SALMONID PHENOLOGY, MICROEVOLUTION, AND GENETIC DIVERSITY IN A WARMING ALASKAN STREAM

A

THESIS

Presented to the Faculty

of the University of Alaska Fairbanks

in Partial Fulfillment of the Requirements

for the Degree of

DOCTOR OF PHILOSOPHY

By

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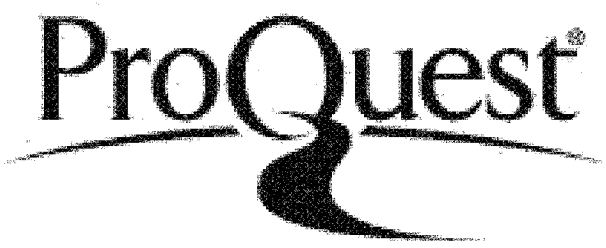
Fairbanks, Alaska

December 2012



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SALMONID PHENOLOGY, MICROEVOLUTION, AND GENETIC VARIATION

IN A WARMING ALASKAN STREAM

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ABSTRACT

Climate change is a formidable challenge for fish and wildlife conservation because it will directly influence the ecology and evolution of wild populations. Though climate-induced temporal trends in phenological events are common in many populations, there remains considerable uncertainty in the patterns, mechanisms, and consequences of phenological shifts. To address this, and clarify how climate change has impacted salmonid migration timing and microevolution in a warming (0.34°C per decade) Alaskan stream, long-term demographic and genetic data were used to answer these questions: how has migration timing changed in multiple salmonid species; what sources of variation influence migration timing; are changes in migration timing a result of microevolution; and does migration timing and change in migration timing influence intra-population genetic variation? For most salmonid species, life stages, and life histories, freshwater temperature influenced migration timing, migration events occurred earlier in time (mean = 1.7 days earlier per decade), and there was decreasing phenotypic variation in migration timing (mean 10% decrease). Nonetheless, there were disparate shifts in migration timing for alternative life history strategies indicative of biocomplexity. Population abundances have been stable during these phenotypic changes ($\lambda \approx 1.0$), but adult salmon availability as a nutrient resource in freshwater has decreased by up to 30 days since 1971. Experimental genetic data spanning 16 generations in the odd-year pink salmon population demonstrate that earlier migration timing is partly due to genetic changes resulting from selection against late-migrating fish and a three-fold decrease in this phenotype. However, circadian rhythm genes hypothesized to influence migration timing in Pacific salmon showed no evidence of inter-generational selective change. Migration timing itself influences the distribution of genetic variation within pink salmon, as there were genetic differences between early- and late-migrating fish. Despite shifts in migration timing, genetic structure and the genetic effective population size were both stable across years, indicating that in the absence of demographic change patterns of genetic diversity are resilient to climate change. These findings indicate that climate change has significantly influenced the ecology and evolution of salmon

populations, which will have important consequences for the numerous species, including humans, who depend on this resource.

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ACKNOWLEDGEMENTS

First and foremost, I thank my primary advisor David Tallmon. In addition to being a constant source of valuable scientific wisdom, Dave was infinitely flexible and patient with me through my own maturation as a young professional. In addition to aiding my development as a research scientist, Dave was eager to guide me through the world of grant-writing, peer-review, mentoring, dealing with the public/media, and generally honing my understanding of how humans understand the world and why that is important. Simply, I would have never finished this without his help. My co-advisor Mark Lindberg was similarly great. Mark's applied points of view on all aspects of my research were a welcome perspective. I genuinely regret that I was unable to find a way to incorporate more of his quantitative skills into this dissertation. Tony Gharrett was in many ways my third co-advisor. Many of the most exciting findings in this dissertation are due to his willingness to collaborate and share extraordinary data, his helpful hand in the lab, and stringent application of scientific rigor (and forcing me to always double my sample sizes!). My final committee member, Milo Adkison, was always willing to impart his shrewd understanding of salmon ecology, statistics, and study design. I am indebted to his and my entire committee's commitment to making scientific progress through well-developed hypotheses and meticulous analyses.

There is a long list of entities that helped fund this research, and to whom I am extremely grateful. Alaska NSF EPSCoR, the North Pacific Research Board, Alaska Sustainable Salmon Fund, IAB Summer Research Fellowship, and Alaska Sea Grant College Program all generously contributed to this work in various fashions. There are also the many individuals who help guide confused graduate students like myself through the numerous intangibles of graduate education especially Carol Piser, Jolene Troup, Gabrielle Hazelton, and many others – thank you! I also thank the other scientists who helped with various aspects of this research. John Joyce and Jesse Echave were generous with knowledge, effort, data, and most importantly humor. Without Lisa Eby's inspiration there is no way I would have ever done this. I appreciate the fact that members of the Gharrett lab (Rachel, Mike, Chris, Pat, Katie, Sara) were incredibly

flexible and helpful in accommodating my occasional lab work. Other scientists who commented on drafts of the chapters within this dissertation or provided advice include L. S. Mills, M. McPhee, A. Whiteley, K. O'Malley, C. Funk, F. Allendorf, A. Werthheimer, and B. Wing. Finally, Scott Vulstek and Tyler Linderoth were outstanding lab mates – both were extremely knowledgeable and fun guys to have to spend hours and hours of grueling time in the lab with (and go fishing with). I also need to thank Tyler for helping collect some of my genetic data.

Of course, there are the numerous people not involved with science that I owe many thanks. Thanks Mom, Dad, and Devin for being the greatest and most supportive family members. All of my friends, I am indebted to the respite you have provided. Most of all, Lily: thanks for always making me laugh, being so encouraging, and just being the best friend someone could ask for.

CHAPTER 1: INTRODUCTION

Introduction

Human-induced global change will dramatically increase extinction rates, decrease biodiversity, and alter ecosystem interactions, all of which will have significant consequences for fish and wildlife populations (Chapin et al. 2000, May 2010, Walther 2010, Woodward et al. 2010, Bellard et al. 2012). Given the importance of terrestrial and marine ecosystem services for human society and the importance of biodiversity for supporting ecosystem functioning (Chapin et al. 2000, Worm et al. 2006), these issues are of considerable scientific and social concern. While many aspects of human-induced global change will negatively impact the persistence of wild populations (e.g. Sala et al. 2000, Jelks et al. 2008), climate change is a primary threat to biodiversity (Heller and Zavaleta 2009), especially in combination with other stressors (Hulme 2005, Brook et al. 2008, Woodward et al. 2010). As such, climate change represents a unique and difficult challenge for wildlife and fisheries management, largely due to the substantial uncertainty in many important factors including: future climate projections, habitat changes, population level responses, ecological interactions, and community and ecosystem dynamics (Allison et al. 2009, Both et al. 2009, Heller and Zavaleta 2009, Martin et al. 2011, Nichols et al. 2011, Polasky et al. 2011, Bellard et al. 2012).

Recent climate change has had substantial impacts on organismal ecology, and evidence for climate-induced distributional and phenological changes within populations is widespread across all biological levels (Parmesan and Yohe 2003, Root et al. 2003, Parmesan 2006). These changes are necessary for the persistence of many populations, because distributional and phenological shifts can allow populations to continue to occupy their climatic niche (Bellard et al. 2012). While some species may be able to shift their spatial distribution, increased habitat fragmentation, limited dispersal abilities, and endemism are likely to reduce or eliminate the dispersal capabilities of many species (Peters and Darling 1985, Travis 2003). Researchers have argued that assisted translocation is a viable management solution (Hoegh-Guldberg et al. 2008, Thomas

2011), but translocations can be detrimental to the target population and to ecosystems as a whole (Tallmon et al. 2004, Ricciardi and Simberloff 2009).

Alternatively, adaptive phenological shifts are a mechanism by which populations can persist *in situ*. Phenotypic plasticity – phenotypic expression based on environmental conditions – is likely to be responsible for many observed phenological changes (Gienapp et al. 2008). It is a crucial (but poorly understood) mechanism of population response to environmental change (Nussey et al. 2007, Auld et al. 2010, Merilä 2012). However, resilience conferred by phenotypic plasticity is limited because (1) reaction norms may become non-adaptive in novel environments outside the range of present plasticity thresholds; (2) indirect cues (e.g. photoperiod, temperature, river flow-regime etc.) may cease to be informative about future conditions; and (3) plasticity decreases individual fitness in highly stochastic and unpredictable environments (Schlaepfer et al. 2002, Ghalambor et al. 2007, Visser 2008, Reed et al. 2010a, Reed et al. 2010b).

Because of limitations to phenotypic plasticity and spatial dispersal, rapid microevolution as a result of natural selection (i.e. changes in gene frequencies resulting in phenotypic changes that increase fitness) will be crucial for the persistence of many populations (Reed et al. 2010b, Hoffman and Sgro 2011). There is increasing evidence that adaptive microevolution in nature can occur rapidly (Hendry and Kinnison 1999, Reznick and Ghalambor 2001, Carroll et al. 2007, Hendry et al. 2008), particularly in response to strong directional selection such as human harvest (Kuparinen and Merilä 2007, Allendorf et al. 2008, Hard et al. 2008), and experimental studies have demonstrated that adaptive microevolution can allow populations to persist during rapid and extreme environmental changes (e.g. Bell and Gonzalez 2011). Unfortunately, there is a paucity of empirical evidence for rapid genetic adaptation to global climate change (Gienapp et al. 2008), which has made it extremely difficult to evaluate when this phenomenon may influence population dynamics and consequently affect the probability of persistence for wild populations.

For many species phenology – the timing of seasonal life history events – is thought to be adapted to optimum environmental conditions because life history events

can directly influence individual fitness (Visser and Both 2005, Miller-Rushing et al. 2010). Climate change is likely to alter optimum environmental conditions by shifting the timing of seasonal events (Forrest and Miller-Rushing 2010) and many species will have to adjust their phenology to match environmental conditions produced by climate change (Schlaepfer et al. 2002, Cotton 2003, Visser 2008, Saino et al. 2011). Consequently, it is thought that seasonally influenced life history traits (e.g. migration timing) are the traits most likely to adapt via evolution by natural selection to the shifting environmental conditions caused by climate change (Bradshaw and Holzapfel 2008). Migration timing is particularly likely to adapt via microevolution because this trait is often under genetic control (Liedvogel et al. 2011) and can influence individual fitness in many populations (e.g. Marra et al. 1998, Both and Visser 2001, Dickerson et al. 2005).

The long-term demographic and genetic data necessary to test this hypothesis are extremely rare and therefore, assessments of the evolutionary responses of organisms to climate change are uncommon, and the biological mechanisms underlying any observed change (genetic vs. plastic) poorly understood (Gienapp et al. 2008, Hansen et al. 2012). Consequently, there is a critical gap in our understanding of how populations are responding to climate change, especially the role of microevolution in promoting population resilience through adaptive genetic change.

The rarity of high-quality long-term data have also made it difficult to determine the processes that induce variability in climate-induced phenological change and the consequences of these changes for populations and ecosystems (Diez et al. 2012). Despite considerable research on relationships between climate and phenology (e.g. Cotton 2003, Root et al. 2003, Parmesan and Yohe 2003, Thackeray et al. 2010, Diez et al. 2012), little is known about how life history variation affects climate-induced phenological change and reciprocally, how phenological shifts influence phenotypic diversity (Schoener 2011). Investigations into the population-level consequences of climate-induced phenological change have almost entirely focused on demographic parameters (e.g. population abundance, survival, reproductive success; Miller-Rushing et al. 2010, Saino et al. 2010). But, climate change may also affect population genetic

diversity within species (Rubidge et al. 2012), which can have wide-ranging impacts on persistence and ecological dynamics (Frankham 2005, Kinnison and Hairston 2007, Hughes et al. 2008). Therefore, it will be necessary to understand the relationship(s) between climate change, phenotypic diversity, evolutionary dynamics, and population demography in order to determine and prioritize where and how conservation and management actions should be taken.

Pacific Salmon, Climate Change and Biocomplexity

Because of their economic importance (e.g. Woodby et al. 2005), unique and significant ecological role (Willson and Halupka 1995, Gende et al. 2002, Moore and Schindler 2008, Hocking and Reynolds 2011), cultural value to indigenous groups (O'Neil 2007), and the extinction risk faced by many populations (Nehlsen et al. 1991), Pacific salmon (*Oncorhychus* spp.) are of significant concern across their historical range. Salmonids are poikilothermic and may be especially sensitive to climate warming because their biology and life history events are directly influenced by temperature conditions in the marine and freshwater environment (Groot and Margolis 1991, Quinn 2005). As a result, there is substantial interest in how climate change may affect salmonid fishes and the degree to which salmonid populations may be able to respond adaptively (Battin et al. 2007, Reiman et al. 2007, Crozier et al. 2008, Waples and Hendry 2008, Bryant 2009, Williams et al. 2009).

Climate change may have a particularly strong influence on the adaptive timing of migration events for adult and juvenile salmonids (Crozier et al. 2008, Taylor 2008). Migration timing is a heritable trait that is presumably adapted to local thermal conditions in freshwater rivers, streams, lakes, and also the ocean (Taylor 1991, Smoker et al. 1998, Hodgson and Quinn 2002, Quinn 2005, Hard et al. 2008). Changing water temperatures in oceanic and freshwater environments may affect salmon migration timing by altering environmental cues, or the fitness benefits/costs of certain phenotypes associated with specific migratory timing (Crozier et al. 2008).

In salmon, migration timing can influence reproductive traits such as energy allocation and reproductive lifespan (Hendry et al. 1999), individual reproductive success (Dickerson et al. 2005, Anderson et al. 2010), patterns of genetic diversity (Hendry and Day 2005), and ultimately population dynamics and persistence (Wright and Trippel 2009, Reed et al. 2011). Moreover, timing of juvenile emigration is strongly related to survival to adulthood in Chinook salmon (*Oncorhynchus tshawytscha*), steelhead trout (*Oncorhynchus mykiss,* Scheuerell et al. 2009) and Atlantic salmon (*Salmo salar*, Kennedy and Crozier 2010). The strong relationship between salmonid ecology and migration timing, coupled with the high heritability of phenological traits in salmonids (median = 0.51, Carlson and Seamons 2008), suggests that migration timing is a key trait that may respond via microevolution to climate change (Crozier et al. 2008). This is supported by data showing that migration timing has undergone rapid microevolution in translocated populations of salmonids (Quinn et al. 2000, O'Malley et al. 2007).

Biocomplexity – biological variation within and between populations – plays an important role in determining how Pacific salmon populations respond to environmental variation. Populations or population complexes with greater biocomplexity exhibit more stable dynamics and greater resilience to deterministic and stochastic environmental change (Hilborn et al. 2003, Greene et al. 2010, Moore et al. 2010, Schindler et al. 2010). Biocomplexity can exist within populations in the form of distinct heritable phenotypes such as early- and late-migrating fish, and/or alternative life history strategies that differ in their maturation schedules (Schindler et al. 2010, Greene et al. 2010). It is unknown how this variation influences phenological and microevolutionary responses to climate change, but given that individuals expressing diverse life histories are subject to different selective pressures and environmental heterogeneity (Groot and Margolis 1991, Smoker et al. 1998, Quinn 2005), biocomplexity may play an important role in influencing how salmonids respond to climate warming. Such empirical information would provide valuable support for the theory that biocomplexity and genetic diversity should be primary targets of conservation protection in rapidly changing environments (Hilborn et al. 2003, Frankham 2005, Hughes et al. 2008).

Project Objectives, Questions, and Hypotheses

The primary purpose of this project was to address research gaps in our understanding of climate-induced changes in the ecology and evolution of fish and wildlife populations. Specifically, my research objectives were to (1) describe long-term patterns in migration timing for multiple salmonid species in a warming stream; (2) test whether observed changes in migration timing are due to microevolution; and (3) describe how migration timing and changes in migration timing influence intra-population genetic diversity. To achieve these goals I used an invaluable long-term data set from Auke Creek, Alaska, where there has been a complete census of multiple salmonid species and life histories during multiple life stages, historically archived genetic samples and genetic data, and a complete collection of multiple environmental variables. Additionally, a wealth of previous research on the salmonid populations in Auke Creek (reviewed in Smoker et al. 1998) acted as a substantial knowledge base from which it was possible to identify valuable research questions.

In CHAPTER 2, I identified patterns in migration timing for Dolly Varden char (*Salvelinus malma*), coastal cutthroat trout (*Oncorhynchus clarki clarki*), pink salmon (*Oncorhynchus gorbuscha*), coho salmon (*Oncorhynchus kisutch*), and sockeye salmon (*Oncorhynchus nerka*). I used the daily counts of each species, life stage, and life history to estimate temporal trends in the median date of migration timing and trends in phenotypic variation in migration timing. I also used an information theoretic approach to determine which abiotic factors influence migration timing. Given that salmonid populations are locally adapted to thermal regimes, I hypothesized that freshwater and oceanic temperatures would influence migration timing and that there would be temporal trends in migration timing due to rapid warming in Auke Creek itself. Because alternative life histories in salmon populations are subject to different selective pressures and environmental conditions, I hypothesized that there would be intra-specific variation in temporal trends and environmental drivers of migration timing. Additionally, I hypothesized that changes in the central tendency of migration timing would be

associated with changes in phenotypic variation because migration timing is highly heritable in salmonid populations.

The purpose of CHAPTER 3 was to determine if shifts toward earlier migration timing in adult, odd-year pink salmon are due to genetic changes (i.e. microevolution). To do so, I used genetic data spanning 17 complete generations, including neutral genes, circadian rhythm genes, and an experimental genetic marker for late-migration timing (Lane et al. 1990, Gharrett et al 2001). Given that migration timing is heritable and influences several aspects of biology within this population (e.g. development rates, juvenile and adult survival, and reproductive success; Hebert et al. 1998, Smoker et al. 1998, Gharrett et al. *in submission*), I hypothesized that changes in migration timing may be due to genetic changes as a result of climate-induced natural selection.

In CHAPTER 4, I described intra- and inter-annual patterns of genetic variation in the odd-year pink salmon population. I tested if migration timing itself influences the distribution of genetic variation within the odd-year pink salmon population, estimate the genetic effective population size to measure evolutionary potential, and examine whether changes in migration timing reflect changes in genetic diversity. I hypothesized that heritability in migration timing influences patterns of within-population genetic variation, and that the Type III life history curve present in pink salmon populations can substantially reduce the genetic effective population size relative to numerical abundance. Because climate-induced phenological change can influence aspects of demography (e.g. survival and reproductive success) and the interaction of individuals within a population, I hypothesized that changes in migration timing can affect patterns of within-population genetic differentiation as a result of migration timing and the genetic effective population size.

This research is the result of close collaborations with other scientists who generously shared data, lab supplies, equipment, and space, obtained funding, offered wisdom, edited drafts of manuscripts, and aided in sample collections. They are included as co-authors for publication of the manuscripts that make up CHAPTERS 2-4. This research was conducted under UAF IACUC protocols #09-34 and 228569-1.

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CHAPTER 2: EARLIER MIGRATION TIMING, DECREASING PHENOTYPIC VARIATION, AND BIOCOMPLEXITY IN MULTIPLE SALMONID SPECIES[1]

Abstract

Climate-induced phenological shifts will play an important role in determining population, evolutionary, and ecological dynamics; but our understanding of these phenomena is hampered by a lack of long-term demographic data. We use a multi-decade census of 5 salmonid species representing 14 life histories in a warming Alaskan stream to address the following key questions about climate change and phenology: how consistent are temporal patterns and drivers of phenology for similar species and alternative life histories; are shifts in phenology associated with changes in phenotypic variation; and how do phenological changes influence the availability of resource subsidies? For most salmonid species, life stages, and life histories, freshwater temperature influences migration timing – migration events are occurring earlier in time (mean = 1.7 days earlier per decade), and intra-annual variation in migration timing is decreasing (mean 10% decrease). The magnitudes of temporal trends in migration timing were not correlated with changes in intra-annual phenotypic variation, suggesting that these components of the phenotypic distribution have responded to environmental change independently. Despite commonalities across species and life histories, there was important biocomplexity in the form of disparate shifts in migration timing and variation in the environmental factors influencing migration timing for alternative life history strategies in the same population. Overall, adult populations have been stable during these phenotypic and environmental changes ($\lambda \approx 1.0$), but the temporal availability of salmon as an ecosystem service and resource in freshwater has decreased by up to 30 days since 1971 due to changes in the median date of migration timing and decreases in intra-annual variation in migration timing. These novel observations advance our

[1] Kovach, R. P., Joyce, J. E., Echave, J. D., Lindberg, M. S., & Tallmon, D. A. Earlier migration timing, decreasing phenotypic variation, and biocomplexity in multiple salmonid species. Accepted *PLoS ONE* pending minor revision.

understanding of phenological change in response to climate warming, and indicate that climate change has influenced the ecology of salmon populations, which will have important consequences for the numerous species that depend on this resource.

Introduction

Along the coasts of the northern Pacific Ocean, salmonids (*Oncorhynchus* and *Salvelinus* spp.) are a vital link between marine and terrestrial ecosystems, and provide a massive source of nutrients to coastal food webs [1,2]. Salmonids are also harvested for commercial, recreational, and subsistence purposes and are a sustainable fishery in Alaska [3] that has supported human coastal communities and cultures for millenia. An important aspect of salmonid biology that may be influenced by climate change is migration timing because this trait is closely adapted to local environmental conditions, particularly temperature [4], and influences individual fitness by affecting survival and reproductive success [5-8]. Due to their important ecological role [1,2] and predictable migratory timing [9], many species are thought to have adapted their phenologies to correspond with the presence of adult salmon in freshwater [10,11]. Thus, changes in this trait may have substantial ecological ramifications [1,2,12], and understanding the response of salmonids to climate change is imperative for conserving functional coastal human and ecological communities [2,6]. Here, we use a multi-decade census to identify key patterns and processes affecting migration timing across multiple salmonid species, life histories, and life stages. In so doing, we address important research gaps concerning the phenological responses of organisms to climate change.

In addition to other factors such as harvest [13] or hatchery supplementation [14], reports of changes in migration timing for salmon species, including pink (*O. gorbuscha*), sockeye (*O. nerka*), and Atlantic salmon (*Salmo salar*) [8,13,15-19], may be due to environmental change, including climate warming [8,17,19]. Previous studies have been limited to single species, providing little opportunity for comparison between species and alternative life history forms in the same species, which could help resolve confounding

explanations for factors affecting timing or highlight important inter- and intra-population variation.

Recent evidence suggests phenological trends among similar species occupying the same habitats can differ substantially [20]. The degree to which this holds true within and across populations as a result of different life history strategies is unknown. Intra-population heterogeneity in response to climate change could potentially be an important aspect of biocomplexity [3] for species such as Pacific salmon where individuals pursuing alternative life histories use and respond differently to environmental variation [9]. Similarly, quantifying the relationship between environmental variation and phenological variation for multiple species and life histories occupying the same habitats is rarely performed but may provide insight into biocomplexity within species and across populations [e.g. 3]. On a larger scale, understanding how these changes influence ecosystem processes and ecosystem services remains a critical research gap [21]. Phenotypic changes can have substantial ecological impact [22], particularly for species such as salmon that act as key components and drivers of ecosystem dynamics [1, 23].

Temporal trends in the mean/median of phenological events have been well documented for many species [e.g. 24-26]. But it is unknown whether there are trends in phenotypic variation (V_P) within populations and if trends in V_P are independent of, or correlated with, changes in the mean phenotype [27]. Changes in V_P may be caused by shifts in allele frequencies [28], intra-generation selection [23], or environmentally dependent trait expression (i.e. plasticity, [29]). Ultimately, V_P is the basis for evolutionary change and is necessary for long-term persistence under changing environmental conditions [30], making it a critical but neglected aspect of ecological and conservation research. Because salmonid migration timing has a high heritability [31] and directional selection decreases V_P [28], we predicted that directional changes in migration timing would be correlated with decreases in V_P. Alternatively, increased phenotypic variation can be evidence of exposure to novel environmental conditions and the expression of increasingly diverse phenotypes as a result of plasticity [29].

