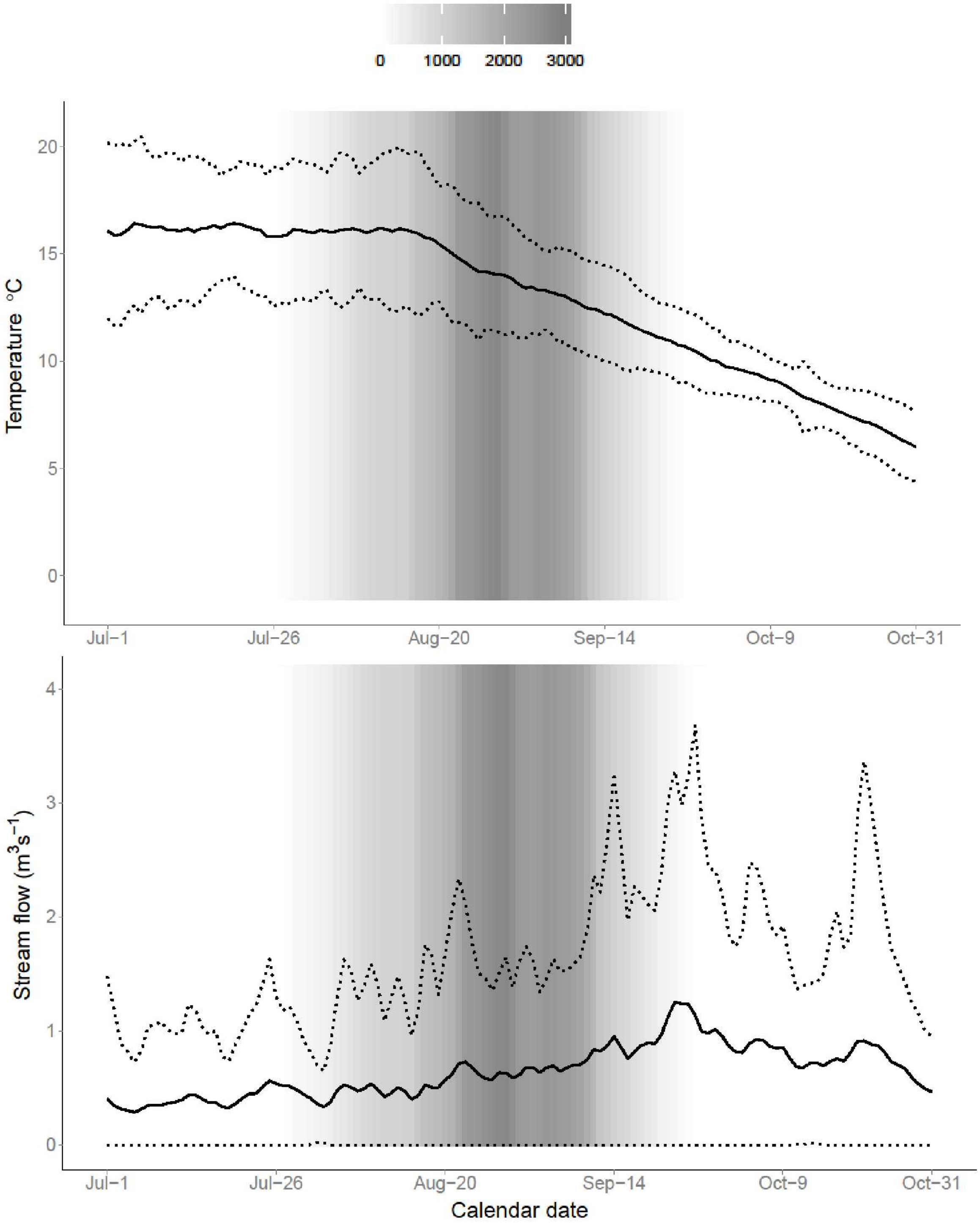

Figure 1.2 - Observed stream temperature and reconstructed stream flow of Auke Creek during late-summer and fall (1984-2012). Daily means (solid black lines) and 95% prediction intervals (dotted black lines) are depicted for each environmental attribute. The historic mean abundance of spawning pink salmon in the stream on each day is depicted by a gradation scheme. The daily abundance of spawners is based on a stream life of seven days.

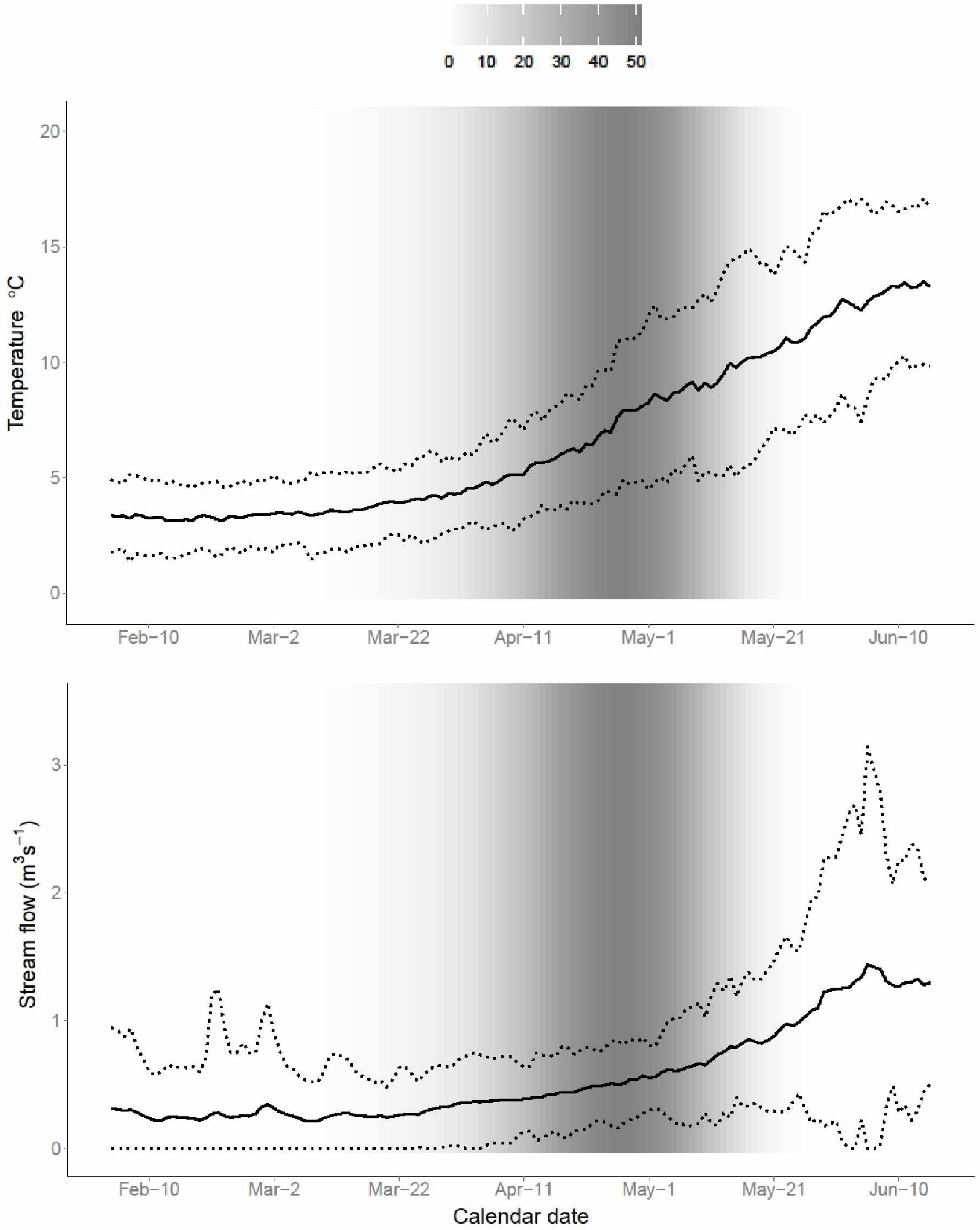

Figure 1.3 - Observed temperature of Auke Bay and reconstructed stream flow of Auke Creek during late-winter and spring (1983-2011). Daily means (solid black lines) and 95% prediction intervals (dotted black lines) are depicted for each environmental attribute. The historic mean abundance of Pink Salmon fry (1000s) in Auke Bay on each day is depicted by a gradation scheme. The daily abundance of fry was based on a nearshore residency period of fifteen days.

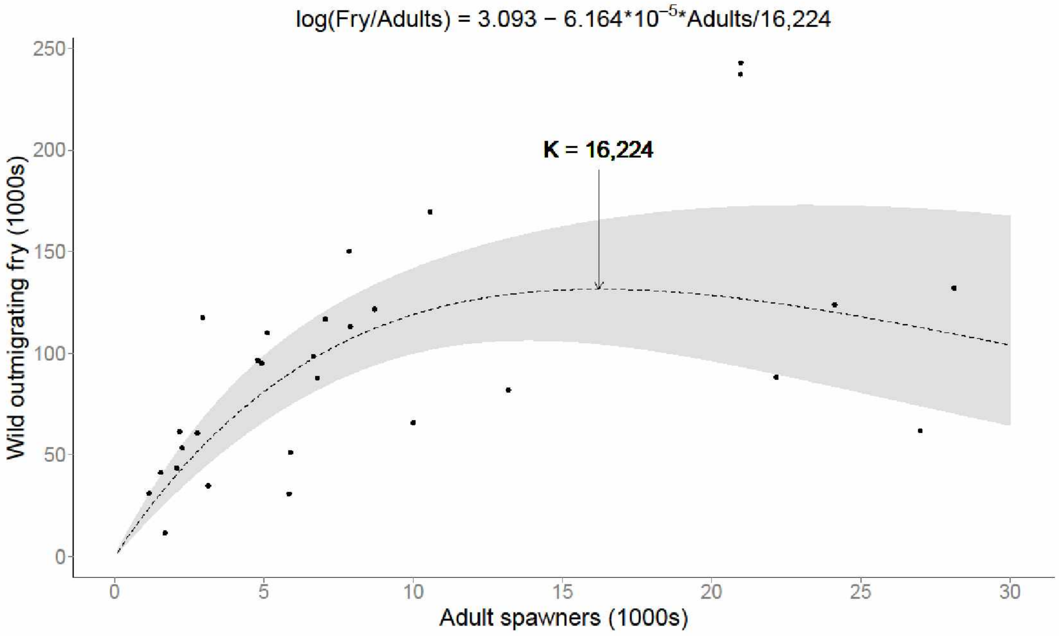

Figure 1.4 - Abundance-based model of freshwater productivity of Auke Creek Pink Salmon (R^2 = 0.542; P = 5.371*10^{-6}). The shaded region depicts the 95% confidence interval.

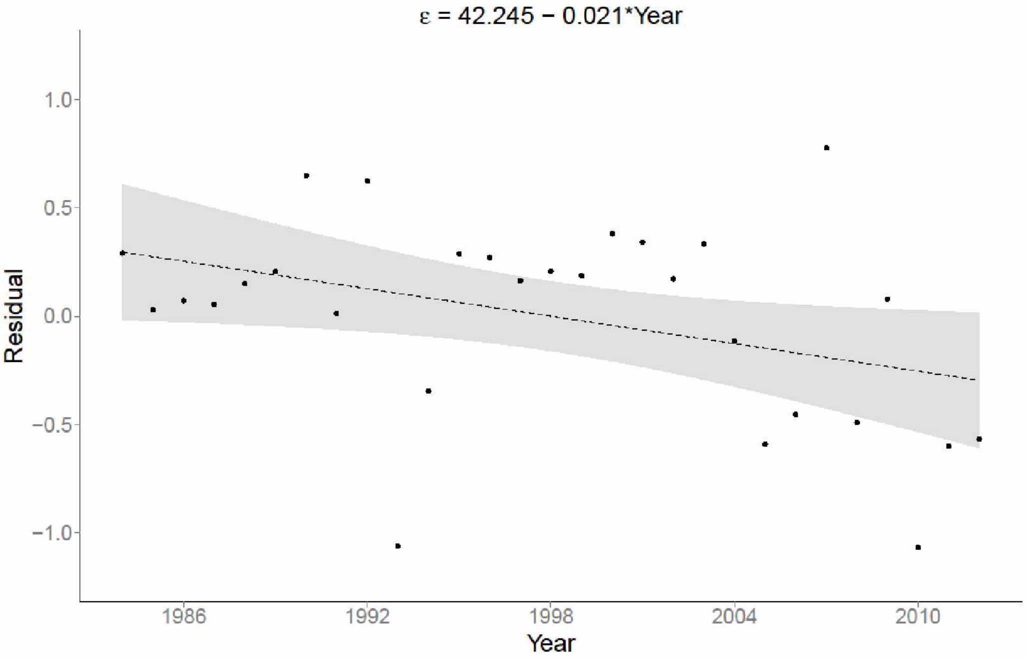

Figure 1.5 - Environmentally-based model of freshwater productivity of Auke Creek Pink Salmon (R^2= 0.148; P = 0.039). The response variable was the residuals from the abundance-based model of freshwater productivity. The model included year of adult migration. The shaded region depicts the 95% confidence interval.

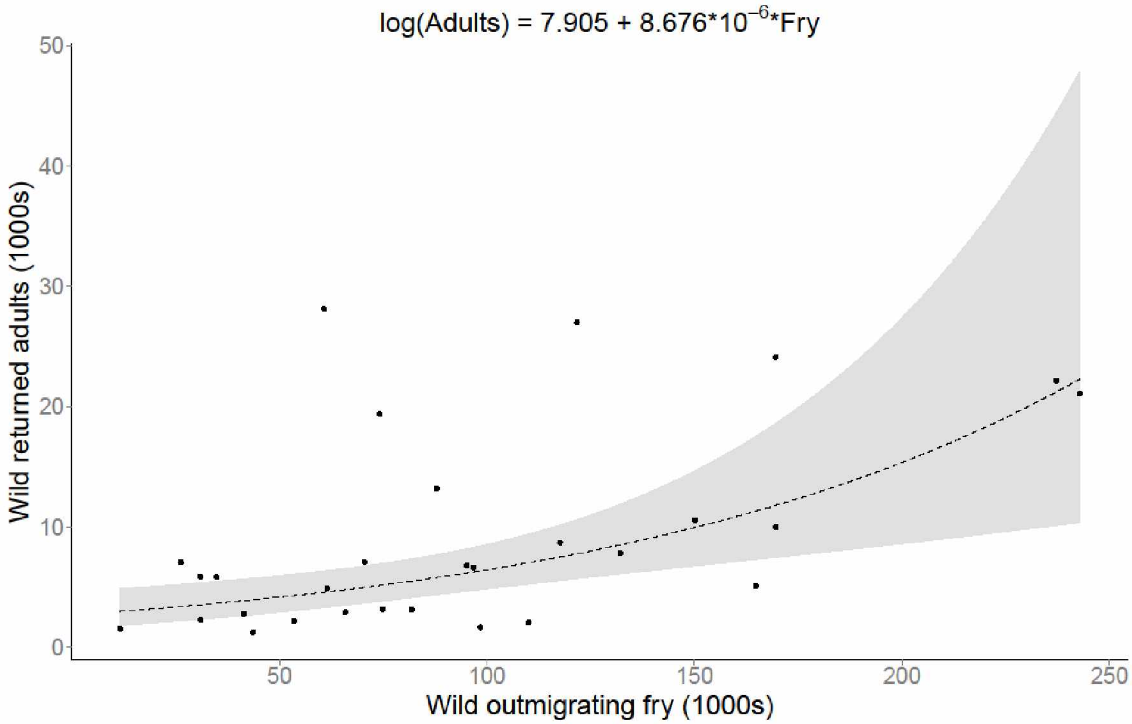

Figure 1.6 - Abundance-based model of marine productivity of Auke Creek Pink Salmon ($R^2 = 0.317$; $P = 0.002$). The shaded region depicts the 95% confidence interval.

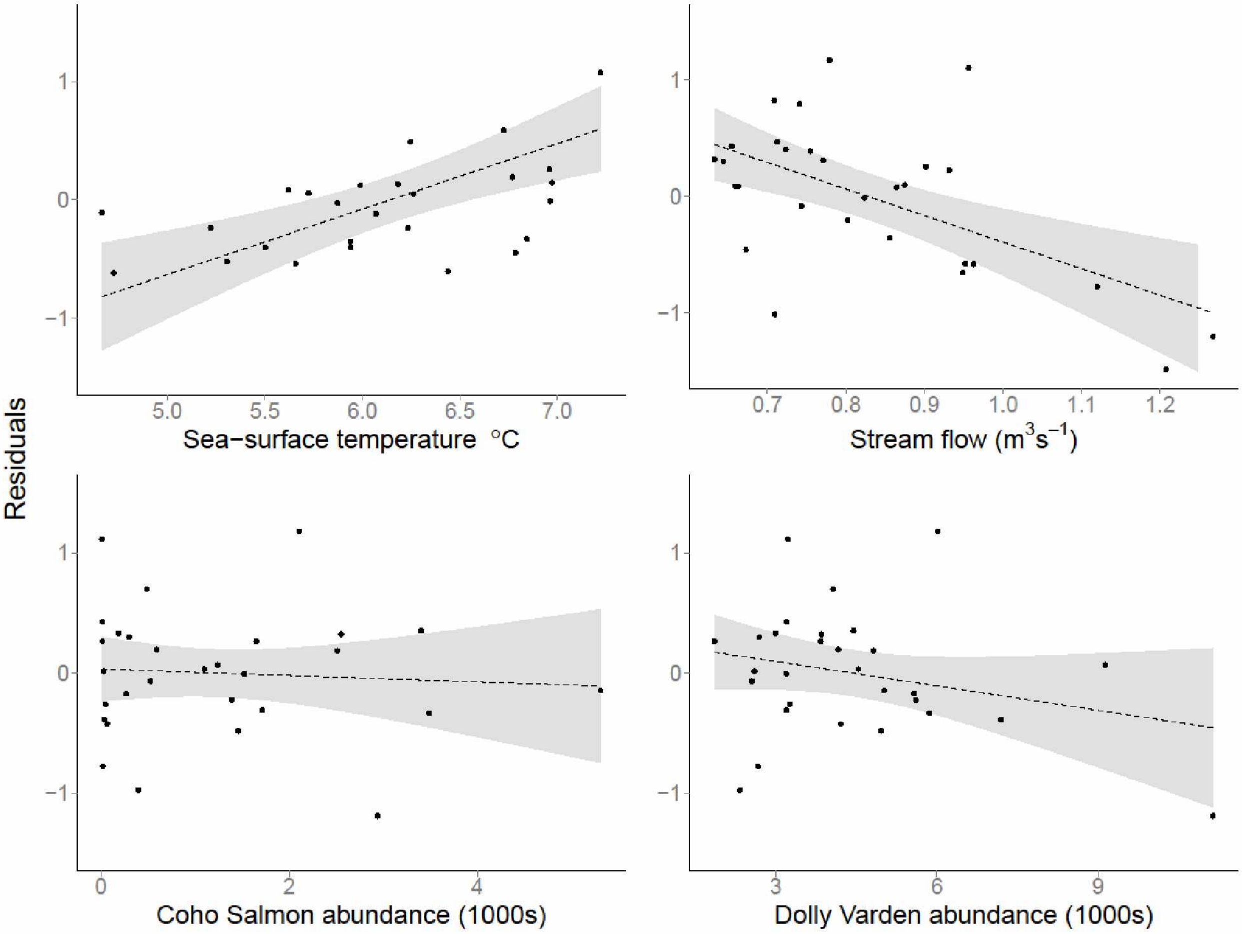

Figure 1.7 - Univariate plots depicting the relationship between marine productivity and each of the environmental covariates that were supported by the most parsimonious models. In each plot, the response variable is the residuals from a model of marine productivity that was based on the other covariates. Hence, these plots capture the relationship between a given covariate and marine productivity after other important environmental sources of variation have been accounted for. The shaded regions depict the 95% confidence intervals.

Chapter 2 - Evolution of phenology in a salmonid population: a potential adaptive response to climate change[1]

Abstract

An important issue in evolutionary research is whether wild populations can evolve at rates sufficient to keep pace with climate change. Many populations have responded to climate change through shifts in the timing of life history events, but it is often unclear whether these phenological changes represent phenotypic plasticity or genetic adaptation. One of the first demonstrations of contemporary evolution of phenology in a wild salmonid population was provided by monitoring of experimentally manipulated allozyme marker alleles in a temporally structured population of pink salmon (*Oncorhynchus gorbuscha*). The marker alleles, which were changed by selective breeding of late-spawning salmon, were stable during the first four generations of monitoring before exhibiting abrupt changes in adult salmon in a single generation. These observations suggested that recent trends toward earlier migration time in this stream have a genetic basis. Here, we extend that research by using observations of the late marker alleles in juvenile salmon to demonstrate that these genetic changes in migration time were caused by a strong selective event during the oceanic phase. The selective event appeared to be driven by early warming of the nearshore marine environment and consequent increased survival of early-migrating fry relative to late-migrating fry. These results suggest that a trend towards warmer spring sea-surface temperatures has conferred an adaptive advantage to earlier-migrating fish. Despite rapid evolution of phenology, this population has maintained historic levels of adult recruitment. Collectively, these results indicate that local adaptation of phenology may support resilience to climate change in temporally structured populations.

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[1] Manhard, C.V., J.E. Joyce, and A.J. Gharrett. Evolution of phenology in a salmonid population: a potential adaptive response to climate change. Formatted for submission to *Oikos*.

Introduction

An understanding of the mechanisms that drive phenotypic change is essential to predicting whether wild populations can respond adequately to periodic environmental fluctuations and persistent climatic trends. Of particular importance to population persistence is whether evolution in response to new selective pressures can occur swiftly enough to prevent irreversible declines in abundance or genetic vitality. The importance of evolutionary rates has long been recognized and, accordingly, the topic has been examined by numerous studies, many of which have reported microevolution in contemporary populations (Losos et al. 1997, Svensson 1997, Hendry and Kinnison 1999, Carroll et al. 2007). Collectively, these studies suggest that patterns of microevolutionary change in response to changing environmental conditions may be apparent over relatively short time series. However, evidence of adaptive microevolution in response to temporal environmental variability remains scarce, primarily because it is generally difficult to determine whether an observed phenotypic change is the consequence of phenotypic plasticity, which is characterized by environmentally-induced variation of a given genotype, or genetic adaptation, which is characterized by differential fitness of distinct genotypes in a given environment (Gienapp et al. 2008). Genetic monitoring, a technique in which neutral molecular markers that are associated with phenotypes are tracked through time in order to identify adaptive genetic changes in relation to a changing environment, provides a means of distinguishing between adaptive and plastic responses. Hansen et al. (2012) proposed that, in order to convincingly demonstrate adaptive genetic change based on molecular markers, a researcher must ensure that (1) the monitored traits exhibit sufficiently high heritability; (2) the traits are relevant to specific selective agents; (3) the traits are monitored over time; (4) selection is tested; (5) shifts in traits are consistent with the expected response to environmental changes; and (6) replacement by a genetically different population has been ruled out.

One mechanism by which plants and animals may respond to a changing environment is by shifting the timing of life history events, known as phenology, in order to track optimal environmental conditions in time (Bellard et al. 2012). Adaptive phenology arises because temporal environmental variability often creates windows that are optimal for reproduction and growth, and failure to time life history stages with their appropriate windows can have deleterious fitness consequences (Visser and Both 2005, Miller-Rushing et al. 2010). Phenology is of primary importance for migratory species, such as Pacific salmon (*Oncorhynchus* spp.), which must initiate an array of physiological, morphological, and behavioral changes at precise times during their life cycle (reviewed by Groot and Margolis 1991, Taylor 1991). The precision with which salmonids time migration can produce variability of phenology within populations, which is maintained by adaptation to seasonal environmental conditions (Smoker et al. 1998, Quinn et al. 2000, Fillatre et al. 2003, Gharrett et al. 2013). For example, investigations into the locus *Ots Clock1b*, a circadian rhythm gene that may be associated with detection of seasonal changes in day length, have demonstrated latitudinal allele frequency clines that ranged from weak in pink (*O. gorbuscha*) and coho (*O. kisutch*) salmon to strong in Chinook (*O. tshawytscha*) and chum (*O. keta*) salmon, thereby revealing a possible fingerprint of local adaptation of phenology (O'Malley et al. 2010). Comparisons of seasonally distinct migratory groups of Chinook salmon that spawn in the same river, which demonstrated significant genetic divergence at three circadian clock genes, but not neutral markers, provided additional evidence of local adaptation of phenology (O'Malley et al. 2013). The tight coupling of salmonid ecology and phenology, along with the generally high heritability of phenological traits in salmonids (median $h^2 = 0.51$; Carlson and Seamons 2008), suggests that salmonid populations can respond to periodic environmental fluctuations or persistent climatic trends through microevolution of phenological traits.

