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ARTICLE

Evolution of phenology in a salmonid population: a potential adaptive response to climate change

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Abstract: Accumulating evidence has indicated that many fish populations are responding to climate change through shifts in migration time, but genetic data identifying the role of evolution in these shifts are rare. One of the first demonstrations of evolution of migration time was produced by monitoring allozyme alleles that were experimentally manipulated to genetically mark late-migrating pink salmon (*Oncorhynchus gorbuscha*). Here, we extend that research by using observations of the marker alleles in fry to demonstrate that these changes in migration time were caused by directional selection against the late-migrating phenotype during the oceanic phase of the salmonid life cycle. The selective event, which appeared to be driven by early vernal warming of the nearshore marine environment and consequent decreased survival of late-migrating fry relative to early-migrating fry, decreased the late-migrating phenotype from more than 50% to approximately 10% of the total fry abundance in only one generation. These demographic changes have persisted over the subsequent 13 generations and suggest that a larger trend toward earlier migration time in this population may reflect adaptation to warming sea-surface temperatures.

Résumé : Si une accumulation d'observations indique que de nombreuses populations de poissons réagissent aux changements climatiques en modifiant le moment de leur migration, les données génétiques qui établissent le rôle de l'évolution dans ces modifications sont rares. Une des premières démonstrations de l'évolution du moment de la migration a été produite en suivant des allèles d'allozymes ayant fait l'objet de manipulations expérimentales pour marquer génétiquement des saumons roses (*Oncorhynchus gorbuscha*) à migration tardive. Nous élargissons ces recherches en utilisant des observations d'allèles marqueurs chez des alevins afin de démontrer que ces changements du moment de la migration ont été causés par une sélection directionnelle contre le phénotype à migration tardive durant la phase océanique du cycle de vie des salmonidés. L'évènement de sélection, qui semble avoir été provoqué par un réchauffement vernal précoce du milieu marin sublittoral et la baisse conséquente de la survie des alevins à migration tardive par rapport à celle des alevins à migration précoce, s'est traduit par une diminution du phénotype à migration tardive de plus de 50 % à environ 10 % de l'abondance totale des alevins en une seule génération. Ces changements démographiques ont persisté durant les 13 générations subséquentes et donnent à penser qu'une tendance plus vaste vers un moment de migration plus précoce dans cette population pourrait refléter une adaptation à la hausse des températures de la surface de la mer. [Traduit par la Rédaction]

Introduction

A primary mechanism by which animal populations can respond to environmental change is by shifting the timing of life history events, known as phenology, to track optimal environmental conditions in time (Bradshaw and Holzapfel 2006, 2008; Bellard et al. 2012). Phenology is of critical importance for migratory fish, such as Pacific salmon (*Oncorhynchus* spp.), which must initiate an array of physiological, morphological, and behavioral changes at precise times during their life cycle (reviewed by Groot and Margolis 1991). The precision with which salmonids time migration can constrain gene flow between seasonally distinct spawning segments, thereby enabling local adaptation of phenology (Quinn et al. 2000; Fillatre et al. 2003; Gharrett et al. 2013). Evidence of local adaptation of phenology in salmonids has been provided by comparisons of seasonally distinct groups of Chinook salmon (*Oncorhynchus tshawytscha*) that spawn in the same river, which demonstrated significant genetic divergence at three circadian clock genes, but not at neutral markers (O'Malley et al. 2013). The tight coupling of salmonid ecology and phenology, along with the generally high heritability of phenological traits in salmonids (median h^2 = 0.51; Carlson and Seamons 2008), suggests that salmonid populations may respond to periodic environmental fluctuations or persistent climatic trends through contemporary evolution of phenology.