Using a 30-47 year census at a permanent weir, we estimated trends in migration timing for five species of salmonids: pink salmon, coho salmon (*O. kisutch*), sockeye salmon, Dolly Varden char (*Salvelinus malma*), and coastal cutthroat trout (*O. clarkii clarkii*). Considerable life history variation (e.g. age at maturity, age at migration to saltwater, semelparity vs. iteroparity) within and among these salmonid species (See Text S1, [9]), and data for different life stages (adult vs. juvenile) provide a unique opportunity to understand the influence of life history variation on phenological change. Using these data we address several questions: (1) has inter-annual migration timing changed in those species and life histories occupying Auke Creek; (2) what factors appear to play a role in determining migration timing; (3) are there temporal trends in V_P in migration timing that are correlated with changes in the average phenotype (4); how variable are phenological responses and the environmental factors influencing phenology across different species, life-stages, and life-histories; (5) have changes in the central tendency and variance in migration timing altered the availability of salmon as an ecosystem service?

Methods and Materials

Study Site

Auke Creek is a small, lake-outlet stream near Juneau, Alaska (Fig. S1) that has undergone rapid warming since 1971 (Fig. 1). In 1980 a permanent weir was constructed on Auke Creek just above the Auke Bay high tide mark. The weir is a National Oceanic and Atmospheric Administration (NOAA) facility, and NOAA employees conduct and oversee operations. All immigrating and emigrating salmonids are captured and counted at the weir. Prior to 1980, pink salmon juveniles were captured with fyke nets, while adult salmon (coho, sockeye and pink) were captured with a semi-permanent gated weir. While all individual fish have been counted since 1980, in years prior to 1980 very high stream flows may have reduced the efficiency of the traps in some instances (but only for coho salmon). However, this source of error was very small (only a few fish were undetected), and the trends in coho migration timing are robust to deleting the pre-1980 data. Therefore, a complete census is available for all individuals beginning in 1980,

with some species and life histories having datasets spanning up to 47 years in length (See Text S1 and Table S1 for additional details). Figure S2 depicts historical and recent annual temperature profiles for Auke Creek and dates of migration timing for each species.

Because these data were collected over a 50-year time frame, much of this work predates animal care policies. The University of Alaska's Institutional Animal Care and Use Committee (IACUC) has approved the collection of recent data under a variety of different protocols. Additionally, the weir is required to operate under a Fish Resource Permit from the Alaska Department of Fish and Game, a permitting process by which the State of Alaska ensures that research does not adversely effect fish populations. The annual operational plans are essentially unchanged, and therefore the historical data were collected in a manner that complies with contemporary animal care policies. All individual fish were captured, counted, and released unharmed either upstream or downstream of the permanent weir depending on the direction of their migration.

Data analyses

Linear regression was used to estimate trends in the median date of migration timing and to measure how V_P has changed over time. For the latter analysis, the response variable was the number of days over which the central 95% of migrating fish returned to or migrated out of Auke Creek. Table S1 provides the sample size (number of years) for each species and life history as well as the estimates from the linear regression of migration timing and V_P vs. year. To quantify the relationship between temporal trends in the median date of migration timing and V_P, we calculated the correlation between responses across all species and life histories using Spearman's rank correlation. In order to directly test the hypothesis that larger temporal trends in migration timing are positively correlated with decreasing V_P, we standardized the estimates of the temporal trends by taking the absolute value of each response, calculated a mean of the absolute values, and then subtracted this mean value from each absolute

value ($| b_i | - \frac{1}{n}\sum | b_i |$, where the b_i are the estimates of change in migration timing and n is the total number of estimates).

We used several analyses to describe temporal changes in the distribution of adult salmon in freshwater. We estimated the change in the peak period that adult salmon are available in Auke Creek by performing a linear regression of the difference between the median dates of adult coho and sockeye salmon migration vs. year (i.e. the earliest and latest migrating species respectively). An identical analysis was performed on the number of days between the first and last 100 salmon to enter freshwater as a response variable. Finally, linear regression was used to estimate the temporal trend in the cumulative number of days that adult salmon migrate into Auke Creek. For the response variable we summed across pink, coho and sockeye salmon the number of days over which the central 95% of migrating fish returned to Auke Creek in each year. We did not include jacks – male salmon that mature at least one year earlier than all other adult salmon and are much smaller in body size (See Text S1) – in these analyses.

We estimated population growth rate for adult salmon species (coho, sockeye, and pink) because the interaction between abundance and migration timing constrains the harvest allotted to fisheries and the impact of salmon on ecosystems through bioturbation and marine-derived nutrient subsidies [1,2]. We used the exponential growth state-space method [32] to estimate population growth rate from 1971 – 2010 for adult pink, sockeye and coho (1971-2009) salmon, and we report discrete-time estimates (i.e. lambda). This method estimates population growth rate based on the observed numerical abundances of adult salmon across time.

We used an information theoretic approach based on Akaike's Information Criterion adjusted for sample size (AIC_C) to identify models that best described inter-annual variation in migration timing for the various species and life histories in Auke Creek [33]. We used simple linear regressions with *a priori* defined covariates that we hypothesized influenced migration timing (Fig. S3, See Text S1). Median date of migration timing for each species/life history was used as the response variable. We combined data from even- and odd-year pink salmon to increase sample size, given that

these populations demonstrated similar temporal trends in migration timing (Fig. 2), and should respond somewhat similarly to environmental variation.

A variety of environmental variables, some of which have been monitored in Auke Creek (stream temperature and precipitation/streamflow), were used in these analyses (Text S1). We also included variables representing biological (density) and oceanic conditions (Pacific Decadal Oscillation and sea surface temperature, Text S1). We standardized predictor variables by subtracting the mean from each value and dividing by the standard deviation. We compared the importance of each predictor variable by summing the AIC_C weights from each model that included a given covariate and had a $\Delta AIC_C < 10$ [33,34]. Identical candidate model sets (Table S2) were used for all reproductively mature fish, including jacks, migrating into Auke Creek to facilitate comparison between species and life histories. Similarly, we used identical candidate model sets (Table S3) for all fish migrating from Auke Creek to the ocean; but we did not include density in models for pink salmon because juvenile density interactions in freshwater are negligible for this species. We primarily considered additive models, but included interactive effects where we hypothesized they may be important. Specifically, we hypothesized that the influence of water temperatures on migration timing may have changed over time due to temporal shifts in migration timing and/or increasing water temperatures. This could arise from novel or increased selective pressures on migration timing, or different patterns of phenotypic plasticity in novel environments [29]. For example, juvenile migration timing may have historically been negatively correlated with water temperatures, but early migration timing due to warming streams may be disadvantageous, because there may be a selective advantage not to respond to increasing stream temperatures (e.g. [6]).

In addition to environmental variables, we included time (year) as a potential covariate for all species (See Text S1 for additional details on study populations). We included year as a surrogate for unmeasured environmental change and/or as a variable representing directional evolutionary change in migration timing, which has been observed in other salmon populations [35]. We tested covariates for pair-wise

correlations (i.e. multicollinearity; Table S4), and found that our candidate variables were minimally correlated [33,36]. All data analyses were performed in R (The R Development Core Team 2010).

Results

We observed earlier migration timing from saltwater to freshwater (Fig. 2A) in odd-year pink salmon adults (slope (b_1) = -0.253, SE = 0.12), even-year pink salmon adults (b_1 = -0.331, SE = 0.11), coho salmon adults (b_1 = -0.418, SE = 0.07), coho salmon jacks (b_1 = -0.307, SE = 0.06), and sockeye salmon jacks (b_1 = -0.305, SE = 0.16) but not sockeye salmon adults (b_1 = 0.192, SE = 0.13). Migration from freshwater to the ocean showed a similar shift toward earlier migration timing (Fig 2A), because point estimates for Dolly Varden (b_1 = -0.070, SE = 0.12), cutthroat trout (b_1 = -0.119, SE = 0.12), odd-year pink salmon (b_1 = -0.494, SE = 0.15), even-year pink salmon (b_1 = -0.273, SE = 0.17), age 2 sockeye salmon (b_1 = -0.14, SE = 0.16), age 2 coho salmon (b_1 = -0.091, SE = 0.09) were all negative. However, point estimates for age 1 coho (b_1 = 0.070, SE = 0.09) and sockeye salmon (b_1 = 0.105, SE = 0.15) were positive. V_P in migration timing has decreased for many species and life history types (11 of 14, Fig. 2B), but the magnitude of temporal trends in the median date of migration timing was not correlated with decreases in V_P (Spearman's Rank r = 0.055, P = 0.852).

The greatest change in migration timing from saltwater to freshwater was for adult coho salmon (Fig. 3A), which are now migrating into Auke Creek approximately 17 days earlier than they did 40 years ago. Freshwater temperatures during migration were positively related to migration timing for all species, except for sockeye jacks (b_1 = -4.16, SE = 2.54). Similarly, there was a positive relationship between migration timing and the date of peak stream flow during the migration for all species and life histories except for sockeye jacks (b_1 = -1.198, SE = 1.92). Therefore, most individuals appear to avoid migrating during high temperatures and low flows (i.e. migrate during high flows and cooler temperatures). Overall, there was earlier migration from saltwater to freshwater in

all species and life history types except for adult sockeye salmon (Fig. S3, Table 1 and S2).

Although stream temperatures had a strong influence on migration timing, other variables were important. Interactive terms between year and stream flow and year and temperature were important for both sockeye jacks and coho jacks respectively. However, a model for coho jacks that included year and sea-surface temperature was equally supported ($\Delta AIC_C = 0.001$), and is more parsimonious (2 vs. 3 parameters). In Table 1, we present estimates from the more parsimonious models. For sockeye jacks, a model that only included year was well supported by the data ($\Delta AIC_C = 0.7$) and had fewer parameters (3 vs. 1). Oceanic conditions were included in the best-fit models for pink salmon, sockeye adults and coho jacks (Table 1). However, the model that did not include PDO for pink salmon was equally supported by the data ($\Delta AIC_C = 0.04$), and year and temperature were more important than oceanic conditions when compared across models (Fig. S3). Sea-surface temperatures had opposite relationships with migration timing for sockeye adults and coho jacks; sockeye migration timing was negatively related to sea surface temperature but the opposite was true to coho jacks (Table 1). Finally, there was intra-specific variation in phenological trends for sockeye salmon, where alternative life histories (adults and jacks) show contrasting temporal trends in migration timing. Adult sockeye are migrating later in time while sockeye jacks are migrating earlier in time (Fig. 2).

Migration timing from freshwater to the ocean is influenced by stream temperature and stream temperatures are increasing (Fig 1A, Fig. S3, Table 2, Table S3). In the most extreme case, odd-year pink salmon juveniles are now leaving Auke Creek for the ocean approximately 19 days earlier than in 1974 (Fig. 3B). Stream temperature during peak outmigration to the ocean was the best overall predictor, and had a negative relationship with timing of outmigration for all juvenile Pacific salmon, cutthroat trout, and char (Fig. 2, Table 2 and S3). Dolly Varden and cutthroat trout were the most sensitive to variation in water temperature in terms of their migration timing. Based on best-fit models predicting migration timing, both species migrate into freshwater 4.62 (*SE*

= 0.67) and 4.9 (SE = 0.67) days earlier (respectively) for each 0.506 °C (one standard deviation in the variability of stream temperatures during trout and char migrations) increase in temperature. Cumulative stream temperatures during the growth period from the previous year (or incubation period for pink salmon) did not appear to be strongly related to migration timing, but there was a detectable effect for age 2 coho and age 1 sockeye salmon (warmer temperatures were associated with earlier dates of migration timing). Despite the commonalities, age 1 and 2 juvenile sockeye and coho salmon demonstrate contrasting trends in migration timing; in both species age 2 juveniles make up an increasing proportion of the outmigrants (Fig. S4) and are migrating earlier, whereas age 1 juveniles are decreasing in relative abundance and migrating later.

There was also a strong relationship between juvenile pink salmon migration timing and freshwater temperature, and which results in pink salmon migrating into saltwater nearly one week earlier for each 1.33 °C (one standard deviation) increase in water temperature. Generally, year was a poor predictor of run timing across species and life histories, but there was negative linear trend in pink salmon migration timing. As noted elsewhere, this trend is partially due to changes in adult migration timing, because migration timing of juvenile pink salmon can be influenced by the timing of adult spawning [19]. Nonetheless, when adult migration timing from the previous year (b_1 = 0.323, SE = 0.11) is included in the best-fit model predicting migration timing of pink salmon from freshwater to saltwater (Temperature + Year + Year_lag), year remains an important variable in the model based on effect size (b_1 = -0.210, SE = 0.07). This *post-hoc* model [37] also had a considerably better fit (AIC_C = 218.931) than a model that included adult migration timing in the previous year, but not year itself (Temperature + Year_lag; AIC_C = 226.869). In other words, the trend toward earlier migration timing for juvenile pink salmon does not appear to be entirely due to changes in adult migration timing.

The estimated trends in V_P indicate that 11 of 14 salmonid life histories are migrating over a shorter range of dates. In the most extreme case of decreasing V_P, odd-year adult pink salmon are now migrating over a period of time that is on average 13 days

shorter (from 46 to 33 days) than it was forty years ago (Fig. 3C, b_1 = -0.340, SE = 0.12). The average response across all species and life histories was a 10.2% (SE = 6.1%) decrease in V_P, and the greatest single change was a 41% decrease in V_P in age 2 sockeye salmon. Alternative life histories in coho salmon (in both adults and juveniles) had contrasting trends in V_P. Coho jacks (b_1 = 0.366, SE = 0.09) and age 1 smolts (b_1 = 0.214, SE = 0.08) are both migrating over a longer period of time, while age 2 smolts (b_1 = -0.192, SE = 0.13) are migrating over a shorter period of time, and coho adults (b_1 = -0.002, SE = 0.13) have demonstrated almost no change in migration timing. Excluding even-year juvenile pink salmon, there was little evidence for correlations between the number of days that fish migrate into or out of freshwater and numerical abundance (Pearson's r = -0.401 – 0.449). The number of days over which even-year juvenile pink salmon migrated into saltwater was positively correlated with abundance (Pearson's r = 0.769). However, there was no temporal trend in the natural logarithm of juvenile pink salmon abundance (b_1 = 0.045, SE = 0.04), suggesting that the trend in V_P for this life history is independent of the effects of abundance (i.e. the trend in V_P is not due to a change in abundance).

As a result of the changes in median dates of migration timing, the range of dates over which adult salmon return to spawn in Auke Creek and are available as a resource in freshwater has decreased from 79 to 55 days (b_1 = -0.618, SE = 0.18). The decrease in the range of dates in which all adult salmon return is even greater when considering the number of days between the first 100 and last 100 salmon to enter freshwater (b_1 = -0.697, SE = 0.14). Finally, the cumulative number of days per year that adult salmon migrate past the weir into freshwater has decreased (b_1 = -0.792, SE = 0.23) by approximately 31 days. However, the abundances of salmon have not decreased and population growth rates are stable. We observed that population growth rate (λ) was close to the replacement level of 1.0 and the associated 95% confidence intervals (CI) all encompassed 1.0 for odd-year pink salmon λ=1.064 (0.740,1.530), even-year pink salmon λ=1.059 (0.700,1.603), sockeye salmon λ=0.971 (0.853,1.106), and coho salmon λ=0.997 (0.969, 1.027).

Discussion

Our results show that the migration timings of Auke Creek salmonids have changed across multiple species and life history types toward an earlier migration that takes place over a narrower range of dates. Although the temporal availability of adult salmon has been reduced, their abundances remain stable. Earlier migration timing of salmon from saltwater to freshwater is opposite to what we would predict given that adult salmon in Auke Creek migrate in the fall and prefer cooler water temperatures (Table 1). Earlier migration timing has also been observed in Columbia River, Fraser River, and Bristol Bay sockeye salmon [13,15,16]. Environmentally induced changes in salmon migration timing have now been observed across the northern Pacific Ocean, raising the possibility that these general patterns may be due to large-scale environmental change such as climate warming. The ubiquity of shifts toward earlier migration timing across species, life stages, and life histories within this study location is indicative of biological response to a common phenomenon. While changes in migration timing have been implicated in reduced fitness in some salmonid populations [15,38], populations of adult salmon in Auke Creek have been stable.

At our study location, the only species not migrating earlier were adult sockeye. Unlike pink and coho, sockeye do not reproduce immediately after entering Auke Creek, but instead mature in Auke Lake for up to a month before spawning in August [39]. As a result, they appear to have more plasticity in their migration timing ($P < 0.001$, pair-wise F tests for equality of inter-annual variation in migration timing), and appear to be more strongly influenced by local environmental variation, particularly stream flows, which can be prohibitively low during peak periods of sockeye migration. Whether Auke Creek sockeye are actually reproducing earlier in time is unknown.

Other factors including salinity [40,41], harvest [13], or hatchery activities [14], appear to influence migration timing in other salmon populations. In Auke Creek, hatcheries have not had persistent directional effects on these populations because augmentation only occurred over brief periods of time for pink salmon (1973-1981) and

sockeye salmon (1988-1992). In years with hatchery returns, the percentage of hatchery fish relative to wild fish varied widely for both sockeye (1%-61%) and pink salmon (1-68%), but hatchery fish were not included in our analyses. The effects of commercial fisheries are much more complicated in Southeast Alaska than in other more well studied areas (e.g. Bristol Bay [13]) because of the immense variability in the geography and biology of the salmon populations in this region [42], and the fact that harvest is not terminal (i.e. commercial harvest does not occur at or near the mouth of Auke Creek). Harvest rates are not known for pink and sockeye populations (sockeye harvest has been closed in Auke Bay since 1980), but commercial fisheries appear to harvest approximately 40% of Auke Creek coho (unpublished data). For all species, the fisheries occur on mixed stocks migrating thru distant purse seine, drift gillnet, and trolling fisheries, making it unlikely that harvest-induced directional selection on migration timing is persistent for these adult salmon populations. Simply, harvest occurs but it is more likely to be stochastic than deterministic as has been observed elsewhere (See also Text S1). This is strongly supported by the fact that we observed temporal trends in migration timing for jacks as well as adult salmon, and neither commercial nor recreational fisheries target jacks.

The fact that juvenile salmonids develop more quickly and migrate to saltwater earlier with increasing stream temperatures is well documented [e.g. 8,43,44, and reviewed in [45] for sockeye], and is assumed (at least historically) to be an adaptive behavior allowing juvenile salmon to exploit peak resource availability in estuarine environments [e.g. 9,19]. Based on our results, this relationship between freshwater temperature and migration timing also holds for mature and juvenile trout and char, both of which are iteroparous. Both species appear to migrate out of Auke Creek approximately 9-10 days earlier for each 1.0 °C increase in water temperature. Reproductively mature cutthroat trout that leave Auke Creek immediately move to other freshwater streams to spawn, suggesting that stream temperature may be acting as a migratory cue or may influence the timing of this life history event [46]. Also, cutthroat trout and Dolly Varden can feed heavily on juvenile salmon that are also migrating into

saltwater, and it is believed that their migrations may be timed to coincide with the availability of juvenile salmon resources [e.g. 47]. This raises the possibility that "phenological cascades" may be occurring, or will occur, among salmon, trout, and char migrating from freshwater to saltwater. Overall, projections of rapidly warming temperatures [48] imply that substantial changes in migration timing from freshwater to saltwater are likely. Future changes in migration timing to salt water are concerning because of the potential for juvenile salmon to experience trophic mismatches in the oceanic environment, that is, arriving in the ocean before their primary food resource, zooplankton, has undergone its spring bloom [6-8,19].

The decrease in V_P for pink salmon (all life stages), sockeye salmon (all life stages and life histories), coho salmon (age 2 smolts), and Dolly Varden, has broad implications because of the importance of phenotypic variation and biocomplexity to Pacific salmon population stability [3,49-52]. Migration timing from freshwater to saltwater can strongly influence juvenile salmon survival [7,8,53], but optimal migration timing varies from year to year [7]. Reductions in the window of time that salmonids migrate into saltwater may decrease the probability that migration events coincide with optimal conditions. Similarly, Dolly Varden migrations to the marine environment are also occurring over a shorter period of time, and there may be some risk migrating fish will fail to coincide with peak food resources [54].

It is possible the observed phenotypic changes have influenced evolutionary and population dynamics [55-57], especially given the high heritability of phenology in salmonids (median $h^2 = 0.51$, [31]). We predicted larger decreases in V_P with greater temporal shifts in migration timing, which could be taken as evidence for directional selection and possible genetic response to this selection. Instead, we found no correlation between changes in V_P and changes in the median date of migration timing, suggesting that these two aspects of the phenotypic distribution have responded to climate warming independently. A notable exception was in odd- and even-year pink salmon, where fish from both populations are migrating earlier and over a reduced period of time. Along with these observations, genetic data indicate that there has been a genetic change for

earlier migration timing in the odd-year pink salmon population [58]. The degree to which these phenotypic changes, including changes in V_P, are a function of microevolution as opposed to phenotypic plasticity is a critical unknown for the other populations at Auke Creek and for the many phenological changes observed elsewhere [59]. Directional changes, coupled with high heritability, suggest that evolution by natural selection may have occurred in some of these populations, and empirical research on other salmon populations supports this possibility [35]. However, there is a strong plastic component to migration timing and development in salmonid fishes [e.g. 6,43,60], and an alternative, but plausible explanation is that the observed shifts are entirely due to environmentally induced trait expression [61]. In fact, the only 3 trends toward increased phenotypic variation (coho jacks, age 1 coho, cutthroat) were fairly strong, potentially indicative of novel phenotypic expression due to plasticity [29]. It may be possible to test these hypotheses with further experiments and/or analysis of temporal genetic data [62].

Despite commonalities in temporal trends in migration timing and V_P, an important observation is that in a single stream different life history types within a population can have disparate phenological responses to climate change (i.e. biocomplexity, Fig. 2A, Fig. 2B). Opposite temporal trends among life histories within species were observed for both median date of migration timing (sockeye adults vs. jacks and age 1 vs. 2 sockeye and coho smolts) and V_P (age 1 vs. 2 coho salmon smolts). Additionally, different environmental variables influenced migration timing for different species and life histories (Fig. S3, Tables 1 and 2). The disparity between sockeye adults and jacks may be due to the positive relationship between adult sockeye migration timing and the Pacific Decadal Oscillation [63], or that sockeye jacks may be less influenced by high stream temperature and low flow due to their smaller body size [64]. This latter idea is supported by the fact that freshwater environmental variables had little influence on migration timing for sockeye jacks.

The contrasting patterns of temporal changes in phenology for age 1 and 2 sockeye and coho salmon smolts are interesting given the common effect of water temperature on these life histories. Smolts of different age classes differ in their timing

of outmigration to saltwater (Text S1) and are subject to different environmental conditions (e.g. streamflow) due to rapid changes in springtime hydrological cycles [65]. In some years, age 1 smolts migrate after peak springtime flows and their migrations may be constrained by low stream flows until rain events increase stream discharge. Temporal trends toward an increasing proportion of age 2 smolts (Fig. S4) are another indicator that complex environmental and/or genetic changes may be occurring within these populations. Generally, the largest/oldest fish (age 2) tend to outmigrate earlier and have higher marine survival (e.g. [66] and see Text S1). Therefore, if selection is occurring, it may be acting on migration timing or age at outmigration, either of which could produce the observed trends [23,67]. It does appear that selection and/or some unidentified environmental change may be leading to these shifts in timing and age structure. This is because increasing water temperatures in Alaskan lakes have been shown to increase juvenile salmon growth [68,69], which should in turn lead to faster maturity and therefore an increasing prevalence of age 1 fish [e.g. 70]. This pattern is opposite to what we observed, suggesting some other mechanism may be influencing these populations. Regardless of process, these observations highlight the conservation value of preserving life history variation in the face of uncertainty. Our present biological knowledge is insufficient to adequately predict how climate change will impact many populations [20] and how various life histories will influence population persistence [3,49].

Changes in adult salmon migration at Auke Creek timing since 1971 have reduced the time period that salmon are available in freshwater as an ecosystem service and resource subsidy by nearly a month. These results have significant implications for salmon management because commercial, subsistence, and recreational fisheries are partially determined by an accurate knowledge of migration timing [13,50]. Also, compressed migration distributions within and between salmon species could potentially increase density dependence as a result of competition for spawning areas and therefore act to decrease population abundance. Because the phenologies of other organisms are adapted to the timing of salmon spawning, these changes have the potential to influence

other ecological interactions (e.g. mayfly emergence, plant-pollinator interactions, wildlife behavior [1,10,11,71]) and both human and non-human consumers of adult salmon will need to adjust their behavior to continue to consume Auke Creek salmon.