Pink salmon that spawn in Auke Creek, Alaska provide an example of a population that exhibits genetically determined variability in phenological traits. The Auke Creek pink salmon population is comprised of even- and odd-year brood lines, whose complete genetic segregation is maintained by the strict two-year life cycle that pink salmon exhibit throughout their natural range (Heard 1991). Within each brood line, spawning adults return to Auke Creek in two distinct segments; 'early' spawners typically return between mid- and late August, whereas migration of 'late' spawners typically occurs between early and mid-September (Taylor 1980). The structuring of the adult spawning run is maintained by the high heritability of migration time within each spawning segment ($h^2 = 0.4$; Smoker et al. 1998). Moreover, early- and late-run pink salmon exhibit genetically based differences in embryonic development rate, a phenological trait that influences juvenile outmigration timing (Hebert et al. 1998).

Experimental manipulations of a neutral marker locus have provided the basis for genetic monitoring of the Auke Creek pink salmon population. Artificial breeding was used to change the allele frequencies of an isolocus pair in late-migrating fish in 1979. The marking event led to significant differences between the early- and late-spawning segments at the late-migration marker locus (LMML) in the odd-year brood line, which persisted throughout the 1980s (Gharrett et al. 2001). An absence of genetic differences at the LMML was first observed in adults returning to Auke Creek in 1991, and observations of subsequent spawning runs in 1993, 2001, and 2011 confirmed that the allele frequencies at the LMML no longer differed significantly between pools of early- and late-migrating fish, thereby suggesting that the temporal structure of the odd-year brood line has decreased in extent. Furthermore, significant changes in the frequency of the marker alleles were observed throughout the entire brood line in 1991, which indicated that the observed demographic changes were the result of anomalously low fitness of late-migrating fish from broodyear 1989 (Kovach et al. 2012). While that study produced evidence of natural selection against late-migrating fish, an important factor that must be considered is that extensive hatchery propagation occurred in Auke Creek during the 1970's. Hatchery juveniles were often unmarked, making it difficult to evaluate the demographic consequences of propagation. There exists the possibility that preferential use of late-migrating fish as broodstock artificially inflated the late-spawning segment. Consequently, the declines in the abundance of late-migrating fish in the 1980s may represent a return to a natural population state. Nonetheless, observations of the genetic marker can provide insight into the mechanisms underlying evolution of phenology.

From a combination of census and genetic data collected from outmigrating juveniles during the 1980s and early 1990s, we evaluated the ecological basis of changes in the temporal genetic structure of the odd-year brood line. Additionally, daily census data collected from pink salmon that spawn within the Hugh Smith Lake system in Southeast Alaska were examined in order to determine whether environmental processes operating over large spatial scales have produced changes in run composition similar to those observed in Auke Creek. Importantly, because Auke Creek and Hugh Smith Lake are separated by several hundred miles, the signatures of large-scale climate change may differ at these two locations. Hence, the absence of a parallel response in these systems would not necessarily indicate that the recent demographic changes at Auke Creek are localized in nature. The primary questions that these data addressed were (1) Have juvenile Auke Creek pink salmon exhibited changes in the LMML and, if so, are the changes indicative of enhanced gene flow between spawning segments, selection against late-run fish, or both? (2) Is the anomalously poor fitness of late-run fish from broodyear 1989 the result of poor reproductive success or low marine survival? (3) Do environmental factors that are relevant to lifetime fitness provide evidence of an ecological mechanism of evolution? and (4) Has the distribution of migration time of pink salmon homing to the Hugh Smith Lake system exhibited a shift toward earlier dates and decreased duration as has been observed in Auke Creek?

Methods

Field and laboratory methods

Study site - Auke Creek, a 323-m outlet of Auke Lake that drains into Auke Bay, Alaska (Figure 2.1), is a migratory corridor and spawning habitat of pink salmon which has produced returns of adults that ranged from 1,500 to 28,000 fish over the past 40 years. Within each of the genetically isolated odd- and even-year lineages, migration of spawning adults has historically followed a bimodal distribution consisting of two distinct segments of fish that are separated by approximately two weeks (Taylor 1980). The U.S. National Marine Fisheries Service operates Auke Creek Research Station, a permanent fish-counting weir and experimental salmon hatchery located at the head of tidewater, which enables complete censuses of spawning adults and outmigrating juveniles.

Genetic marking of late-migration timing - A selective mating experiment was conducted in 1979 with the goal of incorporating a putatively neutral genetic marker into the late-spawning segment of the odd-year brood line in Auke Creek (Lane et al. 1990). During the spawning migration of 1979, skeletal muscle samples were collected from 3,906 pink salmon that entered Auke Creek after 15 September and, therefore, represented the latest-migrating fish that year. Each sample was subsequently genotyped by electrophoretically screening the malate dehydrogenase (*MDH-B1,2**) isolocus pair (Allendorf and Thorgaard 1984). Adults (n = 407) which had an *MDH-B1,2*70* allele but not an *MDH-B1,2*130* allele were artificially spawned, and the resulting embryos were incubated in the Auke Creek Hatchery. Genetically marked juveniles (*n* = 178,219) were released into Auke Creek between 7 April and 7 May in 1980 to coincide with the peak of outmigration of wild fry. Interbreeding between experimentally-marked returning adults and wild adults during the spawning period of 1981 produced changes in the allele frequencies of *MDH-B1,2** in the late-spawning segment; the frequency of the *70** allele increased from 0.027 in 1979 to 0.134 in 1983, and the frequency of the *130** allele decreased from 0.022 in 1979 to 0.010 in 1983. Monitoring of migrating adults revealed that allele frequencies at *MDH-B1,2** were stable and differed between samples of early- and late-migrating fish in each odd brood year from 1983 to 1989, thereby demonstrating that the experimental manipulations effectively marked genetic material that is correlated with late-migration time.

Census and genetic data - During each spring from 1984-1992, daily counts were made of all pink salmon fry that passed the Auke Creek weir during their downstream migration. Daily samples of fry (typically 10-20 fish) were collected for genotype determination. Genotyping was accomplished by using starch gel protein electrophoresis to resolve allozyme banding patterns at the LMML (*MDH-B1,2**).

Auxiliary environmental data - The Auke Bay Laboratories Climatological Series is a data set consisting of daily environmental records that have been maintained at the Auke Bay Marine Station since February 1963. These data include measurements of stream temperatures of Auke Creek and sea-surface temperatures of Auke Bay. Seasonal variation in stream temperature is associated with differences in freshwater productivity between early- and late-spawning adults (Fukushima and Smoker 1997), and interannual variation in sea-surface temperature is important to marine survival of juveniles (Manhard, Chapter 1). Hence, these environmental data may provide evidence of an ecological mechanism underlying phenological changes.

Auxiliary biological data - Parallel shifts in phenology were evaluated with a 32-year time series of daily counts of pink salmon that home to the Hugh Smith Lake system (Figure 2.1). Hugh Smith Lake, located southeast of Ketchikan on mainland Southeast Alaska, covers a surface area of approximately 3.2 km^2 and reaches a maximum depth of 120 m. The lake empties into the Boca de Quadra inlet via Sockeye Creek, a 50 m long stream that serves as a migratory corridor for pink, coho, and sockeye (*O. nerka*) salmon migrating upstream to Hugh Smith Lake and its inlets. The two major inlets of Hugh Smith Lake are Buschmann Creek, which flows northwest 4 km to the head of the lake, and Cobb Creek, which flows north 8 km to the southeast head of the lake. Located at the outlet of Hugh Smith Lake is a permanent salmon weir, which has been operated from mid-June to November by the Alaska Department of Fish and Game (ADF&G) since 1982. The majority of pink salmon that enter Sockeye Creek spawn upstream of the weir, presumably in the major inlet streams and their tributaries (S. Heinl, ADF&G; personal communication). Although this weir is operated to monitor sockeye and coho salmon populations, complete daily counts of adult pink salmon migrations have been opportunistically documented since 1982. During that time period, annual migrations of adults have typically ranged from 4,000 to 20,000 fish.

Statistical methods

Graphical and statistical comparisons of temporal patterns - Because the *MDH-B1,2*130* allele underwent relatively small manipulative changes and exhibited allele frequencies close to zero, it provided minimal power for detecting genetic changes. Consequently, the statistical methods that we used to characterize the temporal patterns of the LMML included only the *MDH-B1,2*70* allele, which we refer to as the late-migration marker allele (LMMA). Temporal patterns of the LMMA frequencies were monitored during five odd-year juvenile outmigration periods (1984-1992). Graphical comparisons of five-day running averages of frequencies of the LMMA were made among brood years to describe interannual changes in genetic differences between spawning segments. A two-sample test of equality of proportions, in which juveniles were classified as early or late migrants based on whether they were sampled at the weir before or after the historical median outmigration date (20 April), was used to determine whether early- and late-migrating fry exhibited significant genetic differences at the LMMA. Only data from the calendar dates that included the first and last 100 fish sampled during each migration period were included in the test.

Overall late-marker allele frequency - Interannual patterns in the overall (population-wide) frequency of the LMMA in the odd-year brood line can provide insight into whether allele frequency changes are most likely caused by increased gene flow between spawning segments or reduced fitness of the late-spawning segment. Gene flow between spawning segments within the stream should have caused the frequency of the LMMA to decline within the late-spawning segment, but remain stable overall. Conversely, reduced fitness of the late-spawning segment should have caused a reduction in the overall frequency of the LMMA. Estimating the overall frequency of the LMMA and its statistical certainty is complicated by the variation of daily return numbers and unequal sample representation throughout the migratory periods. These issues were addressed with a parametric bootstrap algorithm that resampled alleles at the LMML (1,000 iterations) in running five-day pools of genetic samples collected throughout each of the juvenile outmigrations. The algorithm divided the migration distribution each year into *n* five day periods, beginning on the first date in which genetic samples were collected. Within each i^{th} period, the maximum likelihood frequency of the LMMA (f_i) was estimated from the genetic samples that were collected during that period. Each iteration of the algorithm began with a random draw of alleles (x_i) from a binomial distribution:

$$x_i \sim Bin(f_i, a_i)$$

where a_i was the number of alleles that were sampled within a period (2 * the number of genetically sampled fish). In each year, the overall frequency of the LMMA (f) was estimated as:

$$f = \frac{\sum_{i=1}^{n} \frac{x_i}{a_i} 2N_i}{\sum_{i=1}^{n} 2N_i}$$

where N_i was the census number of outmigrating juveniles that were observed during a period. The bootstrap simulation provided a point estimate and 95% confidence interval for the overall LMMA frequency during each year of genetic monitoring.

Stock separation algorithm - A stock separation algorithm (Pella and Milner 1987), which used allele frequencies of the LMML in collections of early- and late-migrating adults from 1983 as a baseline, estimated the total number of fish that belonged to the early and late segments in each adult and juvenile migration. The baseline consisted of 564 adults collected on 10 and 18 August and 161 adults collected on 21 September, which represented the unmarked early segment and the marked late segment respectively. Statistical replication of the stock separation algorithm was accomplished with a non-parametric bootstrap simulation that resampled alleles (20,000 iterations) in both the baseline and in running five-day pools of fish. On each iteration of the bootstrap simulation, an expectation-maximizing algorithm estimated the proportion of fish that belonged to each segment by comparing running five-day averages of allele frequencies of genetic samples to allele frequencies of the baseline. The estimated proportion of fish from each segment was then multiplied by the census number of fish that were observed on that date to produce daily estimates of the number of early and late fish. This process was repeated on each day of the migration period. These daily estimates were used to construct plots of the migration distribution of the early and late segments each year. The bootstrap simulation provided point estimates and 95% confidence intervals for the annual numbers of fish from each segment, dates of migration mid-points of each segment, and temporal differences between the two segments. Furthermore, the bootstrap simulation provided point estimates and 95% confidence intervals for the number of juveniles produced per spawner (freshwater productivity) and the number of returned adults per outmigrating juvenile (marine survival) in each segment. Comparisons of freshwater productivity and marine survival between spawning segments and among brood years provided insight into the ecological mechanisms underlying demographic changes in Auke Creek during the genetic monitoring period.

Environmental conditions - Graphical depictions of environmental conditions during the genetic monitoring period were used to provide a qualitative evaluation of potential ecological mechanisms. Demographic plots of the daily number of adults from each segment that were engaged in spawning in Auke Creek were constructed with the estimates from the stock separation algorithm and under the assumption that the average stream life was seven days (Fukushima and Smoker 1997). Thermal-gradient plots of stream temperatures were superimposed over the demographic plots to depict the conditions encountered by early- and late-spawning adults.

The initial marine residence period, which typically occurs in the nearshore environment, is critical to the survival of juvenile salmon (e.g. Beamish and Mahnken 2001, Farley et al. 2007). Observations of tagged juvenile pink salmon in Auke Bay have demonstrated that the duration of this residence period varies seasonally and interannually. For instance, in one year, the duration of nearshore residency ranged from twenty-nine days in fry tagged on 1 April to seven days in fry tagged on 13 May (Mortensen et al. 2000). Given the uncertainty in the duration of nearshore residency, we focused on the first few days in Auke Bay, where juveniles are likely most susceptible to mortality from predation and starvation. Demographic plots of the number of juveniles from each segment that were residing in the nearshore habitat of Auke Bay on each day were made with estimates from the stock separation algorithm and included only fish that had outmigrated from Auke Creek within a week of that date. Thermal-gradient plots of sea-surface temperatures, which were superimposed over the demographic plots, provided a graphical depiction of the conditions that juveniles encountered during their first week in the nearshore environment.

Migration of Hugh Smith pink salmon - Daily counts of migrating adult pink salmon from the Hugh Smith Lake system (1982-2013) were used to produce graphical depictions of the typical migration distribution in the even- and odd-year brood lines. Temporal trends in the time and duration of the migration were quantified over the 32-year time series by estimating general linear models. The time of migration in a given year was defined as the median adult migration date, whereas the duration of the migration period was defined as the number of days separating the dates on which first 5% and 95% of the adult migration passed the weir. Estimates of trends in these migration characteristics enabled us to evaluate whether there have been changes in migration time in the Hugh Smith Lake system that parallel those observed in Auke Creek.

Results

Graphical and statistical monitoring of the late-marker allele - Daily samples of juveniles revealed that, during each of the four odd-year juvenile outmigrations from 1984-1990, there was a rapid increase in the LMMA frequency near the mid-point of the migration (Figure 2.2). In each of these outmigration periods, the LMMA frequency was approximately 6-7 percent higher in juveniles sampled from the late segment than in the early segment, which was a highly significant difference ($P << 0.001$). A substantial decline in the LMMA frequency was first evident in outmigrating juveniles during the spring of 1992. Although there remained a significant difference in the LMMA frequency between samples of early- and late-migrating juveniles ($P = 0.021$), the frequency was only 2 percent higher in late-migrating fish, which was an appreciable decline from previous brood years.

The temporal characteristics of the LMMA in adult pink salmon were described previously (Kovach et al. 2012). We briefly present those results here so that comparisons can be made between adults and juveniles. Returning adults exhibited patterns in the LMMA that were similar to those observed in juveniles during the monitoring period. In each of the three odd-year adult migrations from 1985-1989 a substantial increase in the LMMA frequency occurred after the mid-point of the migration (Figure 2.3), and the frequency of this allele was 6-7 percent higher in late-migrating adults than in early-migrating adults, which was a significant difference in each instance ($P << 0.001$). However, beginning in the adult migration of 1991, there was a marked decline in the magnitude of the increase in this allele over the migration period, and the allele frequencies of samples of early- and late-migrating adults were nearly identical that year ($P = 0.820$).

The results of the bootstrap analysis demonstrated that the overall frequency of the LMMA declined substantially in adults that returned to Auke Creek in 1991 after being relatively stable during the previous three migration periods (Figure 2.4). Similarly, the overall frequency of the LMMA declined substantially in juveniles that outmigrated from Auke Creek in 1992 relative to the previous four outmigration periods, in which the overall frequency of this allele was relatively stable.

Patterns in population demography - The stock separation algorithm produced estimates of early and late components of the juvenile outmigration. During the four odd-broodyear outmigration periods from 1984-1990, the late-migrating segment accounted for an estimated 51-68% of the total fry abundance. Beginning in the outmigration of 1992, the proportion of late-migrating fry declined to only 12% of the total fry abundance. Bootstrap replication produced point estimates and standard errors for marine survival and freshwater productivity in the early- and late-migrating segments of each odd-brood year from 1983-1989 (Figure 2.5). In each of the four brood years, marine survival of late-migrating fry was significantly lower than that of early-migrating fry. This discrepancy was particularly pronounced in broodyear 1989, where the survival rate of late-migrating fry was only 1.4 percent. This survival rate was lowest of the four brood years and was less than one tenth of the survival rate of early-migrating fry from the same brood year. Conversely, freshwater productivity of late-migrating adults was significantly higher than that of early-migrating adults in each odd-brood year from 1983-1987 (Figure 2.5). Broodyear 1989 was distinct in that the mean bootstrap estimate of freshwater productivity of late-migrating adults was similar to that of early-migrating adults. However, aspects of the sampling design produced a high standard error for the estimated freshwater productivity of the late run that broodyear.

Environmental conditions - The estimated temporal distributions of spawning activity in the early and late segments indicated that, while the median migration dates of the two segments were separated by 19, 11, and 15 days during the first three years of monitoring, there was some overlap in their distributions (Figure 2.6). In the fourth year of monitoring, the early and late segments shared the same median migration date and the temporal distributions of spawning activity were indistinct. Seasonal stream temperature patterns varied substantially among years. During 1985 and 1991, stream temperatures dropped below 14 °C in mid-August, which was in advance of peak spawning activity. Conversely, stream temperatures did not fall below 14 °C until 1 September in 1987 and 15 September in 1989, and early-migrating fish were therefore exposed to potentially stressful average temperatures of 15.4 and 15.8 °C in 1987 and 1989 respectively. The 1989 adult migration was unique in that it was bimodal; an initial pulse of fish began entering the stream on 4 August, and a second pulse began entering the stream in large numbers on 21 August. The combination of high stream temperatures and early stream entry caused adults from the first pulse to experience an average temperature of 16.5 °C during their stream life. With the exception of the anomalously warm year in 1989, late-migrating fish did not enter Auke Creek in large numbers until temperatures had dropped below 14 °C and therefore experienced average stream temperatures that ranged between 12.0 and 13.3 °C in each of those years. In 1989, however, late-migrating fish experienced an average stream temperature of 14.2 °C despite a late median migration date that occurred on 8 September.

The estimated migration distributions of juveniles indicated that the median outmigration dates of the early and late segments differed by 12 and 15 days in 1984 and 1986, whereas they differed by only 6 and 7 days in 1988 and 1990. This was reflected in the initial marine residency periods of the early and late segments, which exhibited considerably higher overlap in the last two years of monitoring as compared to the first two years (Figure 2.7). Because of their earlier outmigration dates, early juveniles encountered cooler temperatures in Auke Bay than juveniles from the late segment in all observed years. The outmigrations of 1986 and 1990 are particularly noteworthy because of the contrasting temperature profiles experienced by early- and late-run fry in those years. In 1986, sea-surface temperatures remained below 5 °C through April, which caused early-run fry to experience an average temperature of 5.4 °C. Late-run fry, conversely, began to enter Auke Bay in peak numbers during a warming event in the beginning of May and experienced an average temperature of 8.8 °C. The thermal advantage experienced by late-run fry in 1986, which was largest of the first four years of monitoring, may have diminished the survival advantage of early-run fry; the survival rate of late-run fry was 60% of that observed in early-run fry that year, which was the highest relative survival rate estimated over the monitoring period. During the spring of 1990, an early warming period that began on 15 April coincided with the peak outmigration of the early segment. Consequently, early fry experienced an average sea-surface temperature of 7.0 °C during their first week in Auke Bay. Conversely, the peak outmigration of the late segment that spring coincided with a period of cooling sea-surface temperatures, and late fry experienced an average temperature of 7.7 °C. Although late fry experienced a positive temperature discrepancy relative to early fry in 1990, the discrepancy was substantially smaller than in the first three years of monitoring. The absence of a pronounced thermal advantage of late-run fry in 1990 may have enhanced the survival advantage of early-run fry. This was supported by the fact that the survival rate of late-run fry was only 8% of that observed in early-run fry, which was the lowest relative survival rate over the monitoring period.

Migration of Hugh Smith pink salmon - Annual plots of the temporal distribution of adult pink salmon migrating into the Hugh Smith Lake system revealed little evidence of multimodality in the even- or odd-year brood line, thereby suggesting that the migration into this system typically occurs in a single, primary pulse (Figure 2.8). General linear models of the temporal characteristics of the adult migration detected a significant trend towards earlier median migration dates ($P = 0.04$) and a moderately significant trend toward shorter adult migration periods ($P = 0.06$) in the even-year brood line (Figure 2.9). In the odd-year brood line, negative slopes were estimated for both the date and duration of the adult migration, but neither was statistically significant.

Discussion

One of the few documented instances of microevolution in a wild population came from a study of pink salmon that spawn in Auke Creek, Alaska, which used a neutral genetic marker to demonstrate that changes in the temporal characteristics of the adult spawning migration were due to genetic evolution (Kovach et al. 2012). Here, we expanded this research by incorporating observations of the same genetic marker, which were collected from juvenile pink salmon during their spring seaward migration. During the first four years of genetic monitoring, the patterns of the LMMA in juveniles paralleled those that were observed in adults. Both life history stages exhibited a pronounced increase in the frequency of the LMMA beginning near the midpoint of the migration, and fish sampled from the early and late segments of the migration displayed significantly different allele frequencies, thereby demonstrating considerable genetic structure in both life history stages (Figures 2.2, 2.3). The presence of parallel seasonal patterns of this allele in adults and juveniles demonstrates that the phenology of these two life history stages is tightly coupled and indicates that natural selection upon one stage would likely produce an evolutionary response in the other. While the fingerprint of the LMMA was strong in juveniles outmigrating during spring of 1990, the adult migration of 1991 was the first instance after genetic monitoring began in which a mid-season increase in the LMMA frequency or significant allelic differences between samples of early- and late-run fish was not observed. Similarly, the magnitude of the mid-season increase in the LMMA frequency declined considerably in the juvenile outmigration of the following spring (1992). Hence, it appears as though this population experienced genetic changes in phenology at two different life history stages and that this evolutionary event first occurred during the oceanic phase of broodyear 1989. Subsequent observations of this population in 1993, 2001, and 2011, which also failed to demonstrate a fingerprint of the LMMA in late-run fish, suggests that these genetic changes have persisted over multiple generations (Kovach et al. 2012).

While it is evident that microevolution occurred in this population during the period of genetic monitoring, the potential driving forces are numerous and may include natural selection, genetic drift, and gene flow. Environmental conditions that modulate stream entry date can create opportunity for gene flow between spawning segments in some years. In order to address this possibility, we used a bootstrap approach to evaluate the overall frequency of the LMMA in juveniles from the odd-year brood line under the expectation that gene flow alone would cause allele frequency changes within spawning segments while producing minimal changes in the brood line as a whole. We observed that the overall frequency of the LMMA (Figure 2.4) was stable during the first four years of genetic monitoring, before undergoing a substantial decline in 1992, which was consistent with the pattern observed in adults. Collectively, these results indicate that gene flow between spawning segments is probably not a primary driver of the observed changes.

The plausibility of genetic drift and interpopulation gene flow as drivers of microevolution have also been evaluated in this population. Estimates of the effective population size (N_e) can yield insight into expected changes due to genetic drift. Observations of genotype data over 23 microsatellite loci produced estimates of N_e in the odd-year brood line that ranged between 788 and 6005 adults, depending on the estimation method and observed time frame (Kovach et al. 2013a). This range of possible N_e values suggests that we might expect a change of 0.003-0.007 in the LMMA frequency due to genetic drift alone. However, the observed change between the adult migrations of 1989 and 1991 was substantially higher than this range (ΔLMMA = 0.020). Observations of another putatively neutral control locus, which exhibited minimal genetic change during the monitoring period, provided additional evidence that genetic drift was not nearly strong enough to produce the observed genetic changes (Kovach et al. 2012). Gene flow was evaluated with LMMA frequencies from nearby pink salmon populations in an island-continent model. The model estimated that the migration rate (m) necessary to produce the observed genetic changes ranged from 0.69-0.85 (Kovach et al. 2012), which was substantially higher than estimates of straying rates of Auke Creek pink salmon ($m = 0.02$-0.04; Mortensen et al. 2002) and gene flow between proximate populations and Auke Creek ($m = 0.0015$; Gharrett et al. 2001). These results suggest that gene flow from other populations was probably inadequate to produce the observed changes. Hence, the most probable cause of the genetic changes was a natural selection event in brood year 1989, which resulted from relatively low survival of late-run fish during their oceanic phase.

The stock separation algorithm, which produced estimates of the number of early- and late-run fish during the adult and juvenile migrations, presented an opportunity to examine freshwater productivity and marine survival of each spawning segment during the genetic monitoring period (Gharrett et al. 2013). Bootstrap estimates revealed that freshwater productivity of late-run fish was significantly higher than that of early-run fish during the first three years of genetic monitoring (Figure 2.5). This is consistent with the ecology of this population, in which redds of early-run fish incur heavy mortality due to scouring and mechanical disturbance by spawners arriving later in the season (Fukushima et al. 1998). Indeed, early- and late-run fish exhibit different life history characteristics, including stream life (Fukushima and Smoker 1997) and embryonic development patterns (Joyce 1986), which are probably adaptations to spawner densities. Despite these life history adaptations, early-run fish generally appeared to have lower fitness in the freshwater environment.