In summary, this long-term, multi-species, multi-life history data set allowed us to make several novel insights into the process of phenological change in response to climate warming for a suite of species and life history stages. By examining migration timing across multiple species and life stages, we found that salmonids in Auke Creek are generally migrating earlier during a period of environmental warming. The dramatic decrease in V_P across most life histories indicates a general trend at this study site. However, this statistic is almost never reported in other studies of phenological change (but see [27]), making it difficult to generalize to other studies [21]. Researchers should report changes in V_P, given that it is a critical parameter that may indicate the long-term capacity for populations to persist during changing environmental conditions [30,72,73]. Importantly, a majority of the contrasting temporal trends in migration timing were intra-specific as opposed to inter-specific. Therefore, heterogeneity in phenological changes within species can be as great as variation between species occupying the same habitat. The phenological changes observed in this stream have reduced the temporal distribution and availability of an important ecosystem service and source of marine derived nutrients, a trend that will influence other aquatic and terrestrial species. At present, it seems impossible to predict how long these populations will remain stable if the observed trends in migration timing and environmental warming continue.

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Tables

Table 2.1. Parameter estimates from the best-fit and most parsimonious model for migration timing from saltwater to freshwater for each species and life history type. Standard errors for each estimate are in parentheses. Labels for the covariates are Year = year, T = temperature during peak migration, P = Pacific Decadal Oscillation, S = sea surface temperature, F = stream flow. All covariates are standardized except for Year.

	Parameter					
Species	Y	T	P	S	F	R^2
Pink salmon	-0.34 (0.07)	3.13 (0.86)				0.45
Coho adults	-0.37 (0.07)	1.61 (0.74)			1.75 (0.82)	0.64
Coho jacks	-0.29 (0.06)			1.48 (0.71)		0.45
Sockeye adults		3.35 (1.64)	4.10 (1.64)	-2.67 (1.74)	3.35 (1.68)	0.30
Sockeye jacks	-0.31 (0.16)					0.09

Table 2.2. Parameter estimates from the best-fit and most parsimonious model for migration timing from freshwater to saltwater for each species and life history type. Standard errors for each estimate are in parentheses. Labels for the covariates are Year = year, T = temperature during peak migration, Tlag = temperature during the previous years developmental period, D = density. Models are additive unless specified otherwise (e.g. Y * Tlag).

	Parameter					
Species	Year	T	TD	D	Y*Tlag	R^2
Pink salmon	-0.29 (0.07)	-6.43 (0.74)				0.76
Coho age 1		-2.54 (0.57)		-1.08 (0.56)		0.48
Coho age 2		-3.22 (0.46)	-1.05 (0.52)			0.75
Sockeye age 1	0.09 (0.14)		-13.53 (4.55)		0.59 (0.18)	0.33
Sockeye age 2		-3.90 (1.20)				0.27
Dolly Varden		-4.62 (0.67)				0.62
Cutthroat trout		-4.90 (0.67)				0.65

Figures

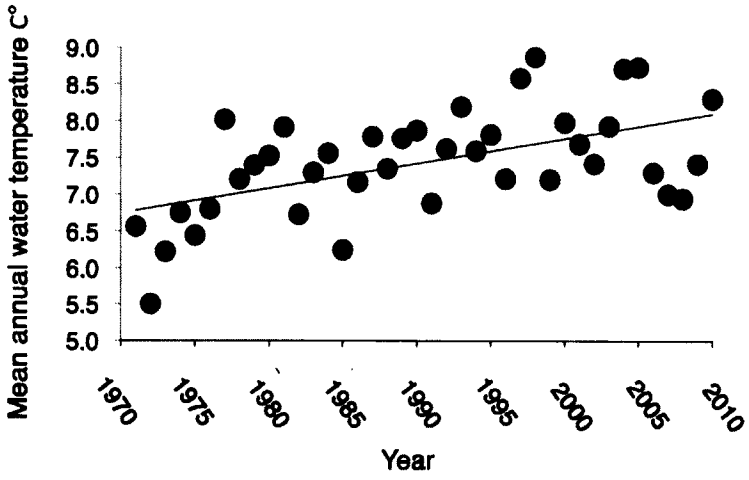

Figure 2.1. Mean annual water temperature in Auke Creek. Alaska.

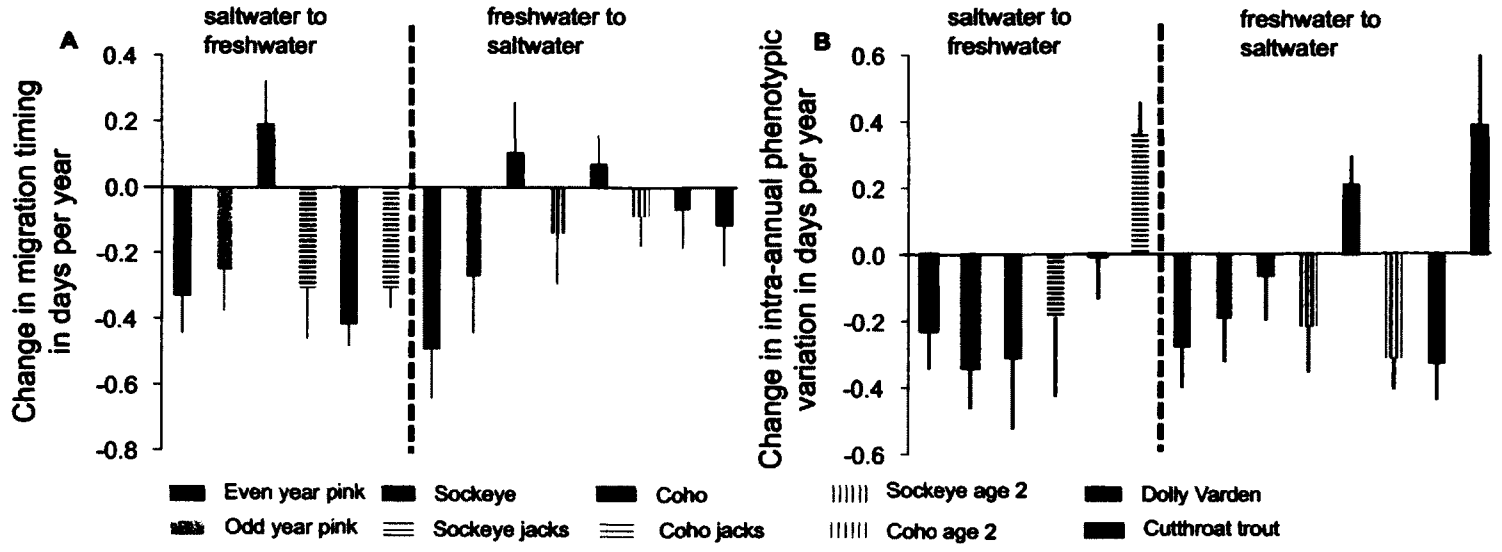

Figure 2.2. Change in migration timing for salmonids in Auke Creek, AK. (a) Temporal change in median date of migration timing. The color key for the different species is located below the figures. For both panels, the error bars represent standard errors, and the dashed line separates estimates of temporal trends in migration timing from saltwater to freshwater (left side), from temporal trends in migration timing from freshwater to saltwater (right side). (b) Change in intra-annual variation in migration timing (V_P) estimated from linear regression of the number of days over which each life history type migrates into or out of Auke Creek vs. year.

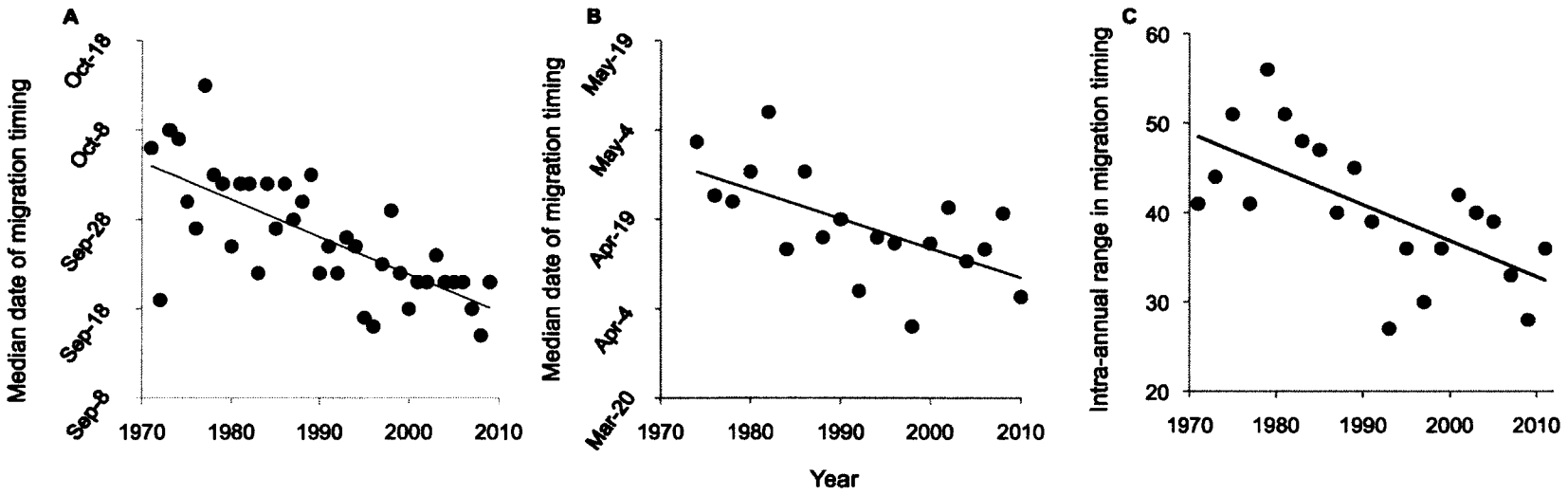

Figure 2.3. Examples of temporal changes in the median date of migration timing and V_P. These plots show the greatest trends toward earlier migration timing from saltwater to freshwater (adult coho, A), freshwater to saltwater (juvenile odd-year pink salmon, B) and decreasing intra-annual range in migration timing (adult odd-year pink salmon, C). The lines are the fitted regressions. Please note that odd-year (referring to year of adult spawning) juvenile pink salmon migrate in even years.

Supplementary Information

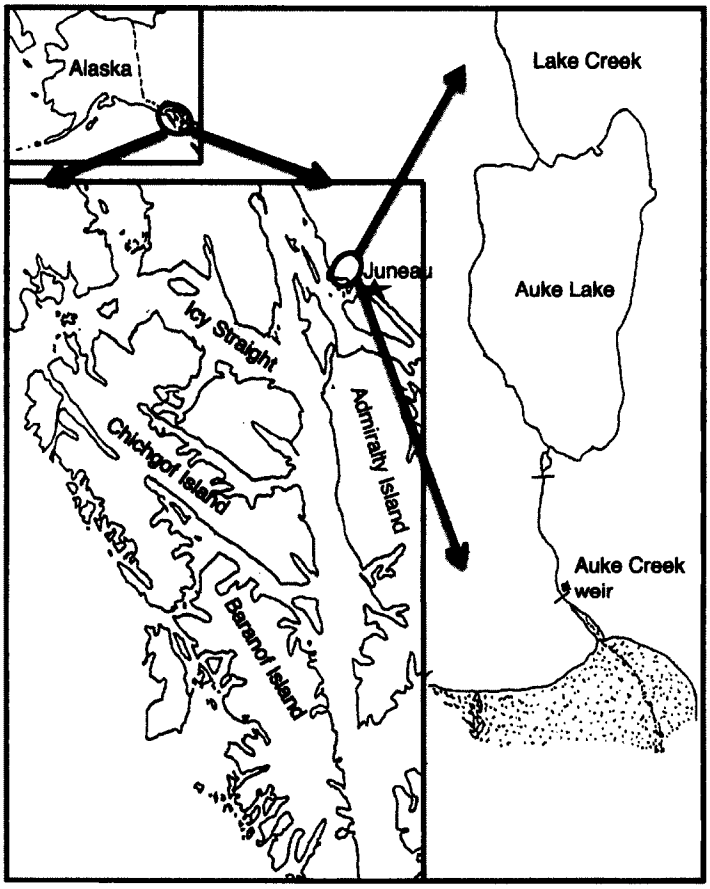

Figure 2.S1. Map of the study area in relation to Southeast Alaska and the entire state of Alaska

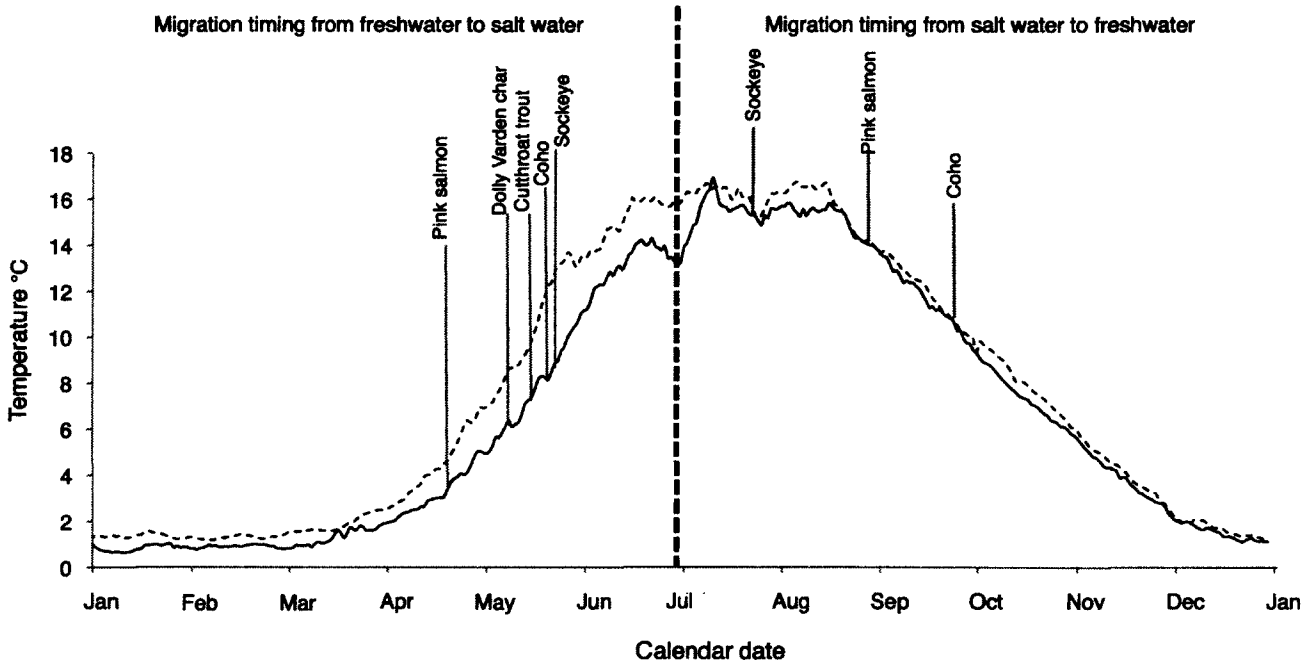

Figure 2.S2. Average dates of salmon migration timing and average daily water temperatures (°C) for Auke Creek Alaska. The solid line represents the average daily water temperature from 1971-1980 and the dashed line represents the average daily water temperature from 2001-2010. The average date of migration timing is labeled for each species. Alternative life histories (e.g. sockeye adults and jacks) within a species and life-stage are combined for greater clarity, and the average date of their migration timing is presented. The vertical lines from the temperature trends to the species description represent the average of the median dates of migration across the time series. Lines to the left of the dashed vertical line are for migration timing from freshwater to saltwater, and lines to the right depict migratory timing from saltwater to freshwater.

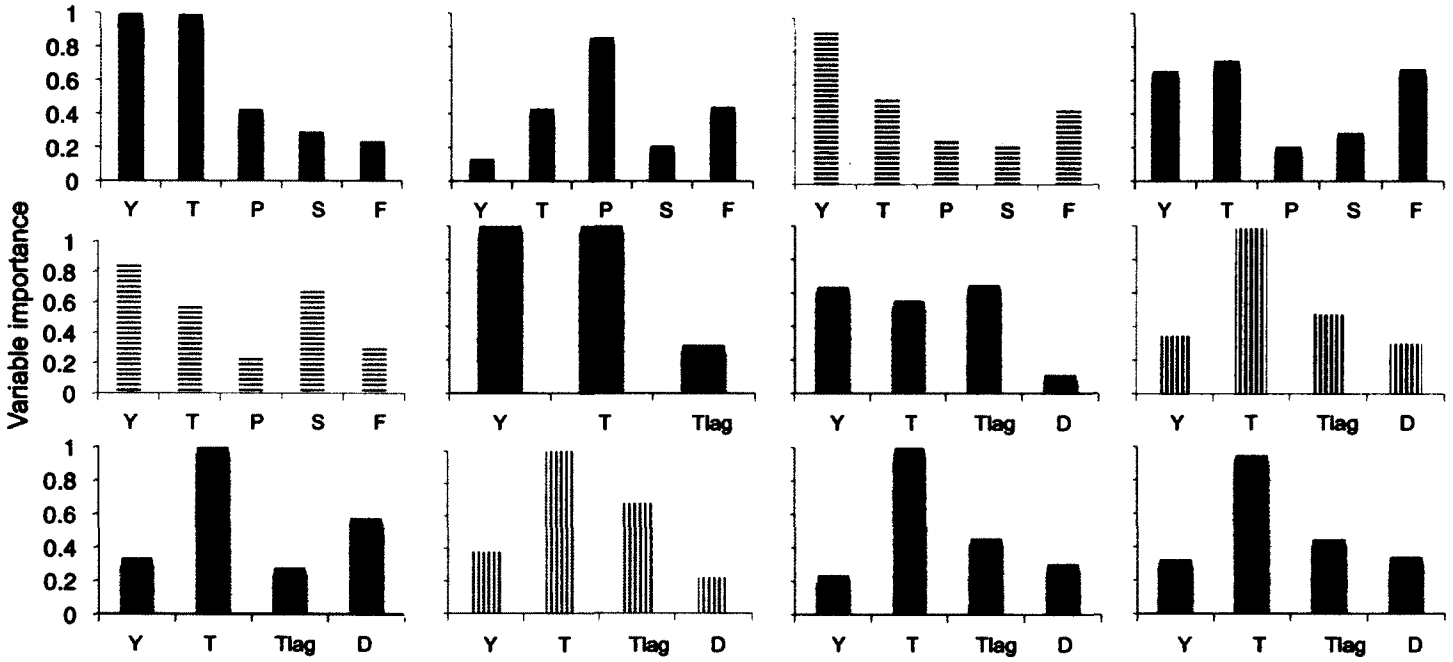

Figure 2.S3. Relative support for variables used to predict migration timing for each species and life history. Variables are depicted on the x-axis by Y = Year, T = Stream temperature during peak migration, P = Pacific decadal oscillation, S = Sea surface temperature, F = Peak stream flow, Tlag = Average stream temperature during the growth and development period, D = conspecific density (See SI text). The y-axis is the sum of the AIC_C weights from each model that included a given covariate and had a ΔAIC_C <10, and represents the relative support for each variable. Variables with relative importance = 1 are present in all best-supported models. For each species and life history, all candidate variables are included on the figure, even those with relatively less support. See Figure 1 caption for description of figure colors (Blue = Pink salmon even- and odd-years combined). Shaded panels represent migration timing from freshwater to saltwater.

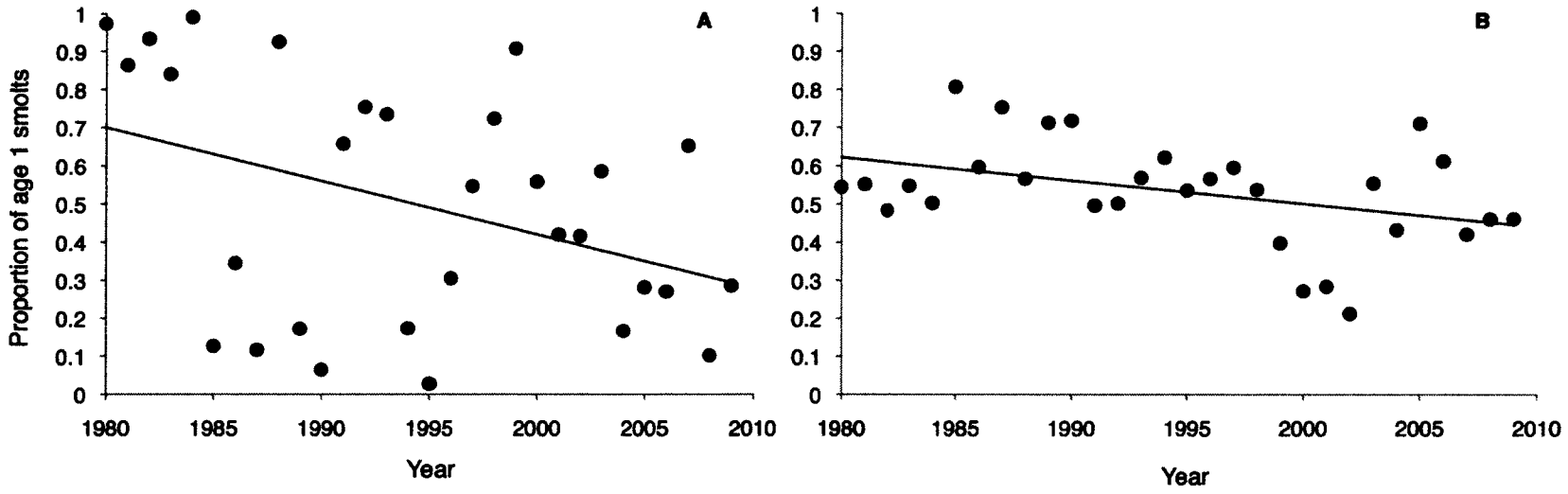

Figure 2.S4. Proportion of age 1 smolts vs. time for sockeye (A) and coho (B). The fitted lines are linear regressions of the proportion of smolts vs. time (A, $b_1 = -0.014$, $SE = 0.006$) (B, $b_1 = -0.006$, $SE = 0.\ 0.002$) salmon.

Text S1

Auke Creek salmonids: life history description and data preparation

Pink salmon

Pink salmon (*Oncorhynchus gorbuscha*) have the simplest life history of the salmonids in Auke Creek. Pink salmon have a strict semelparous two-year life cycle. The result of this life history is that odd- and even-year pink salmon have undergone different demographic and evolutionary histories and are reproductively isolated (Churikov & Gharrett 2002), and we treated them as independent populations. Odd- and even- year populations refer to the year in which adults spawn. Pink salmon enter Auke Creek to spawn in late July through the middle of September. The following spring, juveniles migrate to the ocean. Pink salmon spend approximately one and a half years in the ocean before returning to Auke Creek to spawn. Although pink salmon have a simple life cycle, they demonstrate important biocomplexity and local adaptation in the form of migration timing diversity (Smoker et al. 1998, Gharrett et al. 2001).

Alexansdottir and Mathisen (1982) documented selective harvest impacts on Southeast Alaska pink salmon run timing and population productivity on a broad regional scale spanning the time period from 1926-1944. Specifically, fish traps focused tremendous harvest pressure on early returning mixed stock fisheries in the major migratory paths of pink salmon in Southeast Alaska. Since 1945 there have been regulations to ensure that harvest occurs across the migratory timing of pink salmon in an effort to reduce potential selective effects.

Coho Salmon

Coho salmon (*Oncorhynchus kisutch*) are semelparous and migrate into Auke Creek to spawn from September through October. Juveniles spend either one or two years in Auke Lake before migrating to the ocean as smolts in May and June. The amount of time spent in freshwater is a function of both genetic and environmental influences (Quinn 2005). The largest smolts (age 2) tend to migrate earliest while smaller smolts (age 1) migrate later in the season. Scale samples for aging are collected from

approximately 50 individuals (each day) from 4-12 days of the outmigration. For each year, we used logistic regression and scale aging data to estimate the proportion of age 1 and age 2 smolts that emigrated each day. The term for an effect of time in the logistic model was significant ($P < 0.05$) in 27 out of 30 years, suggesting that this model was an adequate representation of the differences in migration timing between age 1 and age 2 smolts. In years in which time did not influence age proportions (1982, 1983, 1985), daily proportions of age 1 and 2 smolts were estimated from the overall mean of the scale aging data. Excluding these data points had negligible effects on the results.

As an alternative life history, some male coho salmon spend one summer (approximately one half year) in the ocean before returning to Auke Creek to spawn. Individuals with this life history are referred to as "jacks". Other coho salmon spend one and a half years in the ocean before returning to Auke Creek. In Alaska, Auke Creek is the only location that provides complete counts of jacks. Therefore, Auke Creek is critical to investigations that attempt to understand how biocomplexity influences demographic and evolutionary trajectories for this species. Importantly, alternative life histories in Pacific salmon are often a function of both genetic and environmental variation (Quinn 2005, Hutchings 2011).

Sockeye salmon

Auke Creek sockeye salmon (*Oncorhynchus nerka*) juvenile life history and phenology are similar to those of coho salmon (see above). Scale samples are also collected from sockeye salmon smolts, and we used these data to estimate proportions of age 1 and age 2 smolts, as described above. The sample size (number of days that scales were collected and aged) was insufficient (< 4 days) to fit a logistic model in five years. In the remaining years, time significantly ($P < 0.05$) influenced intra-annual age proportions in 21 of 25 years. In years where the time was not a significant effect, it was largely due to a strong cohort effect, where either age 1 or age 2 smolts were much more abundant than the other age class. As described above, we used mean age proportions when samples sizes were limited or when a logistic model did not fit the data.

Adult sockeye salmon are semelparous and enter Auke Creek in July and August. Similar to coho, some male sockeye adopt the "jack" alternative life history. Jacks spend slightly more than one year in the ocean. Other sockeye spend between 2-5 years in the ocean before they return to Auke Creek as reproductively mature adults. To estimate the trend in phenotypic variation we did not use data from 1963, 1964, and 1968-1970 because sampling did not include the latest migrating fish and therefore negatively biased the data in these years. Generally, the vast majority of fish migrate during 1-2 days; but this bias does not influence the value for the median date of migration timing as long as the peak dates are sampled, which they were.

Dolly Varden

Dolly Varden char (*Salvelinus malma*) are fall spawning, iteroparous fish, which migrate into Auke Creek throughout the fall. It is unknown if Dolly Varden successfully spawn in the system, though adult fish are present and appear to be reproductively mature based on phenotypic characteristics. Reproductively immature fish also migrate into Auke Creek during the fall and use Auke Lake as a wintertime refugium. Most fish migrate back to the ocean the following spring, usually during April and May. The number of times they migrate back and forth from freshwater to saltwater and the duration of time they spend in either habitat appears to be variable and is generally unknown. Only data on migration of Dolly Varden to saltwater are available.

There is a strong, positive relationship between timing of emigration and individual size, in which the largest individuals migrate earliest. Large cohort effects could potentially obscure different trends in migration timing for different age classes if they are not accounted for. A proportion of fish was measured (fork length to the nearest 5 mm) on most days during the course of the Dolly Varden emigration. We used these data to estimate overall age proportions with logistic models based on size categories. Logistic models significantly ($P < 0.05$) fit the data in 28 of 30 years. Based on visual inspections of the length frequency distributions in multiple years and our basic understanding of the biology of Dolly Varden in Auke Creek, we used three size

categories; 1-180mm, 181-320mm, and >320mm. For each year, we estimated the proportion of the smallest and the largest size classes with two separate logistic regressions, where logistic models estimated the proportion of small (or large) fish on each date relative to the proportion of all other fish. We then estimated the proportion of individuals in the middle size class by subtracting the estimated proportions of the largest and smallest size classes from 1.0. We conducted linear regressions of the trend in phenology and trait variation vs. time from both the estimated age proportion data and the pooled (all size classes) data. There were minimal quantitative or qualitative differences between the results, so we presented only the pooled data in the primary findings. Dolly Varden are used for subsistence purposes and are economically valuable because of recreational fisheries, but they are not targeted in commercial fisheries.

Coastal cutthroat trout

Coastal cutthroat trout (*Oncorhynchus clarki clarki*) are iteroparous and have a very complex life history. Similar to Dolly Varden, cutthroat trout primarily use Auke Creek and Auke Lake as a wintertime refuge. It is unknown if they reproduce in the system. Cutthroat trout may spend varying amounts of time in the system, from one winter to multiple years. Most cutthroat trout migrate into Auke Creek during the fall and leave the system during the spring. Similar to Dolly Varden, the largest individuals migrate earliest in each year. These individuals are often reproductively mature and migrate to various other stream systems near Auke Creek to spawn (Jones and Seifert 1997). Because large individuals are generally reproductively active (as opposed to migrating to the ocean to feed on marine resources), we assumed that these ecological differences might be reflected in terms of different temporal trends in migration timing.

Data on cutthroat trout migration timing to saltwater were available beginning in 1980. Length was measured for almost every individual emigrating from Auke Creek, except in 1980, 1981, 1983, 1985, and 2001. Based on visual inspections of length frequency distributions and our basic knowledge of cutthroat trout biology in Auke Creek, we used these data to separate the cutthroat trout data into three size classes (small

<191mm, medium 191-320mm, large >320mm). Because nearly every individual fish is measured, we did not use logistic regressions to estimate size (age) proportions. Similar to Dolly Varden, using age structure data as opposed to the entire pooled size distribution did not change the results of the analysis. The pooled cutthroat trout emigration data are presented in the primary findings. Cutthroat trout are used for subsistence purposes and are economically valuable because of recreational fisheries, but they are not targeted in commercial fisheries.

Environmental covariates used for model selection analyses:

Water temperature

Low stream flows and high stream temperatures appear to be negatively correlated with migration timing in adult salmon that migrate after peak annual summer water temperatures (Robards & Quinn 2002, Quinn 2005, Goniea et al. 2006). Daily stream temperatures have been measured at Auke Creek since 1963. Daily stream flow data have only been measured intermittently at Auke Creek; however, intra-annual stream temperature variation is strongly correlated with stream discharge (r = -0.646, Fukushima and Smoker 1997). Therefore, stream temperature was used as an index of local abiotic conditions at the site of spawning/migration for adult salmon. Average stream temperatures during the peak period of migration timing were used to describe local conditions encountered by the majority of adult migrating pink salmon (August 20 – September 10), coho salmon (September 15 – October 10), and sockeye salmon (July 1 – July 31).

We hypothesized that temperature could influence migration timing from freshwater to saltwater by two mechanisms. First, temperature may influence migration timing by influencing cumulative growth and development in freshwater such that warm temperatures over an extended period of time increase growth and development causing individual fish to migrate earlier (Groot & Margolis 1991, Quinn 2005). Second, many of the important physiological and developmental changes associated with migration from freshwater to saltwater occur immediately prior to the migratory event (Groot & Margolis

1991, Hodgson et al. 2006). Therefore, temperatures during the time period immediately prior to migration may be important in determining inter-annual variation in timing. Also, migration timing from saltwater to freshwater is associated with the temporal availability of food resources (pink, sockeye, coho salmon, Dolly Varden char, immature cutthroat trout) or spawning (mature cutthroat trout). Therefore, temperatures immediately prior to and during the migration event may act as a cue for phenological events in other species (i.e. prey) or environmental conditions in other locations. To approximate the first mechanism we used average stream temperatures during the previous year. Specifically, average stream temperature from June – March was used for Dolly Varden, cutthroat trout, sockeye, and coho salmon and temperatures from September – February were used for pink salmon. For the second mechanism, we used average stream temperatures during the period of peak outmigration timing for each species (April for pink salmon and May for all other species). Though there was marginal correlation ($r = 0.5$) between these variables, we included both in linear models because they are not redundant in a statistical or biological sense (Burnham & Anderson 2002).

Stream Flow

Based on our own observations, we hypothesized that sockeye salmon (adults and jacks) and pink salmon migrate into Auke Creek during the first major flow event during their peak reproductive period. To obtain values for this covariate we used precipitation records because precipitation is highly correlated with stream flow (one-day lag $r = 0.865$). NOAA has maintained a nearly complete record of daily precipitation near the mouth of Auke Creek since 1963. During the peak migratory time period for sockeye and pink salmon, we used the first date that 2 inches of rain were measured during a four-day period or the middle date from the 7-day period with greatest precipitation, whichever came first. Stream flows during the coho migration are generally higher, and we used the middle date from the 7-day period with greatest precipitation as a covariate for this species (adults and jacks). Our variable for peak flow was only marginally

correlated with stream temperature (Pearson's correlation $r = 0.327$ pink, $r = 0.088$ coho, $r = 0.144$ sockeye salmon).

Oceanic conditions

Oceanic conditions during the spring and summer before spawning can influence migration timing (Hodgson et al. 2006, Crozier et al. 2011, Mundy & Evensen 2011), potentially by influencing reproductive development (Groot & Margolis 1991), or acting as an indirect cue/indicator for migration conditions in estuaries or freshwater (e.g. Dahl et al 2004, Hodgson 2006). It is unclear if temperature has a gradual effect over longer periods of time (and by necessity larger geographic space) or if conditions immediately prior to migration and during the final and most dramatic physiological changes are of most importance. Sea-surface temperatures were obtained from the International Comprehensive Ocean-Atmosphere Data Set. Values were obtained from two 1° x 1° grid boxes located at 58°-59°N and –(134°-136°W). Overall mean values for the month that each salmon species migrates into Auke Creek were computed from data within these grids (pink salmon = August, coho = September, sockeye = June and July). This location includes the area immediately around Auke Bay and the ocean area used by each salmon species in their final migration towards Auke Creek.

The Pacific Decadal Oscillation (PDO) is a composite measure of oceanic temperature conditions and is correlated with sea-surface temperatures and productivity in the North Pacific (Mantua et al. 1997). Therefore, the PDO provides an index of broad scale oceanic conditions that may influence migration timing. PDO measurements were obtained from the University of Washington (http://jisao.washington.edu/pdo/PDO.latest). The mean of the monthly PDO values from March-June were used for sockeye salmon, April-July were used for pink salmon and May-August for coho salmon. This time period is important for final somatic and gametic development and is related to migration timing in sockeye salmon across the Northern Pacific (Hodgson et al. 2006).

Density

Density dependent growth and survival for salmonids in freshwater is well documented (Quinn 2005, Groot & Margolis 1991). We hypothesized that increased density could delay migration timing from freshwater to saltwater if growth and development are limited by density interactions (Reed et al. 2010). We used the overall number of migrating fish of each species as our measurement of density.

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Table 2.S1. Results from the linear regressions of timing of migration and intra-annual trait variation vs. year. Regression describes which data were used in the analysis, n = sample size, b = slope from the regression, SE(b) = standard error of the slope, L CI = lower 95% confidence interval, U CI = upper 95% confidence interval, r^2 = coefficient of determination, P = P value of the regression analysis.

Species/life history	Regression	n	b	SE(b)	R^2	P
Pink salmon adults	Median	20	-0.253	0.12	0.19	0.057
odd year	Range	20	-0.340	0.12	0.30	0.013
Pink salmon adults	Median	20	-0.331	0.11	0.32	0.010
even year	Range	20	-0.227	0.12	0.17	0.068
Sockeye adults	Median	47	0.192	0.13	0.05	0.147
	Range	47	-0.311	0.21	0.05	0.150
Sockeye jacks	Median	40	-0.305	0.16	0.09	0.059
	Range	36	-0.189	0.24	0.02	0.432
Coho adults	Median	39	-0.418	0.07	0.51	0.000
	Range	39	-0.002	0.13	0.00	0.990
Coho jacks	Median	40	-0.307	0.06	0.39	0.000
	Range	39	0.366	0.09	0.29	0.000
Pink salmon fry	Median	19	-0.273	0.17	0.13	0.132
even year	Range	19	-0.278	0.12	0.24	0.034
Pink salmon fry	Median	19	-0.494	0.15	0.39	0.004
odd year	Range	19	-0.214	0.14	-0.02	0.139

Table 2.S1 continued

Coho smolts age 1	Median	30
	Range	30
Coho smolts age 2	Median	30
	Range	30
Sockeye smolts age 1	Median	30
	Range	30
Sockeye smolts age 2	Median	30
	Range	30
Dolly Varden	Median	31
	Range	31
Cutthroat trout	Median	31
	Range	31

0.070	0.09	0.02	0.419
0.214	0.08	0.19	0.016
-0.091	0.09	0.04	0.314
-0.192	0.13	0.07	0.147
0.105	0.15	0.02	0.498
-0.064	0.13	0.01	0.631
-0.140	0.16	0.03	0.377
-0.312	0.09	0.29	0.002
-0.070	0.12	0.01	0.558
-0.327	0.11	0.24	0.005
-0.119	0.12	0.03	0.338
0.392	0.24	0.08	0.115

Table 2.S2. Model selection results for migration timing from saltwater to freshwater. AIC_C values for the models predicting the median date of migration timing from saltwater to freshwater for reproductively mature Pacific salmon. The model with the lowest AIC_C is highlighted in yellow. *Y* = year, *T* = temperature during migration, *P* = PDO, *S* = sea surface temperature, *F* = peak stream flow.

Model	Pink salmon	Coho adults	Coho jacks	Sockeye adults	Sockeye jacks
Null	271.66	261.62	256.69	372.42	314.18
Y	262.43	235.79	239.19	372.30	312.47
T	268.00	263.37	258.79	369.51	313.55
PDO	272.96	263.57	258.64	367.68	316.18
SST	273.62	259.74	254.90	373.54	316.29
PF	267.30	244.94	254.47	372.40	315.88
Y+T	252.42	230.65	239.16	371.17	313.31
Y+P	264.42	238.01	241.38	369.26	314.27
Y+S	264.34	233.31	237.04	373.69	314.69
Y+F	261.38	230.82	241.41	372.43	314.38
T+P	267.36	265.24	260.87	365.48	315.62
T+S	269.69	261.96	256.53	369.75	315.75
T+F	266.37	247.07	256.60	370.26	315.49
P+S	274.68	261.56	256.76	369.50	318.40
P+F	268.90	247.07	256.58	366.31	318.05
T+F	269.52	246.90	255.03	372.22	318.05
*Y * T*	254.45	229.86	**237.04**	372.69	313.06
*Y*F*	262.98	230.06	243.74	374.30	**311.77**
Y+T+P	**252.38**	232.47	241.15	367.83	315.25
Y+T+S	253.57	230.91	238.63	371.77	315.64
Y+T+PF	254.38	**228.24**	241.35	371.98	315.42
Y+P+S	266.20	235.63	239.20	371.23	316.58
Y+P+F	263.51	233.17	243.73	368.16	316.41
Y+S+F	263.66	231.56	238.53	372.63	316.65
T+P+S	269.57	263.88	258.69	366.74	317.93
T+P+F	266.64	249.24	258.88	365.10	317.74
T+S+F	268.01	249.22	256.85	369.09	317.74
Y+T+P+S	254.27	232.78	240.71	369.23	317.66
Y+T+P+F	254.68	230.33	243.44	367.58	317.58
Y+T+S+F	255.63	230.14	239.90	371.25	317.79
Y+P+S+F	265.82	234.04	240.78	369.05	318.77
T+P+S+F	268.85	251.51	259.22	**365.02**	320.11
Y+T+P+S+F	256.68	232.32	241.95	367.65	320.05

Table 2.S3. Model selection results for migration from freshwater to saltwater. AIC_C values for the models predicting the median date of migration timing from freshwater to saltwater for the various species and life histories. The model with the lowest AIC_C is highlighted in yellow. *Y* = year, *T* = temperature during migration, *TD* = temperature during the developmental period leading up to migration, *D* = conspecific density (See covariate descriptions).

Model	Pink salmon	Coho age 1	Coho age 2	Sockeye age 1	Sockeye age 2	Dolly Varden	Cutthroat trout
Null	275.96	171.93	174.42	206.28	207.83	200.35	202.65
Y	267.50	173.36	175.46	207.93	209.12	202.11	203.79
T	240.02	158.58	138.88	205.00	200.44	172.46	172.34
TD	262.53	170.10	166.04	207.71	209.91	194.23	196.23
D		171.07	176.55	208.08	209.82	200.37	203.75
Y+T	225.68	158.99	139.04	206.55	201.96	174.74	174.11
Y+TD	258.57	170.33	168.16	209.73	211.42	196.52	198.39
Y+D		173.09	176.23	210.03	211.03	202.34	205.58
T+TD	238.59	160.54	136.94	203.39	200.96	172.88	172.62
T+D		156.97	141.08	206.19	201.27	173.68	173.00
TD + D		169.01	168.33	209.93	211.94	195.01	198.25
Y * T	228.04	161.44	141.33	202.93	203.19	177.16	176.30
Y * TD	256.36	172.68	170.62	201.31	209.49	198.73	200.82
T*TD	239.98	162.99	138.79	205.75	201.07	175.28	175.08
Y+T+TD	226.97	160.54	138.22	205.66	201.83	175.30	174.89
Y+T+D		159.45	140.39	208.23	202.29	176.13	175.38
Y+TD+D		171.46	170.26	212.16	213.44	197.46	200.67
T+TD+D		158.99	139.33	205.63	202.72	174.39	174.00
Y+T+TD+D		161.63	140.35	208.15	203.31	176.99	176.64

Table 2.S4. Pearson product moment correlations between environmental variables and time (Year) for those data used to predict migration timing for each life history and species. All values above the double horizontal line are for data used to predict median date of migration timing into freshwater from the ocean, and values below are for data used to predict salmonid migration timing from freshwater into saltwater. T refers to water temperatures during peak migration timing, F refers to flows during peak migration timing, PDO refers to values of the Pacific Decadal Oscillation, SST refers to sea-surface temperature, and TD refers to temperatures during developmental periods in freshwater (see Text S1 for more information). The label "All" refers to data used for all species and life histories migrating into saltwater except for pink salmon.

	Year	Flow	PDO
Pink T	0.1492029	0.3269946	0.2577341
Coho T	0.3070841	0.0883677	0.2485382
Sockeye T	0.3703783	0.1443037	0.1126701
Pink F	0.02640422		-0.07258238
Coho F	-0.3127213		-0.03700868
Sockeye F	-0.5028006		-0.1098793
Pink PDO	0.1532378	-0.07258238	
Coho PDO	0.05972725	-0.03700868	
Sockeye PDO	0.3022053	-0.1098793	
Pink SST	0.03254216	0.1684941	0.3125356
Coho SST	-0.08595782	0.4070197	0.09457787
Sockeye SST	-0.0712369	0.3015931	-0.1616757

	Year	Temp
Pink T	0.1734565	
All T	0.3263582	
Pink TD	0.3374658	0.5330291
All TD	0.480725	0.5431625

CHAPTER 3: GENETIC CHANGE FOR EARLIER MIGRATION TIMING IN A PINK SALMON POPULATION[1]

Summary

To predict how climate change will influence populations, it is necessary to understand the mechanisms, particularly microevolution and phenotypic plasticity, which allow populations to persist in novel environmental conditions. Although evidence for climate-induced phenotypic change in populations is widespread, evidence documenting that these phenotypic changes are due to microevolution is exceedingly rare. In this study, we use 32 years of genetic data (17 complete generations) to determine whether there has been genetic change toward earlier migration timing in a population of pink salmon that shows phenotypic change; average migration time occurs nearly 2 weeks earlier than it did 40 years ago. Experimental genetic data support the hypothesis that there has been directional selection for earlier migration timing, resulting in a substantial decrease in the late migrating phenotype (from >30% to <10% of the total abundance). From 1983-2011 there was a significant decrease – over three fold – in the frequency of a genetic marker for late migration timing, but there were minimal changes in allele frequencies at other neutral loci. These results demonstrate there has been rapid microevolution for earlier migration timing in this population. Circadian rhythm genes, however, did not show any evidence for selective changes from 1993-2009.

Introduction

It is becoming increasingly apparent that adaptive microevolution can occur rapidly in wild populations [1-4]. Nonetheless, there is a paucity of empirical evidence for rapid adaptive microevolution (i.e., genetic change) in response to climate warming, largely because it is unclear if many climate induced phenotypic changes have a genetic basis or are due to phenotypic plasticity [5]. In other words, observed phenotypic changes may be due to the same genotypic distribution producing a new phenotypic

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[1]Kovach, R. P., A. J. Gharrett & D. A. Tallmon. 2012. Genetic change for earlier migration timing in a pink salmon population. *Proceedings of the Royal Society B* 279:3870-3878.

distribution (plasticity). Appropriate methods, including genetic data or quantitative genetic designs, will help clarify the influences of plastic and genetic adaptations to climate change [6-9] and help predict and quantify the impacts of global change on ecosystems and biodiversity. This information is critically important given the proliferation of evidence suggesting that life history traits are changing in many populations as a response to global climate change [e.g. 10,11].

Generally, migration events are timed to coincide with environmental conditions that maximize individual fitness, and many species will have to change their migration timing to match new environmental conditions produced by climate change [12,13]. Changes in migration timing for Pacific salmon populations may be particularly necessary [14,15], because salmonid phenological events – the timing of seasonal life history events – are often highly adapted to local thermal conditions in freshwater rivers, streams, lakes, and also the ocean [16-18]. Phenological traits are generally heritable in salmonid populations (median $h^2 = 0.51$, [19]), and it is hypothesized that microevolutionary changes in migration timing may be one mechanism that would allow salmon populations to persist under climate warming [14,20,21]. A general trend toward earlier migration timing observed in many salmonid species and populations [22-26] supports this hypothesis, but molecular genetic evidence for microevolution toward altered migration timing is non-existent.

In this study we use phenotypic data on migration timing, archived genetic samples, and data from a marker locus, the allele frequencies of which were experimentally altered more than 30 years ago, to determine whether change in migration timing in a population of pink salmon has a genetic basis (i.e., microevolution). Although rare, experimental genetic data in salmon populations can provide a tool by which genetic changes can be tracked in natural populations [27]. Specifically, we observed that both even- and odd-year adult pink salmon that spawn in a warming Alaskan stream (Fig. 1) are migrating into freshwater earlier and are migrating over a shorter period of time (Fig. 2, 3 [15,26]). Due to a strictly semelparous, two-year life cycle, pink salmon have the potential for rapid rates of adaptive evolution relative to other salmon species (in terms of

number of years). The combination of high trait heritability, short generation time, and observed phenotypic change provides a suitable context to study evolutionary change over a contemporary and relatively short time frame.

In many vertebrate species, maturation schedule and migration timing are influenced by endogenous circadian or circannual rhythms that are driven by photoperiod [28-30]. Photoperiod also appears to be a primary cue that initiates adult maturation and migration timing in Pacific salmon [18,31]. Recently, researchers have identified crucial molecular components of the circadian rhythm cycle in salmon, including genes in *Clock* (a transcription factor) and *Cryptochrome* (an inhibitor) [32,33]. *OtsClock1b* has been used to detect Chinook salmon population structure that was not evident from neutral microsatellite locus data [32]. Latitudinal clines in *OtsClock1b* allele frequencies exceed neutral expectations for Chinook salmon, chum salmon (*O. keta*), and pink salmon (*O. gorbuscha*) and indicate that local adaptation may be responsible for patterns of clock gene frequencies across geographical space [34,35]. Additionally, *OtsClock1b* and *Cryptochrome2b* map to genomic regions that explain variation in growth and development in juvenile coho salmon (*O. kisutch*, [33]). To test our hypothesis that there has been genetic change for earlier migration timing, we used >30 years of temporal genetic data (17 complete generations) from the odd-year population and predicted that there would be a significant decrease over time in a neutral genetic marker manipulated to alter allele frequencies in the late migrating portion of the population as well as evidence of directional selection at circadian rhythm genetic loci.

Methods

Study site

Auke Creek is a small, lake-outlet stream near Juneau, Alaska. There have been complete daily counts (census) of all adult pink salmon migrating into Auke Creek since 1971. Some experimental hatchery activity occurred in the 1970's; since that time, however, there has been little hatchery activity. Historically, the distribution of migration timing of the Auke Creek pink salmon population was moderately bimodal and had

relatively distinct early and late migrating population components that were separated by approximately 20 days (Fig. 3, [36,37]). This bimodality in the migration distribution was associated with distinct phenotypic differences. Toward the end of August returning adults tended to be very "dark" and in advanced states of maturity; beginning in September, ocean fresh "bright" individuals would arrive signifying the beginning of the late migration [38].