Poor spawning efficiency has been observed in association with high stream temperatures (> 15 °C) during the adult migration in Auke Creek (Fukushima and Smoker 1997). Furthermore, it is thought that stream temperatures must be sufficiently low (typically < 14 °C; Velsen 1987) during and after the spawning period in order for embryos to survive and develop. These observations indicate that exposure to high stream temperatures in 1989 (Figure 2.6) may have caused poor freshwater productivity in the initial pulse of early-run adults. This idea was supported by the fact that the outmigration of early-run juveniles during the following spring was late and truncated relative to previous years, thereby indicating that few offspring were produced from the first pulse of spawners. Interestingly, the two spawning segments produced similar numbers of fry per spawner in 1991, which was a substantial departure from the previous three years. The median migration date of the late run coincided with peak abundances of early-run spawners that year, and it is possible that the relatively poor freshwater productivity of late-run spawners resulted from competition with early-run spawners. Without their characteristic fitness advantage in the freshwater environment, late-run fish from broodyear 1991 appeared to incur further demographic declines.

We observed consistently higher marine survival of early-run fish relative to late-run fish during each year of the genetic monitoring period (Figure 2.5). In order to understand the basis of these differences, it is necessary to consider the ecological factors that determine marine survival of pink salmon in this system. Juveniles transition from Auke Creek to the estuarine environment of Auke Bay shortly after emerging from the gravel. This early marine residence period, which is characterized by high and variable mortality, is a primary determinant of marine survival (Manhard, Chapter 1). Much of our understanding of the early marine ecology of Auke Creek pink salmon came from a study that examined how physical conditions, plankton abundance, and predator densities influenced survival and growth of juveniles in Auke Bay over four brood years (Mortensen et al. 2000). The study observed that the primary factor controlling growth was temperature and found minimal evidence that prey abundance limited growth during the middle of the outmigration period. However, growth rates of the earliest migrants appeared to be limited by both low temperatures and prey abundance, while the latest migrants, which entered Auke Bay in May, exhibited declines in growth rates despite encountering favorable sea-surface temperatures and high zooplankton densities (Figure 2.10). This pattern may have been caused by heavy grazing by early-run fry or other competitors (e.g. juvenile herring, capelin) and resultant changes in the size composition of zooplankton. Substantial declines in the quality of prey would likely constrain the growth potential of late-run fry and potentially cause some of them to starve. Another important observation from that study was that the abundance of predators of juvenile pink salmon [e.g. coho salmon (*O. kisutch*), Dolly Varden trout (*Salvelinus malma*), sculpin (family Cottidae)] was relatively low in early spring before increasing dramatically in late April and throughout May. Predation is likely a large component of early marine mortality of pink salmon, and a primary mechanism linking early marine growth to survival is provided by the size-selective mortality hypothesis, which asserts that larger fry are less vulnerable to predators, potentially because of their increased swimming speed and because their girth provides protection against gape-limited predators (Parker 1971, Hargreaves and LeBrasseur 1986). Late-run fry are smaller than early-run fry throughout the nearshore residence period (Mortensen et al. 2000) because of their shorter marine growth period and, potentially, because they are at a disadvantage in competing with earlier migrants for food. Collectively, our knowledge of the early marine ecology of Auke Creek pink salmon suggests that the latest outmigrating fry may suffer higher mortality from starvation and size-selective predation than fry that enter Auke Bay during the middle of the outmigration period.

We have established the oceanic phase as the period in which substantial genetic changes initially occurred, and we have demonstrated that, while early-migrating fish consistently exhibited higher marine survival during this stage, the lifetime fitness difference between these spawning segments was probably mitigated by higher freshwater productivity of late-run fish during the first three years of monitoring. Furthermore, we have presented evidence that the consistently higher marine survival of early-run fry reflects a size advantage that results from being on the leading edge of the migration. The magnitude of this size advantage is probably modulated by seasonal sea-surface temperature patterns which influence the growth of juvenile salmon, as well as their planktonic prey. Hence, size-dependent mortality is a potential temperature-mediated mechanism by which genetic changes in migration time may occur. Demographic plots of the fry outmigration in the spring of 1990 indicated that there was considerable overlap in the nearshore residence periods of early- and late-migrating fry (Figure 2.7). Additionally, because of a period of early warming in Auke Bay that coincided with the peak outmigration of early-run fry, late-run fry appeared to experience a relatively small thermal advantage that spring. Hence, there was potential for anomalously high size-selective predation on late-run fry from broodyear 1989. This is supported by the observation that marine survival of early-run fish was more than ten times that of late-run fish in broodyear 1989, which was substantially larger than in the previous years of monitoring.

Our results suggest that early warming of Auke Bay favored higher fitness of early-migrating fry in the spring of 1990. Significant trends toward earlier migration times in both brood lines (Kovach et al. 2012) and warmer spring sea-surface temperatures in Auke Bay (Figure 2.11) have been observed in this system over the past four decades, and the ecological mechanism that we detailed provides a potential link between these trends. Because low sea-surface temperatures limit growth of early-migrating fry, we might expect earlier vernal warming to cause the optimal growth window to occur earlier in spring. Furthermore, although we do not have information on trends in zooplankton phenology in Auke Bay, trends toward earlier onsets of spring zooplankton blooms have been observed in other systems (Edwards and Richardson 2004, Thackeray et al. 2010), and earlier zooplankton blooms would likely reinforce a shift in the optimal window. Observations of tagged juveniles have provided some evidence that the growth rates of the latest migrants are limited by prey availability, which supports the idea that a shift in the optimal growth window could cause changes in phenology. However, the coupling of the phenology of the marine and freshwater stages suggests that fitness gains from exploitation of more favorable growth conditions in early spring would be met with fitness losses from exposure to stressfully warm stream conditions early in the spawning season. Hence, we might expect the migration distribution to become truncated rather than merely shifted in time. This idea is consistent with the significant trends toward truncated adult migration periods that have been observed in both brood lines over the past four decades (Kovach et al. 2012).

Thus far we have provided evidence of evolution of phenology of Auke Creek pink salmon, which may have resulted from warming sea-surface temperatures. Given that these climatic changes are probably linked to processes operating over a large spatial scale, it is possible that parallel responses are occurring in other salmon populations. The Hugh Smith Lake system is located approximately 270 miles southeast of Auke Creek (Figure 2.1) and supports a pink salmon population on which daily counts have been made over a 32 year period, making it one of the best available sources for evaluating temporal trends in pink salmon migrations. While the Hugh Smith Lake system consists of a considerably larger expanse of riverine habitat than Auke Creek, it supports runs of spawning adults that are comparable in size. Hence, natural selection arising from competition for spawning habitat may be weaker in this system. This was evident in the fact that, unlike the characteristic multimodal migration at Auke Creek, the migration into Hugh Smith Lake typically occurred in a single pulse (Figure 2.8). Despite this apparent lack of temporal run structure, we observed significant trends towards earlier and truncated migrations in the even-year broodline (Figure 2.9), which paralleled those observed at Auke Creek. The odd-year broodline exhibited insignificant trends toward earlier and truncated migrations, but the powers of those tests were low (median migration date = 0.06; migration span = 0.22), indicating that a much longer time series would be required to produce a reasonable chance of detecting significant trends in this brood line. Our demonstration of parallel phenological changes in a geographically separate system suggests that other salmonid populations may evolve earlier life history schedules in order to exploit warming conditions. Indeed, the literature concerning phenological changes in anadromous salmonids is growing, and earlier migration times have now been documented in Auke Creek coho salmon (Kovach et al. 2013b), Columbia River steelhead trout (*O. mykiss*: Robards and Quinn 2002), Bristol Bay sockeye salmon (*O. nerka*: Quinn et al. 2007), and Atlantic salmon (*Salmo salar*) in Northern Ireland (Kennedy and Crozier 2010).

Depending on the species and location, different patterns of phenological change in response to climate warming might be expected in salmonid populations. Expected environmental changes include increasingly stressful stream temperatures in late summer, which should favor later adult migration times, and earlier vernal warming, which should favor earlier fry outmigration times. The pattern of phenological change in a given population is influenced by the relative strength of selection at these two critical life history stages, along with the phenotypic plasticity of traits associated with migration time. Because pink salmon do not have an extended freshwater growth period prior to outmigrating, they are probably more vulnerable to size-selective predation than species such as coho and sockeye salmon, which typically reside in freshwater for one or more years. Consequently, environmental factors that condition vulnerability to predation in pink salmon (e.g. temperature, food availability) should have a large bearing on fitness, so that selection of juvenile outmigration time is particularly strong. Furthermore, pink salmon generally spawn in the lower reaches of coastal streams and therefore have a relatively short in-stream migration. This life history characteristic reduces the duration of exposure to stressful stream conditions prior to spawning, potentially reducing the relative strength of selection against early adult migration time. Another important consideration is that, in lower latitude salmonid populations, where spawning adults frequently encounter stream temperatures that are close to their thermal threshold, high levels of pre-spawning mortality (i.e. fish kills) and poor survival of embryos likely results in intense selection against early migrating fish. Auke Creek is located close to the latitudinal center of the North American range of pink salmon and these events are less frequent than in lower latitude streams, such as those of the Pacific Northwest. Finally, and possibly most importantly, the pattern of phenological change in a given population is, in part, an outcome of the amount of adaptive life history variation that it supports. In Auke Creek pink salmon, both brood lines harbor fine-scale genetic variation in phenology, and it is this genetic structure that has provided the basis for contemporary evolution. In populations where this adaptive variation does not exist, the limited scope of phenotypic plasticity may provide the only means of shifting phenology in response to climate change.

Conclusions

We have presented an example of microevolution of phenology in a wild population, which meets all of the criteria necessary to demonstrate adaptive genetic change (Hansen et al. 2012). Despite apparently strong natural selection against late-run fish and resulting demographic changes, the odd-year brood line has exhibited sustained levels of adult recruitment over the past 17 generations. Similarly, the even-year brood line, which has exhibited parallel changes in phenology, has also sustained its recruitment levels over the same time period (Kovach et al. 2013b). This suggests that fine-scale local adaptation of phenology has supported sustained productivity of Auke Creek pink salmon in the face of a rapidly changing climate and underscores the importance of biocomplexity to the resilience of wild populations (Hilborn et al. 2003, Greene et al. 2010). Heritable variation in phenology has been demonstrated in wild populations over a wide range of taxa, and individuals within these populations often possess traits that are locally adapted to environmental conditions characteristic of their life history schedules (e.g. birds: Møller 2001, Sheldon et al. 2003; fish: Hendry et al. 1999, Quinn et al. 2000; plants: Weis and Kossler 2004, Hall and Willis 2006). Our results suggest that the pace of contemporary evolution may be sufficiently high to enable some of these populations to adapt to climate change without suffering from significant declines in productivity and emphasize the importance of tailoring management programs to recognize and conserve genetic diversity.

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Figures

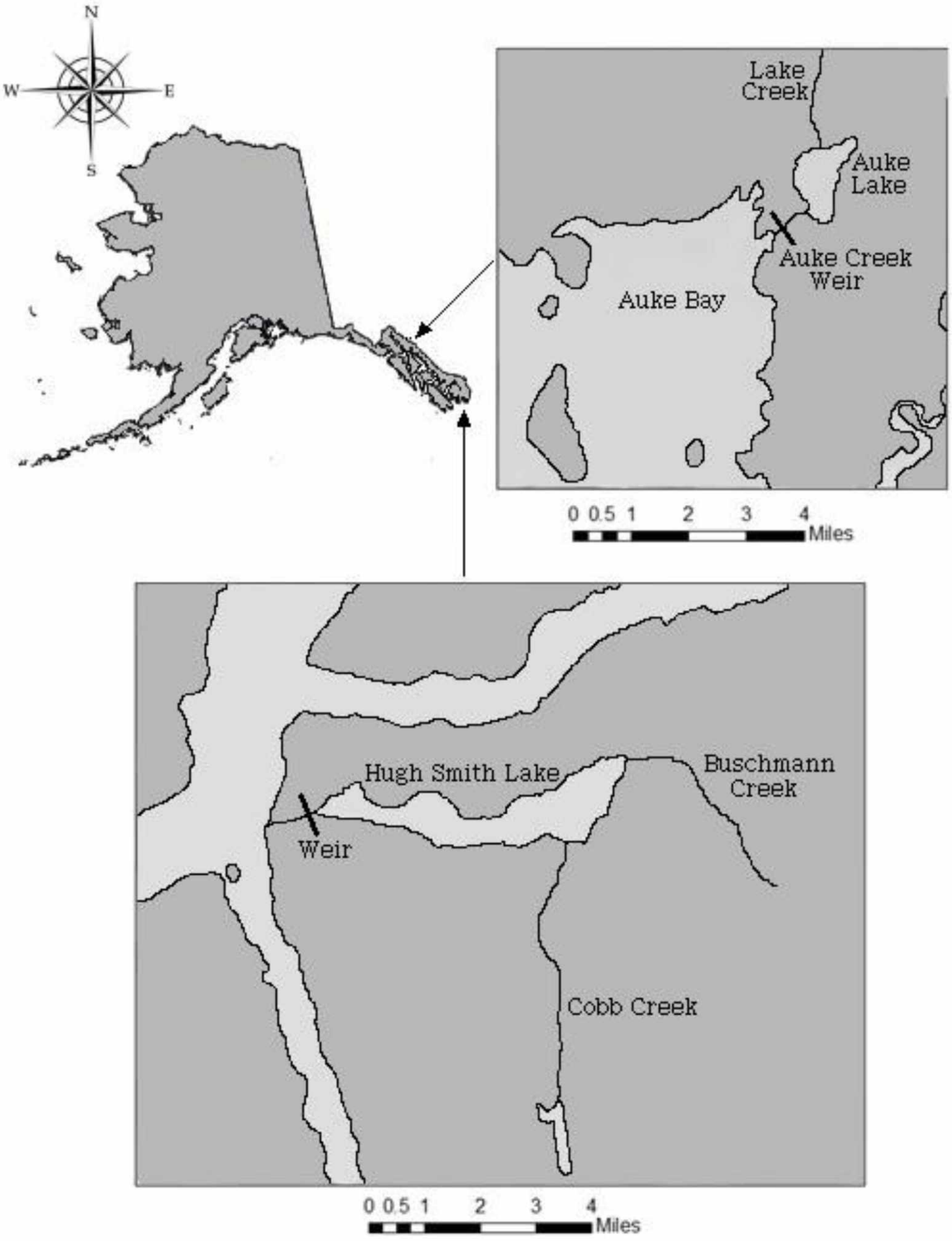

Figure 2.1 - Maps of the Auke Lake (top panel) and Hugh Smith Lake (bottom panel) systems. The locations of salmon weirs are denoted by bold black lines.

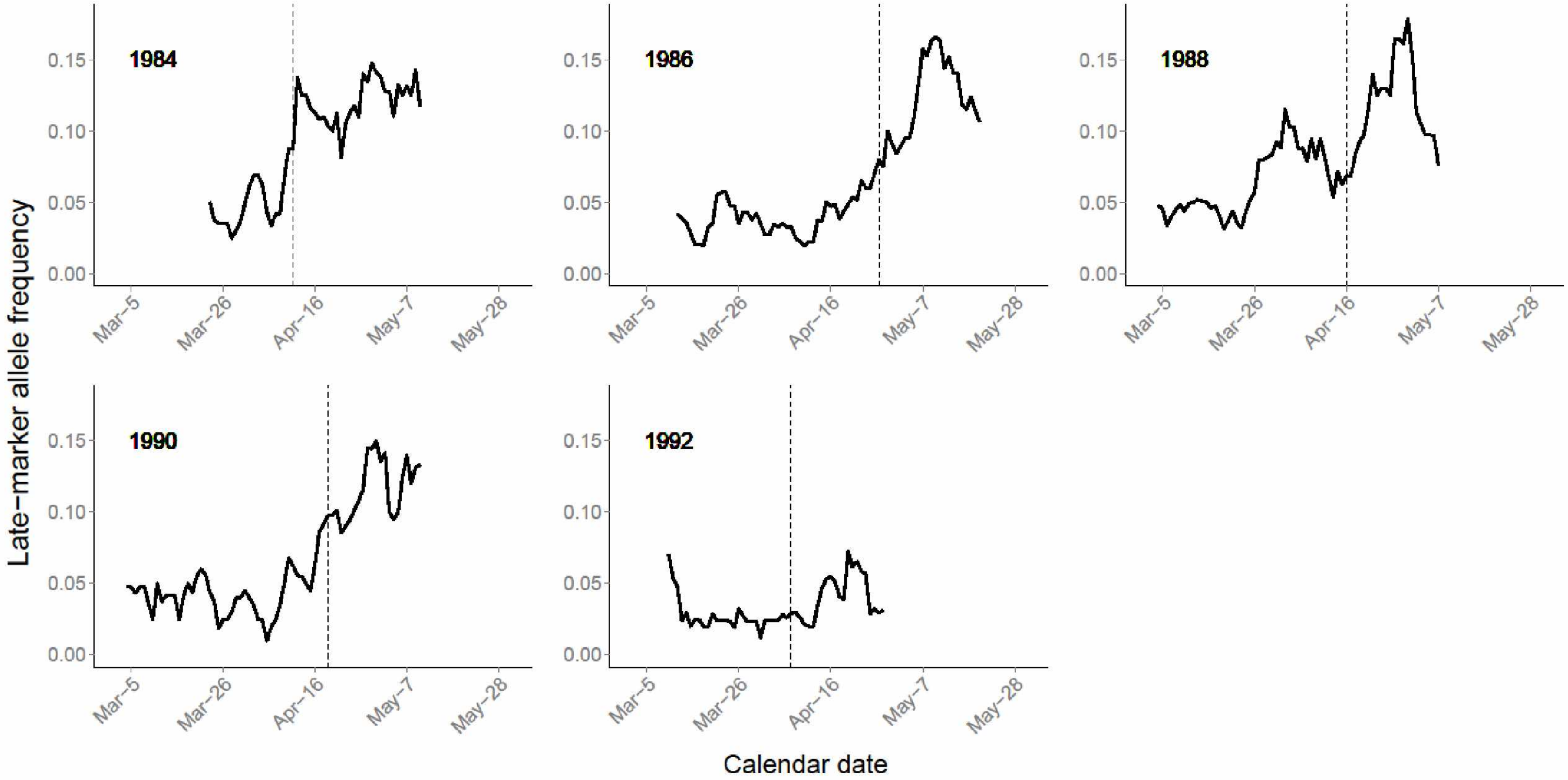

Figure 2.2 - Five-day running averages of the frequency of the late-migrating marker allele in juvenile pink salmon sampled throughout the duration of each odd-broodyear outmigration from 1984-1992. Median outmigration dates are indicated by a dashed line.

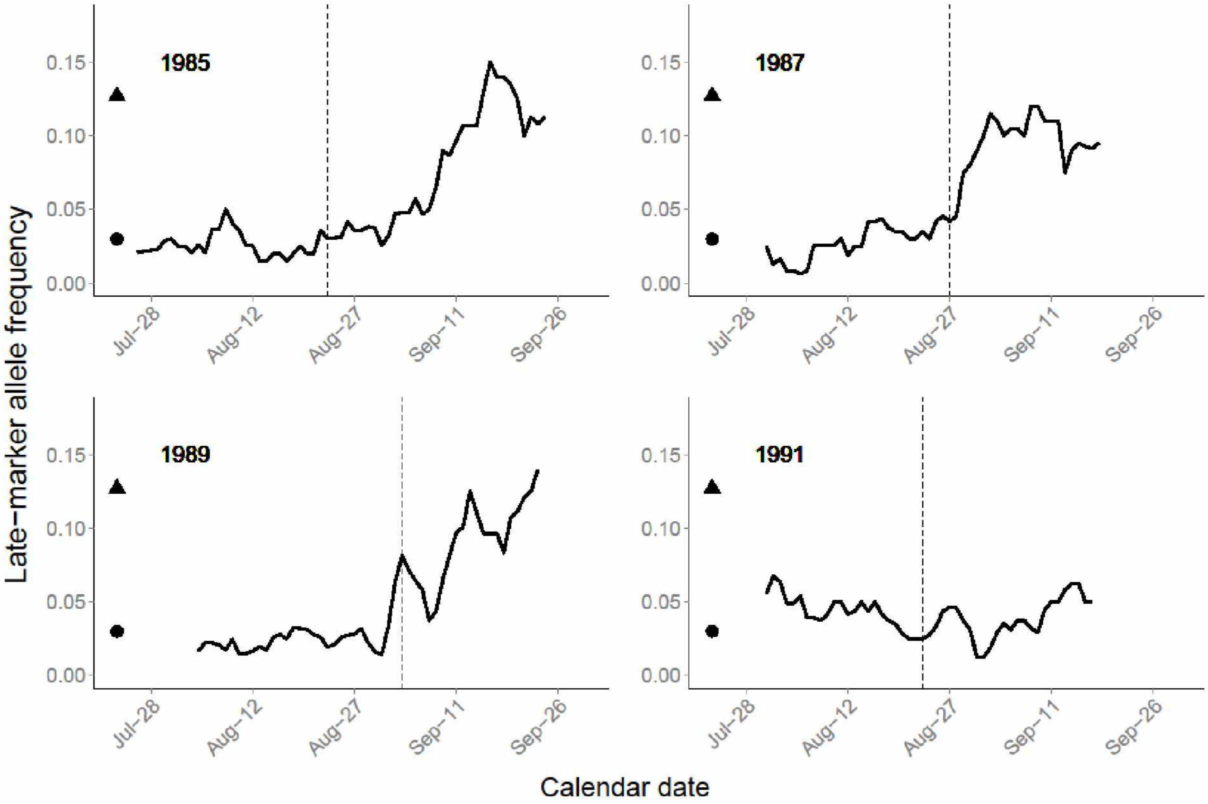

Figure 2.3 - Five-day running averages of the frequency of the late-migrating marker allele in adult pink salmon sampled throughout the duration of each odd-year migration period from 1985-1991. Median migration dates are indicated by a dashed line, and the 1983 late-marker allele frequencies are depicted for early (circle) and late (triangle) fish.

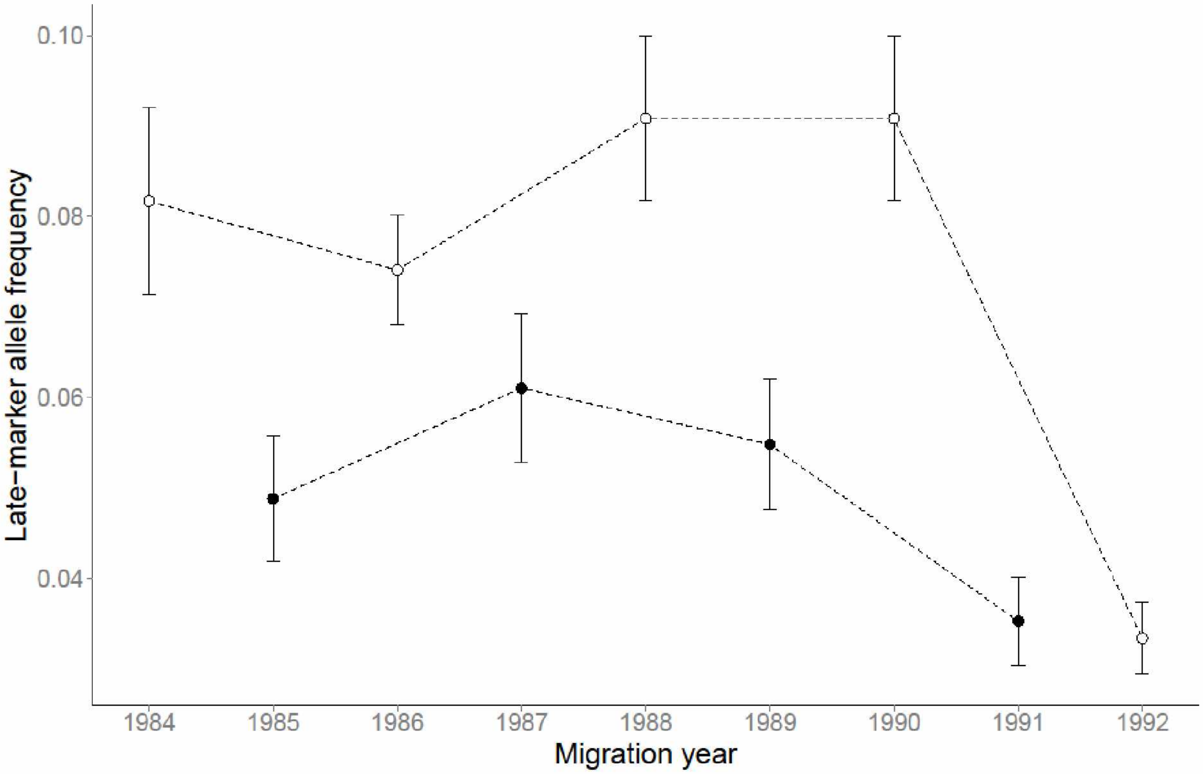

Figure 2.4 - Annual bootstrap estimates (20,000 iterations) of the overall frequency of the late-migrating marker allele (*MDH-B1,2*70*) in migrating adult (solid circle) and juvenile (open circle) pink salmon at Auke Creek. Errors bars are the 95% confidence intervals of each estimate.

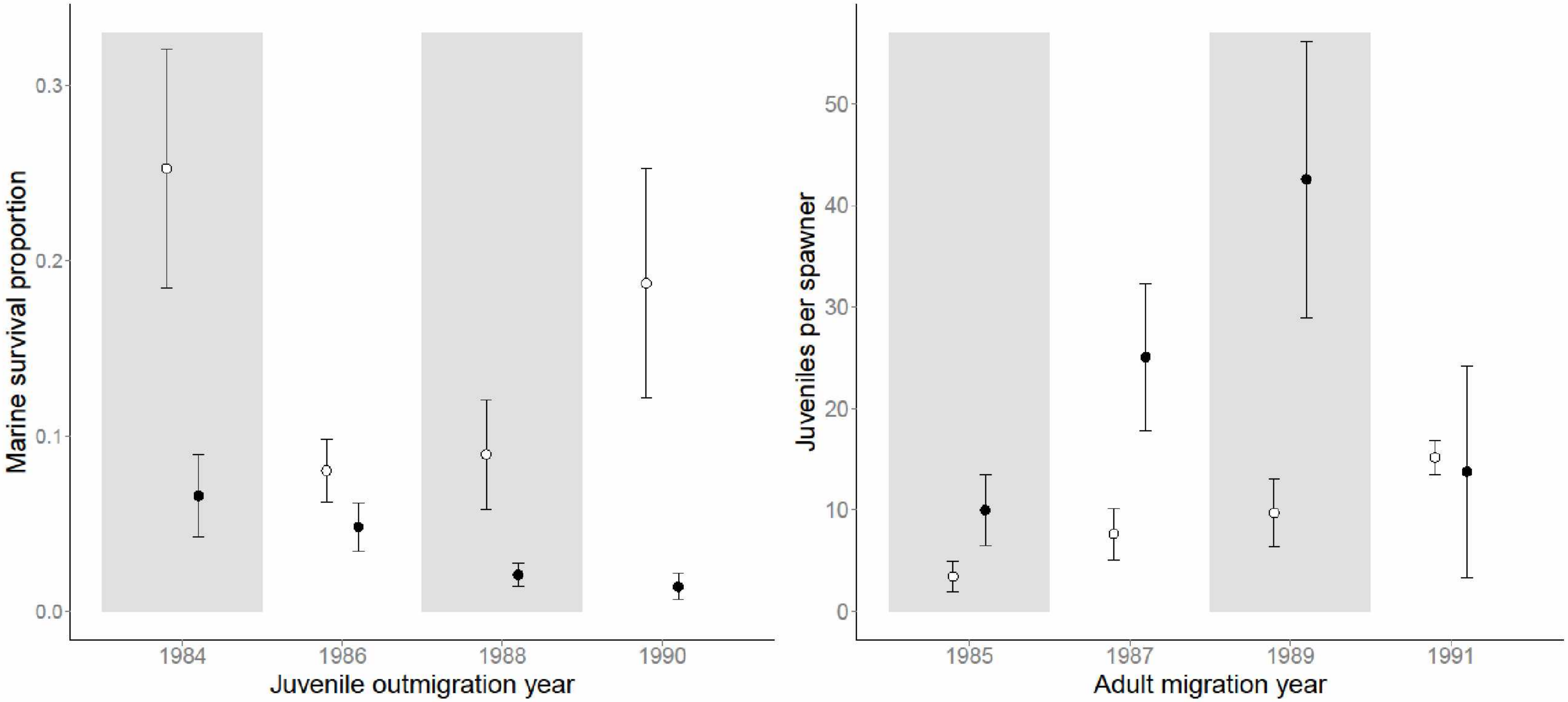

Figure 2.5 - Bootstrap estimates (20,000 iterations) of marine survival and freshwater productivity (emigrating fry per spawner) over four brood years of early- (open circle) and late- (closed circle) migrating pink salmon. Error bars are the 95% confidence intervals of each estimate. Shading emphasize the comparison of runs within each brood year.

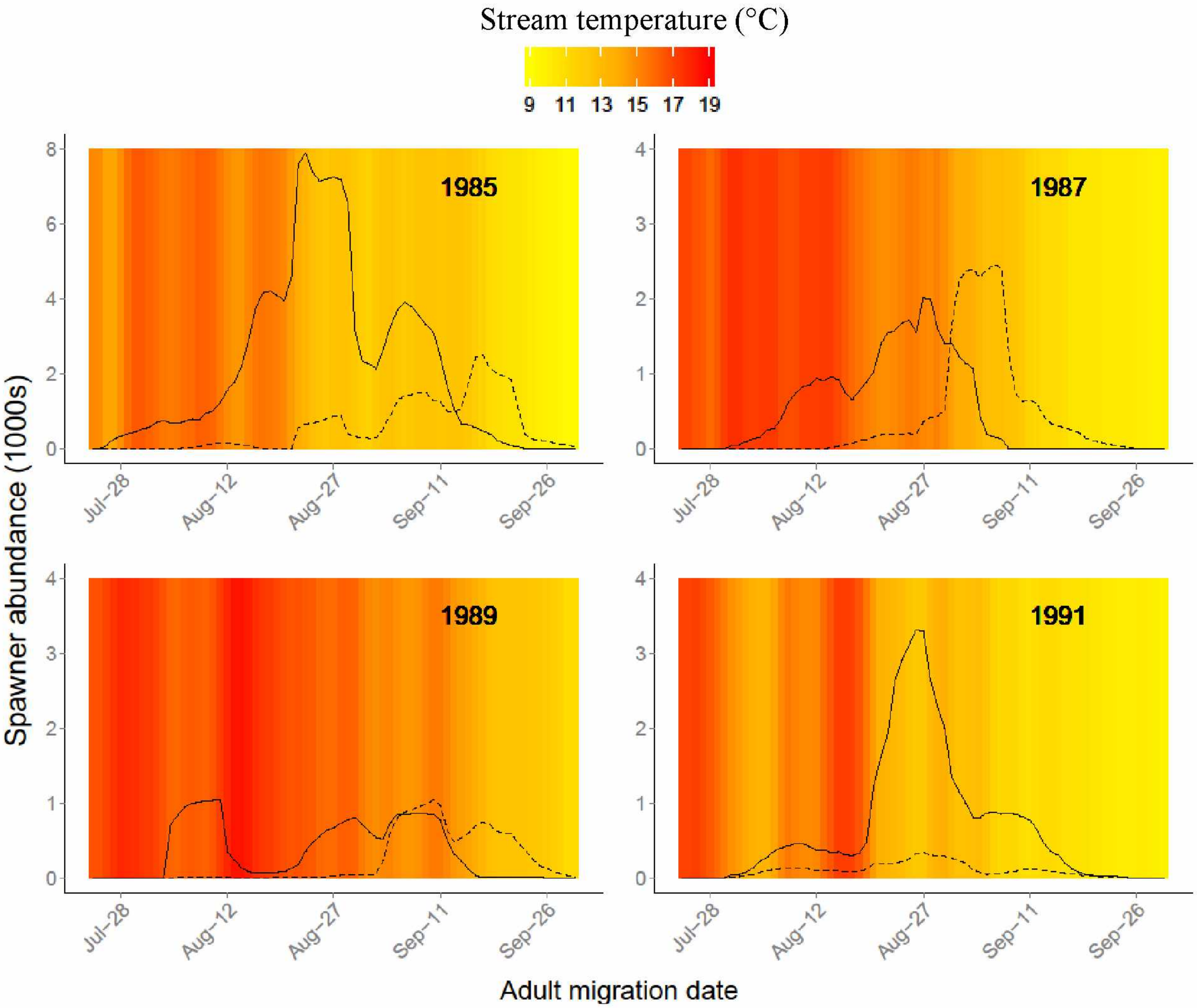

Figure 2.6 - Stream temperatures experienced by spawning pink salmon from the early (solid line) and late (dashed line) subpopulations during the genetic monitoring period. Subpopulation assignment was made with a stock separation algorithm that used allele frequencies at the late-marker locus, and abundance estimates are based on a stream life of seven days. Three-day running averages of Auke Creek temperatures (°C) are depicted by a gradation scheme.

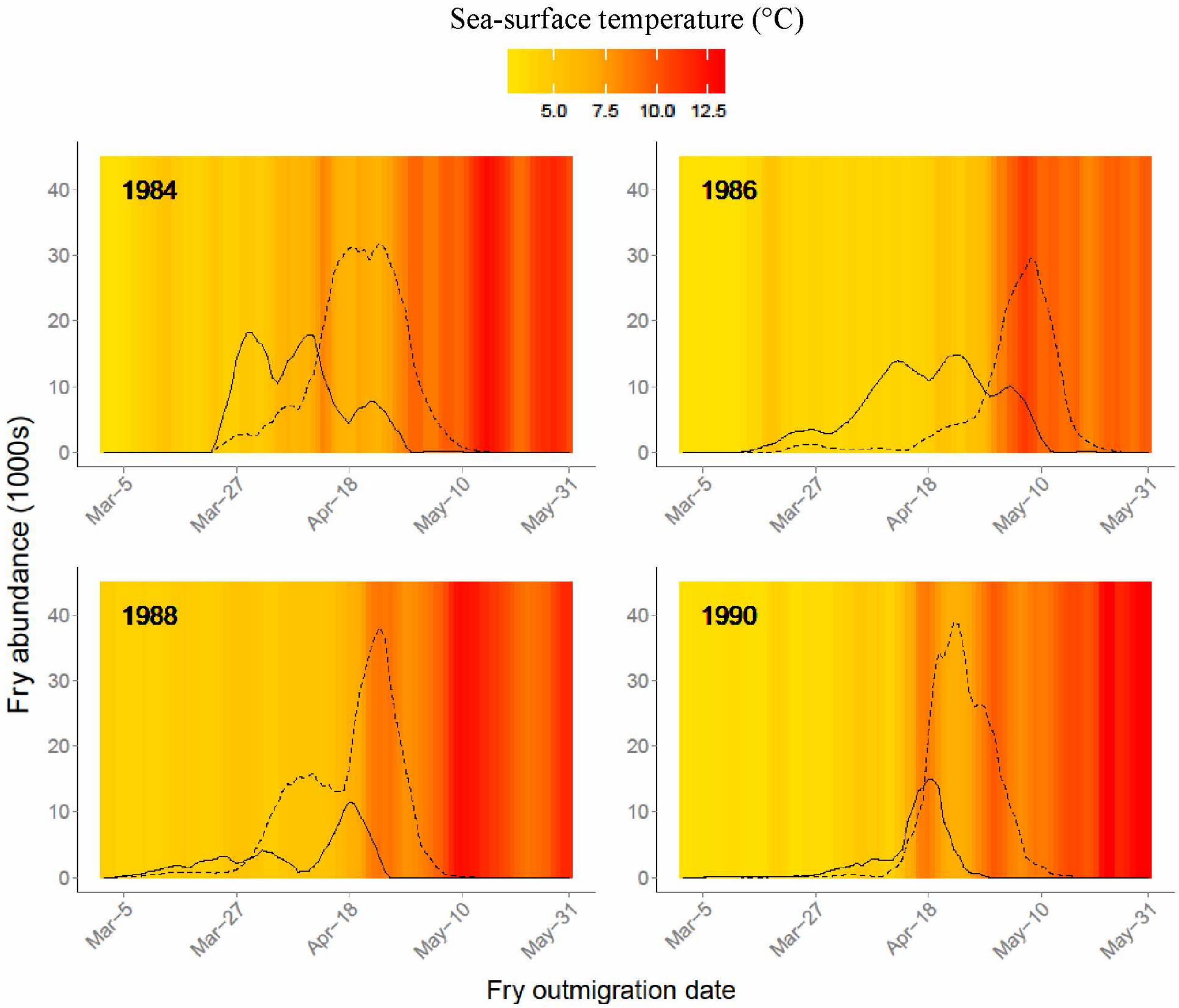

Figure 2.7 - Sea-surface temperatures experienced by juvenile pink salmon from the early (solid line) and late (dashed line) subpopulations during the genetic monitoring period. Subpopulation assignment was made with a stock separation algorithm that used allele frequencies at the late-marker locus, and abundance estimates include only fish that were in the first seven days of their marine residency. Three-day running averages of Auke Bay sea-temperatures (°C) are depicted by a gradation scheme.

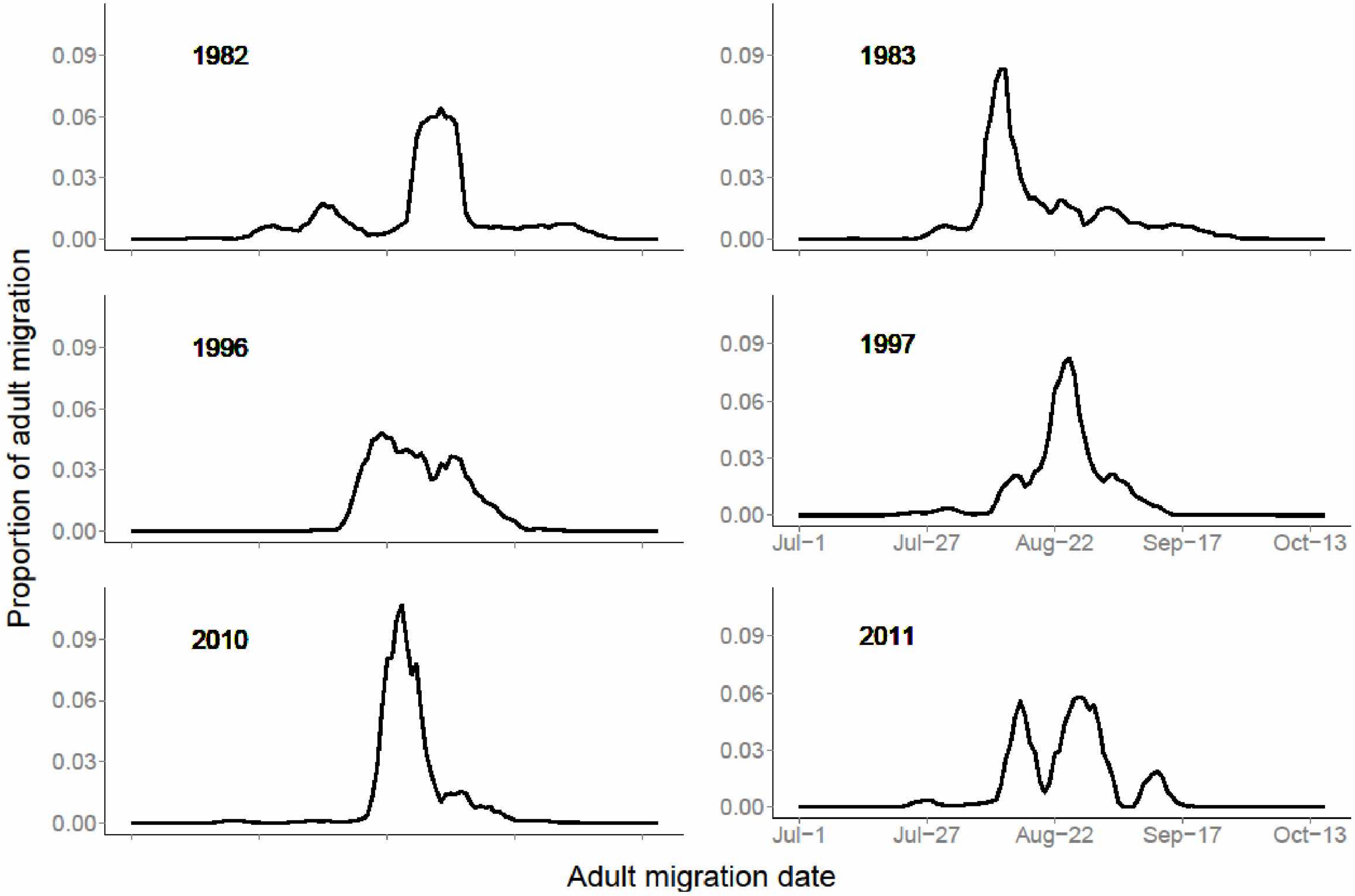

Figure 2.8 - Running five-day averages of the proportion of the adult pink salmon migration that passed the Hugh Smith Lake weir on each day of a given year. Individual years were selected to represent the beginning, middle, and end of the 32-year time series. The even- and odd-year brood lines are depicted on the left and right columns respectively.

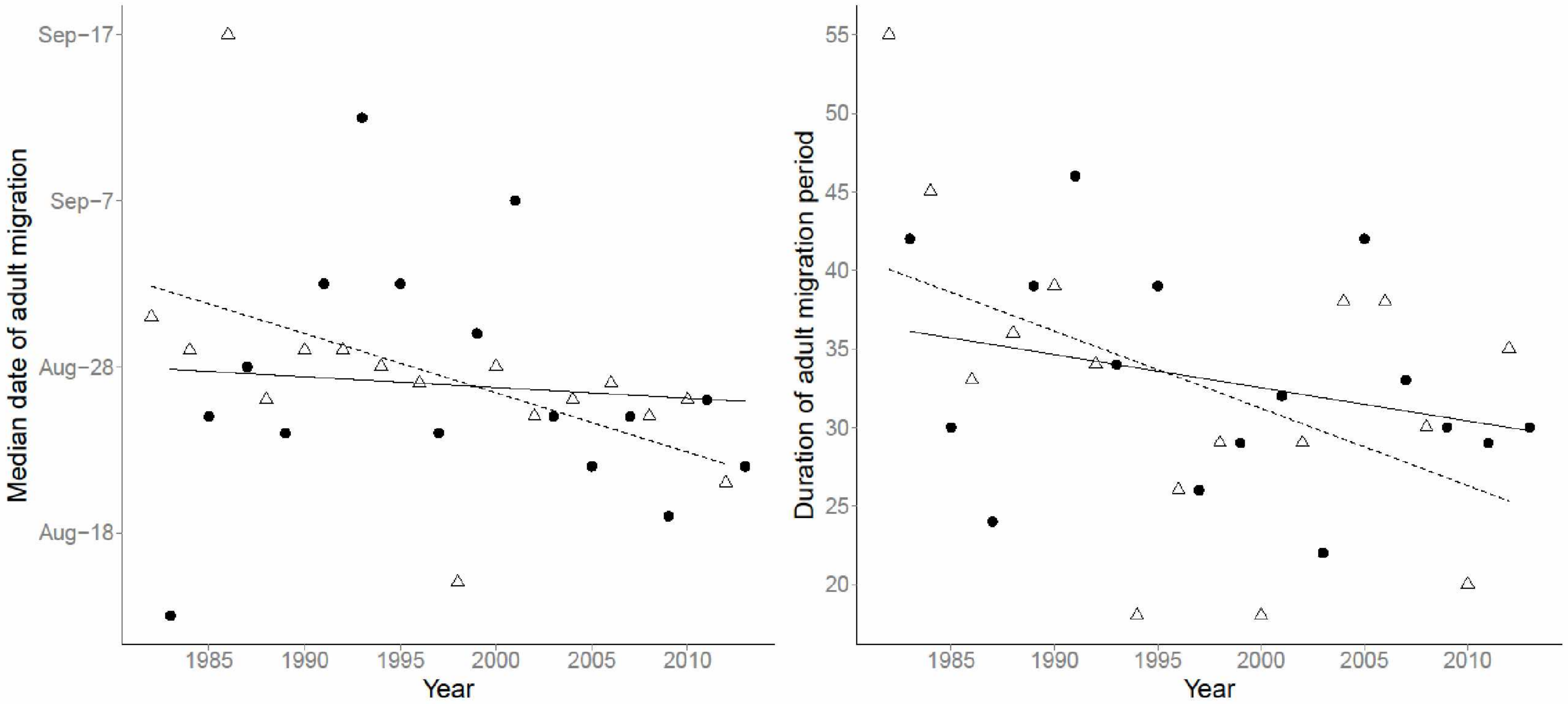

Figure 2.9 - Temporal trends in the median date and duration of the adult migration for odd- (closed circles, solid line) and even- (open triangles, dashed line) year pink salmon homing to the Hugh Smith Lake system.

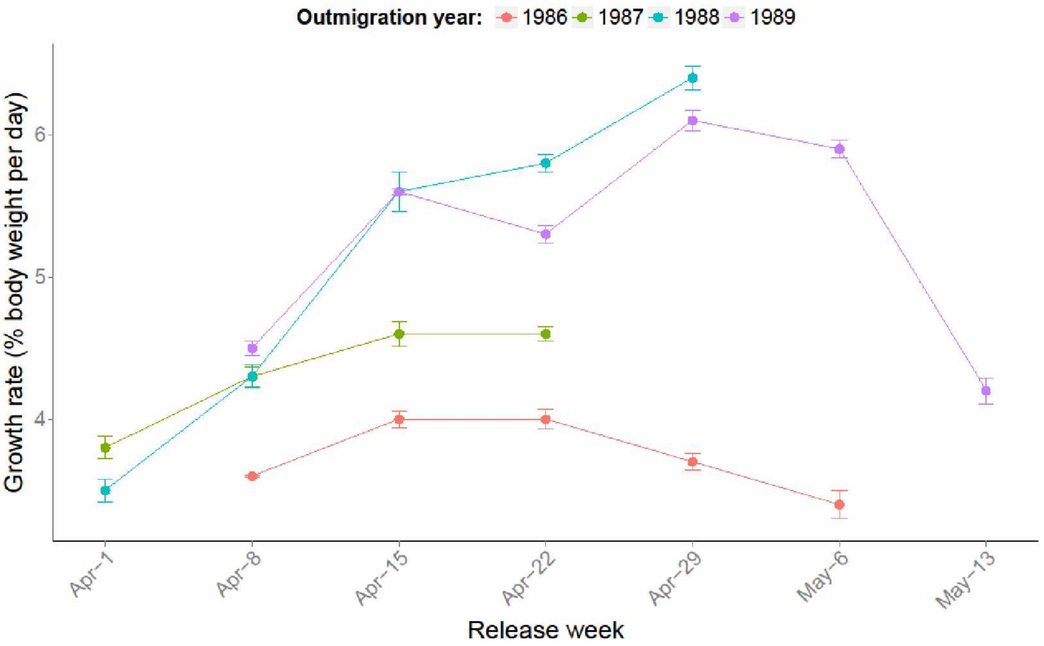

Figure 2.10 - Estimated growth rates with standard error bars for Auke Creek pink salmon during their nearshore residence period. Estimates were obtained from a previous tagging study that monitored four brood years (Mortensen et al. 2000).

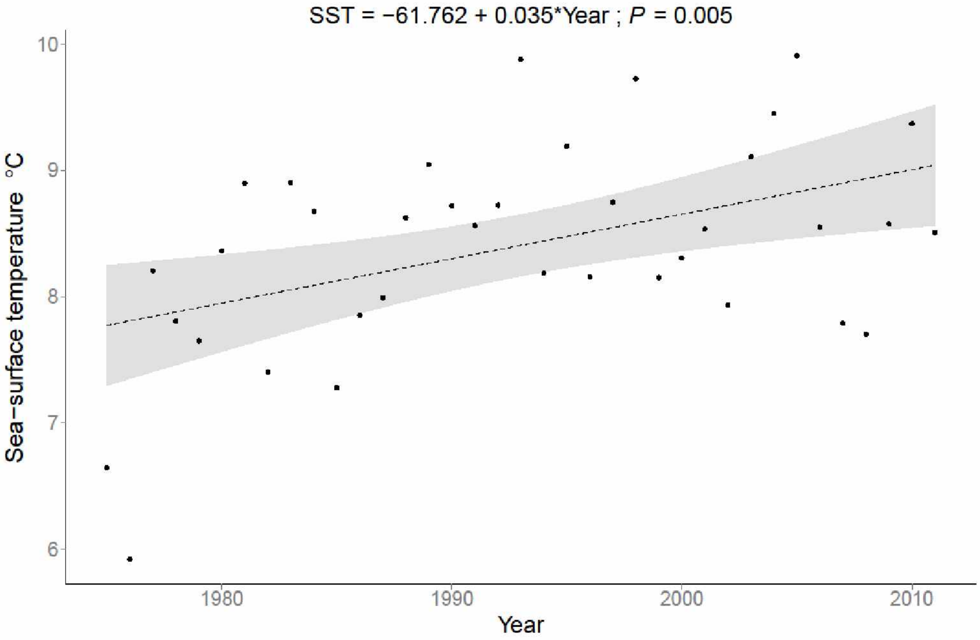

Figure 2.11 - Temporal trend in spring sea-surface temperature of Auke Bay (1975-2011). Sea-surface temperatures were averaged over the period of 1 March - 30 June. The shaded area depicts the 95% confidence interval.