Experimental genetic marker for late migration timing

Experimental manipulations in 1979 introduced a putatively neutral genetic marker into the late migrating portion of the odd-year Auke Creek pink salmon population. A neutral marker was used so that it would be possible to genetically track late migrating individuals without influencing their fitness. Selective breeding was used to alter the frequency of two neutral alleles so that late migrating fish were genetically differentiated from earlier migrating fish. Specifically, individuals that migrated into Auke Creek after September 15 were used in the genetic marking experiment (i.e., the latest migrating individuals). A large effective population size ($N_E \sim 400$) was used, and there was no evidence for natural selection at this locus after the 1979 marking event or genetic heterogeneity between the pre- and post-experimental populations at other allozyme loci (See [27,38] for details on the experimental design). Within the late migrating portion of this population, the frequency of the **70* allele at the *MDH B1,2** allozyme locus was substantially increased from 0.056 in 1979 to 0.256 in 1983. Additionally, the frequency of the **130* allele was decreased in the latest migrating individuals from 0.046 in 1979 to 0.023 in 1983 (the third allele **100* changed by necessity due to these manipulations). From 1981-1989 the allele frequencies at this locus did not substantially change (i.e. stayed at pre-experimental levels) in the early migrating portion of the population (**70* = 0.04-0.05; **130* = 0.04-0.05, Fig. 4), and experimental allele frequencies in late migrating fish remained stable and differentiated from early migrating fish (**70* = 0.20-0.30; **130* = 0.01-0.03). Therefore, these alleles genetically marked late migrating individuals and allow us to infer whether changes in

the migration timing distribution are due to changes in the genotype for migration timing. Hence, selection toward earlier migration timing should change the frequency of these alleles toward the frequencies of the early run fish, thus confirming our prediction. The experimental manipulations led to allele frequency differences between early- and late-migrating fish that were substantially larger for the **70* allele than for the **130* allele. We frequently refer only to the **70* allele with the notation LMMA for "late migration marker allele". When we refer to the entire locus we use LMML for "late migration marker locus."

Genetic data

To obtain allele frequency data for the LMML and a control locus not associated with the late portion of the population, approximately 5-30 (generally 10) fish were sampled each day from fish migrating into Auke Creek, Alaska in 1983, 1985, 1987, 1989, 1991, 1993, 2001, and 2011 (but see additional details below). All fish were sampled for skeletal muscle tissue as they passed through the weir, except for samples in 1993. In 1993, samples were collected from newly dead carcasses on each day that fish mortalities (i.e. post-spawning) were observed. Starch gel protein electrophoresis was used to resolve allozyme banding patterns [27]. Data were obtained from fish in 1983 (N = 645), 1985 (N = 587), 1987 (N = 459), 1989 (N = 524), 1991 (N = 507), 1993 (N = 550), 2001 (N = 490), and 2011 (N = 606). Allele frequencies were obtained for the allozyme locus *G3PDH-1** in 1979 (N = 179), 1981 (N = 203), 1983 (N = 726) and 2011 (N = 551). *G3PDH-1** is not associated with migration timing and was used as a comparison (selectively neutral control [39]) to the LMML allele frequencies.

Microsatellite data were obtained from approximately 10 individuals sampled every other day during the migration in 1993, 2001 and 2009. Approximately 160-190 individuals were genotyped at each locus in each year (Supplementary Table 1). DNA was extracted from all samples with the protocol described in [40], and was amplified at 23 putatively neutral microsatellite loci and three candidate loci that are part of the circadian rhythm gene complex (Supplementary Table 1). PCR amplification used

optimized, locus-specific temperature profiles and a QIAGEN Multiplex PCR Kit (QIAGEN, Valencia, CA). PCR products were visualized on a LI-COR 4300 DNA Analyzer. Allele sizes were estimated with SAGA Generation 2 software (LI-COR, Lincoln, NE). All data are available from Dryad (doi:10.5061/dryad.m3c53).

Data analyses

Multiple approaches were used to describe temporal changes in intra-annual variation in the allele frequencies at the LMML over time. For each year, simple graphical comparisons of 5-day running allele frequency averages of the LMMA were used to track changes in genetic differentiation over time. A binomial *t*-test was used to test for significant genetic differences at the LMMA between early and late migrating individuals. We used data from the calendar dates that included the first and last 100 fish sampled in each year.

Gene flow between early- and late- migrating fish could erode the genetic structure introduced by the marking effort. Thus, we estimated the overall frequency of the LMMA, because gene flow alone should not change the overall frequency of the allele, whereas demographic changes that reduced the late run would decrease the frequency of this allele. Obtaining an unbiased estimate of the overall frequency and associated uncertainty at the LMMA across the entire migration timing distribution is complicated because of strong genetic differentiation between early- and late-migrating fish, unequal abundance during different portions of the migration timing distribution, and unequal sample representation across the migration timing distribution.

A parametric bootstrap approach that included the genetic and daily census data was used to resolve these issues. Specifically, in each year the migration timing distribution was systematically separated into five-day "subsamples" starting with the first date that genetic samples were collected. We calculated maximum likelihood allele frequency estimates (f) for each period (i, $i = 1, 2, 3...j$) and then drew random parametric bootstrap samples from a binomial distribution, $x_i \sim Bin(f_i, n_i)$, where n_i are the number of alleles sampled (2 * number of individuals sampled) in each period. For

each year, an overall allele frequency estimate was obtained with $F = (1/(2*\sum_i^j N_i))\sum_i^j \alpha_i$, where $\alpha_i = x_i/n_i * 2 * N_i$ and N_i is the census number of adult fish migrating into Auke Creek during the same 5-day period. One thousand bootstrap replicates were performed and 95% confidence intervals were calculated by excluding the most extreme 0.025% of the smallest and largest values. For 1993, we used census data from 8 days prior to the date of the genetic samples (obtained from carcasses), because this approximates the duration of freshwater life for Auke Creek pink salmon [41]. In 1983, samples were taken on only three days roughly corresponding to the beginning, middle, and end of the migration. To estimate the overall allele frequency for this year, we equally allocated maximum likelihood allele frequency estimates between the sampled dates for each period. We used weighted allele frequencies at *G3PDH-1** in 1979, 1981, and 1983 from data in [42], and used the approach described above to estimate frequencies for 2011. We detected two alleles at *G3PDH-1** and report estimates for the less abundant allele. We did not replicate these analyses at ***130* because the manipulative change in frequency was small (~0.02) and the allele frequencies are very close to 0 (the boundary), resulting in very little power to detect small changes.

Importantly, inter-population gene flow could also influence the frequency of the LMMA, but estimates of contemporary gene flow between pink salmon in Auke Creek and other nearby locations are low (proportion of migrants in each generation m = 0.0015, [27]). Estimates of direct immigration/straying are also low (m = 0.02-0.036, [43,44]). The average frequency of the LMMA in other populations ranges from 0.059 in the most proximate populations (approx. 1-6 km distance) to 0.057 when including populations up to 30 km away [42].

A bootstrap simulation based on allele frequencies at the LMML was used to estimate the total number of fish that belong to the early and late segments of the Auke Creek pink salmon population (essentially a mixed stock analysis). Expectation maximization algorithms [45] were used to allocate fish to the early or late portion of the population by comparing daily running averages of estimated allele frequencies to allele

frequencies from a baseline population (in our case 1983 because this is the first return of post-experimental fish that randomly mated in the wild [46]). Specifically, the simulation uses running 5-day allele frequency averages (e.g days 3,4,5,6,7) to estimate the composition, in terms of origin (early vs. late migrating fish), of the middle date (5). The census number of fish migrating into Auke Creek on that day is multiplied by the estimated contribution of early and late migrating individuals to yield the estimated daily return of early and late migrating fish. This same procedure is performed for each day of the migration to estimate the total contribution of early- and late-migrating fish to the total abundance. Statistical replication is performed through non-parametric bootstrapping of the empirical data. This method makes use of the clear genetic difference between the marked and unmarked portions of the population in 1983 to estimate total population contribution of each phenotype in each subsequent year. The simulation also estimates the median date of migration timing for the early and late migrating portions of the population. For all allozyme analyses, data from the LMML were treated as if the locus was diploid [38].

The population genetic parameter $F_{TEMPORAL}$ was used to describe and compare change in allele frequencies at the nuclear loci. This parameter measures differences in allele frequencies between two samples [47] and is a powerful method to detect genetic changes in populations [9,48]. Changes in allele frequencies at candidate loci that exceed changes at neutral microsatellite loci are evidence of directional selection at this or closely linked quantitative trait loci. To test for directional selection at candidate loci, we used genetic outlier tests [49]. LOSITAN [50] and Bayescan [51] were used to generate estimates of $F_{TEMPORAL}$ and compare these estimates between putatively neutral and candidate microsatellite loci. These are frequentist and Bayesian approaches, respectively. Essentially, these methods attempt to differentiate signals for natural selection from those of genetic drift. Other methods to detect selection at genetic loci exist, but LOSITAN and Bayescan have the lowest type I and II error rates [52]. The recommended settings for LOSITAN were used for all analyses, including an additional 20,000 simulations. In Bayescan, we used 100,000 iterations of burn-in, 20 pilot runs, a

thinning rate of 15 iterations, and retained 8,000 iterations of the MCMC chain to ensure convergence of the posterior distributions with minimal MCMC chain autocorrelation.

Results

Throughout the 1980's the frequency of the LMMA in the latest migrating fish differed from those of the early migrating fish ($P < 0.001$; Fig. 4). Specifically, the allele frequency in samples of fish collected before September 1 was approximately 0.04-0.05 and was 0.21-0.26 in samples collected from the latest migrating fish. Beginning in 1991, this pattern completely disappeared and the LMMA frequency did not differ significantly between early and late migrating fish ($P = 0.69$). A lack of genetic differentiation at the LMMA after 1989 was confirmed in 1993 ($P = 0.91$), 2001 ($P = 0.27$), and 2011 ($P = 0.85$). There was a strong decrease – approximately three-fold – in the total frequency of the LMMA across the entire migration timing distribution from 0.131 ($SE = 0.016$) in 1983 to 0.043 ($SE = 0.008$) in 2011 (Fig. 5). The frequency of the LMMA was relatively stable during the 1980's, but decreased rapidly and significantly ($P < 0.05$) between 1989 and 1993. The frequency of this allele has been relatively constant since 1993.

This rapid change in the LMMA contrasts with the stable frequencies of the *200* allele at the control locus *G3PDH-1*, which changed very little (Fig. 5); its frequency was 0.098 ($SE = 0.022$) in 1979, 0.108 ($SE = 0.011$) in 1981, 0.101 ($SE = 0.011$) in 1983, and 0.109 ($SE = 0.013$) in 2011, which suggests that genetic drift had minimal effects on *G3PDH-1**, and hence the entire population, during this time period. These results support the hypothesis of directional selection for earlier migration timing. The altered allozyme frequencies at the marker locus in the late migrating portion of the population changed substantially during the study period, but such changes were not observed at another locus.

Results from the bootstrap simulation demonstrate that during the 1980's the late migrating genetically marked component of the population accounted for 27-39% of the total abundance (Table 1). This proportion decreased rapidly after 1989 and was

approximately 5% (SE = 2.4%) in 2011. Because of the loss of intra-annual genetic differentiation by time at the LMML after 1989, the simulation was unable to differentiate the early and late migrating population, as demonstrated by the overlap in the median dates of migration timing (Table 1). Therefore, estimates from 1991–2011 should be used with caution. However, the primary pattern is clear and consistent with the other results; the late run portion of the population used to be an important component of the total population abundance, but is no longer. However, overall abundance has not changed (see Discussion).

Conversely, genetic outlier analyses did not provide any evidence for selection at the candidate loci associated with circadian rhythms. Results from LOSITAN indicate that none of the 26-microsatellite loci used in this study appear to be under directional or balancing selection (Supplementary Fig. 1a). Locus-specific estimates of $F_{TEMPORAL}$ were low (0-0.007) across all loci, suggesting that the combined effect of genetic drift and selection has been weak at these loci. Nonetheless, it is noteworthy that the candidate locus *Cryptochrome2b* had the highest $F_{TEMPORAL}$ value of all loci (0.007). This locus had the lowest expected heterozygosity (0.044) and, therefore, was located in the region of the plot in which selection is the most difficult to detect (i.e., has the widest 95% confidence regions). Similarly, results from Bayescan suggest that directional selection is not acting at any of these loci; but there may be mild balancing selection at *Ots101* (Supplementary Fig. 1b).

The small values of $F_{TEMPORAL}$ and relatively large N_E (N_E = 271 [53]) at these microsatellite loci indicate little genetic drift in this population and support the idea that the radical changes in the LMML over the course of the study were due to selection against the late migrating portion of the population. For example, $F_{TEMPORAL}$ was 0.025 at the LMML from 1989-1993 (Waples 1989), a value that is over three times greater than that observed at any of the microsatellite loci (though the time periods are not overlapping). We used LMMA frequencies from other populations in an island-continent model to estimate the migration rate (m – the percent of the population that are immigrants [54]) that would be necessary to achieve the observed changes in the LMMA

in the Auke Creek population from 1989-1991. Depending on the scale of the analysis (only including nearby locations vs. more distant populations) or whether the analysis was restricted to late migrating fish, *m* would need to be 0.69 to 0.85 to satisfy the observed genetic changes. For populations around Auke Creek, these values are 19-24 times higher than the largest demographic estimate of *m* (i.e. dispersal, [42]), and 460-566 times larger than genetic estimates of *m* [27]). As such, migration is not a likely explanation for the observed genetic changes in the LMML.

Discussion

In order to understand whether phenological shifts in a population of pink salmon were due to microevolution, we used genetic data collected from 1979 to 2011 and observed evidence for genetic change associated with shifts towards earlier migration timing. Data from the LMML demonstrated that there was a significant decrease – at least three-fold – in the late migrating portion of the odd-year Auke Creek pink salmon population. This provides evidence of a rapid microevolutionary change in this population, which has proven exceptionally elusive in other studies [5,9]. The trend towards earlier migration timing in this population does not appear to be anomalous, because it is replicated in the even-year population that uses the same freshwater habitat, and in other salmonid species and life histories (Fig. 2,3, [23-26]). Importantly, another recent study that used a modeling approach determined that microevolution for earlier migration timing has occurred in a population of sockeye salmon in the Columbia River [20]. Together, these results provide compelling evidence that recent climate change has influenced the evolutionary dynamics of salmonid populations and their adaptation via migration timing to their respective habitats.

The LMML indicated that a major selection event occurred between 1989-1993. Although we do not know the specific selective pressures that led to earlier migration timing in this population, stream temperatures during peak migration timing in 1989 were the second highest on record, and we observed substantial genetic changes at the LMML in the progeny from this spawning generation. Migrating pink salmon appear to avoid

high stream temperatures; given the trend in migration timing, changes in the genetic marker, and increasing stream temperatures in Auke Creek [26], it appears that earlier migrating fish may have higher fitness in warmer years. Adaptations-by-time [55] for different thermal regimes and biotic interactions are well documented for this population, and there is evidence that early migrating adult fish are adapted to warmer conditions at multiple life stages and life history events (e.g. juvenile developmental rates and migration timing, and adult migration timing, life-span and breeding date) [37,41,56,57]. These patterns of local adaptation result in strong temporal structuring of the population [37]. Another possible explanation is that warm stream temperatures may have caused reproductive overlap (hence gene flow) between early and late migrating fish, and the resulting evolutionary changes are due to outbreeding depression.

Stream temperatures during peak migration approach upper lethal limits in some years [18], which could potentially act as a constraint to further microevolution. However, there are no temporal trends in migration timing for the first 5% ($P = 0.854$), and first 25% ($P = 0.102$) of the migration timing distribution. This observation and data at the LMML suggests that there has been a truncation of the migration timing distribution and strong selection against the latest migrating fish, resulting in the near elimination of this phenotype. The rapid genetic changes imply that climate-induced selection on life history traits may not result in gradual evolutionary shifts. Rather, selection events may be extreme, episodic, and have severely different consequences for different phenotypes (i.e. near elimination of the late-migrating phenotype). Interestingly, the median phenotype appears to have undergone a continuous shift towards earlier timing (as opposed to rapid truncation in one generation), indicating that plasticity must also be responsible the observed change in migration timing.

Temporal structuring due to migration timing complicates whether this represents the evolution of a single population, or selection against one population and a demographic response (increase in abundance) in another. Like other salmon populations, pink salmon in Auke Creek are probably best described as a single population exhibiting intra-population genetic structure due to heritable differences in

migration timing [55]. This is corroborated by weak, but statistically significant genetic differentiation between early and late migrating fish at neutral loci (i.e. there is gene flow between reproductively isolated components of the migration timing distribution) [42, 53].

Interestingly, the progeny of early migrating adult pink salmon historically (1970's - 1980's) had lower early marine survival than progeny of later migrating fish [40,58]. To better understand these phenotypic and evolutionary changes it would be valuable to determine whether this pattern is still present, or if these populations are stable because of increased fitness (compensation) at some other population vital rate (e.g. reproductive success in freshwater). Additionally, it is unclear if these changes could lead to future trophic mismatches between juvenile pink salmon and the availability of marine resources [15]. Despite the fact that Auke Creek has undergone significant warming and there have been substantial phenological shifts, both odd- and even-year pink salmon populations are stable [26] and population abundance in 2011 was the second highest on record. Given that changes in migration timing can influence population dynamics, it seems plausible that the observed changes in migration timing have allowed these populations to remain resilient to environmental change [59].

Selection was not detectable in the circadian rhythm genes we used in this study. It is possible that these loci do not directly influence migration timing [60]. Along the same lines, migration timing is likely a complex quantitative trait influenced by many genes. If selection did not act on our candidate loci for migration timing, then it must have acted on other loci that influence migration timing. However, archived genetic samples were only available dating back to 1993 and it is possible that selection occurred at circadian rhythm genes before this date. This is hinted at by the LMML data that showed a decrease in the late migrating phenotype/genotype between 1989-1993. Alternatively, sampling more selectively neutral markers would increase our power to detect subtle differences in genetic change (e.g. changes at *Cryptochrome2b*).

Genetic variation for migration timing is an important aspect of biocomplexity in Pacific salmon populations that decreases population stochasticity [61]. Along with the

shift in the distribution of migration timing and loss of the late migrating component of the population, there is no longer distinct bimodality in the distribution of migration timing in the even- or odd-year populations. We no longer observe the clear phenotypic distinction between early and late migrating individuals that was once present in the system [27,37]. Apparently, the very late migrating phenotype has been greatly reduced or potentially lost. Though microevolution may have allowed this population to successfully track environmental change, it may have come at the cost of a decrease of within-population biocomplexity – the loss of the late-run [61]. This is not a surprising result; by definition directional selection will decrease genetic variation. However, it does highlight the importance of maintaining sufficient genetic and phenotypic variation within populations in order for them to have the ability to respond to environmental change. In this particular population, genetic and phenotypic variation have allowed for evolutionary changes in an important life history trait, the result of which is that this population is persisting through rapid temperature warming. These findings are an important empirical advancement toward understanding the process of climate-induced microevolution in wild populations.

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Tables

Table 3.1. Estimates of the abundance (*N*) of the early and late (genetically marked) portions of the population. *M* is the estimated median date of migration timing of the early and late migrating portions of the population based on the number of days after July 1. *PLR* is the proportion of the overall population composed of late migrating fish.

Year	Run	*N*	SE (*N*)	*M*	SE (*M*)	*PLR*	SE (*PLR*)
1985	*Early*	17619.11	972.38	52.52	0.09		
	Late	6494.89	972.38	68.67	2.51	0.27	0.04
1987	*Early*	4812.46	375.12	51.77	0.50		
	Late	3052.54	375.12	61.86	0.17	0.39	0.05
1989	*Early*	3403.25	223.64	56.92	1.25		
	Late	1596.75	223.64	69.70	1.87	0.32	0.04
1991	*Early*	5668.26	226.46	53.35	0.09		
	Late	937.74	226.46	53.35	1.78	0.14	0.03
1993	*Early*	1545.25	53.37	67.64	0.27		
	Late	137.75	53.37	67.20	4.48	0.08	0.03
2001	*Early*	6959.42	217.64	59.46	0.17		
	Late	569.58	217.64	58.03	1.15	0.08	0.03
2011	*Early*	25634.18	650.21	51.04	0.59		
	Late	1347.82	650.21	53.92	6.99	0.05	0.02

Figures

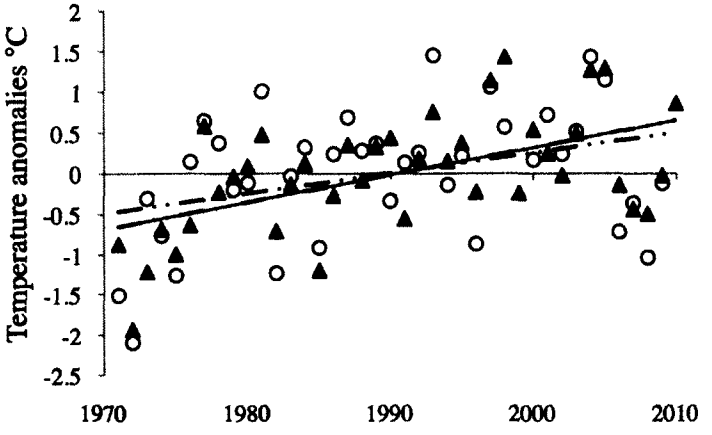


Figure 3.1. Yearly mean temperature anomalies for stream temperature in Auke Creek (black triangle, solid line), and ambient temperature at Auke Bay (open circle, dashed line), Alaska.

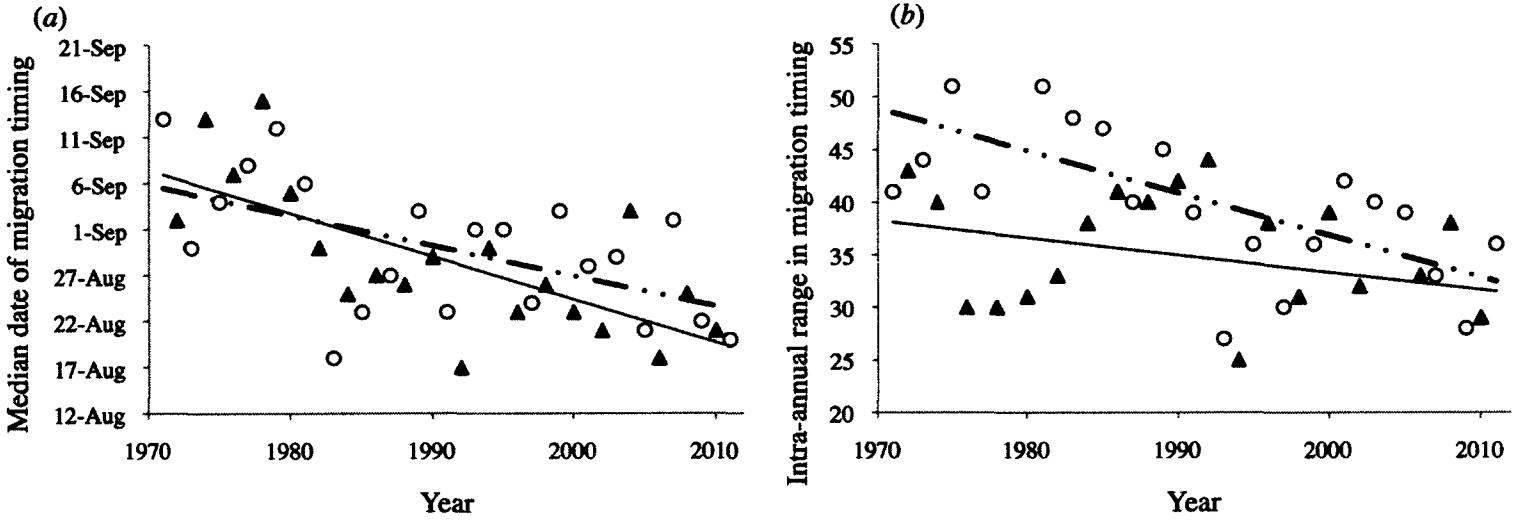


Figure 3.2. Change in migration timing of pink salmon in Auke Creek, Alaska. (*a*) Median date of migration timing vs. year for odd- (open circle, dashed line) and even-year (closed triangle, solid line) pink salmon populations. (*b*) Phenotypic variance in migration timing vs. year for odd- and even-year pink salmon (symbols as above). Phenotypic variance was measured as the number of days over which the central 95% of the migration timing distribution entered Auke Creek.