Chapter 3 - Local adaptation of phenology revealed in out crosses between spawning segments of a salmonid population[1]

Abstract

Local adaptation has been demonstrated in spatially or temporally distant animal populations but seldom in proximate populations. To address the scale of local adaptation in Pacific salmon (*Oncorhynchus* spp.), two generations of hybrids between temporally separated spawning segments were made in a population of pink salmon (*O. gorbuscha*) and compared with controls to test for declines in marine survival that resulted from outbreeding depression and to evaluate the genetic architecture underlying adult migration time. Marine survival was generally similar between control and hybrid lines, which suggested that the effect of outbreeding upon marine survival was minimal at such a fine scale of genetic divergence. In contrast, Bayesian mixed-effects models revealed that adult migration times in hybrid lines were intermediate to those of controls and additive sources of genetic variation were significant, which indicated that these spawning segments exhibit additive genetic differences in migration time. Similarly, a line cross analysis revealed that an additive model best described the genetic architecture of adult migration time. The implications of these results are that (1) local adaptation can facilitate genetic divergence of life history traits between proximate subpopulations; (2) artificial relaxation of natural barriers to gene flow can cause maladaptive shifts in life history traits; and (3) wild populations may harbor fine-scale adaptive variation that supports productivity and sustainability.

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[1] Manhard, C.M., J.J. Hard, M.D. Adkison, W.W. Smoker, and A.J. Gharrett. Local adaptation of phenology revealed in out crosses between spawning segments of a salmonid population. Formatted for submission to *Molecular Ecology*.

Introduction

Genetic variation in breeding time has been demonstrated in populations spanning a wide range of taxa. Individuals within these populations often have life history traits that are locally adapted to environmental conditions characteristic of their breeding time (e.g. birds: Møller 2001; Sheldon *et al.* 2003; fish: Smoker *et al.* 1998; Hendry *et al.* 1999; Quinn *et al.* 2000; plants: Weis and Kossler 2004; Hall and Willis 2006). Fidelity of breeding time constrains gene flow between population segments, which facilitates genetic divergence through local adaptation, provided the effects of divergent selection exceed those of genetic drift and gene flow, and there is sufficient genetic variability in the traits under selection (Kawecki and Ebert 2004). By optimizing fitness in individuals that breed at different times, local adaptation staggers the use of resources over time, which may increase the carrying capacity of resource-limited populations (Gharrett *et al.* 2013). Additionally, local adaptation promotes genetic variation of phenology, which can enable populations to sustain productivity during climate changes (Greene *et al.* 2010; Schindler *et al.* 2010). Hence, local adaptation is likely an important component of the productivity and sustainability of populations that support genetic variation in breeding time.

Because of their characteristic ability to home to spawn in their natal habitat with high temporal precision and the wealth of life history variation that they exhibit over their extensive range, anadromous Pacific salmon have been the focus of many studies on local adaptation (Fraser *et al.* 2011). The high fidelity with which salmon typically home enables adaptation to local niches within heterogeneous ecological landscapes, while constraining gene flow between diverging salmon populations. This pattern of population divergence due to local adaptation is probably the primary means by which many genetically distinct populations have come to exist in each of the anadromous Pacific salmon species (Carvalho 1993). Indeed, local adaptation is well documented in salmonid populations separated by large geographic distances (Taylor 1991). However, with the use of stock transfers and hatchery propagation to enhance salmon fisheries or rehabilitate wild salmon populations, there is concern that introgression of non-adapted genetic material into wild populations will cause a decline in the number of locally adapted populations, thereby reducing diversity that is a crucial buffer against inexorable environmental changes (Waples 1991; Eldridge and Naish 2007; Naish *et al.* 2008). Furthermore, interbreeding between locally adapted populations can reduce fitness in hybrids, a phenomenon known as outbreeding depression (Lynch 1991; Gharrett *et al.* 1999; Edmands 1999; Gilk *et al.* 2004).

Outbreeding depression manifests through two different mechanisms that can occur independently or jointly. Outbreeding between populations from different local environments can depress fitness by disrupting fitness-related genotype-by-environment interactions (Edmands 2007). This mechanism, which is termed ecological outbreeding depression, acts on first and subsequent generations of hybrids (Lynch 1991). Alternatively, outbreeding between genetically isolated populations can disrupt complexes of genes at epistatic loci, leading to a decline in fitness. Epistatic gene complexes can arise through joint selection for multiple loci during local adaptation and random drift (Lynch 1991). Populations may evolve different epistatic gene complexes under similar selection pressures because random drift participates in determining the genetic material that is available for co-adaptation (Lenski and Travisano 1994) and because favorable allele combinations are maintained by natural selection. Epistatic outbreeding depression typically does not manifest until the second generation or later (Emlen 1991) because epistatic gene complexes are maintained in the gamete contributed by each parent.

Pink salmon that home to Auke Creek, Alaska provide an excellent model system for examining adaptive divergence between isolated populations. The strict 2-year anadromous life cycle of pink salmon (Anas 1959; Turner and Bilton 1968) has resulted in genetically distinct odd- and even-year brood lines in Auke Creek. In a study of two generations of hybrids between the even- and odd-brood lines, reduced survival relative to controls was observed in F_2 hybrids only, which indicated that outbreeding depression had resulted from disruption of epistatic gene complexes (Gharrett *et al.* 1999). Similarly, more pronounced outbreeding depressive effects were observed in the second generation of hybridization between pink salmon from Auke Creek and spatially distant (~1000 km) Pillar Creek, Kodiak, Alaska (Gilk *et al.* 2004). These studies demonstrated that large temporal and spatial barriers have enabled populations of salmon to diverge, most likely through some combination of local adaptation and genetic drift, and that removal of such barriers can have detrimental effects on overall fitness. However, there has been a dearth of investigations into fine-scale adaptive differences that may arise between proximate salmon populations.

This study attempts to provide insight into the scale at which local adaptation occurs by examining out crosses between pink salmon from temporally distinct subpopulations that spawn in the same habitat at Auke Creek, but do so at different times during a spawning season. Comparisons were made between controls and two generations of hybrids between these subpopulations in order to look for evidence of outbreeding effects on two traits that contribute to lifetime fitness: marine survival and adult migration time. While ecological outbreeding depression may be evident in F_1 hybrids, epistatic outbreeding depression does not usually occur until the second generation of hybridization. Hence, contrasts of marine survival between controls and hybrids over two generations provided the opportunity to investigate whether outbreeding has disrupted genotype-by-environment interactions or co-adapted gene complexes that have been structured by local adaptation. Also of interest was the degree of variability of outbreeding effects on marine survival among families. Higher variability of marine survival among families would be expected to increase the variance of family size. This is important because effective population size (N_e), a measure of the ability of a population to perpetuate its genetic variation into subsequent generations, generally decreases as variance of family size increases (Crow and Kimura 1970). To address this possibility, parentage analysis was used to estimate family-specific marine survival rates, and variation in those rates was compared between controls and hybrids. Adult migration time was compared between controls and hybrids within each generation of this experiment in order to determine whether this life history trait has been shaped by local adaptation to the seasonally distinct environmental regimes encountered by the early- and late-spawning segments. Because adult migration time is closely coupled with fitness in salmon (Dickerson *et al.* 2005; Scheuerell *et al.* 2009) and highly heritable in the Auke Creek pink salmon population (Smoker *et al.* 1998), hybrids may exhibit maladaptive shifts in this trait. In addition, a line cross analysis was performed in the second generation of this experiment in order to investigate the primary sources of genetic variation underlying adult migration time. The primary questions that these data addressed are (1) Does outbreeding depression result in reduced marine survival of F_1 or F_2 hybrids relative to their respective control lines? (2) Do variances of family-specific marine survival rates differ between control and hybrid lines? (3) Does outbreeding result in a shift in adult migration time, such that hybrids exhibit migration times intermediate to early- and late-spawning controls? and (4) To what extent does additive genetic variation contribute to adult migration time and are other sources of genetic variation (e.g. dominance) important?

Methods

Field methods

Auke Creek, a short (323 m) outlet steam of Auke Lake that drains into Auke Bay, is a spawning ground and migratory corridor of a temporally structured population of pink salmon. Genetically isolated even- and odd-year brood lines exist at Auke Creek, and within each brood line there are temporally separate early- and late-spawning segments whose peak migration dates have been separated historically by about two weeks (Taylor 2008). Located at the mouth of Auke Creek and at the head of tidewater are a permanent counting weir and experimental salmon hatchery, which are operated by the U.S. National Marine Fisheries Service. Early- and late-migrating pink salmon were collected at the weir and artificially spawned at the hatchery in the summers of 2005 and 2006 to create F_1 hybrid and control lines, which were propagated into the F_2 generation by artificially spawning returning F_1 progeny that were collected at the weir in 2007 and 2008. The breeding design was structured to enable the development rates of hybrids to be compared with those of early and late controls (Echave 2010). In order to minimize environmental effects, each control line was incubated in the same thermal regime as its hybrid counterpart; since early and late controls were spawned on separate days, this required the creation of separate early and late hybrid lines. The run source (i.e. early vs. late) of each experimental cross was defined by the run source of the female parent. For instance, an early-female by late-male cross was considered an early hybrid. The first generation breeding design was accomplished by using gametes collected from early-run males and females (22 August 2005; 3, 4, and 5 August 2006) to produce early controls in each brood year; semen collected from the early-run males in 2005 was also cryopreserved to create late hybrids in the odd-brood line with eggs collected from late-run females on 7 September 2005. We did not produce a late hybrid line in the even-brood line because cryopreservation of semen from early-run males in 2006 failed. We used cryopreserved semen collected from late-run males two brood years prior (11 September 2001; 6 and 9 September 2002) and eggs collected from early- and late-run females on the aforementioned dates to produce early hybrids and late controls in each brood year. Because of our failure to produce late hybrids in 2006, we did not release a corresponding late control line in the even-brood line.

Identical methods were used to rear first and second generation progeny. Each full-sib cross was equally divided between two randomly selected compartments within stacks of modified vertical incubation trays (FAL^{TM}; Marisource, Milton, WA). Control and hybrid embryos were incubated in separate stacks at the Auke Creek hatchery in ambient temperature water that was pumped from the creek and treated twice a week with dilute formalin (1:6000 in static water) for 1 hour to inhibit growth of fungus and bacteria. The source water was delivered to the stacks by a single PVC pipe, which was split into two separate pipes of approximately one foot in length just prior to entering the incubation stacks in order to ensure that embryos of controls and hybrids incubated in a common environment. Developing embryos were incubated until they were ~5% yolk by weight, whereupon the fish were anesthetized by immersion in an aqueous MS-222 (Tricaine Methanesulfonate) solution (100 mg/liter) for approximately 3 minutes. Anesthetized fish were immediately marked with an experiment-identifying adipose fin excision and contrasting pelvic fin excisions to distinguish controls from hybrids. Controls and hybrids were concurrently released into Auke Creek in April, at the peak of natural pink salmon outmigration, and all returning adults were collected at Auke Creek weir during late summer, as they migrated into the stream, and examined for the absence of an adipose or pelvic fin to determine if they belonged to one of our experimental lines. All marked fish were removed from the population, and unmarked fish were returned to Auke Creek above the weir. Tissue samples were obtained from each marked adult by clipping the axillary process at the base of the remaining pelvic fin. During the summers of 2007 and 2008, marked adults were tagged with numbered Floy^{TM} (Floy Tag Inc., Seattle, WA) tags and held captive until full maturity. A randomly selected sample of those fish was used as broodstock for the second generation crosses. Marked fish that were not chosen as broodstock were euthanized by cranial concussion followed by exsanguination.

Laboratory methods

Tissue samples from experimental broodstock and their returned progeny were stored in numbered vials of preservative solution (Seutin *et al.* 1991) and refrigerated at -22 °C. We isolated total genomic DNA with DNeasy Blood and Tissue kits (QIAGEN, Inc., Valencia, CA).

Five microsatellite loci (*Ogo1a* [Olsen *et al.* 1998]; *Oki10* [Smith *et al.* 1998]; *One102* [Olsen *et al.* 2000]; *One109* [Olsen *et al.* 2000]; and *OtsG311* [Williamson *et al.* 2002]) were chosen to unequivocally assign parental pairs to progeny. Amplification and separation of target fragments of microsatellite loci were accomplished with methods that are detailed in the supplementary materials.

Statistical methods

Parentage - Microsatellite genotype information was used to assign parental pairs to returning adults with PROBMAX (Version 1.2; Danzmann 1997), which uses exclusion analysis based on known parental mating combinations. Parentage assignment was confirmed when the genotype of an individual was consistent with those of a prospective parental pair for all five loci (10/10 alleles). In instances where a near perfect match (9/10 alleles) was observed between an individual and a pair of parents, an additional pair of microsatellite loci (*Ots103* [Small *et al.* 1998] and *Ots208* [Greig *et al.* 2003]) was used to confirm the assignment of those parents. Hence, a threshold of 13/14 alleles (92.9%) over seven loci was used for parentage assignment. Parentage information was used to confirm that returning adults belonged to our experiment, to determine the experimental line to which each returning adult fish belonged, and to assign fish to their respective full-sib families.

Marine survival - Log-linear analysis of first and second generation marine survival was conducted in SYSTAT (SYSTAT Software, Inc. 2004) to quantify interactions between run, cross, and survival. A statistical power analysis was conducted in R (R Core Team 2015) with the package 'pwr' to determine the minimum proportionate decrease in marine survival that would be needed to observe a statistically significant difference between controls and hybrids, given our release numbers.

Family-specific marine survival - A Bayesian hierarchical analysis was used to quantify family-specific marine survival in the first and second generation. The hierarchical framework has the advantage of enabling families with small release numbers to absorb some of the inferential strength of families in which many fry were released. In order to simplify the computations, the control and hybrid experimental lines in each brood year were treated as independent experiments, which were modeled separately. The computational load was further reduced by including only families in which 100 or more fry were released in the hierarchical models. The Bayesian approach produces posterior distributions of parameters of interest, which enables straightforward comparisons of parameters between experiments. Samples from the posterior distribution of each parameter were drawn with the Markov chain Monte Carlo (MCMC) algorithm, which was performed by using the package 'R2WinBUGS' (Sturtz *et al.* 2005) to call WinBUGS (Lunn *et al.* 2000) from R. The likelihood of observing y_{ij} returned adults from the i^{th} family and within the j^{th} experimental line followed a binomial distribution:

$$y_{ij} \sim Bin(p_{ij}, n_{ij})$$

where p_{ij} is the marine survival proportion of n_{ij} released fry from a family. The hierarchical framework specifies that the marine survival proportions of individual families are drawn from a common distribution that is specific to a given experimental line. This assumption is based on the idea that, within an experimental line, offspring from different families should exhibit marine proportions that are similar because of experimental treatment, but different because of environmental and genetic effects that contribute to marine survival. Specifically, the logit (i.e. log-odds ratio) of the survival proportion of each family (l_{ij}) followed a normal distribution:

$$l_{ij} = \log\left(\frac{p_{ij}}{1 - p_{ij}}\right)$$

$$l_{ij} \sim N(\mu_j, \tau_j)$$

where the hyperparameters that govern the distribution of the logits are the mean (μ_j) and the inverse (τ_j) of the variance, which WinBUGS accommodates as the variance parameter in normal distributions. Non-informative, yet proper priors were used for the hyperparameters μ_j and τ_j. A normal zero-mean prior with a large variance was used for μ_j, and a gamma prior with parameters that yield a large variance was used for τ_j:

$$\mu_j \sim N(\mu = 0,\ \sigma^2 = 1\ x\ 10^6)$$

$$\tau_j \sim Gam(\alpha = 0.001, \beta = 0.001)$$

The overall mean survival proportion of the j^{th} experimental line (p_j) and its standard deviation on the logit scale (σ_j), were calculated as functions of μ_j and τ_j:

$$p_j = \frac{\exp(\mu_j)}{1 + \exp(\mu_j)}$$

$$\sigma_j = \sqrt{1/\tau_j}$$

Comparisons of the posterior distributions of the parameter p_j among experimental lines provided a complement to the log-linear analysis. Because the parameter σ_j provided an index of variation in survival among families, comparisons of its posterior distributions among experimental lines enabled us to evaluate whether we could expect outbreeding to cause a decline in N_e. In order to determine an appropriate burn-in period, two separate chains with slightly different starting values were constructed for each model, and convergence was evaluated by the Gelman-Rubin convergence diagnostic $\hat{R}$ (Gelman and Rubin 1992), which compared variance within and between chains. A thinning interval of 10 was used for each model in order to reduce autocorrelation among posterior samples. Plots of posterior predictive probabilities, which were calculated for each family within each experimental line, were constructed to determine whether each hierarchical model adequately captured the variation in marine survival.

Adult migration time - Components of variation of adult migration time of first and second generation experimental lines were quantified with linear mixed-effects models under a Bayesian framework. This modeling framework was robust to our migration time data, which were unbalanced because of unequal marine survival among families. Additive genetic components of variation arising from covariance among siblings were estimated as random effects, while components of variation arising from experimental treatment (e.g. type of cross) were estimated as fixed effects. Samples from the posterior distribution of each effect were drawn with the MCMC algorithm, which was conducted in R with the package 'MCMCglmm' (Hadfield 2010). This analysis was performed separately for the early and late experiments in each generation in order to avoid confounding the effects of run source with those of cross. The adult migration data were comprised of incomplete and non-equivalent experimental blocks, which precluded reliable estimation of a block effect or of sire-by-dam interaction. Consequently, those terms were not included in the model. The linear mixed-effects model that describes all pertinent fixed and random effects on adult migration time within a brood year (2005, 2006, 2007, or 2008) and run (early or late) was:

$$y_{ijkl} = \mu + C_i + D_{ij} + S_{ik} + \varepsilon_{ijkl}$$

where y_{ijkl} is the Julian date of weir passage (i.e. migration time) of an individual. The overall mean migration time is μ, C_i is the fixed effect of the i^{th} cross (hybrid or control), D_{ij} is the random effect of the j^{th} dam within the i^{th} cross, S_{ik} is the random effect of the k^{th} sire within the i^{th} cross, and ε_{ijkl} is the residual random error associated with the l^{th} replicate of the j^{th} dam and k^{th} sire within the i^{th} cross. Non-informative, yet proper priors were used for the fixed and random effects. A normal, zero-mean prior with a large variance was selected for C_i:

$$C_i \sim N(\mu = 0,\ \sigma^2 = 1\, x\, 10^{10})$$

The inverse-gamma is the classical prior distribution for variance components, but this prior can cause inefficient sampling of the posterior distribution of small variances. To address this, a method known as parameter expansion (Gelman *et al.* 2008; Browne *et al.* 2009) was used to give more flexibility to the MCMC algorithm by partitioning each random effect R_t into two independent components:

$$R_t = \alpha \eta_t$$

$$\alpha \sim N(0, 1000)$$

$$\eta_t \sim N(0,\ \sigma_\eta^2)$$

$$\sigma_\eta^2 \sim InvGam(0.5, 0.5)$$

Each model was run for 500,000 iterations with a burn-in period of 20,000, and a thinning interval of 50 was used to reduce autocorrelation among posterior samples. Convergence was tested by comparing two chains that were run with different starting values, and posterior predictive probabilities were estimated in order to evaluate model performance. A useful feature of the MCMCglmm function is that it enables computations of the posterior distribution of functions of variance components. For instance, the proportion of variation that is attributable to covariance among maternal (P_D) and paternal (P_S) siblings can be calculated as functions of the posterior values of the variance components:

$$P_D = \frac{D_{ij}}{D_{ij} + S_{ik} + \varepsilon_{ijkl}}$$

$$P_S = \frac{S_{ik}}{D_{ij} + S_{ik} + \varepsilon_{ijkl}}$$

This gives the posterior distributions of meaningful parameters that describe how additive genetic components contribute to adult migration time. In each model, the statistical significance of the fixed effect of cross was evaluated simply by determining whether the 95% Bayesian credible interval of its posterior distribution included zero. However, since variance components are constrained by their prior distribution to exceed zero, the interpretation of their statistical significance was not as straightforward. One method of interpreting variance components is to use the deviance information criterion (DIC), which is a Bayesian metric that counterbalances the gain in inferential strength penalized against the added complexity of incorporating an additional model term. Since smaller DIC values are indicative of a better model fit, the difference in the DIC between a full model and one with the variance component of interest removed (ΔDIC) was calculated and included in the model summary. In general, a ΔDIC value of five is considered substantial, and a ΔDIC value of greater than ten is adequate to rule out the model with the higher DIC (Spiegelhalter *et al.* 2002).

Line cross analysis - Because returning F_1 fish carried a mark that denoted the type of cross but not the run source, some F_1 hybrids (early control x late control) were produced in the second generation of the odd-broodline experiment. Consequently, F_1 and F_2 hybrid lines were reared with early and late control lines that year (Supplementary Materials; Table S2). The existence of F_1 hybrids presented the opportunity to use a line cross analysis to evaluate the genetic architecture underlying adult migration time. Line cross analyses provide a framework for revealing the underlying genetic causal components of phenotypic differences between subpopulations for traits of interest. A line cross analysis is conducted by comparing experimentally reared lines consisting of differing fractions of genetic material from each of the subpopulations under observation. Adopting the nomenclature of Lynch and Walsh (1998), the early and late control lines are referred to as the parental lines (P_1 and P_2 respectively). The types of genetic parameters that can be estimated in a line cross analysis are determined by the number of lines, and the four lines present in the second generation of the odd-broodline experiment (P_1, P_2, F_1, and F_2) provide sufficient degrees of freedom to test for composite additive and dominance effects. The joint scaling procedure described in Lynch and Walsh (1998) was used to perform the line cross analysis. This procedure involves first testing the fit of a null model with additive effects only. If the additive model is rejected by a chi-square test, a higher order null model with additive and dominance effects can be tested. A statistical power analysis was performed for the null model with a bootstrap simulation. New data were simulated by drawing a new mean for each line from a normal distribution with mean and variance equal to the weighted mean of the line ($\bar{Z}_j$) and its standard error respectively; the null model was then re-fit with the new data. The statistical power was estimated as the percentage of times in which the null model was rejected over 10,000 iterations. The statistical power analysis was conducted over different scenarios in which the number of full-sib families present in each line ranged from 1 to 100.

Results

Log-linear analysis of marine survival - We captured 176 marked pink salmon from broodyear 2005 at the Auke Creek weir during the 2007 adult migration and 122 marked fish from broodyear 2006 during the 2008 migration. Parentage analysis conclusively linked 169 (96%) and 112 (92%) first generation progeny to parental pairs from 2005 and 2006 respectively. Type of cross (control or hybrid) was determined for each experimental fish, and all cross designations were concordant with documented fin excision marks. Based on the number of released fry and recovered adults (Supplementary Materials; Table S1), the marine survival proportions of experimental fish from broodyear 2005 (0.38%) and broodyear 2006 (0.32%) were each less than one tenth of the marine survival proportion of wild pink salmon fry from the same brood year. In broodyear 2005, marine survival was 0.12% lower in hybrids than in controls and 0.20% lower in late- than in early-run fish, and the log-linear analysis (Table 3.1) revealed significant interaction between survival and run source ($P = 0.001$), survival and type of cross ($P = 0.042$), and among survival, run source, and type of cross ($P = 0.046$). Conversely, marine survival was only 0.05% lower in hybrids than in controls from broodyear 2006, and the log-linear analysis did not reveal a significant interaction between survival and type of cross ($P = 0.465$).

Pink salmon adults from second generation crosses were captured at the Auke Creek weir during the summers of 2009 and 2010. During those migration periods we captured 826 and 552 marked adults from broodyears 2007 and 2008 respectively. Parentage analysis conclusively linked 803 (97%) and 541 (98%) F_2 progeny to parental pairs from 2007 and 2008, respectively. However, there was uncertainty regarding run source (early or late) of broodstock used for the second generation crosses, and some of the returned experimental fish from the first generation that were used as broodstock did not have distinct pelvic fin marks that were necessary to determine type of cross. Consequently, some crosses that were created in the second generation were not relevant to the log-linear analysis. In total, 606 and 521 experimental fish from broodyears 2007 and 2008 were used to analyze marine survival (Supplementary Materials; Table S2). The marine survival proportion of experimental fry from broodyear 2007 (1.53%) was approximately one fifth of that observed in wild fry, and in broodyear 2008 (1.39%) it was less than one tenth of that observed in wild fry. In broodyear 2007, marine survival was 0.14% higher in hybrids than in controls and 0.18% higher in late- than in early-run fish, but the log-linear analysis (Table 3.1) did not reveal significant interaction between survival and run source (P = 0.161), survival and type of cross (P = 0.270), or among survival, run source, and type of cross (P = 0.752). In broodyear 2008, marine survival was 0.13% lower in hybrids than in controls, but the log-linear analysis did not reveal a significant interaction between survival and type of cross (P = 0.383).