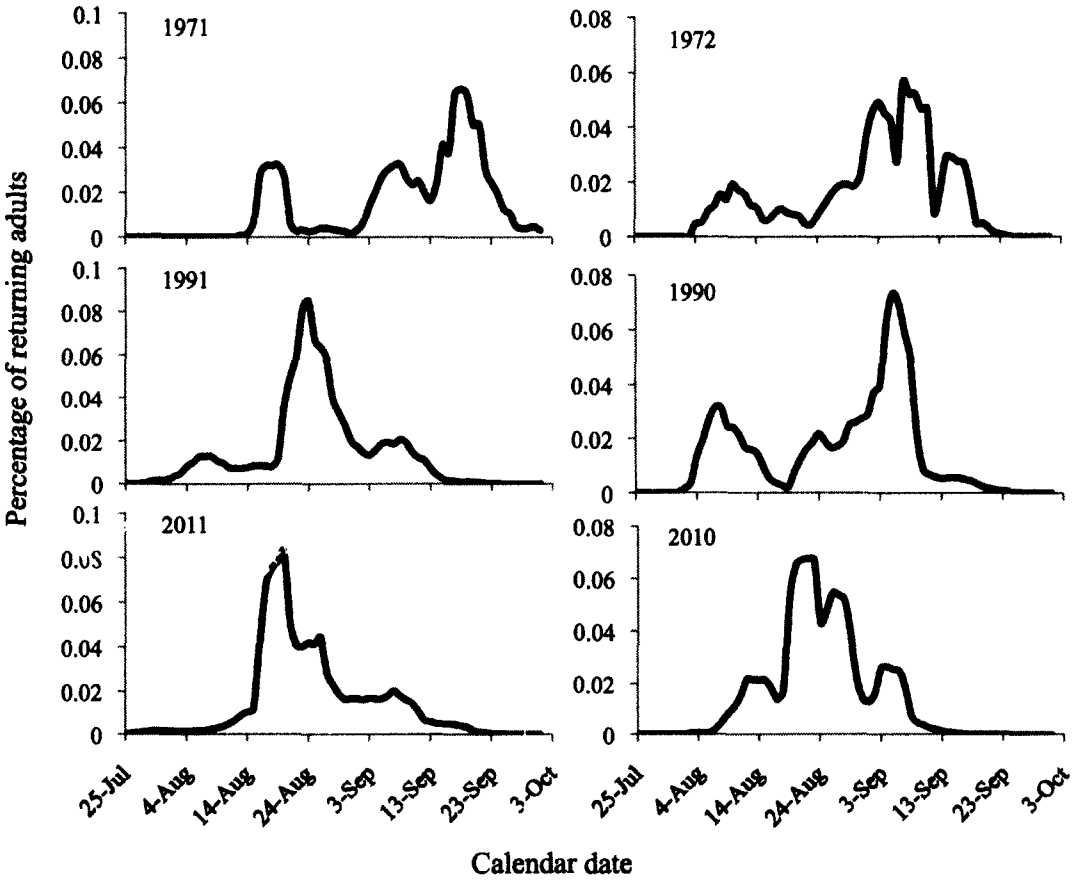


Figure 3.3. Pink salmon migration timing distributions from three years representing the beginning, middle and end of the time series for the odd- and even-year populations. The data series are five day running averages of the total percentage of migrating adults on each day. The odd-year population is on the left and even-year is on the right.

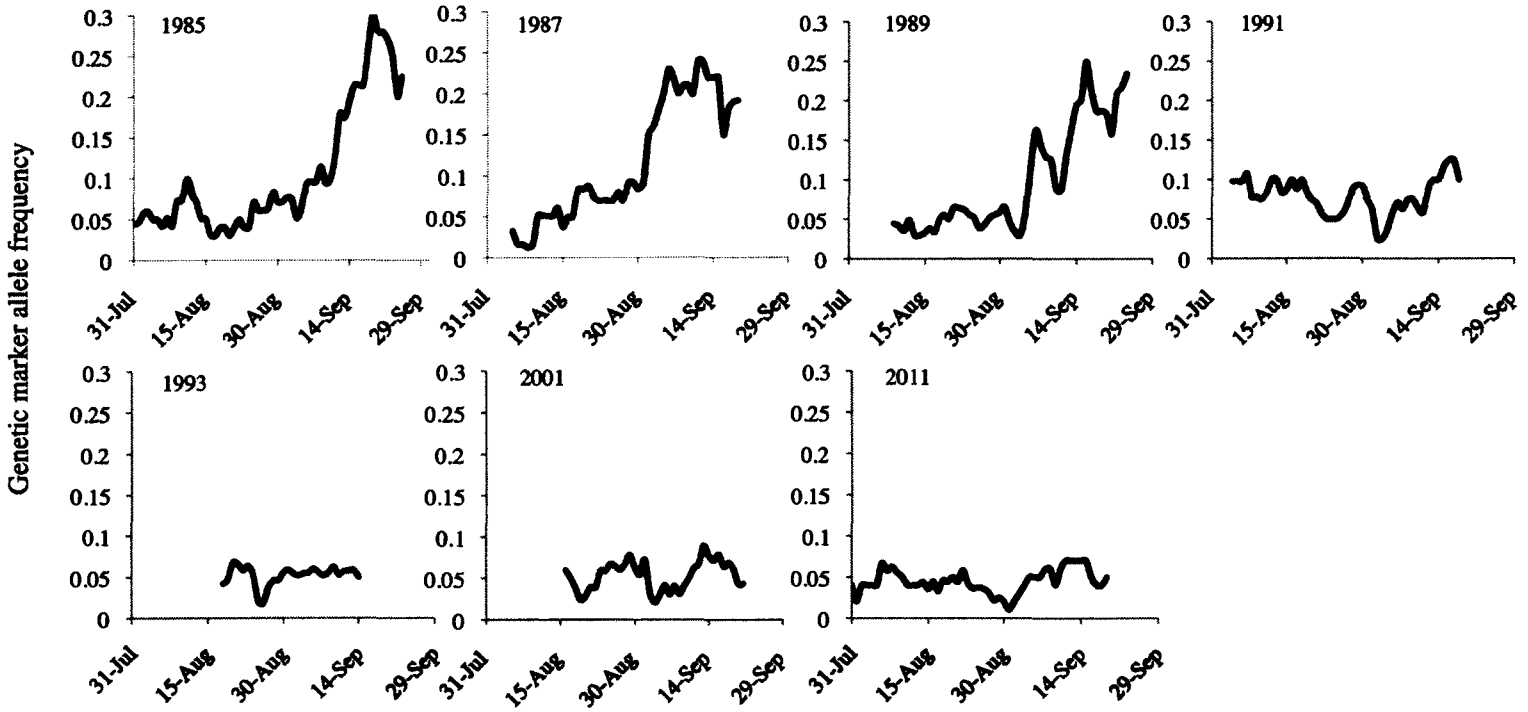


Figure 3.4. 5-day running averages of the frequency of the late migration marker allele (LMMA). 1983 is not included because samples were only taken on three days.

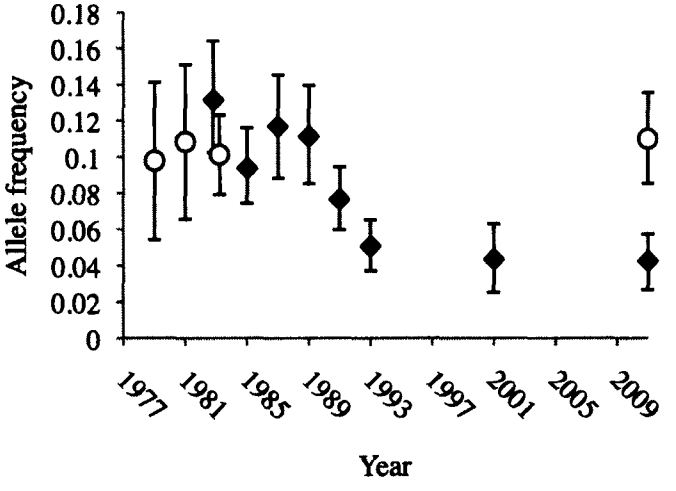
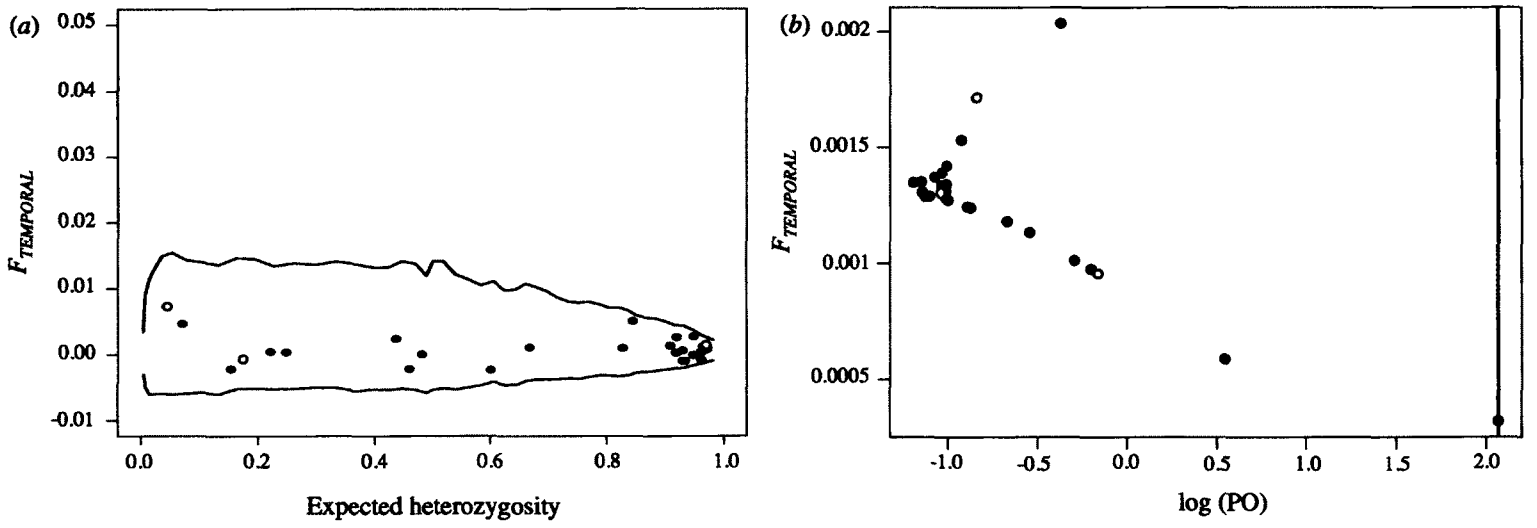


Figure 3.5. Overall frequency of the late migration marker allele (LMMA, closed black diamond) and the alternate allele at control locus *G3PDH-1 *200* (open circle) across years. Error bars are the 95% confidence intervals for each estimate.

Supplementary Information

Supplementary Table 3.1. Microsatellite loci used in this study. The values under the year headings are the number of individuals genotyped at each locus.

Locus Name	Reference	1993	2001	2009
Oki10	Smith et al. 1998	152	177	171
Ots1	Banks et al. 1999	153	172	180
Omy1011	Spies et al. 2005	161	179	189
One109	Olsen et al. 2000	150	175	175
Ogo1a	Olsen et al. 1998	150	188	169
Ogo2	Oslen et al. 1998	159	186	171
Ots101	Nelson and Beacham 1999	138	173	175
Ssa197	O'Reilly et al. 1996	152	179	177
OtsClock1b	O'Malley and Banks 2007	153	187	187
One102	Olsen et al. 2000	154	182	183
OtsG311	Williamson et al. 2002	163	186	186
One111	Olsen et al. 2000	163	185	184
One105	Olsen et al. 2000	152	185	179
Oki100	Beachem et al. 2009	131	171	149
One13M	Scribner et al. 1996	158	183	187
Oki200	Beacham et al. 1999	156	181	184
Ots103	Nelson and Beacham 1999	152	184	173
Str60-1	Estoup et al. 1993	161	185	185
Str60-2	Estoup et al. 1993	135	181	158
Ssa20.19-1	Sanchez et al. 1996	252	458	189
Ssa20.19-2	Sanchez et al. 1996	254	466	189
One103	Olsen et al. 2000	253	467	187
Cryp2b	O'Malley et al. 2010	242	467	181
Ogo6	Olsen et al. 1998	241	446	184
Ogo8	Olsen et al. 1998	252	459	188
Cryp3	Kathleen O'Malley pers. com.	207	443	156



Supplementary Figure 3.1. Results of genetic outlier tests between data from 1993, 2001, and 2009 based on (*a*) LOSITAN and (*b*) Bayescan. The circles are the estimates of $F_{TEMPORAL}$ for each locus (open – candidate locus, closed – neutral locus). The black lines in (*a*) are the upper and lower 95% confidence intervals for the null (neutral) region of $F_{TEMPORAL}$ vs. expected heterozygosity. The black line in (*b*) denotes the location of the 5% false discovery rate of the logarithm of the posterior odds vs. $F_{TEMPORAL}$. Loci to the right of this line are potentially subject to balancing or directional selection.

CHAPTER 4: TEMPORAL PATTERNS OF GENETIC VARIATION IN A SALMON POPULATION UNDERGOING RAPID CHANGE IN REPRODUCTIVE TIMING[1]

Abstract

Genetic diversity is necessary for population persistence in a rapidly changing world, but little is known about the distribution of genetic variation within many populations or how genetic diversity has changed as a result of biological responses to climate change. We examined genetic change in a pink salmon population over 16 generations during a period of environmental warming by quantifying the genetic effective population size (N_e) and genetic differentiation due to variation in reproductive timing. We predicted that temporal trends toward earlier reproductive timing and a corresponding loss of phenotypic variation would decrease genetic differentiation based on reproductive timing and N_e. We found significant ($P < 0.05$) genetic differentiation based on reproductive timing and genetic heterogeneity between early- and late-reproducing fish. There was evidence for divergent selection between early- and late-migrating fish at circadian rhythm genes, but results varied over time. Estimates of N_e were large (>1000) and the N_e/N_c ratio was 0.17-0.38. Despite shifts in reproductive timing, there was no evidence for changes in within-population genetic differentiation or N_e over the course of this study. These results suggest that in instances of population stability, genetic diversity may be resistant to climate-induced changes in reproductive timing.

Introduction

Describing and understanding the distribution of genetic variation within populations is fundamental to the management of species, particularly in a rapidly changing world (Allendorf and Luikart 2007). Climate-induced changes in the spatial

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[1]Kovach, R. P., A. J. Gharrett & D. A. Tallmon. Temporal Patterns of Genetic Variation in a Salmon Population Undergoing Rapid Change in Reproductive Timing. *Submitted to Evolutionary Applications on October 22, 2012.*

distribution and reproductive timing of populations can influence numerous aspects of demography including dispersal, survival, reproductive success, and overall abundance, all of which have consequences for the distribution of genetic variation within and among populations (Frankham 1996, Parmesan 2006). For example, reductions in habitat and increasing fragmentation as a result of distributional shifts toward higher elevation can reduce genetic diversity and increase genetic differentiation among populations of alpine mammals (Rubidge et al. 2012). Similarly, phenological changes – the timing of seasonal life history events such as reproduction – could alter patterns of genetic diversity for populations that exhibit genetic differentiation based on differences in reproductive timing (Hendry and Day 2005) or increase variance in reproductive success if intra-population variation in migration timing influences individual fitness. Despite substantial evidence for climate-induced changes in reproductive timing (Parmesan 2006), there is little information documenting how these changes influence microevolution within populations (Franks and Weis 2009).

Since 1971, pink salmon in rapidly warming (Fig. 1A) Auke Creek, Alaska, have undergone a shift towards earlier migration timing into freshwater (nearly two weeks) and have lost nearly 30% of their phenotypic variation in migration timing (Fig. 1B, Taylor 2008, Kovach et al. 2012a). Auke Creek pink salmon reproduce soon after entering freshwater, consequently migration timing reveals reproductive timing. Within this population, adult migration timing is highly heritable, there is a genetic component to developmental rates, and there is evidence for local adaptation based on migration timing for a suite of life-history traits (Hebert et al. 1998, Smoker et al. 1998). Changes in migration timing for this population appear to be due, at least in part, to microevolutionary responses to natural selection against late-migrating fish (Kovach et al. 2012b). Therefore, this population is ideal for exploring how climate-induced changes in reproductive timing can influence genetic diversity.

Ultimately, the ability to adapt to novel environmental conditions is limited by the amount of genetic diversity within a population (Frankham 1995a, Allendorf and Luikart 2007). Loss of genetic diversity can increase probability of extinction because genetic

variability gives rise to alternative phenotypes (e.g. morphologies or behaviors) that can respond to environmental change (Lacy 1997, Frankham 2005). At a larger scale, genetic diversity can influence ecological interactions within and between species, and thereby impact overall ecosystem dynamics (Hughes et al. 2008, Palkovacs et al. 2011), making it a critical component of biodiversity which merits further attention in conservation and natural resource management (Laikre 2010).

One way to measure a population's evolutionary potential and genetic diversity is the genetic effective size of a population (N_e). The N_e of a population is one of the most important parameters in evolutionary and conservation biology (Waples 1989, Frankham 1995) because it describes the rate at which genetic variation is lost, the influence of inbreeding, and the relative strengths of selection and migration in determining allele frequencies (Allendorf and Luikart 2007). In so doing, N_e provides important information about population viability (Frankham 1995b). Many factors can cause a population's N_e to be less than the census population size (N_c) including natural selection, uneven sex ratios, temporal variation in population size, over-dispersed variance in reproductive success, and population age structuring (Frankham 1995b). As such, N_e is a particularly useful parameter because it captures information about genetic and demographic processes.

Little is known about N_e and the N_e to N_c ratio for pink salmon and whether these values are stable over time. Pink salmon have approximately equal sex ratios and non-overlapping generations, and therefore variance in reproductive success (Geiger et al. 1997) and inter-generational fluctuations in N_e (Kalinowski and Waples 2002) should be the primary factors that reduce N_e relative to N_c for this species. Variance in the reproductive success of pink salmon may be highly over-dispersed because competition for spawning areas (i.e., density dependence) can lead to redd superimposition (i.e., destruction of spawning redds and reproductive failure of some adults Groot and Margolis 1991, Fukushima et al. 1998, Quinn 2005). Additionally, in many pink salmon populations including Auke Creek, there is family-correlated marine survival, (Geiger et al. 1997, Geiger et al. 2007), which results in very high survival for some families and

low survival in other families. This results in some families contributing the majority of individuals to the next generation, while many other families contribute few or no progeny. Overall, these aspects of pink salmon ecology indicate that this species may have highly depressed N_e relative to N_c in many populations. Whether evolutionary shifts in migration timing have further increased reproductive variance as a result of natural selection, or increased density dependence owing to a compressed migration distribution, is unknown for the Auke Creek population.

Describing genetic population structure within and between populations is another way to quantify genetic diversity. Understanding within- and between-population genetic structure is critical to understanding the evolutionary and demographic forces influencing a population and for making informed management decisions (Waples 1998, Waples and Gaggiotti 2006). Whereas genetic structure between populations is a well-described phenomenon, much less attention has been given to within-population genetic structure resulting from phenotypic differences among individuals (Hendry and Day 2005). As a result of high heritability in migration timing (median $h^2 = 0.51$, Carlson and Seamons 2008), salmonid populations often exhibit significant intra-annual genetic differentiation based on reproductive timing (McGregor et al. 1998, Fillatre et al. 2003, Hendry and Day 2005). The Auke Creek pink salmon population historically exhibited temporal population structuring, including significant genetic differentiation at selectively neutral and experimentally manipulated allozyme loci (McGregor et al. 1998, Gharrett et al. 2001), based on migration timing (Smoker et al. 1998, Gharrett *unpublished data*).

With genetic data spanning the period from 1979-2009 (16 complete generations) we address four questions regarding patterns of intra- and inter-annual genetic diversity for this pink salmon population: (1) What is the magnitude and pattern of genetic differentiation and temporal autocorrelation in allele frequencies due to variation in migration timing? (2) Are genetic differences between early- and late-migrating fish larger at circadian rhythm genes than at selectively neutral loci? (3) What are N_e and the N_e to N_c ratio for odd-year pink salmon in Auke Creek? (4) Have N_e and patterns of population genetic differentiation based on migration timing changed in concert with the

significant changes in reproductive timing observed in this population? We predicted there would be significant genetic differentiation between early- and late-migrating fish, but that the magnitude of differentiation would decrease from 1979-2009 as a result of changes in migration timing and loss of phenotypic variation. Loss of differentiation could arise via genetic admixture as a result of a compressed spawning distribution, and/or as a result of decreased genetic variation due to a strong reduction in the late-migrating phenotype (i.e., truncation of the migration timing distribution). We also predicted that there would be divergent selection at circadian rhythm genes between early- and late-migrating fish because this population demonstrates strong local adaptation as a result of migration timing (Hebert et al. 1998, Smoker et al. 1998). In other salmon species and populations these genes appear to influence migration timing and development (O'Malley et al. 2007, O'Malley et al. 2008, O'Malley et al. 2010a, O'Malley et al. 2010b). For N_e, we predicted that N_e/N_c would be less than values observed in other species due to variation in abundance, strong density dependence, and family correlated survival, and that directional selection toward earlier migration timing may have increased reproductive variance and thereby decreased N_e over time.

Methods

Study site, population and genetic data

Pink salmon have a strictly semelparous, two-year life cycle that produces distinct odd- and even-year populations within a stream (Aspinwall 1974). This study focuses on the odd-year pink salmon population, which has been censused during its spawning migration into Auke Creek, Alaska, since 1971. From 1971-2011 the abundance of pink salmon varied widely in Auke Creek, from N_c = 1,548 (1995) to N_c = 28,127 (1999), but the population is stable and population growth rate is at the replacement level ($\lambda \approx 1.0$, Kovach et al. 2012a). Tissue samples that had been archived were analyzed for this study, in conjunction with genetic data from another study of this population that took place from 1979-1983 (McGregor 1983, McGregor et al. 1998).

Fish were sampled as they migrated through the Auke Creek weir (2001 and 2009) or from recent (< 24 hours) carcasses (1993). Genetic samples were collected from 10 fish every other day so that 180~200 fish were genotyped in each year. Each fish was genotyped at 23 microsatellite loci, three of which (*OtsClock1b, Cryp2b, Cryp3*) are located within the *Clock* and *Cryptochrome* circadian rhythm genes that are correlated with migration timing and development rate in other salmonid species and populations (O'Malley and Banks 2008, O'Malley et al. 2010a, O'Malley et al. 2010b). Complete descriptions of tissue sampling and microsatellite genotyping were presented in Kovach et al. (2012b). We checked for deviations from Hardy-Weinberg predictions by using a pseudo-exact test and tested for significant pair-wise linkage disequilibrium between loci in GENEPOP (Raymond and Roussett 1995).

Data analysis

Genetic structure based on migration timing

We calculated G''_{ST} (Meirmans and Hedrick 2011) between the earliest and latest migrating fish in 1979, 1981, 1983, 1993, 2001, and 2009. Estimates of G''_{ST} from 1979-1983 were based on allele frequencies from 11-12 allozyme loci (McGregor 1983, McGregor et al. 1998). Sample sizes varied between loci, run components (early or late), and year, but averaged ~100 for both early and late migrating fish from 1979-1983, and ~50 for both early- and late- migrating fish from 1993-2009. We used G''_{ST} as our measurement of effect size because it is relatively insensitive to the substantial differences between allozyme and microsatellite loci in mutation rates and the numbers of alleles (Hedrick 2005, Meirmans and Hedrick 2011). For the microsatellite data, we used GenoDive (Meirmans and Van Tienderen 2004) to calculate G''_{ST} and associated 95% confidence intervals by bootstrapping over loci. Because we did not have genotypic data (only allele frequencies and sample sizes) for the allozymes, we calculated G''_{ST} manually and obtained 95% confidence intervals by bootstrapping over loci in the boot package in Program R (R Development Core Team 2009). To test the hypothesis that genetic differentiation based on reproductive timing has changed across years and potentially

declined as a result of changes toward earlier reproductive timing and decreasing phenotypic variation, we compared 95% confidence intervals for G''_{ST} between years. This method is more conservative than directly testing for a significant difference between two estimates, but with large numbers of molecular markers, this is a powerful method to detect genetic change in a population (Schwartz et al. 2007).