Hierarchical model of family survival - Family-specific marine survival proportions from 194 families from broodyear 2005 and 112 families from broodyear 2006 were used in the Bayesian hierarchical analysis of first generation marine survival (Table 3.2). The hierarchical models produced estimates of the posterior distributions of the mean survival proportion (p_j) and logit-scale deviation (σ_j) of each experimental line. The parameter p_j enabled comparisons of survival among experimental lines and therefore provided a complement to the log-linear analysis, while σ_j provided an index of among-family variance in marine survival. Comparisons of the posterior distributions of σ_j among experimental lines enabled us to evaluate whether we could expect outbreeding to cause a decline in N_e. In broodyear 2005, 95% Bayesian credible intervals (BCI) indicated that the marine survival percentage (p_j x 100) of early controls (0.45 – 0.77%) exceeded that of early hybrids (0.09 – 0.36%); although the mean survival percentage of late controls was lower than that of late hybrids, the large overlap in credible intervals (controls 0.08 – 0.32% vs. hybrids 0.12 – 0.60%) suggested that these two lines had similar survival percentages. In broodyear 2006, early controls (0.19 – 0.38%) and early hybrids (0.18 – 0.47%) had similar marine survival percentages. With the exception of the early control line from broodyear 2005, the posterior distributions of p_j overlapped substantially among all F_1 experimental lines. In broodyear 2005, the logit-scale standard deviation of marine survival was similar between early controls (0.03 – 0.63) and early hybrids (0.04 – 1.64), and between late controls (0.05 – 1.60) and late hybrids (0.03 – 1.75). Similarly, in broodyear 2006, the logit-scale standard deviation did not differ between early controls (0.11 – 1.08) and early hybrids (0.03 – 0.93).

In the second generation of the experiment, family-specific marine survival proportions from 65 families from broodyear 2007 and 69 families from broodyear 2008 were used in the Bayesian hierarchical analysis (Table 3.3). In broodyear 2007, Bayesian credible intervals indicated that mean marine survival percentages were similar between early controls (1.13 – 1.64%) and early hybrids (1.06 – 1.77%), and between late controls (1.01 – 2.18%) and late hybrids (1.13 – 1.89%). Similarly, in broodyear 2008, early controls (1.08 – 1.51%) and early hybrids (0.75 – 1.53%) had similar marine survival percentages. Collectively, the posterior distributions of p_j overlapped substantially among all F_2 experimental lines. In broodyear 2007, the logit-scale standard deviation of family-specific marine survival was similar between early controls (0.07 – 0.49) and early hybrids (0.03 – 0.61), and between late controls (0.03 – 0.76) and late hybrids (0.25 – 0.75). In broodyear 2008, early controls (0.25 – 0.61) and early hybrids (0.13 – 0.97) had similar logit-scale standard deviations.

Mixed-effects model of migration time - Data from 169 returned adults from broodyear 2005 and 112 adults from broodyear 2006 were used in the analysis of migration time of adults from the first generation of this experiment (Table 3.4). Samples (n = 9,600) were drawn from the posterior distributions of three parameters of interest: the fixed effect of cross (C_i) and the random effects of dam (D_{ij}) and sire (S_{ik}). In broodyear 2005, the mean posterior estimate of adult migration date was 3.6 days later in early hybrids relative to early controls, and 3.8 days earlier in late hybrids relative to late controls (Figure 3.1). Bayesian credible intervals (95%) indicated that the effect of cross was significant in both the early (0.8 – 6.5) and late (-6.2 – -1.5) experiments of this brood year. Similar results were observed in broodyear 2006 (Figure 3.1), where the mean posterior estimate of adult migration date was 10 days later in early hybrids relative to early controls, and the effect of cross was significant (6.6 – 13.6). Bayesian credible intervals of the random effects of dam and sire in both experiments from broodyear 2005 were broad and included zero, and only the inclusion of the dam term from the late experiment produced a moderate change in DIC (-6.7). In the early experiment from broodyear 2006, only the inclusion of the sire term produced a large change in DIC (-19.2), and the BCI of P_S (6.4 –

56.9%) suggested that the sire term explained a significant amount of the variation in migration time.

Data from 606 returned adults from broodyear 2007 and 521 adults from broodyear 2008 were used in the analysis of migration time of second generation adults (Table 3.5). As was done in the first generation, samples (n = 9,600) were drawn from the posterior distributions of C_i, D_{ij}, and S_{ik}. In broodyear 2007, the mean posterior estimate of adult migration date was 4.4 days later in early hybrids relative to early controls and 4.9 days earlier in late hybrids relative to late controls (Figure 3.2). Bayesian credible intervals indicated that the effect of cross was significant in both the early (1.6 – 7.5) and late (-9.7 – -0.3) experiments. Similar results were observed in broodyear 2008 (Figure 3.2), where the mean posterior estimate of adult migration date was 7.4 days later in early hybrids relative to early controls, and the effect of cross was significant (4.8 – 10.1). In broodyear 2007, large changes in DIC accompanied the inclusion of the dam term in the early experiment (-11.1) and the sire term in the late experiment (-14.4), and the BCI of P_D in the early experiment (2.0 – 28.9%) and P_S in the late experiment (2.8 – 32.8%) suggested that these terms explained a significant amount of the variation in migration time. In the early experiment of broodyear 2006, only the inclusion of the sire term produced a large change in DIC (-9.3); the BCI of V_s, however, had a lower limit close to zero (0.0 – 29.9%).

Line cross analysis - A line cross analysis was conducted to evaluate migration time of adults from the second generation odd-broodline experiment. The number of families that had at least one returning adult ranged from 6 in the P_2 line to 37 in the F_2 line. The weighted mean Julian dates of migration in the F_1 and F_2 lines were similar and approximately intermediate to those of the P_1 and P_2 lines (Supplementary Materials; Table S3). The estimated additive composite effect from the additive null model was significant ($\widehat{\alpha_1^c}$ = -3.460; SE = 0.537). The chi-square test of the additive null model was not significant (P = 0.352) and, hence, a higher order model that incorporated directional dominance was not tested.

Discussion

Local adaptation is recognized as an important driver of genetic divergence between populations, but the spatial and temporal scale at which it occurs has not been thoroughly investigated. To address this, we looked for evidence of local adaptation in two generations of experimental hybrid lines created by outbreeding pink salmon from early- and late-spawning subpopulations that home to Auke Creek, Alaska. Specifically, we looked for evidence of outbreeding effects in hybrid lines by examining two components of lifetime fitness: marine survival and adult migration time. Adult migration time, which would be expected to be optimized to the different environmental regimes encountered by early- and late-spawning fish, is probably closely related to reproductive success in this population (Fukushima *et al.* 1998; Smoker *et al.* 1998; Gharrett *et al.* 2013). Control lines, created by crossing individuals from the same spawning segment, served as a reference from which outbreeding effects could be resolved. The magnitude and pattern of outbreeding effects across two generations of hybridization provided an indicator of the genetic architecture underlying local adaptive differences between these subpopulations.

Marine survival - Reduced survival of experimental fish relative to wild fish was observed in both generations, which was consistent with hatchery and marking effects observed in prior experiments at Auke Creek (e.g. Lane *et al.* 1990). Various aspects of the culturing process might adversely impact developing fry and consequently reduce survival in the marine environment. In addition, the comparatively benign hatchery environment may favor survival to emigration of many embryos that would have perished during exposure to the harsher natural environment. Hence, delayed mortality may reduce marine survival. To account for this, controls and hybrids were reared under identical incubation conditions and released at the same time, which gave each fish an equal opportunity to prepare for the marine environment. Therefore, any discrepancies in marine survival rates between controls and hybrids were most likely the result of differential performance in the marine environment rather than experimental treatment.

The log-linear analysis of marine survival revealed that marine survival of even-year broodline F_1 hybrids between early- and late-run pink salmon was similar to that of controls. The power of this test, however, was low (0.11). An F_1 hybrid marine survival rate of 0.17%, approximately 0.6 of that observed, would be required for this test to attain a power of 0.8. In contrast, marine survival of F_1 hybrids was significantly lower than controls in the odd-year brood line. The reason for this unexpected result appears to be the anomalously high marine survival of early controls from broodyear 2005. Consistent with this, the posterior distributions of line-specific survival, which were estimated as part of the Bayesian hierarchical model of family survival, had considerable overlap among five of the six experimental lines from the first generation; only the early controls from the odd-year brood line exhibited positive divergence (Tables 3.2, 3.3). The mechanisms contributing to the anomalously high marine survival rate in this experimental line are unknown and, although considerable care was taken to eliminate systematic differences in survival arising from experimental treatment, the possibility that experimental biases underlie this observation cannot be eliminated. The lack of similarly high marine survival in the two other control lines, relative to their hybrid counterparts, suggests that we have no reasonable basis for concluding that one generation of outbreeding between the early and late subpopulations influenced the likelihood of surviving the marine stage. This is not a surprising result, given that previous studies conducted on local adaptation in highly segregated pink salmon populations revealed that depression of marine survival generally did not occur in the first generation of hybridization (Gharrett *et al.* 1999; Gilk *et al.* 2004). Furthermore, although early- and late-run juveniles transitioning to the nearshore marine environment generally encounter seasonal differences in growth conditions and predator abundance (Mortensen *et al.* 2000), differences in traits that confer adaptation to the marine environment have not yet been characterized in these subpopulations. Hence, there is no theoretical basis to support the potential for ecological outbreeding depression of marine survival, and this is the mechanism that would be most likely to cause depression of this trait in first generation hybrids.

In the second generation experiments, the log-linear analysis did not reveal a significant difference in marine survival between controls and F_2 hybrids in either the odd- or even-year brood line but, once again, the statistical power was low. In order to attain a power of 0.8, an F_2 hybrid marine survival rate of approximately 1% would have been required in both brood lines; this value was only 0.63 and 0.77 of that observed in the odd- and even-year brood lines respectively. Similar to the first generation, the posterior distributions of line-specific marine survival from the Bayesian hierarchical models had extensive overlap among all six second-generation experimental lines. Collectively, the results of these analyses did not provide evidence of effects of outbreeding on marine survival in F_2 hybrids. These results contrast those of experiments on populations of pink salmon separated by large temporal or spatial barriers to gene flow, which revealed significantly lower marine survival in F_2 hybrids than in controls (Gharrett *et al.* 1999; Gilk *et al.* 2004). Those experiments detected reduced survival almost exclusively in the second generation, which suggested that outbreeding had disrupted local adaptation primarily by the segregation of co-adapted gene complexes (i.e. epistatic outbreeding depression). This hypothesis was particularly well supported in the experiment that examined hybridization between the even- and odd-year brood lines; because individuals from the two brood lines spawn in the same habitat and are likely to encounter, on average, similar environmental conditions, there was little reason to expect hybridization to disrupt genotype-by-environment interactions. However, without regular exchange of migrants, natural selection and genetic drift may drive the formation of distinct co-adapted gene complexes within those isolated populations. Conversely, there is opportunity for interbreeding between the early- and late-spawning segments of Auke Creek, depending on annual environmental characteristics (e.g. stream flow and temperature) that determine the date of creek entry of adults. Regular exchange of migrants probably constrains the development of distinct co-adapted gene complexes (Edmands 1999), which could explain why experiments that examined local adaptation at large scales of genetic isolation yielded results that differed from those of this experiment, which examined local adaptation at a fine scale.

Family survival - Although two generations of outbreeding did not produce evidence of differences in family survival between controls and hybrids, outbreeding between spawning segments may still have an impact on population viability if outbreeding effects manifest differently among hybrid families. That is, if marine survival of some families is impacted by outbreeding more so than others, an increase in variance of family size and a corresponding decline in N_e could occur. To address this, posterior inference of family-specific marine survival proportions was made within the framework of the previously outlined Bayesian hierarchical models. The Bayesian hierarchical models of first- and second-generation marine survival indicated that, while some brood years appeared to display more variability in family survival than others, there was no apparent association between variability in family survival and type of cross (i.e. control or hybrid). This observation was supported by the posterior distributions of an index of among-family variance in marine survival (σ_j), which had considerable overlap among experimental lines. Hence, the results of this analysis did not provide any evidence of increased variance of marine survival in hybrids between the early- and late-spawning segments.

Adult migration time - Adult migration time was examined over two generations of out crosses to look for evidence of local adaptive differences between early- and late-run pink salmon. Analysis of causal components underlying variation in adult migration time was accomplished with Bayesian mixed-effects models. The results of the first and second generation analyses were consistent; the posterior distributions of migration time of controls and hybrids were distinct in each of the six contrasts. Since the families from each experiment were reared in a common environment, these results indicate that there is a genetic basis for the differences in adult migration time between controls and hybrids. In both generations, the pattern of migration time observed among different crosses was consistent with initial expectations based on local adaptation of this trait: hybrids returned to Auke Creek later than controls in the early experiment of both brood lines, and hybrids returned earlier than controls in the late experiment of the odd-year brood line (Figures 3.1, 3.2). Sire and dam components of variance were more pronounced in the second generation than in the first; three out of the six variance components that were estimated in the second generation experiments accounted for a significant amount of phenotypic variation in migration time, indicating that additive genetic effects contribute to variation in this trait. The discrepancies between generations probably resulted from differences in sample size. Poor marine survival of first generation fish resulted in few returned adults (Supplementary Materials; Table S1). Consequently, the analysis of first generation migration time was based on relatively few observations. This was evident in the Bayesian credible intervals (BCI) of the dam and sire components in the first generation, which were generally broad (Table 3.4), thereby reflecting low statistical certainty in the estimates of these variance components. For example, the BCI of the percentage of phenotypic variation explained by covariance among paternal siblings (P_S) ranged from near zero to over 50% in broodyear 2006. This is not surprising, given that estimates of additive genetic effects are often associated with high standard errors, which may require many observations to compress them (Falconer and Mackay 1996). In contrast to the first generation, higher marine survival rates in the second generation resulted in more returned adults (Supplementary Materials; Table S2), and there were more observations available for estimating additive genetic variance components. This was evident in the fact that BCIs for these variance components were generally narrower in the second generation than in the first (Table 3.5).

Our results demonstrate intermediate phenotypic expression of adult migration time in hybrids relative to controls over two generations of outbreeding and indicate that there is a strong genetic basis for this trait. Furthermore, the pattern of trait expression was consistent with genes influencing migration time in an additive manner. This is reinforced by the fact that, when large numbers of observations were available, significant dam and sire components of variation in this trait were detected. The line cross analysis provided further support of this observation by demonstrating that significant additive composite effects contribute to adult migration time and suggesting that a model with only additive effects was sufficient to explain the genetic architecture underlying this trait. However, the power of a line cross analysis is, in part, a function of the number of families that are included in the experimental design. This experiment was not designed for a line cross analysis, and some of the experimental lines had few representative families (e.g. P_2 and F_1). Consequently, the standard errors of the weighted mean migration times were high in those lines (Supplementary Materials; Table S3). Indeed, a statistical power analysis revealed that the power of the chi-square test of the null additive model was only modest (0.41). This means that, even if directional dominance effects contribute to this trait, the experimental design only yielded a moderate chance of detecting them. The use of a balanced design with the same average number of families per line would have increased the power to 0.5. In order to attain a statistical power of 0.8, the average number of families per line would have had to have been nearly doubled.

Because optimal adult migration time is probably involved in maximizing reproductive success in this population (Gharrett *et al.* 2013), the observed shift in this trait may have provided the basis for ecological outbreeding depression. A significant source of mortality in developing pink salmon embryos is mechanical agitation resulting from redd disturbance by subsequent spawners (Fukushima *et al.* 1998). The bimodal migration distribution of Auke Creek pink salmon enables early-run embryos to develop to a mechanically-resistant developmental stage known as epiboly (Ballard 1973) before the arrival of late-run spawners about two weeks later, thereby reducing mortality from redd disturbance (Smoker *et al.* 1998). The adaptive significance of embryonic development rate is supported by common-garden experiments that have revealed that early- and late-run embryos exhibited genetically-based differences in development patterns (Hebert *et al.* 1998) and that early-run embryos completed epiboly faster than late-run embryos (Joyce 1986). Moreover, early-run embryos from the latter experiment required approximately two weeks to complete epiboly, which is consistent with the two weeks that have historically separated the peaks of the early- and late-spawning segments; the implication is that the embryonic development trajectory is adapted to the time of egg deposition in the early-spawning subpopulation. Hence, our demonstration of intermediate migration date and, by extension, intermediate egg deposition time in hybrids suggests that outbreeding between these spawning segments can disrupt local adaptation by rendering hybrid embryos more prone to mortality from superimposition by late-spawning adults.

Our results complement those of a study on adaptation of embryonic development time in first generation hybrids between early- and late-run pink salmon (Echave 2010) which was conducted as part of the same experiment. That study demonstrated that embryonic development time differed between controls and hybrids in a significant, compensatory way. Furthermore, although dam and sire components of variation of development time were significant, their interactions were not, which indicated that adaptation of this trait has primarily exploited additive genetic variation. Early-run fish appear to compensate for higher water temperatures during incubation by slowing their development rate relative to late-run fish after reaching epiboly (Joyce 1986; Hebert *et al.* 1998); this compensation mechanism results in delayed emergence of early-run fry and a shortened gap in migration time between early- and late-run fry. Delayed emergence may provide an adaptive advantage for early-run fry by synchronizing their transition to the nearshore environment with more favorable growth conditions, and a shortened temporal gap between early- and late-migrating fry may provide beneficial effects of predator saturation.

Conclusions

The results of previous research on Auke Creek pink salmon, when considered alongside those presented here, provide a lucid demonstration of local adaptation of migration and embryonic development time to the seasonally distinct environments encountered by the early- and late- spawning segments. The implications are that fine-scale genetic structure has an important influence on the mean fitness level in this population, which make it unique to the outbreeding experiments conducted at Auke Creek. Furthermore, since spawning habitat limits the number of progeny that can be produced within the short and narrow confines of Auke

Creek, genetically-determined temporal structure likely enhances the carrying capacity of each brood line by staggering the use of this resource over time (Smoker *et al.* 1998; Gharrett *et al.* 2013). Hence, it is likely that erosion of the temporal barrier that separates these spawning segments would cause decreased productivity of this population. Similar temporal structure is likely to be an important component of the population dynamics of other animal populations in which breeding habitat is limited. Fine-scale local adaptation may also promote and maintain biodiversity that enhances the ability of populations to sustain productivity as the climate changes. This hypothesis is supported by studies that have demonstrated how adaptive variation of life history traits confers resilience to climatic fluctuations by enhancing the likelihood that some individuals within a population will carry traits that are well-suited to future environmental regimes (Hilborn *et al.* 2003; Greene *et al.* 2010; Schindler *et al.* 2010). Adaptive variation is often impossible to resolve without genetic analyses, yet failure to maintain it could be detrimental to the productivity, biodiversity, and sustainability of wild populations. Our results suggest that prudent management of wild populations should be conducted not only with regard for genetic and phenotypic variation that arises from isolation of populations by great distance or time, but also fine-scale variation.

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Tables

Table 3.1 - Log-linear analysis of marine survival in outbred first- and second-generation pink salmon. Separate analyses were performed for the odd- and even-year brood lines in each generation. Chi-square tests of independence were used to test for interactions of three terms: type of cross, run source, and marine survival rate.

	Interaction								
	Cross * Survival			Run * Survival			Cross * Run * Survival		
Brood year	χ^2	df	*P*	χ^2	df	*P*	χ^2	df	*P*
2005	4.146	1	0.042	11.538	1	0.001	3.972	1	0.046
2006	0.530	1	0.465						
2007	1.98	1	0.161	1.21	1	0.270	0.10	1	0.752
2008	0.781	1	0.383						

Table 3.2 - Bayesian hierarchical analysis of family-specific marine survival of outbred first-generation pink salmon. Posterior means, 95% Bayesian credible intervals (BCI), and the effective number of posterior samples are listed for two parameters: mean survival proportion (p_j) and the logit-scale standard deviation of marine survival (σ_j) of the j^{th} experiment line.

			p_j				σ_j			
Line	Brood year	No. fam.	Mean	BCI (2.5%)	BCI (97.5%)	Sample	Mean	BCI (2.5%)	BCI (97.5%)	Sample
Early control	2005	66	0.0060	0.0045	0.0077	30000	0.2109	0.0281	0.6329	3200
Early hybrid	2005	42	0.0022	0.0009	0.0036	4700	0.7054	0.0439	1.6430	4300
Late control	2005	60	0.0019	0.0008	0.0032	10000	0.7740	0.0522	1.5960	61000
Late hybrid	2005	26	0.0035	0.0012	0.0060	100000	0.5614	0.0333	1.7530	12000
Early control	2006	69	0.0028	0.0019	0.0038	16000	0.6428	0.1083	1.0830	8700
Early hybrid	2006	43	0.0032	0.0018	0.0047	27000	0.2850	0.0286	0.9312	10000

Table 3.3 - Bayesian hierarchical analysis of family-specific marine survival of outbred second-generation pink salmon. Posterior means, 95% Bayesian credible intervals (BCI), and the effective number of posterior samples are listed for two parameters: mean survival proportion (p_j) and the logit-scale standard deviation of marine survival (σ_j) of the j^{th} experiment line.

			p_j				σ_j			
Line	Brood year	No. fam.	Mean	BCI (2.5%)	BCI (97.5%)	Sample	Mean	BCI (2.5%)	BCI (97.5%)	Sample
Early control	2007	22	0.0138	0.0113	0.0164	10000	0.2732	0.0692	0.4909	9100
Early hybrid	2007	15	0.0142	0.0106	0.0177	3500	0.2198	0.0302	0.6075	7400
Late control	2007	6	0.0153	0.0101	0.0218	10000	0.2099	0.0271	0.7643	10000
Late hybrid	2007	22	0.0150	0.0113	0.0189	1900	0.4666	0.2484	0.7504	10000
Early control	2008	49	0.0130	0.0108	0.0151	10000	0.4132	0.2481	0.6069	2300
Early hybrid	2008	20	0.0114	0.0075	0.0153	10000	0.5012	0.1254	0.9731	7800

Table 3.4 - Bayesian mixed models of adult migration time of outbred first-generation pink salmon. The effective number of samples from the posterior distribution and 95% Bayesian credible intervals (BCI) are listed for the fixed effect of cross (C_i), the random effects of dam (D_{ij}) and sire (S_{ik}), and the proportion of variation explained by covariance among maternal ($\boldsymbol{P_D}$) and paternal ($\boldsymbol{P_S}$) siblings. Delta DIC values, representing the change in DIC accompanying inclusion of a term, are listed for each random effect.

	Broodyear 2005 (early experiment)				Broodyear 2005 (late experiment)				Broodyear 2006 (early experiment)			
Term	BCI (2.5%)	BCI (97.5%)	Sample	ΔDIC	BCI (2.5%)	BCI (97.5%)	Sample	ΔDIC	BCI (2.5%)	BCI (97.5%)	Sample	ΔDIC
C_i	0.796	6.468	9600		-6.175	-1.455	9600		6.662	13.597	9600	
D_{ij}	0.000	13.897	9600	0.184	0.000	12.691	9600	-6.627	0.000	9.198	9260	-1.292
S_{ik}	0.000	16.198	9600	-1.672	0.000	5.712	9600	-0.507	0.000	27.381	9266	-19.177
P_D	0.000	0.253	9600		0.000	0.570	9600		0.000	0.206	9241	
P_S	0.000	0.307	9582		0.000	0.304	9600		0.064	0.569	9323	

Table 3.5 - Bayesian mixed models of adult migration time of outbred second-generation pink salmon. The effective number of samples from the posterior distribution and 95% Bayesian credible intervals (BCI) are listed for the fixed effect of cross (C_i), the random effects of dam (D_{ij}) and sire (S_{ik}), and the proportion of variation explained by covariance among maternal ($\boldsymbol{P_D}$) and paternal ($\boldsymbol{P_S}$) siblings. Delta DIC values, representing the change in DIC accompanying inclusion of a term, are listed for each random effect.

	Broodyear 2007 (early experiment)				Broodyear 2007 (late experiment)				Broodyear 2008 (early experiment)			
Term	BCI (2.5%)	BCI (97.5%)	Sample	ΔDIC	BCI (2.5%)	BCI (97.5%)	Sample	ΔDIC	BCI (2.5%)	BCI (97.5%)	Sample	ΔDIC
C_i	1.560	7.487	9600		-9.723	-0.274	9600		4.844	10.074	9600	
D_{ij}	0.447	13.416	9600	-11.090	0.000	9.124	9600	0.898	0.000	1.623	8960	1.005
S_{ik}	0.000	5.835	9052	0.614	0.980	25.870	9600	-14.439	0.000	9.718	9227	-9.277
P_D	0.020	0.289	9600		0.000	0.128	9600		0.000	0.062	9286	
P_S	0.000	0.134	9600		0.028	0.328	9600		0.000	0.299	9177	

Figures

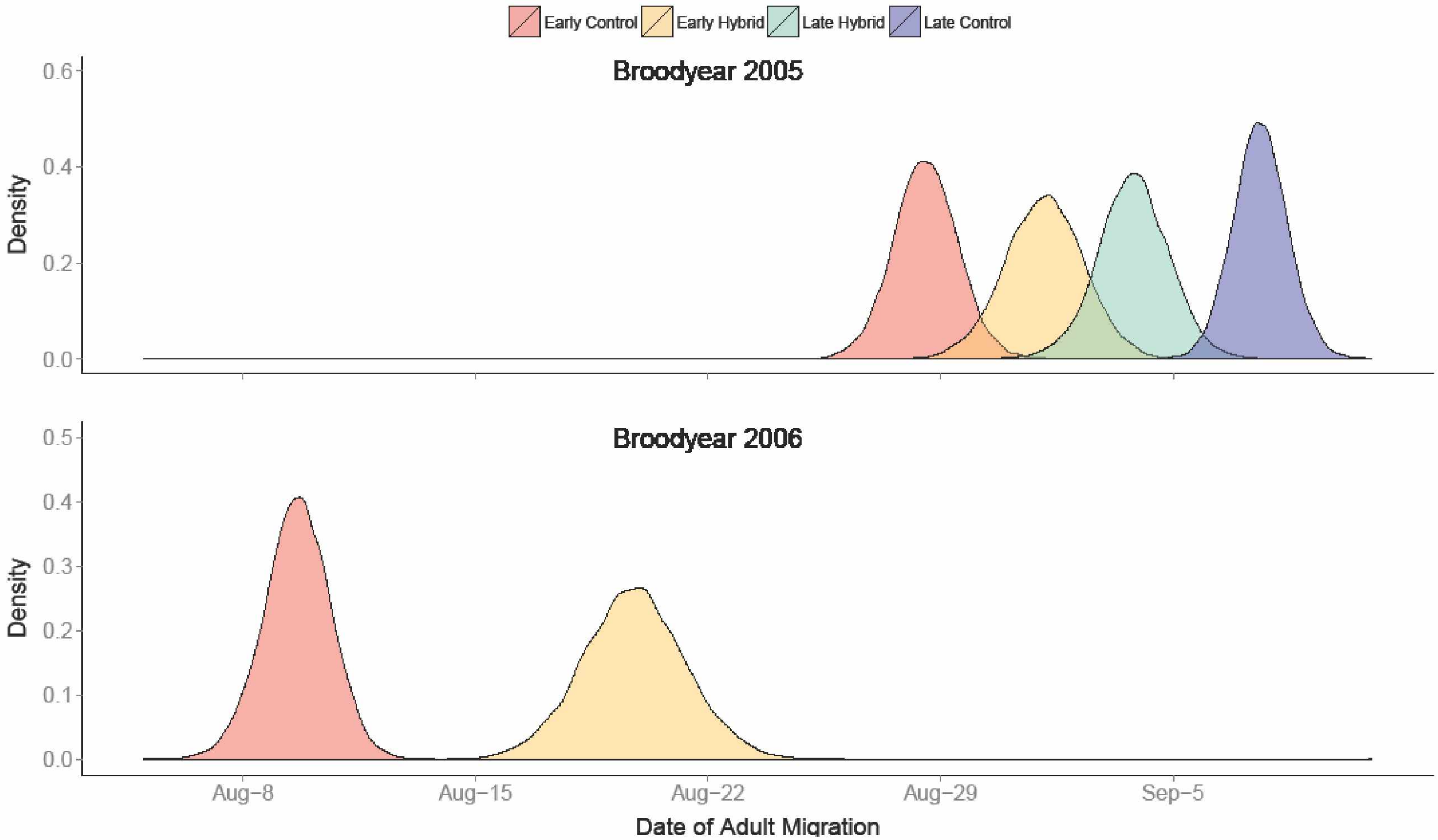

Figure 3.1 - Posterior distributions of mean adult migration time in experimental lines of outbred first-generation Auke Creek pink salmon in the odd- and even-year brood lines. Posterior samples ($n = 9{,}600$) were obtained under a Bayesian mixed model framework.

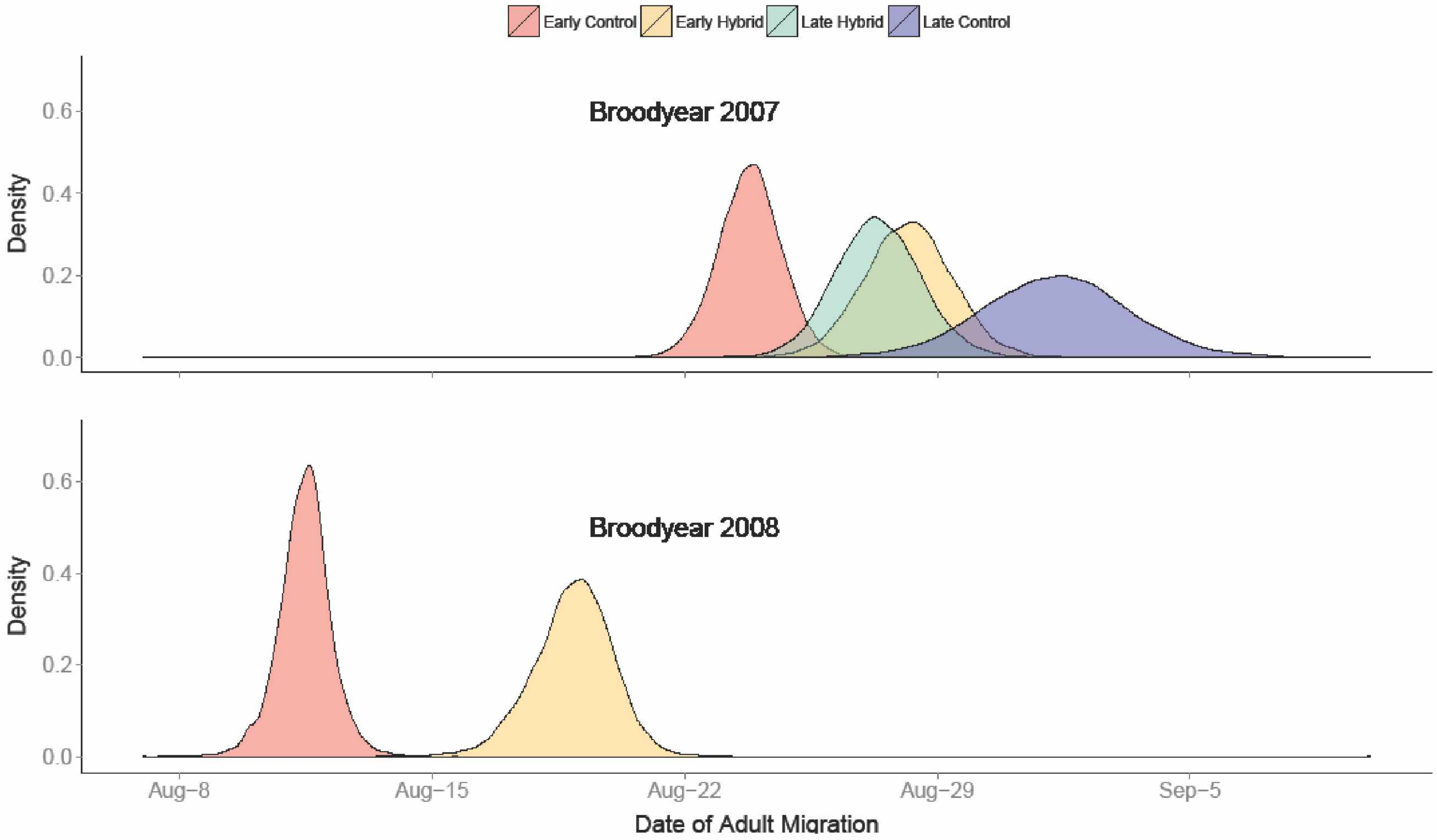

Figure 3.2 - Posterior distributions of mean adult migration time in experimental lines of outbred second-generation Auke Creek pink salmon in the odd- and even-year brood lines. Posterior samples (*n* = 9,600) were obtained under a Bayesian mixed model framework

Conclusions

Introduction

The overall purpose of this research was to describe the scale of local adaptation by observing seasonally structured brood lines of Pink Salmon that spawn in Auke Creek, Alaska. My specific objectives were (1) to identify ecological components of lifetime fitness, (2) to establish an ecological mechanism of contemporary evolution of migration time, and (3) to evaluate the consequences of disrupting fine-scale local adaptation of migration time. In Chapter 1, I used 29 years of local environmental data and censuses of adults and juveniles to identify ecological factors that underlie fitness in the freshwater and marine environments. In Chapter 2, I integrated observations of an experimentally imposed genetic marker in juveniles with previous observations made on adults in order to identify the life history stage at which evolution of migration time occurred and to propose an ecological mechanism for it. In Chapter 3, I used quantitative genetic analyses of migration time in hybrids between early- and late-run Pink Salmon to examine the genetic architecture of migration time and to demonstrate the consequences of relaxing barriers to gene flow. Collectively, these three chapters address each of my research objectives.

Ecological components of lifetime fitness

Chapter 1 provided evidence that compensatory processes related to spawner densities regulate the production of Pink Salmon fry in Auke Creek. Several of these processes, including egg retention arising from competition for spawning substrate and egg mortality resulting from redd disturbances, have been empirically demonstrated in Auke Creek (Fukushima and Smoker 1997; Fukushima et al. 1998). Local adaptation to seasonal differences in spawner densities is evident in the distinct life history traits that are displayed by early- and late-run Pink Salmon, which include stream life and development to epiboly (Joyce 1986; Fukushima and Smoker 1997). These life history adaptations have likely enabled early- and late-run fish to exploit seasonally distinct niches, thereby staggering the adult migration over time and enhancing the aggregate productivity of Auke Creek pink salmon (Gharrett et al. 2013).

Several of the most parsimonious models of freshwater productivity included indices of water quality, but the coefficient estimates for those indices had broad standard errors and, in some cases, had signs that were not consistent with expectations. To account for my inability to detect strong effects of water quality I proposed that, in years of poor stream conditions, losses of freshwater productivity in early migrating fish may be compensated for by gains in freshwater productivity in later migrating fish that encounter more unseeded spawning habitat. Some support for this hypothesis was provided in Chapter 2, where I observed that an early pulse of spawners entered Auke Creek in 1989 during a period when stream temperatures were anomalously high (~16.5 °C). A delayed and truncated outmigration of early-run fry was observed the following spring and indicated that the early pulse of spawners produced few offspring. Despite the apparent failed reproduction of a large number of very early-run spawners that summer, the early run of 1989 produced more fry per spawner than in the previous two years, in which stream temperatures were lower but the adult migration distributions were more compressed. These observations provide support for the hypothesis that the staggering of spawning activity over time increases fry production in this small stream and buffers against fluctuations in environmental conditions.

In contrast to the freshwater phase, there was little evidence of density-dependent processes operating in the marine phase. Although several of the most parsimonious models provided evidence that early marine survival was adversely affected by higher abundances of Dolly Varden charr and Coho Salmon smolts, survival was more closely coupled with environmental factors in the nearshore environment that condition the vulnerability of fry to predators. Consistent with expectations, sea-surface temperatures in the nearshore environment had a substantial influence on marine survival. Sea-surface temperature likely influences growth of juvenile salmon through its direct effects on the physiology of developing fry (Mortensen et al. 1991) and by modulating the growth and abundance of their planktonic prey (Bienfang and Ziemann 1995). This suggests that, because cool sea-surface temperatures and associated low prey abundance are characteristic of Auke Bay in early spring (Mortensen et al. 2000), fry that emerge after vernal warming has commenced should benefit from increased fitness. This idea is supported by the fact that early-spawned embryos exhibit developmental compensation, a genetically determined trait which buffers embryos against warm stream temperatures and increases the likelihood that fry emergence is coincident with vernal warming (Hebert et al. 1998). However, directional selection would likely oppose the persistence of extremely early migration times that result in embryos being exposed to stream conditions in excess of their compensatory scope.

An unanticipated observation in Chapter 1 was the negative relationship between marine survival and stream flows late in the juvenile outmigration period. While the mechanism underlying this relationship remains unclear, it is likely that this period of highly variable stream flows has important implications for the ecology of Pink Salmon fry early in their marine residency. Peak stream flows from Auke Creek are typically observed in late May and early June, which is a period that coincides with the movement of fry from nearshore to offshore waters (Mortensen 2000), and it is possible that heightened stream flows drive this offshore migration. The results of Chapter 1 suggest that late-outmigrating fry that encounter high stream flows generally experience poor survival and, consequently, directional selection may oppose the persistence of extremely late migration times.

The models of lifetime fitness ignored the temporal structure that exists between brood lines and spawning segments in Auke Creek. Given that both brood lines experience, on average, similar environmental conditions, there was little reason to expect that their ecology would systemically differ, so they were modeled together in order to increase the power of these analyses. The early- and late-spawning segments, conversely, encounter different ecological challenges due to their distinct life history schedules. Although it was beyond the scope of the available data, separate models of these spawning segments might have yielded insight into run-specific components of lifetime fitness. As noted before, investigations of seasonal variation in freshwater productivity have demonstrated that compensatory processes have a greater influence on early-spawning fish (Fukushima and Smoker 1997; Fukushima et al. 1998). An important implication of this is that freshwater productivity of early-spawning fish should be generally lower than that of late-spawning fish. This idea was supported by the results of the stock separation algorithm in Chapter 2, which demonstrated that freshwater productivity of early-run fish was substantially lower in three out of the four years of genetic monitoring. Conversely, in the marine environment, there are countervailing environmental factors that must be considered in comparing fitness between spawning segments. Early-outmigrating fry typically encounter cooler water temperatures and reduced zooplankton biomass, but lower predator abundances. Late-outmigrating fry, which typically enter Auke Bay in advance of substantial increases in predator abundance, likely encounter more favorable sea-surface temperatures, but experience a truncated marine growth period prior to the arrival of migratory predators. The net effect of this is that late-run fry are smaller than their early-run counterparts throughout their nearshore residence period (Mortensen et al. 2000). This suggests that early-outmigrating fry should benefit from reduced size-selective mortality and generally exhibit higher marine survival. Support for this idea was provided by the results of the stock separation algorithm from Chapter 2, which demonstrated that marine survival of early-run fish was substantially higher in all four years of genetic monitoring. Hence, it is likely that these life history strategies involve tradeoffs between freshwater and marine productivity. Furthermore, the existence of distinct lifetime fitness components in these spawning segments would likely buffer against productivity losses resulting from periodic environmental fluctuations and create the potential for evolutionary responses to persistent climatic shifts.

Contemporary evolution of migration time

Previous observations of an experimentally imposed genetic marker in adult Auke Creek Pink Salmon demonstrated that natural selection against late-migrating fish caused microevolution of migration time in the odd-year brood line. Furthermore, the demographic changes that resulted from this selection event, which were first evident in broodyear 1989, have persisted over at least thirteen generations (Kovach et al. 2012). In Chapter 2, I extended that research by using observations of this genetic marker in outmigrating fry to demonstrate that the decline in the overall frequency of the late-marker allele was first observed in adults that returned to Auke Creek in 1991. This suggested that an evolutionary event occurred during the marine phase. Furthermore, since genetic drift (Kovach et al. 2012) and interpopulation gene flow (Gharrett et al. 2001; Mortensen et al. 2002) were not sufficiently strong to cause the observed genetic changes, I concluded that natural selection was the most probable agent of evolution. Additional support for this hypothesis came from the observation that estimated marine survival of early-run fry was more than ten times higher than that of late-run fry that outmigrated from Auke Creek in spring of 1990.

Plots of the migration distributions of early- and late-run fry and sea-surface temperatures of Auke Bay during the genetic monitoring period provided a potential ecological mechanism of natural selection. A period of early warming of Auke Bay during spring of 1990, which coincided with the peak outmigration of early-run fry, likely favored increased survival of early-run fry relative to late-run fry. Support for this hypothesis was provided by the results of Chapter 1, which demonstrated that early marine survival covaried with sea-surface temperature, presumably because temperature conditions the vulnerability of fry to size-selective mortality.

The distinct outmigration times of early- and late-run fry produces mixtures of fry of different sizes, which potentially enables natural selection to operate through size-selective mortality. This mechanism may be a driving force behind trends toward earlier and truncated migrations, which have been observed in both brood lines over the past 40 years (Kovach et al. 2012). During this time frame, Auke Bay has exhibited a trend toward warmer spring sea-surface temperatures. Additionally, although temporal trends in the seasonal availability of zooplankton have not been evaluated in this estuary, there is evidence that zooplankton blooms are occurring earlier in other systems (Edwards and Richardson 2004; Thackeray et al. 2010). When considered in the context of environmental changes in Auke Bay, the trend toward earlier migration time might reflect juvenile salmon exploiting more favorable estuarine growth conditions in early spring.

Interestingly, the expected responses to a warming climate are opposing in the freshwater and marine stages. In the freshwater environment, increasingly stressful stream conditions would be expected to favor later stream entry, whereas in the marine environment, a shift in the optimal growth window (e.g. earlier vernal warming, earlier zooplankton bloom) would be expected to favor earlier outmigration. In Chapter 1, I observed that fitness during the marine stage appeared to be more closely coupled to physical conditions than in the freshwater stage, where compensatory processes appeared to drive fry production. The fact that the observed genetic changes in phenology appeared to be primarily driven by physical conditions during the marine stage is, therefore, consistent with the results of Chapter 1.

A significant trend toward earlier and truncated migrations was observed in the even-year brood line of Pink Salmon that home to the Hugh Smith Lake system. This observation was concordant with the idea that the changes in temporal structure observed in Auke Creek are linked to processes operating over a large spatial scale. Similar changes in migration time have been documented in other anadromous salmonid populations spanning multiple species and regions (e.g. Robards and Quinn 2002; Quinn et al. 2007; Kennedy and Crozier 2010). Moreover, earlier timing of life history events has now been observed across a wide range of taxa. These changes in phenology likely reflect a response to the earlier growing seasons that have accompanied climate change (Parmesan 2006; Bradshaw and Holzapfel 2008; Both et al. 2009). While it is unclear whether these phenological shifts generally reflect genetic evolution or plasticity, the results of Chapter 2 suggest that contemporary evolution of phenology is possible in populations that harbor genetically determined life history variation.

The trends in the migratory characteristics of Auke Creek Pink Salmon have coincided with a decline in freshwater productivity over the same time span, which was reported in Chapter 1. The decline may be a direct consequence of temporal compression of the adult migration or, alternatively, it may be related to observed declines in the amount of gravel spawning substrate in Auke Creek. In spite of these reductions in fry production, recruitment of adult Pink Salmon to Auke Creek has remained stable in each brood line over the past 30 years (Kovach et al. 2013). It is possible that losses in fry production have been, in part, compensated for by more favorable growth conditions in Auke Bay and resulting increases in marine survival. A positive temporal trend in marine survival was observed in Chapter 1, but it was not significant over the 29-year time series. The observation that adult recruitment has remained stable indicates that Auke Creek Pink Salmon have sustained historic levels of productivity in spite of substantial environmental changes. Genetic diversity of life history traits has been recognized as an important component of population resilience (Hilborn et al. 2003; Greene et al. 2010). The results of Chapter 2 support that idea and indicate that fine-scale local adaptation can promote such genetic diversity.

Disruption of fine-scale local adaptation

In Chapter 3, log-linear analyses demonstrated that marine-survival rates in two generations of hybrids between early- and late-spawning fish generally did not differ substantially from those of controls. Bayesian hierarchical models, which revealed considerable posterior overlap of mean survival rates among most experimental lines, provided additional support for these observations. Furthermore, the hierarchical models produced no evidence of increased variance of survival rates in hybrids in either generation. Early- and late-run fish encounter different growth conditions during their early marine residence period (Mortensen et al. 2000), and the results of Chapter 1 suggest that these differences are relevant to lifetime fitness. While different lifetime fitness components indicate that there is potential for local adaptation to the nearshore environment, distinct adaptations to this life history stage have yet to be demonstrated in Auke Creek Pink Salmon. Hence, there was no hypothetical basis for ecological outbreeding depression of marine survival in this study, and the similar marine survival rates that were observed between first generation hybrids and controls are therefore not surprising. Conversely, epistatic outbreeding depression has been observed in hybrids between the temporally isolated brood lines of that exist at Auke Creek (Gharrett et al. 1999), thereby demonstrating that this mode of outbreeding depression can manifest in hybrids between populations that encounter, on average, similar environmental conditions throughout their life history. In contrast to the even- and odd-year brood lines, environmentally-induced variation in stream entry date likely creates opportunities for gene flow between the early- and late-spawning segments in some years. Concordantly, genotype data from 24 allozyme loci produced estimates of F_{ST} in the even-year brood line that did not differ from zero (-0.0105; Gharrett et al. 2013), thereby indicating that substantial gene flow does occur between these segments. Regular exchange of migrants can occlude the formation of distinct coadapted gene complexes (Edmands 1999), which could explain why similar marine survival rates were observed between second generation hybrids and controls.

Mixed effects models revealed that additive components of adult migration time, arising from covariation among siblings, were generally present when many observations (i.e. returned experimental fish) were available. Similarly, a line cross analysis revealed that additive composite effects were a significant component of variation in adult migration time and suggested that a model with only additive effects sufficiently described the genetic architecture of this trait. In both generations, the mixed effects models produced posterior distributions of hybrid migration times that were nearly intermediate to those of controls, thereby revealing an outcome of outbreeding that was consistent with genes influencing migration time in an additive manner. Early- and late-spawning fish encounter different ecological challenges in freshwater. As noted earlier, the compensatory processes that were evident in Chapter 1 likely pose greater challenges to early-spawned embryos, which are vulnerable to redd disturbances by late-spawning adults (Fukushima et al. 1998). While early-run embryos appear to have evolved expedient completion of epiboly in response to these compensatory processes (Joyce 1986), the effectiveness of this adaptation is contingent upon early-run embryos being deposited sufficiently far in advance of the arrival of late spawners. This suggests that intermediate arrival on the spawning ground, as was observed in hybrids, and subsequent delayed deposition of embryos is maladaptive in terms of embryo survival. Therefore, the observed shift in migration time of hybrid fish was consistent with ecological outbreeding depression. An implication of these results is that outbreeding depression acts as a postzygotic mechanism of genetic isolation, thereby contributing to the maintenance of fine-scale temporal structure in this small stream. This idea is supported by evolutionary theory, where outbreeding depression in hybrids has been recognized as an important driver of sympatric genetic divergence (Schluter 2000).

Chapter 2 detailed natural selection against late-migrating fish in the odd-year brood line, which resulted in severe demographic declines in the late portion of the migration. Furthermore, this event occurred during a period in which the migrations have become temporally compressed in odd- and even-year fish (Kovach et al. 2012), thereby suggesting that substantial losses of genetically-determined temporal structure have occurred in both brood lines. In spite of these changes, considerable temporal structure was evident in the posterior distributions of adult migration time in the odd-year brood line, which were distinct between early and late control lines in each generation. Although there was no late control line in the even-year brood line, the posterior mean migration times of early hybrids and controls differed by ten days in the first generation and seven in the second, which implies that the even-year brood line also continues to harbor pronounced temporal structure. While I have treated the spawning segments as discrete entities in this dissertation, the phenotypic distribution of migration time in Auke Creek is more realistically regarded as bimodal. Collections of broodstock from the hybridization experiment were made from the earliest and latest arriving fish and represented the tail ends of the distribution. Similarly, collections of broodstock that were used to amplify the late-marker allele were made from the latest arriving fish. It is therefore possible that the selection event that was observed during the genetic monitoring period acted against only the latest migrating fish and the late-spawning segment was largely left intact. This could explain the apparent persistence of temporal structure in Auke Creek.

Management implications

The depth of research conducted at Auke Creek has provided a uniquely detailed level of insight into the ecological and adaptive significance of life history variation in a salmonid population, which has important implications for the management of other salmonid populations. The primary findings from Chapter 1 were that production of fry in Auke Creek is limited by spawning habitat and that survival during the early marine stage is critical to adult recruitment. These results demonstrate that the quality and quantity of spawning and nearshore nursery habitat are critical components of salmonid productivity, and support the vitality of habitat conservation as a component of salmonid management programs. The primary findings from Chapters 2 and 3 were that adaptive life history variation that manifests at even a small scale of genetic isolation can support increased productivity of salmonid populations and provide resilience to climate change. This research focused on local adaptation that occurs in sympatric, yet seasonally separate subpopulations and therefore supports the importance of managing fisheries to conserve seasonal genetic diversity. Furthermore, because this research demonstrates that local adaptation can occur in the presence of substantial gene flow, it suggests that adaptive genetic differences may be found in geographically proximate populations. Hence, this research supports the implementation of hatchery policies that conserve local adaptive variation, such as requiring that broodstock selection is made only from local gene pools and is done so in a manner that captures the life history variation present across the entire migration distribution.