We used multiple methods to describe within-population genetic structure for genotypes collected from 1993, 2001, and 2009. Temporal genetic autocorrelation based on migration timing was estimated using GENALEX V. 6.3 (Smouse and Peakall 1999, Peakall et al. 2003, Peakall and Smouse 2006). If temporal population structure exists, the genetic correlation between individuals decreases as the time period between dates of migration timing increases. This method condenses the genetic data from the microsatellite loci into a matrix of pair-wise individual-by-individual squared genetic distances (Smouse and Peakall 1999) in order to compute correlation coefficients between groups of individuals. We used four-day periods as our distance class (grouped individuals that migrated within four days of each other), and tested for autocorrelation between individuals within distance classes and between individuals in different distance classes (i.e., separated by differences in migration timing). We also investigated the influence of grouping individuals using other distance classes (1 and 2 days) but it had little qualitative effect. For each year, we used 9999 permutations and 999 bootstrap replicates to estimate variance and assess significance. We compared across years the 95% confidence intervals for the correlation coefficients estimated in 1993, 2001, and 2009 to test for inter-annual changes in genetic population structure based on migration timing.

Within-population genetic structure in Auke Creek pink salmon may exist along a gradient of time (isolation by time) or bimodally/multimodally (i.e., an early and late migrating population). To test for distinct population groupings we used program STRUCTURE (Pritchard and Rosenberg 1999) to estimate the number of sub-populations (K) within the overall migration timing distribution. For each year we used 100,000 iterations of burn-in and 500,000 samples from the posterior distribution to estimate the

likelihood of K given the data. We considered $K = 1 - 6$ and averaged the Ln likelihood based on 4 iterations of the MCMC chain.

We used G - tests for genic differentiation in GENEPOP (Raymond and Roussett 1995) to directly test for genetic heterogeneity between non-consecutive quartiles of the migration timing distribution. Quartiles of the migration timing distribution were determined from the census of migrating pink salmon collected at Auke Creek. Samples collected on the day that a particular quartile was reached (e.g., 25 percentile) were allocated to both the first and second quartile. As a result, we did not test for differentiation between adjacent quartiles.

To determine if there has been selection on the three circadian rhythm loci or any of the putatively neutral loci, we used an F_{ST} outlier approach (Beaumont and Nichols 1996). Data from the first and last 10 days of sampling were used to represent the "early" and "late" migrating phenotypes, respectively. For each year, we used LOSITAN (Antao et al. 2008) to test if differentiation (F_{ST}) between early- and late-migrating fish at any particular locus differed from a null distribution of F_{ST} generated from the empirical data assuming an island model of gene flow between early- and late-migrating fish.

N_e and the N_e/N_c ratio

N_e was estimated using the temporal variance in allele frequencies across samples (F_{TEMP}) and approximate Bayesian computation based on summary statistics estimated from single samples (ONeSAMP). We used multiple approaches because each method makes different use of the data, which allows a more robust understanding of N_e and the N_e/N_c ratio (Luikart et al. 2010, Waples and Do 2010). This approach let us better evaluate if the values have changed from 1993-2009. The F_{TEMP} approach requires genetic samples from two time periods and estimates N_e based on the value of N_e that would generate the observed genetic differences between samples (Waples 1989). Samples were available from three time periods, which made it possible to make three N_e estimates (1993-2001, 2001-2009, 1993-2009). NeEstimator 1.3 was used to estimate N_e with the F_{TEMP} approach (Peel et al. 2004). We also estimated N_e with ONeSAMP, which

estimates N_e by making use of eight population genetics summary statistics and compares the observed estimates of the summary statistics to values obtained from simulated Wright-Fisher populations of known N_e (Tallmon et al. 2004, Tallmon et al. 2008). For the prior distribution on N_e we used 100 – 3000. We did not include the circadian rhythm genes in the datasets used to estimates of N_e.

To calculate N_e/N_c we used various combinations of the census values of abundance (N_c) recorded at the Auke Creek weir. For results from ONeSAMP, we used the harmonic mean of N_c for the three generations prior to the N_e estimate because ONeSAMP estimates N_e based on linkage disequilibrium (among other summary statistics), which is influenced by mating events in multiple generations (Waples 2005, Luikart et al. 2010). Non-overlapping confidence/credible intervals of N_e provide evidence that these values have changed during the time period of this study (Schwartz et al. 2007). To calculate N_e/N_c based on N_e values from the F_{TEMP} method, we used the harmonic mean of N_c for the time period spanning from the first sample collection to the generation prior the second sample collection (Waples 2005).

Results

Genetic data

We genotyped Auke Creek odd-year pink salmon at 23 microsatellite loci in 1993, 2001, and 2009, all of which conformed to Hardy-Weinberg proportions or had F_{IS} values near zero (i.e., no evidence of null alleles). Given 23 loci, there were 253 pair-wise tests for linkage disequilibrium in each year, and by chance we expected to observe about 13 significant values (at $\alpha = 0.05$). In each year, the number of significant estimates was ≤ 13 (1993 = 12, 2001 = 9, 2009 = 13). There were no pairs of loci exhibiting significant linkage in all three years.

Genetic structure by migration timing

Intra-annual estimates of G''_{ST} between the earliest and latest migrating fish ranged from -0.003 to 0.010 for data from 1979-2009, but bootstrap 95% confidence

intervals included 0 in each year (Table 1). In each year, there was evidence of significant ($P < 0.05$) positive autocorrelation ($r = 0.005$ in 1993; $r = 0.012$ in 2001; $r = 0.013$ in 2009) between individuals migrating within four days of one another (Figure 2). The majority (20 of 26) of estimates for r were negative for fish that migrated more than 4 days apart from one another, which means that individuals that migrate at different times differ more genetically than expected by chance. The largest single estimate ($r = -0.052$ CI: -0.0231 - -0.0808) was for the maximum distance class (40 day separation) in 2009. Weak negative autocorrelation was significant ($P < 0.05$) in 14 of the 20 estimates before a sequential Bonferroni correction for multiple tests, and significant in 7 of 20 after correction (each data set corrected independently).

There was no evidence of population clustering or substructure revealed by STRUCTURE (i.e., $K = 1$). Based on more sensitive G – tests for genetic differentiation, we were unable to refute the null hypothesis of no genetic differentiation between pairs of quartiles of the migration timing distribution in 1993 (Table 2). After sequential Bonferroni correction there was significant ($P < 0.05$) genetic differentiation between the first and third and between the first and fourth quartiles in 2001. There was also significant differentiation between the first and fourth, and between the second and fourth quartiles in 2009.

Three F_{ST} values exceeded neutral expectation ($\alpha = 0.05$), and in each case it was one of the three-microsatellite loci associated with circadian rhythm genes. However, the outlier loci indicating directional selection between early- and late-migrating individuals differed from year-to-year (Figure 3). In 1993, *OtsClock1b* had F_{ST} values higher than neutral expectation, and in 2009 *Cryp2b* and *Cryp3* had F_{ST} values that exceeded this expectation. With 69 F_{ST} estimates (across all years), we would anticipate approximately three false positives (69 * 0.05) at $\alpha = 0.05$, so these results should be interpreted with caution. Nevertheless, it is notable that the only loci demonstrating outlier behavior were the circadian rhythm genes that we considered *a priori* to be candidates for natural selection.

Genetic effective population size and N_e/N_c ratio

Point estimates of N_e based on F_{TEMP} ranged from 1079-3788 depending on the time period of interest (Table 3). ONeSAMP was only able to estimate N_e based on data from 1993 (N_e = 1256 CI: 788 - 2644). N_e/N_c ratios obtained from the ONeSAMP and F_{TEMP} were alike and point estimates varied from 0.177 to 1.02 across the time periods considered (Table 3). The F_{TEMP} estimates of N_e and N_e/N_c from 1993-2009 appear unrealistically high because the N_e estimate for this time period is greater than the harmonic mean of the census values (3678). Therefore, those results should be treated cautiously. Overall, the harmonic mean of the point estimates for N_e was 1440 (Waples and Do 2010), and for N_e/N_c was 0.29.

Inter-annual changes in genetic differentiation and N_e

Generally, there did not appear to be strong evidence for an inter-annual change in genetic differentiation across the migration timing distribution from 1993-2009 (Figure 2). There were, however, 5 pairs of estimates for the autocorrelation coefficient that did not have overlapping 95% confidence intervals in different years (i.e. the strength of genetic correlation between individuals migrating the same number of days apart from one another varied in different years). The estimate for r for fish migrating within 4 days of each other in 1993 was smaller (r = 0.0054 CI: 0.0016 - 0.0091) than that for 2001 (r = 0.0125 CI: 0.0095 - 0.0154), and the 95% confidence interval for 2009 barely overlapped (r = 0.0125 CI: 0.0091 - 0.0160) with the estimate from 1993, suggesting that positive autocorrelation for fish migrating within 4 days of one another was weaker in 1993. Additionally, three of the non-overlapping estimates were higher than expected positive values of the autocorrelation coefficient (relative to the associated negative value in a different year) for which we have no biological explanation.

The 95% confidence intervals for G''_{ST} in each year overlapped with the 95% confidence intervals for G''_{ST} for every other year. Therefore, there is no evidence for significant differences in the strength of genetic differentiation by migration timing in different years using this summary statistic. This does not support the hypothesis that

there has been a decrease in genetic structure by migration timing due to significant changes in the variance and central tendency of adult migration timing into freshwater. Similarly, the 95% confidence/credible intervals for the point estimates of N_e from ONeSAMP and F_{TEMP} overlapped across all time periods (where estimates were available).

Discussion

We observed subtle genetic differentiation due to variation in migration timing in odd-year Auke Creek pink salmon. Specifically, there was genetic-heterogeneity between non-adjacent quartiles of the migration timing distribution and decreasing genetic correlations between individuals migrating further apart in time. However, the sizes of effects were small and did not result in any distinct population grouping based on allele frequencies. We did not detect a consistent signal across years for divergent selection between early- and late-migrating fish at loci located in circadian rhythm genes, but these genes did show some evidence of divergent selection in some years.

Contrary to our prediction, patterns of within-population genetic differentiation have remained relatively stable since 1979, despite rapid changes toward earlier migration timing and loss of phenotypic variation. Kovach et al. (2012b) noted that there was a selection event against late-migrating fish in 1989, which caused a loss of genetic structure at an experimental genetic marker for late migration timing. At these microsatellite loci, we observed little evidence for genetic differentiation due to migration timing in 1993 (two generations after this event), and less positive genetic correlation for individuals migrating within four days of one another. More recent data (2001 and 2009) demonstrate that the significant genetic differentiation observed in the late 1970's and 1980's (McGregor 1983, McGregor et al. 1998, Gharrett et al. 2001) had been re-established. This suggests that climate-induced selective events may be episodic and lead to short-term changes in neutral genetic structure, but general patterns, at least in this instance, re-emerged.

Alternatively, it may be more difficult to detect subtle differentiation in some years than in others. For example, intra-annual environmental variation (e.g., stream flow) may cause individuals from different portions of the migration timing distribution to migrate earlier or later, resulting in overlaps in migration timing and admixture in the genetic samples collected. This possibility is supported by the fact the number of days over which fish migrated into Auke Creek in 1993 was the lowest on record for this population. Or, biological phenomenon such as strong assortative mating, and/or reduced fitness of progeny from hybridization events between individuals with different reproductive timing are acting within this population. Therefore, it may require sampling multiple generations to detect genetic differentiation based on variation in migration/reproductive timing. Finally, there were no significant changes in estimates of G''_{ST} between early- and late-migrating fish from 1979-2009; but, this method was less sensitive than the autocorrelation and homogeneity tests, and it is possible that we failed to detect very subtle temporal changes in population structure. Unfortunately, this was the best method available to compare differentiation between the allozyme allele frequency data from 1979, 1981, and 1983, and the microsatellite data we collected from samples in 1993, 2001, and 2009.

Our estimates of N_e from F_{TEMP} and ONeSAMP were in fairly close agreement, and the harmonic mean N_e across all estimates was 1440 (Waples and Do 2010). Though the time periods for which the estimates apply are slightly different (Waples 2005), this provides a reasonable value for N_e from 1991-2007. Our harmonic mean estimate is considerably larger than the median N_e of 267 reported in a recent meta-analysis (Palstra and Ruzzante 2008). However, their values are likely to be biased low based on the fact that it is more difficult to estimate larger values of N_e (Waples and Do 2010). Similarly, the harmonic mean N_e/N_c of 0.29 exceeded the median estimate of 0.16 reported in the same study, and the median value 0.11 from Frankham (1995). For pink salmon in this population, family-correlated marine survival can reduce N_e/N_c to approximately 0.5 within a generation (Geiger et al. 1997, Geiger et al. 2007). Fluctuating abundance and over-dispersed variance in reproductive success occurring during reproduction and early

freshwater development appear to further reduce N_e/N_c by by nearly 0.25. To the best of our knowledge, these are the first molecular-based estimates of the N_e/N_c ratio for pink salmon, and these values did not support our prediction that pink salmon have a reduced N_e/N_c ratio compared to other species and salmonid populations (Palstra and Ruzzante 2008). These relatively large N_e values may partially explain the lower than expected patterns of genetic differentiation based on migration timing, as even very small levels of gene flow between fish with different reproductive timing could greatly diminish any genetic signal for differentiation. However, these estimates are somewhat larger than expected given the ecology of this particular salmon population, and in some cases are biologically implausible (e.g. the N_e/N_c value > 1.0). This suggests that these estimates are erroneous or may be approaching a metapopulation N_e value due to gene flow between different populations (Waples 2010).

From 1993-2009, we did not detect a trend in N_e, which is contrary to our prediction that N_e and the N_e/N_c ratio would have decreased from evolutionary shifts in migration timing. One explanation for this observation is that the decline in the late migrating phenotype may have decreased the number of redds of early-spawning fish that are destroyed as a result of superimposition by late-spawning fish (Fukushima and Smoker 1998). However, this form of compensation may have a threshold if intra-annual variation in migration timing continues to decline.

Understanding the factors that limit or decrease genetic diversity will improve our understanding of adaptive potential and therefore persistence in the face of climate change (Frankham 2005, Kinnison and Hairston 2007). This is highlighted in Pacific salmon studies, where life-history variation, presumably due in part to genetic variation (i.e., biocomplexity), has a major impact on population dynamics and resilience (Hilborn et al. 2003, Greene et al. 2010, Schindler et al. 2010). Despite the proliferation of studies demonstrating climate-induced phenological shifts, the effects of these shifts on genetic diversity have received scant attention. We focused on a single population, but changes in phenology can also influence the distribution of genetic variation across populations if it affects interactions among populations and the probability of gene flow (Franks and

Weiss 2009, Rosetto et al. 2011). Surprisingly, patterns of genetic diversity in Auke Creek pink salmon have remained relatively stable and have been resilient to rapid phenological and environmental changes, including a two-week shift in migration timing. Although further studies on other organisms are needed to confirm these results, it would appear that changes in spatial distribution might have a greater influence on genetic diversity (Alsos et al. 2012, Rubidge et al. 2012). Overall, the fact that this population has remained stable and maintained initial genetic diversity while undergoing significant evolutionary change to an important life history trait during environmental warming is a striking demonstration of the value of protecting genetic and phenotypic diversity.

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Tables

Table 4.1. Estimates of G''_{ST} between early and late migrating fish from 1979-2009. LCI is the lower 95% confidence interval and UCI is the upper 95% confidence interval.

Year	G''_{ST}	LCI	UCI
1979	-0.003	-0.007	0.001
1981	0.010	-0.003	0.020
1983	0.001	-0.008	0.006
1993	-0.002	-0.007	0.003
2001	0.005	-0.002	0.015
2009	0.002	-0.006	0.012

Table 4.2. Results (*P* - values) for *G* - tests for genetic differentiation between non-consecutive quartiles of the migration timing distribution. Bold values are significant after Bonferonni correction for multiple tests.

	1993		2001		2009	
Quartile	1	2	1	2	1	2
3	0.078		**0.002**		0.088	
4	0.782	0.369	**0.003**	0.912	**0.034**	**0.009**

Table 4.3. Estimates for the genetic effective population size N_e and the N_e/N_c ratio. Values in parentheses are the lower and upper 95% confidence/credible intervals. For the temporal method (F_{TEMP}), the 2001 value refers to the time period 1993-2001, the 2009 value refers to 2001-2009 and the value with an asterisk refers to 1993-2009.

		N_E	
Method	1993	2001	2009
ONeSAMP	1256 (788, 2644)	∞	∞
F_{TEMP}		1079 (688, 2025)	1266 (782, 2584)
			3788 (1943, 17125)*
		N_E / N_C	
	1993	2001	2009
ONeSAMP	0.215	NA	NA
F_{TEMP}		0.385	0.177
			1.029

Figures

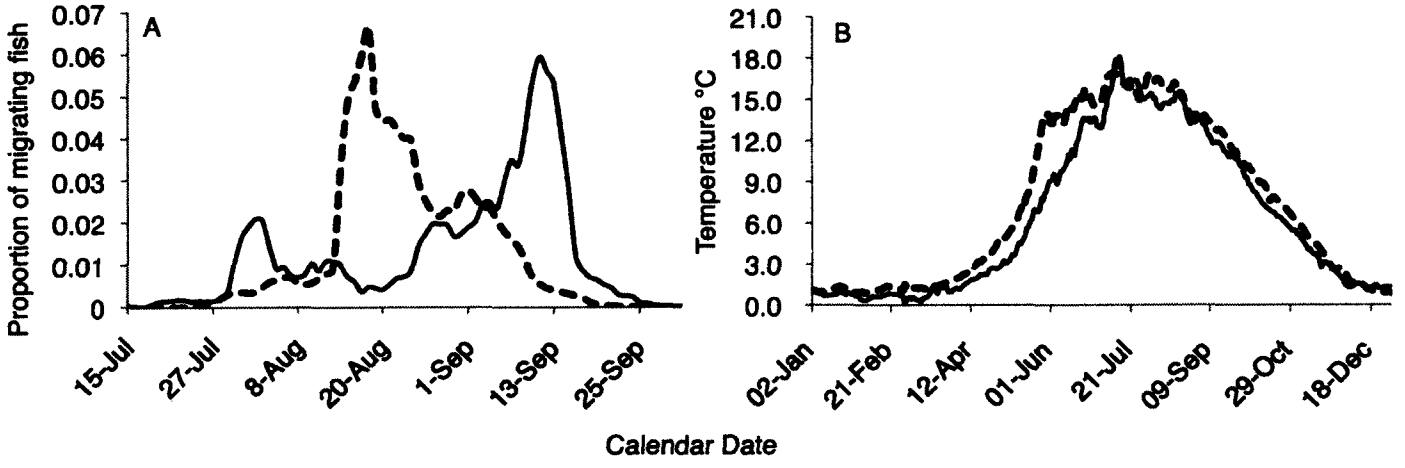

Figure 4.1. The intra-annual distribution of migration timing (reproductive timing) and stream temperature in Auke Creek Alaska. The lines in panel A are the five-day running averages of the proportion of odd-year pink salmon migrating into Auke Creek averaged from 1971-1979 (solid line) and 2003-2011 (dashed line). Panel B depicts the average daily temperature in Auke Creek from 1971-1976, (solid line), and 2006-2010 (dashed line).

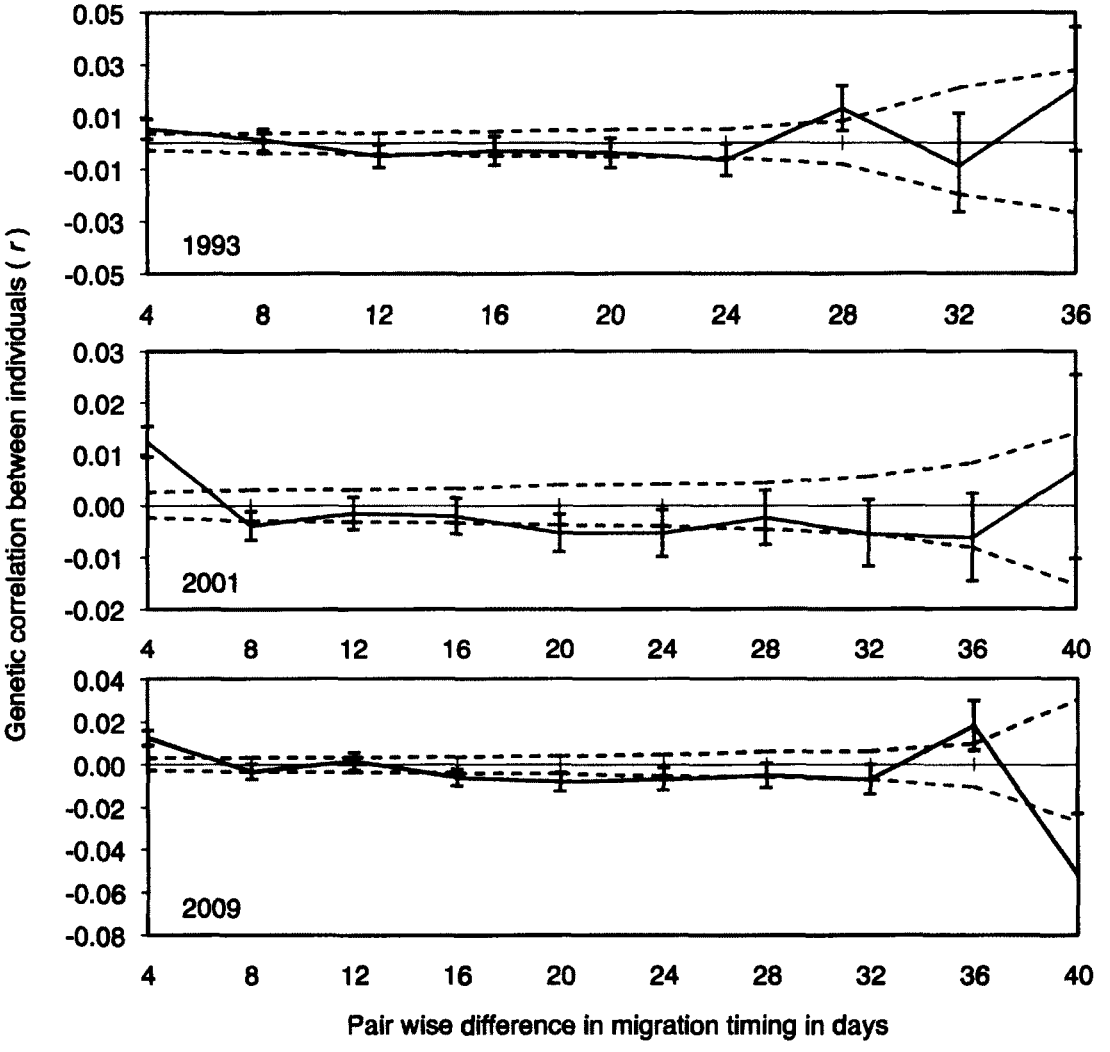

Figure 4.2. Estimates of genetic autocorrelation as a function of the number of days between samples from the migration timing distribution. Dashed lines denote the random expectation 95% confidence areas. Error bars for the point estimates are the 95% bootstrap confidence intervals.

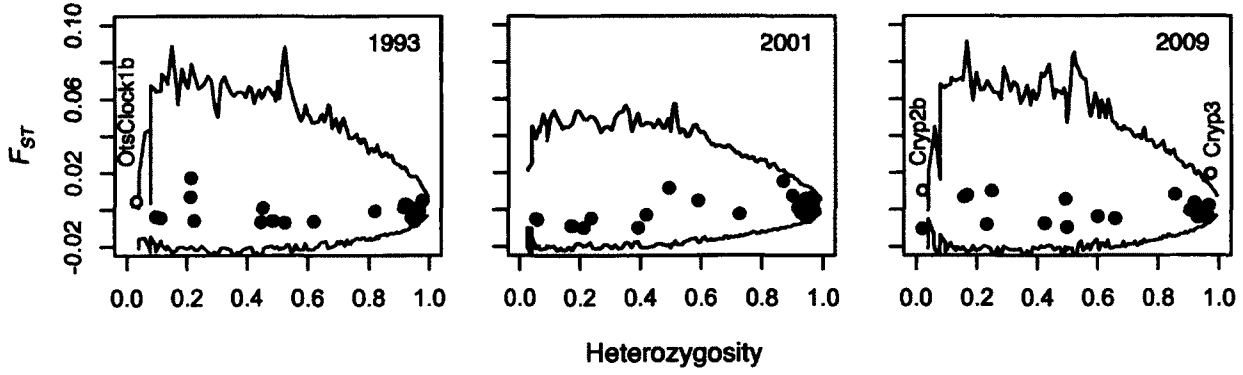

Figure 4.3. Results of intra-annual genetic outlier tests between early- and late-migrating fish based on F_{ST} versus heterozygosity. The circles are the point estimates of F_{ST} for each locus. The black lines denote the neutral 95% confidence intervals for F_{ST}. Each F_{ST} outlier is labeled and marked with an open circle.

CHAPTER 5: CONCLUSIONS

Introduction

The purpose of this research was to describe the patterns, processes, and consequences of climate-induced changes in salmonid migration timing in Auke Creek, Alaska. Specifically, the objectives of this study were (1) to identify and describe patterns of salmonid migration timing, (2) to determine mechanisms underlying changes in migration timing, and (3) to examine how migration timing and changes in migration timing influence intra-population genetic variation. In CHAPTER 2, I used 30-50 years of census data from Auke Creek, Alaska and estimated temporal trends in salmonid migration timing, population abundance, and the temporal availability of salmon as an ecosystem service. In CHAPTER 3, I estimated temporal genetic change at neutral, experimentally altered, and circadian rhythm genes to determine if there has been evolution by natural selection for earlier migration timing in odd-year pink salmon. In CHAPTER 4, I used 23 microsatellite loci and historical allozyme data to estimate intra- and inter-annual patterns of genetic diversity for the same pink salmon population. With the data from CHAPTERS 2-4 I addressed all of the primary objectives for this study.

Climate Change and Salmonid Migration Timing

In CHAPTER 2, I observed a general trend toward earlier migration timing (Fig. 2.2A) and decreasing intra-annual variation (Fig. 2.2B) in migration timing across all species, life stages, and life histories. Stream temperatures were related to migration timing for all species, life stages, and alternative life histories (except sockeye jacks); but the direction of the relationship was opposite for migration timing into freshwater (positive) vs. migration timing into saltwater (negative). Since 1971, mean annual water temperature has increased in Auke Creek (Fig. 2.1, Fig. S2.2) and stream temperatures are predicted to rise throughout the next century (Wolken et al. 2011). Future changes in migration timing appear likely, particularly for migration events from freshwater to saltwater. Despite these general trends, there were multiple examples of disparate temporal trends in migration timing or phenotypic variation for alternative life history

types within the same species. Similarly, there were also instances where different environmental variables were related to migratory timing for different life histories (Table 2.1, Table 2.2, Fig. 2.3). As a result of changes in migration timing, the temporal availability of adult salmon in freshwater has decreased by 24-31 days, but their abundance has been stable ($\lambda \approx 1.0$).

Generally, the observed changes in the median date of migration timing match predictions based on physiological and behavioral relationships with temperature (Beacham and Murray 1990, Roper and Scarnecchia 1999, Kennedy and Crozier 2010, Crozier et al. 2011), and/or empirical observations of changes in migration timing for other salmonid populations experiencing increasing temperatures (Cooke et al. 2004, Juanes et al. 2004, Quinn et al. 2007, Wedekind and Kung 2009). Though researchers have shown that there can be substantial variation in temporal trends in phenological events for similar species occupying the same habitats (e.g. Todd et al. 2011), it appears that life history variation can also influence phenological responses to climate change. In fact, variation in trends in migration timing (sockeye adults and jacks, sockeye age 1 and 2 smolts, coho age 1 and 2 smolts) and phenotypic variation (coho adults and jacks, coho age 1 and 2 smolts) was greater within populations as a result of life history variation, than across species (Fig. 2.2). This variation in migratory behavior as a result of phenotypic diversity indicates that biocomplexity can influence the response of organisms to climate warming.

Decreases in phenotypic variation in migration timing may have important consequences for salmonid population dynamics. Migration timing from freshwater to saltwater can strongly influence survival (Taylor 1980, Scheuerell et al. 2009, Kennedy and Crozier 2010), but optimal migration timing varies from year to year (Scheuerrell et al. 2009). Assuming that inter-annual variability in the timing of optimal conditions is stable, reductions in the window of time that salmonids migrate into saltwater will decrease the probability that migration events coincide with optimal conditions. For adult salmon, migration timing is often highly heritable (Smoker et al. 1998, Dickerson et al. 2005, Carlson and Seamons 2008), and intra-annual variation in migration timing may

reflect additive genetic variation. Therefore, decreases in phenotypic variation may indicate a reduced capacity to evolve in response to environmental warming. I predicted that greater temporal trends in the median date of migration timing would be associated with larger decreases in phenotypic variation, as directional selection reduces phenotypic variation. However, there was no correlation between temporal trends in the mean and variance of the migration timing distribution (Spearman's Rank $r = -0.055$, $P = 0.852$), suggesting that they have responded independently to climate warming. Pink salmon were a notable exception, and both odd- and even-year populations have undergone substantial changes in adult and juvenile migration events toward earlier migration timing and decreased intra-annual variation in migration timing (Fig. 2.2, Fig. 3.2). These changes are highly suggestive of evolution by natural selection, a hypothesis I tested in CHAPTER 3. Unfortunately, changes in phenotypic variation are almost never reported in studies of temporal trends in biological traits, making it difficult to generalize these findings outside of Auke Creek.

Changes in juvenile migration timing have prompted concern over the potential for salmon to experience trophic mismatches during their early-marine residency (Taylor 2008, Crozier et al. 2008), and this appears to have occurred for a population of Atlantic salmon (Kennedy and Crozier 2010). Taylor (2008) argued that Auke Creek pink salmon might have decreased oceanic survival as a result of earlier ocean entry because photoperiod, which is invariable, drives spring phytoplankton blooms in Auke Bay. This may, however, be an over-simplified prediction. In Auke Bay, the timing of zooplankton blooms depends on the spring phytoplankton bloom, but there is substantial variability (peak abundance and biomass can vary by over a month) in the zooplankton bloom due to a variety of abiotic and biotic variables (Coyle et al. 1990, Bienfang and Ziemann 1995). At a larger scale, there is considerable variation in zooplankton blooms across Southeast Alaska due to geography, oceanic patterns, and climate (Weingartner et al. 2009). Weingartner et al. (2009) also note that stratification due to springtime freshwater runoff is a primary factor facilitating phytoplankton blooms. Therefore, phytoplankton blooms may occur earlier in time because of trends towards earlier spring runoff and therefore

stratification across the Pacific coast, including Southeast Alaska (Stewart et al. 2005). Likewise, there is substantial evidence for temporal trends toward earlier zooplankton blooms. Edwards and Richardson (2004) observed a strong relationship between copepod phenology and sea-surface temperature, which resulted in earlier spring blooms for many species. Similarly, a meta-analysis of 138 estimates of zooplankton phenology shows a strong shift (0.38 days earlier per year $SE = 0.007$) toward earlier blooms in the Atlantic Ocean (Thackeray et al. 2010). Freshwater lakes along the Pacific coast show a similar trend toward earlier phenology in some, but not all, copepod species (e.g. Winder and Schindler 2004). Trends toward earlier migration timing may actually be adaptive with respect to potential trends in zooplankton phenology, and the demographic data from Auke Creek support this possibility.

There are no apparent demographic costs due to changes in juvenile migration timing for pink, coho, and sockeye salmon in Auke Creek. There was a shift in age structure towards an increasing prevalence of age 2 coho and sockeye smolts (Fig. S2.3), both of which migrate earlier than age 1 smolts, and the average marine survival for age 2 coho (0.29) and sockeye (0.25) is quite high (S. Smith, unpublished data). Furthermore, there does not appear to be a relationship between timing of pink salmon outmigration and subsequent oceanic survival (R. Kovach, unpublished data). Nonetheless, strong relationships between water temperature and salmonid migration timing to saltwater reported here and elsewhere (Roper and Scarnecchia 1999, Kennedy and Crozier 2010), indicate that migration timing will rapidly change as a result of increasing freshwater temperatures (Morrison et al. 2002, Mantua et al. 2009, Wolken et al. 2011) resulting in an increased risk of a trophic mismatch. Growth and development (both of which influence migration to saltwater) are heritable traits in salmonids (Hebert et al. 1998, Carlson and Seamons 2008) and therefore juvenile migration timing could adapt via evolution as opposed to phenotypic plasticity if selective pressures are present. However, the degree to which migration timing can evolve in juvenile salmon may be constrained by genetic correlations with adult development and maturation rates (Carlson and Seamons 2008) and the fact that development itself may act as a pleiotropic trait in

salmonid fishes (McPhee et al. 2012). That is, selection for late migration timing or slower developmental rates at one life history stage may be offset or restricted by selection for early migration timing or faster rates of development at a different life history stage.

Another concern is that adult salmon will experience elevated freshwater temperatures during their migration and spawning (Stewart et al. 2005, Mantua et al. 2010, Kaushal et al. 2010). Warmer freshwater temperatures are associated with increased pre-spawn mortality and decreased reproductive success (Fukushima and Smoker 1997, MacDonald et al. 2010). Increased stream temperatures, and/or changes in migration timing toward higher temperatures, may threaten the persistence of some salmon populations, particularly in the Fraser River (Martins et al. 2010). Whether this represents a general concern for all salmon populations is complicated by the fact that physiological adaptations to temperature differ substantially between populations (e.g. Eliason et al. 2011), and some species – namely pink salmon – appear to have a much greater scope for tolerating higher temperatures (Clark et al. 2011). It was clear from the model selection analyses that adult salmon (except for sockeye jacks) avoid migrating during high stream temperatures and low stream flows (Table 2.1). Therefore, trends toward earlier migration timing must be due to some other unidentified environmental variable, or natural selection for early-migration timing and/or faster rates of development at some life stage (Crozier et al. 2011, McPhee et al. 2012). To date, warming temperatures and early migration do not appear to have negatively influenced salmon populations in Auke Creek, but whether these populations will remain stable is difficult to predict. If there are adaptive thresholds in these populations as a result of finite genetic variation or limits to phenotypic plasticity (Ghalambor et al. 2007), rapid demographic changes may occur as those thresholds are passed.

Because salmonids are key components of ecosystem dynamics (Willson and Halupka 1995, Gende et al. 2002, Schindler et al. 2003, Hocking and Reynolds 2011), changes in their temporal distribution may have important consequences for other species. The phenology (Moore and Schindler 2010) and seasonal behavior (Willson and

Halupka 1995, Gende et al. 2002, Schindler et al. 2003) of many species along the Pacific coast are adapted to the temporal availability of salmon in freshwater. In some cases changes in migration timing may influence ecological interactions over multiple trophic levels (e.g. Lisi and Schindler 2011), which increases the probability of future trophic mismatches (Both et al. 2009). Therefore, the persistence of other species may depend, in part, on how salmon respond to climate change via shifts in migration timing.

Microevolutionary Responses to Climate Change

Due to the relationship between fitness and migration timing (adult and juvenile) researchers have argued that evolutionary shifts in migration timing may be necessary for salmon populations to persist during climate warming (Crozier et al. 2008, Crozier et al. 2011, Reed et al. 2011). In CHAPTER 3 I determined that shifts toward earlier migration timing and decreased phenotypic variation in the odd-year pink salmon population were due, at least in part, to evolutionary change as opposed to phenotypic plasticity. To date, molecular genetic evidence for climate-induced microevolution in wild vertebrate populations is non-existent, making these results a vital empirical advancement (Gienapp et al. 2008, Hansen et al. 2012).

Specifically, data from an allozyme locus, the allele frequencies of which were experimentally altered to genetically mark late-migrating fish (Gharrett et al. 2001), revealed that there has been a substantial decrease in the very latest migrating phenotype/genotype. From 1983-1989 the frequency of the allele that marked late migrating fish was 0.2-0.3 in fish sampled during the last 10 days of the migration distribution, and 0.04-0.05 in early migrating fish. In recent samples (1993, 2001, 2011) the frequency of the experimental allele is 0.04-0.05 throughout the entire migration distribution (Fig 3.4). Based on these allele frequencies, the genetically marked, late-migrating portion of the population constituted 0.27 (SE = 0.04) - 0.39 (SE = 0.05) of the total abundance from 1983-1989, but from 1991-2011 that value dropped drastically to 0.05 (SE = 0.02) - 0.14 (SE = 0.03; Table 3.1). During the same time period (1979-2011), there were no significant changes at a neutral allozyme locus, indicating that

genetic drift was not responsible for the rapid genetic changes observed at the experimental locus (Fig. 3.4). These data strongly suggest that there was a selective event against late-migrating fish between 1989-1993.

It is unclear what selective pressures led to these evolutionary changes, but stream temperatures during peak migration in 1989 were the second highest on record, and the progeny from this generation showed the greatest genetic changes. Early- and late-migrating pink salmon in Auke Creek have different adaptations to thermal regimes and selection may have favored early-migrating fish because they appear to be adapted to warmer temperatures (Fukushima and Smoker 1997, Hebert et al. 1998, Smoker et al. 1998). The fact that substantial genetic changes occurred over just one or two generations suggests that climate-induced selective events may be extreme and episodic. This selective event appears to have resulted in a truncation of the migration timing distribution, a fact that is supported by a lack of evidence for temporal trends in migration timing for the first 5% ($b_1 = 0.019$, $SE = 0.100$) and first quartile ($b_1 = -0.164$, $SE = 0.098$) of the migration timing distribution.

I also predicted that there would be evidence for natural selection at the circadian rhythm genes *Clock* and *Cryptochrome* (O'Malley et al. 2007, O'Malley et al. 2010), because circadian and circannual rhythms appear to play a role in determining salmon migration timing (Beacham and Murray 1990, Quinn 2005). From 1993-2009 there was no evidence for genetic change that exceeded neutral expectation at any of three circadian rhythm loci or 23 putatively neutral microsatellite loci. Unfortunately, genetic samples were not available prior to 1993, so I was unable to measure genetic changes at these loci during the selective event that occurred between 1989-1993. Whether the circadian rhythm genes used in this study influence migration timing for this population is unknown. However, in CHAPTER 4 I detected some evidence for divergent selection between early- and late-migrating fish at all three circadian rhythm loci (Fig. 4.3). Yet there was no temporal stability in the pattern for divergent selection at these loci across years, indicating that the strength of selection may be weak or that the signal for selection was spurious.

Understanding whether populations can sufficiently adapt to climate change will be crucial for predicting future population dynamics (Reed et al. 2010, Bellard et al. 2012) and an empirical collection of examples where populations undergo adaptive genetic changes in response to climate change will be necessary to understand the conditions under which these evolutionary changes may be likely (or unlikely) to occur. Fortunately, in CHAPTER 3 I was able to use genetic data from an experimental project (Lane et al. 1990) that occurred in 1979. These genetic data were highly unusual in terms of duration and nature. Obtaining quality, long-term, phenotypic, demographic, and genetic data is a large obstacle for documenting, understanding, and predicting adaptive genetic changes in fish and wildlife populations (Gienapp et al. 2008, Hansen et al. 2012).

A valuable data resource to address this issue may be fish and wildlife harvest, or hunter and angler survey databases. These datasets can be substantial (e.g. waterfowl band recoveries, salmon coded wire tag recoveries, hunter check stations, steelhead/salmon punch cards, etc.), and may be particularly useful for understanding changes in phenology, behavior, and age or size distributions (e.g. Eggeman et al. 2009, Valiente et al. 2011). Additionally, harvest databases are often associated with physical sample collections (fish scales and otoliths, duck wings, and goose tails) that can be used for genetic analyses (Schwartz et al. 2007, Hansen et al. 2012, Iwamoto et al. 2012).

It is now possible to genotype individuals at hundreds of loci (Allendorf et al. 2010), which enhances our ability to detect adaptive genetic change (Luikart et al. 2003). Nonetheless, the value of using archived samples and genomic data will be highly dependent on sampling design. The results in CHAPTER 4, and other studies (McGregor et al. 1998, Fillatre et al. 2003, Hendry and Day 2005) demonstrate that there can be significant genetic structure as a result of phenotypic differentiation within populations. If samples are restricted to a portion of the phenotypic distribution in either the historical or contemporary sample collections, failing to account for differentiation may provide misleading results about the magnitude of temporal genetic change.

Determining the specific selective drivers acting on populations, and the adaptive significance of genetic changes themselves, remains a difficult research hurdle (Hansen et al. 2012), a fact that was underscored in CHAPTER 3. Understanding patterns of selection within populations requires long-term field observations or experimental manipulations, as the strength and direction of natural selection can vary widely across years in natural populations (Siepielski et al. 2011). Capture-mark-recapture designs provide an excellent methodology to address questions in evolutionary ecology (Nichols and Kendall 1995, Lindberg 2012) because the estimated parameters (survival, movement, state-transitions, reproduction, etc) can be used to determine selective forces and the strength of selection acting on populations. Overall, a valuable synthesis may be found by combining genetic analyses based on archived samples with long-term capture-mark-recapture surveys (or other demographic analyses) and phenotypic data from harvest databases. Luckily, these data may be available in a number of populations.

Genetic Diversity and Migration Timing

In CHAPTER 4 I addressed objective 3 by examining how migration timing and changes in migration timing influence intra-population genetic variation. To do so, I used genetic data spanning 16 complete generations in the odd-year pink salmon population. To describe patterns of genetic variation in this population I estimated N_e and intra-annual genetic differentiation as a result of differences in migration timing. I found that the harmonic mean $\hat{N}_e$ and $\hat{N}_e/N_c$ from 1991-2007 were 1440 and 0.29 respectively. Within a year, there was genetic heterogeneity between early- and late-migrating fish (Table 4.2) and mild temporal genetic autocorrelation among individuals as a function of days between dates of migration (Fig. 4.2). Because of strong shifts toward earlier migration timing and decreasing intra-annual variation in migration timing, I predicted that there would be decreases in N_e, N_e/N_c, and the strength of genetic differentiation based on migration timing. However, I observed that $\hat{N}_e$ and $\hat{N}_e/N_c$ have been stable, or possibly increasing, from 1991-2007 (Table 4.3), and genetic differentiation due to variation in migration timing has been stable from 1979-2009 (Table 4.1).

Multiple aspects of pink salmon biology suggest that N_e/N_c may be lower in pink salmon than other organisms, and hence N_e may be much smaller than observed abundance. There can be strong density dependence during spawning for pink salmon, leading to the loss of some families due to redd superimposition (e.g. Fukushima et al. 1998). Pink salmon also appear to have family-correlated marine survival, which can strongly skew family size distributions (Geiger et al. 1997, Geiger et al. 2007). Together, these factors can decrease N_e by inflating reproductive variance beyond Poisson expectation (Waples 2002). Pink salmon populations fluctuate widely in size, the result of which is that the N_e across generations is equal to the harmonic mean of the N_e values within each generation (Kalinowski and Waples 2002). Assuming N_e/N_c is constant regardless of N_c (i.e. ignoring the potential for genetic compensation; Ardren and Kapuscinski 2003, Palstra and Ruzzante 2008), N_e across generations for pink salmon may be strongly driven by low values. Interestingly, the harmonic mean estimate for N_e (1440) is fairly large and is considerable greater than the median value ($\hat{N}_e = 260$) across 83 studies reported in a recent meta-analysis (Palstra and Ruzzante 2008). Moreover, the harmonic mean estimate of N_e/N_c (0.29) exceeds the median N_e/N_c values (0.11-0.14) observed in other studies (Frankham 1995, Palstra and Ruzzante 2008). These values indicate that this population is robust to genetic stochasticity and should be able to maintain adequate genetic diversity and evolutionary potential into the future.

Similar to other studies (McGregor et al. 1998, Fillatre et al. 2003, Hendry and Day 2005), I observed that variation in migration/reproductive timing appears to influence the distribution of genetic variation within populations. Although point estimates for genetic autocorrelation by time (r) and genetic differentiation (G''_{ST}) were small, they appear to be biologically meaningful. For example, the mean estimate for G''_{ST} between early and late migrating fish (0.0021, $SE = 0.0018$) is approximately twice the estimates for temporal genetic change via drift ($F_{TEMP} = 0.0008 - 0.0012$) over 4-8 generations. This indicates that for time periods up to 10 generations, migration timing has as much, or more, influence than genetic drift in determining patterns of genetic diversity. Similarly, the estimates for r followed the biological patterns one would

predict for genetic differentiation due to migration timing – namely, positive correlation in allele frequencies for fish migrating close together in time, but negative genetic correlations between individuals separated by 4 or more days in their migration timing (Fig. 4.2). In addition to influencing genetic diversity, population structure as a result of migration timing has important ecological consequences that drive patterns of natural selection and phenotypic variation within salmon populations (Smoker et al. 1998, Hendry et al. 1999, Doctor and Quinn 2009, McPhee et al. 2012). Management actions that selectively impact specific components of a population's migration timing distribution could adversely influence future population persistence by decreasing genetic and phenotypic variation (Wright and Trippel 2009).

Despite rapid changes and loss of phenotypic variation in migration timing, patterns of genetic differentiation as a result of variation in migration timing have been relatively stable based on allozyme data from 1979, 1981, and 1983, and microsatellite data from 1993, 2001, and 2009 (Table 4.1). Similarly, 95% confidence/credible intervals for estimates of N_e and N_e/N_c generally overlapped across time and methods (Table 4.3). Climate-induced changes in the distribution of species can influence within population genetic diversity and genetic differentiation between populations (Rubidge et al. 2012). However, the results from CHAPTER 4 indicate that intra-population patterns of genetic variation are resistant to climate induced changes in phenology. Whether this pattern holds for other populations in unknown. From the perspective of local adaptation, there still appears to be adaptive genetic differences in developmental timing between early- and late-spawning individuals (Echave et al. *in submission*) and additive genetic variation for adult migration timing in odd-year Auke Creek pink salmon (C. Manhard, unpublished data). Therefore, this population appears to have maintained genetic diversity, and consequently evolutionary potential, during a period of significant environmental warming and rapid evolutionary changes in an important life history trait.

Implications

Migration and reproduction are critical life history traits in salmonids that appear to be undergoing rapid temporal changes in response to climate warming (CHAPTER 2). Whether shifts in the timing of these life history events can compensate for environmental changes across the diverse life cycles of these species is unknown, but to date, populations of salmon in Auke Creek are numerically stable. Molecular genetic data demonstrating that there has been microevolution for earlier migration timing in Auke Creek pink salmon (CHAPTER 3) complements model based approaches from two other papers that have documented climate-induced evolution in wildlife populations (Crozier et al. 2011, Karell et al. 2011). Together, these findings emphasize the fact that contemporary microevolution represents a critical aspect of population resilience to climate warming.

In salmon, different populations and/or phenotypes tend to compensate for reductions in the productivity of other populations or life histories (Hilborn et al. 2003, Greene et al. 2010). This form of biocomplexity acts to stabilize population dynamics and increase probability of population persistence (Schindler et al. 2010, Moore et al. 2010). Results from Auke Creek emphasize these findings with respect to intra-population level responses to environmental change. Based on the observation that there can be disparate changes in migration timing for alternative life history strategies within the same population, it appears that climate change has different consequences for diverse phenotypes, and/or alternative phenotypes can respond differently to environmental change. To ensure that populations have the ability to adaptively respond to future climate change, it is critical to protect phenotypic and genetic diversity.

Variation in migration timing (i.e. early- vs. late-migrating fish) has allowed pink salmon to evolve in response to climate warming, the result of which is that odd-year pink salmon have remained numerically resilient to rapid changes in environmental conditions. Here and elsewhere (e.g. Hilborn et al. 2003), it would have been extremely difficult to predict the observed responses of different components of salmon biocomplexity. Therefore, protecting all forms of diversity is critical for ensuring future

population persistence and productivity. The fact that patterns of neutral genetic variation have remained stable during climate-induced evolutionary change in migration timing (CHAPTER 4) suggests that wild populations may be able to adaptively respond to climate warming while maintaining sufficient evolutionary potential for future change. In terms of long-term population stability, this growing body of work suggests that protecting genetic and phenotypic diversity may be as important as maintaining the absolute abundance of populations. This conclusion stands in contrast to how some aspects of fish and wildlife management and conservation is conducted, as policies and practices generally commonly focus on abundance or habitat and can ignore genetic and phenotypic variation (Laikre 2010).

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