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Supplementary Materials

Reconstruction of autumn stream flows of Auke Creek

Daily autumn stream flows of Auke Creek during the study period (1984-2012) were reconstructed with a general-linear model, which was fit with stream flow and rainfall data collected from 1962-75. The reconstruction of autumn stream flow was limited to the months of August-October, a period in which flow regimes vary predictably in accordance with rainfall patterns. Specifically, a power transformation was applied to daily flow rate of Auke Creek (F_t), based on the optimal level of power ($\lambda = 0.5$) selected by a Box-Cox optimization function (Box and Cox 1964), and transformed flow rate (B_t) was modeled as a linear function of daily rainfall (R) on the current and preceding 16 days:

$$B_t = \frac{F_t^{\lambda} - 1}{\lambda}$$

$$B_t = -1.75 + 0.12R_t + 0.16R_{t-1} + 0.11R_{t-2} + 0.08R_{t-3} + 0.06R_{t-4} + 0.05R_{t-5} + 0.04R_{t-6} + 0.03R_{t-7} + 0.02R_{t-8} + 0.02R_{t-9} + 0.02R_{t-10} + 0.02R_{t-11} + 0.02R_{t-12} + 0.01R_{t-13} + 0.01R_{t-14} + 0.01R_{t-15} + 0.01R_{t-16} + \varepsilon_t$$

Each of the 18 coefficients in the linear model were significant ($P < 0.05$; Table A1), and the model accounted for approximately 82% of the variation in daily stream flow in autumn during the observed time frame.

Reconstruction of spring stream flows of Auke Creek

Daily Spring stream flows of Auke Creek during the study period (1983-2011) were reconstructed with a general-linear model, which was fit with environmental data collected from 1962-75 including stream flow, rainfall, and air temperature. The reconstruction of spring stream flows was performed for the months of March-May, a period when melt water from snow pack accumulated over the winter and ice on Auke Lake, along with rainfall, contribute to flow regimes. As was done in modeling fall stream flow, a power transformation was applied to daily flow rate of Auke Creek in spring (F_t), based on the optimal level of power ($\lambda = 0.1$) selected by a Box-Cox optimization function (Box and Cox 1964), and transformed flow rate (B_t) was modeled as a linear function of daily rainfall (R) on the current and preceding 15 days and temperature (T) on the preceding 3 days:

$$B_t = \frac{F_t^{\lambda} - 1}{\lambda}$$

$B_t = -9.38 + 0.02R_t + 0.03R_{t-1} + 0.02R_{t-2} + 0.02R_{t-3} + 0.02R_{t-4} + 0.01R_{t-5} + 0.01R_{t-6} + 0.01R_{t-7} + 0.01R_{t-8} + 0.01R_{t-9} + 0.01R_{t-10} + 0.01R_{t-11} + 0.01R_{t-12} + 0.01R_{t-13} + 0.01R_{t-14} + 0.01R_{t-15} + 0.002T_{t-1} + 0.001T_{t-2} + 0.003T_{t-3} + \varepsilon_t$

Each of the 20 coefficients in the linear model were significant ($P < 0.05$; Table A2), and the model accounted for approximately 70% of the variation in daily stream flow in spring during the observed time frame.

PCR and gel electrophoresis

The polymerase chain reaction (PCR) was used to amplify microsatellite loci. The PCR reaction mixtures were 10 µL volumes: 1 x PCR buffer (50 mM KCl, 10 mM Tris-HCl at pH 9.0); 1.5-3 mM $MgCl_2$; 0.125-0.2 mM each deoxynucleotide triphospate (dNTP); 0.3-0.5 µM each forward and reverse primer (Integrated DNA Technologies, Inc., Coralville, IA); 0.01-0.05 µM labeled primer (Eurofins MWG Operon, Huntsville, AL); approximately 1 unit of generic *Taq* polymerase; and 50-100 ng DNA. The general amplification profile was 1 cycle at 95˚C for 3 min; 30-40 cycles at 95˚C for 30 s, 49-59˚C for 30 s, and 72˚C for 45 s; and 1 cycle at 72˚C for 5 min.

After amplification, PCR products were denatured by adding an equal volume of stop buffer (95% formamide, 0.1% Bromophenol Blue) and heating for 3 minutes at 95˚C. Target fragments were separated by loading approximately 1 µL of PCR product into polyacrylamide denaturing gels containing 6% of a PAGE-PLUS^{TM} 40% concentrate (AMRESCO Inc., Solon, OH), 8 M Urea, and 5X TBE (445 mM Tris-Borate and 10 mM EDTA, pH 8.0), in a reaction catalyzed by ammonium persulfate and TEMED (N,N,N',N'-tetramethylethylenediamine). Electrophoresis was performed in LI-COR automated sequencers (4300^{TM} DNA Analysis System, LI-COR, Inc., Lincoln, NE) in 1X TBE buffer, with running conditions 1,500 V, 40 W, 40 mA, and 45°C plate temperature. Allele sizes were scored by using Saga (Ver. 3.2.1, LI-COR) software to compare allele band patterns with LI-COR IRD700^{TM} or IRD800^{TM} standard ladders (Lincoln, NE).

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Tables

Table S1 - Summary statistics from the linear model of daily stream flow of Auke Creek in autumn as a function of rainfall lagged up to 16 days. Rainfall is donated '*R*' followed by the number of lagged days (e.g. R_{t-5} corresponds to the total daily rainfall from 5 days prior).

Term	Coefficient	Std. error	*t*	*P*
intercept	-1.7510	0.0082	-213.8240	< 2E-16
R_t	0.1157	0.0044	26.2990	< 2E-16
R_{t-1}	0.1607	0.0046	35.0060	< 2E-16
R_{t-2}	0.1116	0.0046	24.3640	< 2E-16
R_{t-3}	0.0765	0.0046	16.6830	< 2E-16
R_{t-4}	0.0603	0.0046	13.1630	< 2E-16
R_{t-5}	0.0472	0.0046	10.2970	< 2E-16
R_{t-6}	0.0393	0.0046	8.5690	< 2E-16
R_{t-7}	0.0340	0.0046	7.4210	1.97E-13
R_{t-8}	0.0244	0.0046	5.3460	1.04E-07
R_{t-9}	0.0209	0.0046	4.5690	5.32E-06
R_{t-10}	0.0193	0.0046	4.2090	2.72E-05
R_{t-11}	0.0175	0.0046	3.8080	0.0001
R_{t-12}	0.0159	0.0046	3.4560	0.0006
R_{t-13}	0.0126	0.0046	2.7480	0.0061
R_{t-14}	0.0091	0.0046	1.9790	0.0480
R_{t-15}	0.0117	0.0046	2.5340	0.0114
R_{t-16}	0.0130	0.0044	2.9560	0.0032

Table S2 - Summary statistics from the linear model of daily stream flow of Auke Creek in spring as a function of rainfall (*R*) lagged up to 15 days and temperature (*T*) lagged up to 3 days.

Term	Coefficient	Std. error	t	P
intercept	-9.4194	0.0078	-1202.34	< 2e-16
R_t	0.019	0.0036	5.323	1.24E-07
R_{t-1}	0.0264	0.0037	7.118	1.99E-12
R_{t-2}	0.0232	0.0037	6.227	6.80E-10
R_{t-3}	0.0218	0.0037	5.837	7.00E-09
R_{t-4}	0.0187	0.0037	5.017	6.14E-07
R_{t-5}	0.0138	0.0037	3.697	0.0003
R_{t-6}	0.0131	0.0037	3.502	0.0005
R_{t-7}	0.0111	0.0038	2.951	0.0032
R_{t-8}	0.0094	0.0037	2.526	0.0117
R_{t-9}	0.0107	0.0037	2.894	0.0039
R_{t-10}	0.0092	0.0037	2.508	0.0123
R_{t-11}	0.0089	0.0037	2.417	0.0158
R_{t-12}	0.0072	0.0037	1.943	0.0522
R_{t-13}	0.0089	0.0038	2.33	0.0200
R_{t-14}	0.0086	0.0039	2.221	0.0266
R_{t-15}	0.0086	0.0038	2.283	0.0227
T_{t-1}	0.0025	0.0005	5.373	9.49E-08
T_{t-2}	0.0011	0.0006	1.827	0.0679
T_{t-3}	0.0032	0.0005	6.841	1.31E-11

Table S3 - Significance values of Pearson's correlation coefficients for each pair of covariates used in the linear models of freshwater productivity. Covariates include adult migration year (Y), median date of migration (M), temperature during migration (T), and stream flow during migration (F_M) and incubation (F_I). Moderately significant values ($P < 0.10$) are shaded.

Covariate:	Y	M	T	F_M	F_I
Y	1.000				
M	0.082	1.000			
T	0.247	0.262	1.000		
F_M	0.283	0.287	0.001	1.000	
F_I	0.990	0.510	0.079	0.284	1.000

Table S4 - Significance values of Pearson's correlation coefficients for each pair of covariates used in the linear models of marine survival. Covariates include juvenile outmigration year (Y), median date of outmigration (M), Coho Salmon smolt (C) and Dolly Varden charr (D) abundance, sea-surface temperature during the spring bloom (T), and stream flow during the spring bloom (F_S) and late outmigration period (F_L). Moderately significant values ($P < 0.10$) are shaded.

Covariate:	Y	M	C	D	T	F_S	F_L
Y	1.000						
M	0.688	1.000					
C	0.389	< 0.001	1.000				
D	0.412	0.023	0.027	1.000			
T	0.165	0.065	0.221	0.692	1.000		
F_S	0.081	0.235	0.475	0.988	0.073	1.000	
F_L	0.064	0.948	0.616	0.224	0.144	0.163	1.000

Table S5 - Coefficients and standard errors (parentheses) of terms from general linear models of freshwater productivity. Covariates include adult migration year (Y), median date of migration (M), Auke Creek temperature during migration (T), and Auke Creek stream flow during migration (F_M) and incubation (F_I). The most parsimonious models are shaded.

Model	AIC	R^2	P	Term 1	Term 2	Term 3	Interaction
Y	38.533	0.148	0.039	-0.021 (0.010)			
M	42.589	0.020	0.460	0.010 (0.014)			
T	43.087	0.003	0.763	-0.020 (0.066)			
F_M	42.282	0.031	0.363	-0.190 (0.206)			
F_I	42.970	0.008	0.656	-0.172 (0.381)			
$Y + T$	40.507	0.149	0.123	-0.021 (0.010)	0.010 (0.063)		
$Y + F_M$	40.205	0.158	0.107	-0.020 (0.010)	-0.109 (0.200)		
$Y + F_I$	40.284	0.156	0.111	-0.021 (0.001)	-0.170 (0.358)		
$M + T$	44.565	0.021	0.757	0.010 (0.015)	-0.010 (0.068)		
$M * T$	45.824	0.046	0.753	0.117 (0.134)	1.514 (1.895)		-0.010 (0.008)
$M + F_M$	43.922	0.043	0.567	0.008 (0.014)	-0.166 (0.213)		
$M * F_M$	44.657	0.084	0.527	-0.013 (0.025)	-13.949 (13.056)		0.059 (0.056)
$M + F_I$	44.258	0.032	0.659	0.011 (0.014)	-0.211 (0.387)		
$F_M + F_I$	44.203	0.034	0.643	-0.179 (0.214)	-0.104 (0.392)		
$Y + F_M + F_I$	42.053	0.162	0.211	-0.020 (0.010)	-0.093 (0.208)	-0.135 (0.372)	
$M + F_M + F_I$	45.765	0.048	0.741	0.009 (0.015)	-0.146 (0.223)	-0.148 (0.403)	

Table S6 - Coefficients and standard errors (parentheses) of terms from general linear models of marine survival. Covariates include juvenile outmigration year (Y), median date of outmigration (M), Coho Salmon smolt (C) and Dolly Varden charr (D) abundance, Auke Bay sea-surface temperature during the spring bloom (T), and Auke Creek stream flow during the spring bloom (F_S) and late outmigration period (F_L). The three models that had the highest probability of being the most parsimonious are shaded.

Model	AIC	R^2	P	Term 1	Term 2	Term 3	Interaction
Y	70.035	0.042	0.284	0.018 (0.017)			
M	69.092	0.073	0.157	-0.028 (0.019)			
C	71.283	0.000	0.943	0.008 (0.109)			
D	68.984	0.076	0.147	-0.103 (0.069)			
T	66.532	0.151	0.037	0.412 (0.188)			
F_S	71.241	0.002	0.835	-0.345 (1.640)			
F_L	64.073	0.220	0.010	-2.150 (0.778)			
$Y + M$	69.999	0.107	0.229	0.017 (0.017)	-0.027 (0.019)		
$Y + C$	71.963	0.045	0.552	0.019 (0.017)	0.028 (0.110)		
$Y + D$	70.127	0.103	0.242	0.015 (0.017)	-0.093 (0.070)		
$Y + T$	64.800	0.254	0.022	0.030 (0.016)	0.505 (0.186)		
$M + F_S$	70.748	0.084	0.320	-0.031 (0.020)	-0.916 (1.643)		
$M + F_L$	63.090	0.297	0.010	-0.029 (0.017)	-2.166 (0.754)		
$C + T$	68.317	0.158	0.108	-0.046 (0.105)	0.432 (0.196)		
$C + F_S$	73.230	0.002	0.974	0.011 (0.112)	-0.368 (1.687)		
$C + F_L$	65.939	0.224	0.037	0.034 (0.098)	-2.177 (0.795)		
$D + T$	65.113	0.246	0.026	-0.114 (0.063)	0.437 (0.181)		
$D + F_S$	70.930	0.078	0.347	-0.103 (0.070)	-0.352 (1.605)		
$D + F_L$	64.953	0.250	0.024	-0.066 (0.065)	-1.961 (0.800)		
$T + F_L$	52.449	0.513	< 0.001	0.596 (0.151)	-2.866 (0.653)		

Table S6 (continued)

Model	AIC	R^2	P	Term 1	Term 2	Term 3	Interaction
$F_S + F_L$	65.786	0.228	0.035	0.775 (1.524)	-2.261 (0.819)		
$Y + C + T$	66.724	0.256	0.057	0.029 (0.016)	-0.026 (0.101)	0.515 (0.193)	
$Y + D + T$	63.956	0.324	0.019	0.026 (0.015)	-0.099 (0.062)	0.515 (0.181)	
$M + F_S + F_L$	65.066	0.297	0.030	-0.028 (0.018)	0.219 (1.525)	-2.197 (0.798)	
$C + T + F_L$	54.243	0.516	< 0.001	-0.034 (0.081)	0.610 (0.157)	-2.857 (0.664)	
$D + T + F_L$	52.479	0.545	< 0.001	-0.068 (0.052)	0.598 (0.149)	-2.673 (0.660)	
$D + F_S + F_L$	66.730	0.256	0.057	-0.064 (0.066)	0.673 (1.530)	-2.063 (0.845)	

Table S7 - Numbers of released fry, adult returns, and proportion returned by brood year, cross, and run in two brood years of Pink Salmon outbred over one generation. Returning odd- and even-year progeny were collected at Auke Creek weir in 2007 and 2008 respectively. Parental run types are abbreviated early (E) and late (L).

			Broodyear 2005			Broodyear 2006		
Dam x Sire	Cross	Run	Fry released	Adult returns	Proportion	Fry released	Adult returns	Proportion
E x E	Control	Early	12,517	75	0.0060	25,294	84	0.0033
E x L	Hybrid	Early	13,047	43	0.0033	9,865	28	0.0028
L x E	Hybrid	Late	7,080	20	0.0028			
L x L	Control	Late	12,084	31	0.0026			

Table S8 - Numbers of released fry, adult returns, and proportion returned by brood year, cross, and run in two brood years of Pink Salmon outbred over two generations. Returning odd- and even-year progeny were collected at Auke Creek weir in 2009 and 2010 respectively. Parental run types are abbreviated early (E) and late (L).

			Broodyear 2007			Broodyear 2008		
(Dam x Sire) x (Dam x Sire)	Cross	Run	Fry released	Adult returns	Proportion	Fry released	Adult returns	Proportion
(E x E) x (E x E)	Control	Early	15,453	223	0.0144	29,729	421	0.0142
(E x L) x (E x L)	Hybrid	Early	3,671	51	0.0139	7,777	100	0.0129
(E x L) x (L x E)	Hybrid	Early	2,939	46	0.0157			
(L x E) x (E x L)	Hybrid	Late	12,473	210	0.0168			
(L x E) x (L x E)	Hybrid	Late	2,228	32	0.0144			
(L x L) x (L x L)	Control	Late	2,898	44	0.0152			

Table S9 - Types of crosses and corresponding line designations used in a line cross analysis of adult migration time in the second generation even-broodyear experiment. The numbers of full sib families and returned adults, and the weighted mean Julian date of adult migration in each line ($\bar{Z}_j$) and its standard error are listed.

(Dam x Sire) x (Dam x Sire)	Line type	No. families	No. adults	$\bar{Z}_j$	$SE(\bar{Z}_j)$
(E x E) x (E x E)	P_1	21	223	235.453	0.415
(L x L) x (L x L)	P_2	6	44	243.545	1.429
(E x E) x (L x L)	F_1	9	109	237.284	0.989
(E x L) x (E x L) (E x L) x (L x E) (L x E) x (E x L) (L x E) x (L x E)	F_2	37	339	238.917	0.611

Figures

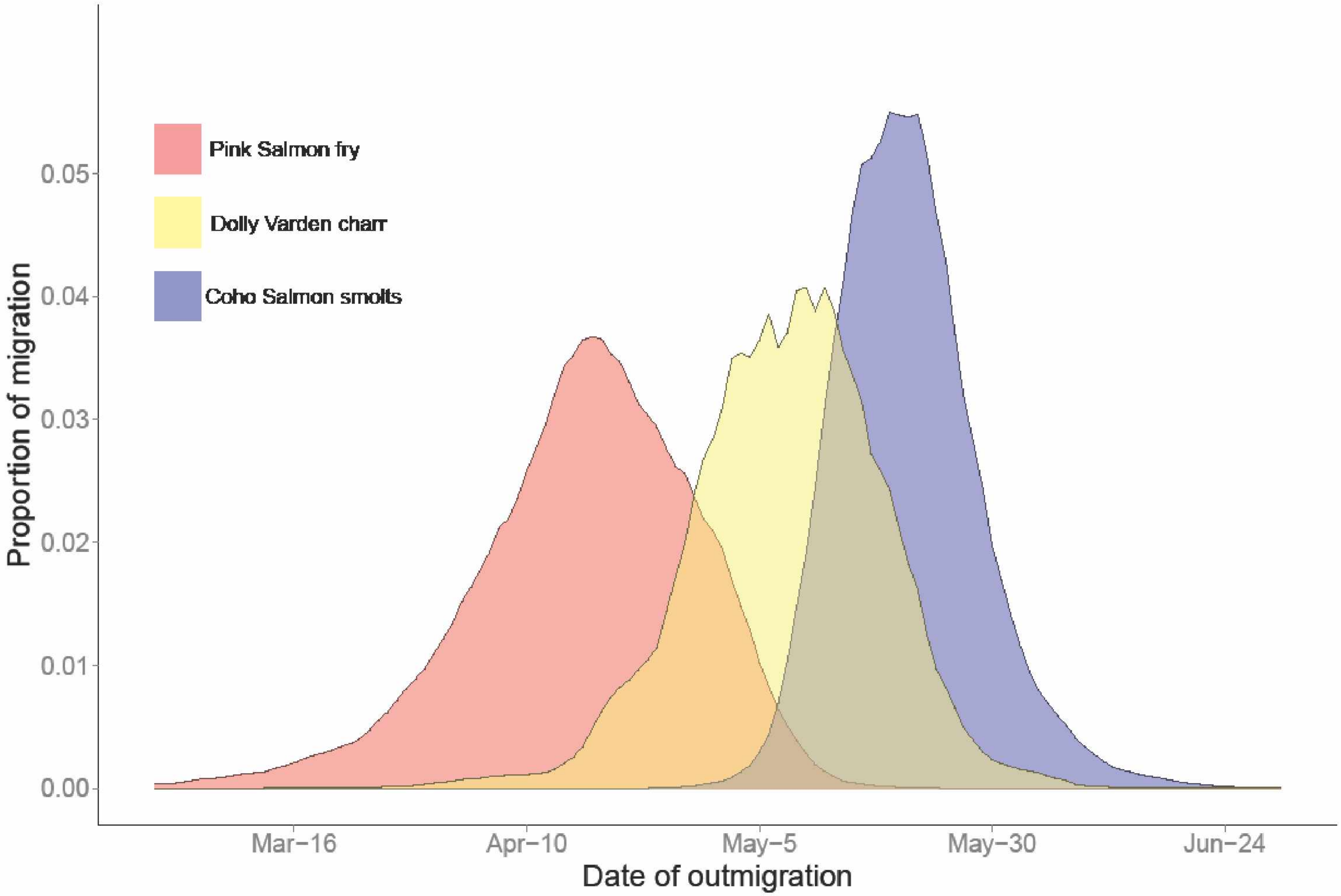

Figure S1 - Average proportion of the total outmigration of Pink Salmon fry, Coho Salmon smolts, and Dolly Varden charr that passed the Auke Creek weir on each date over the time series (1983-2011). Plots were smoothed with 5-day rolling means.

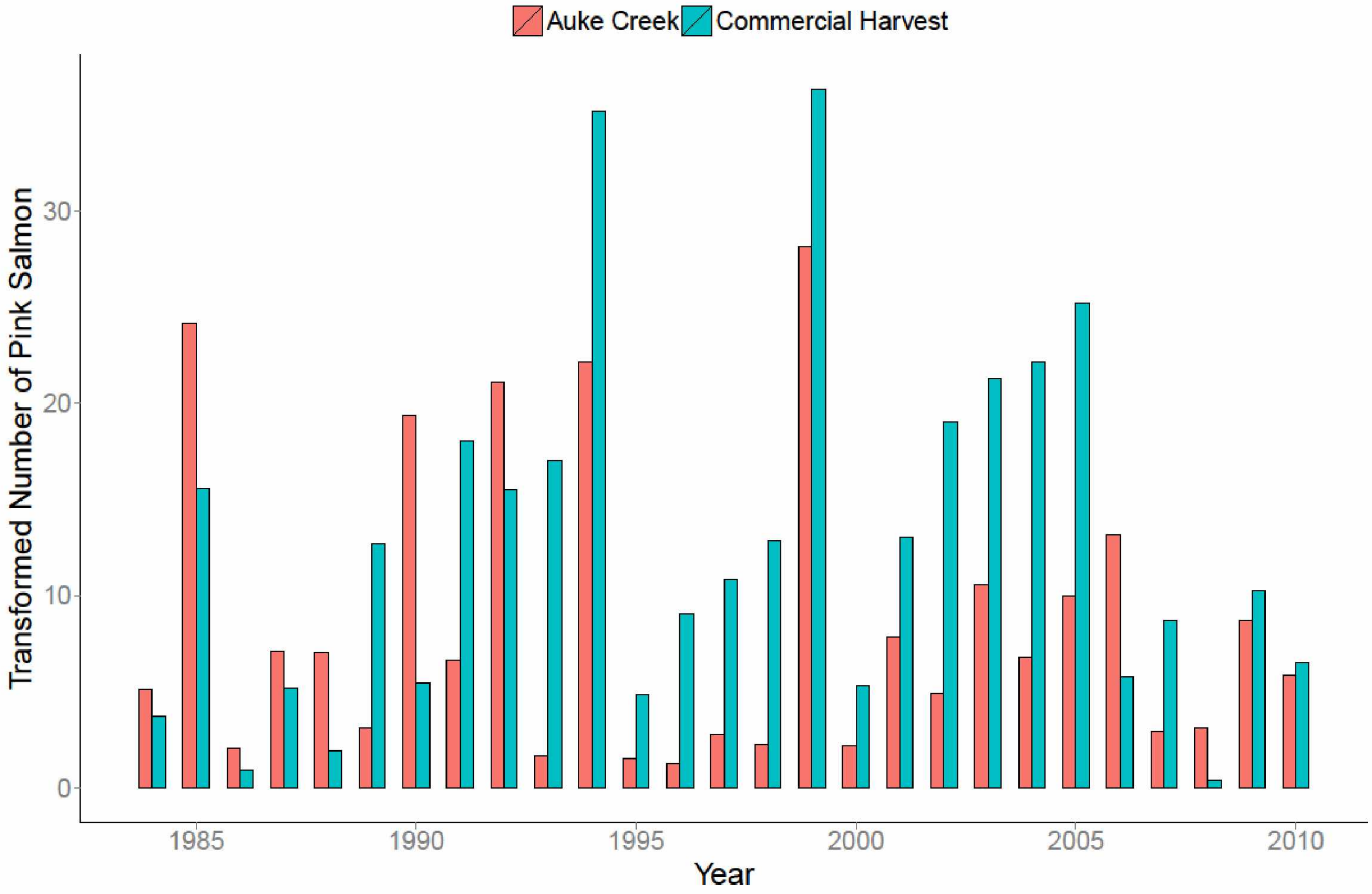

Figure S2 - Annual numbers of adult Pink Salmon returned to Auke Creek and Pink Salmon intercepted in the commercial harvest in Northern Southeast Alaska (1984-2010). Auke Creek Pink Salmon are expressed in thousands, and commercially harvested Pink Salmon are expressed in millions.