An increasing body of literature has indicated that many salmonid populations are responding to climate change through changes in phenology. Earlier migration times have now been documented in populations of pink (*Oncorhynchus gorbuscha*), coho (*Oncorhynchus kisutch*), and chum (*Oncorhynchus keta*) salmon in Southeast Alaska (Kovach et al. 2015); sockeye salmon (*Oncorhynchus nerka*) in the Fraser River (Cooke et al. 2004); steelhead trout (*Oncorhynchus mykiss*) in the Columbia River (Robards and Quinn 2002); and Atlantic salmon (*Salmo salar*) in Northern Ireland (Kennedy and Crozier 2010). There is reason to expect that observed changes in migration time may be adaptive in many salmonid populations. Vernal warming is a primary determinant of the time at which the nearshore marine habitat can support growth of juvenile salmon (Friedland et al. 2000; Mortensen et al. 2000), and the ability of

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juveniles to time their emigrations with the onset of favorable growth conditions is an important component of fitness (Beamish and Mahnken 2001). Therefore, under a scenario in which earlier vernal warming is occurring in the nearshore marine habitat, earlier juvenile emigration times would presumably be adaptive. Because adult migration time is highly heritable in salmonids (Carlson and Seamons 2008), selection that favors earlier juvenile emigration times would be expected to result in earlier adult migration times, and the presence of earlier migration times in both life stages may therefore be a joint response that is being driven by the juvenile stage. However, in populations where adults enter fresh water after stream temperatures peak in late summer, a shift toward earlier adult migration times may be maladaptive, particularly if stream temperatures are warming beyond thermal optima. Such life history trade-offs are probably integral in determining the scope by which salmonid populations can adaptively respond to climate change.

It remains unclear whether the widespread changes in salmonid phenology time generally reflect phenotypic plasticity, which is defined by environmentally induced phenotypic variation of a given genotype, or genetic adaptation, which is defined by differential fitness of distinct genotypes in a given environment (Gienapp et al. 2008). Genetic monitoring, a technique in which putatively neutral molecular markers that are associated with phenotypic traits are tracked through time to identify adaptive genetic changes in relation to a changing environment, provides a means of distinguishing between plastic and adaptive responses. Hansen et al. (2012) argued that convincingly demonstrating adaptive genetic change based on molecular markers requires that (*i*) the monitored traits exhibit sufficiently high heritability; (*ii*) the traits are relevant to specific selective agents; (*iii*) the traits are monitored over time; (*iv*) selection is tested; (*v*) shifts in traits are consistent with the expected response to environmental changes; and (*vi*) replacement by a genetically different population has been ruled out.

Migration time of pink salmon that spawn in Auke Creek, Alaska, is a suitable trait for evaluating adaptive change through genetic monitoring because of its high heritability and because it is likely to respond to changes in water temperature. Genetically segregated even- and odd-year populations of pink salmon (Heard 1991) exist at Auke Creek, and adults within each population return to Auke Creek in two distinct segments; "early-migrating" adults return between mid- and late August, and "late-migrating" adults return between early and mid-September (Taylor 1980). These segments can be thought of as two subpopulations that are partially genetically isolated, with stream temperature modulating the amount of gene flow between subpopulations each year through its influence on stream entry time. The structuring of the spawning migration is maintained in the presence of gene flow by the high heritability of migration time within each of these subpopulations ($h^2 = 0.4$) and by potential dispersive selection against intermediate migration time (Smoker et al. 1998). Migration time is involved in synchronizing the timing of life history events with optimal environmental conditions and may therefore be subjected to selective pressures as water temperatures warm. The importance of water temperature as an adaptive driver is supported by the genetically distinct embryonic development patterns exhibited in the early and late subpopulations (Hebert et al. 1998).

During the past 40 years, trends toward earlier migrations of adults and fry have accompanied trends toward warmer water temperatures in the Auke Creek system. Because water temperature is an important component of phenology in this population, these changes in migration timing may be an adaptive response to climate warming (Taylor 2008). The basis of these changes was evaluated by monitoring an isolocus pair that was experimentally manipulated through artificial selection to genetically mark the late-migrating subpopulation (Lane et al. 1990). This locus pair presumably has little genetic influence on migration time. However, because of its high level of correlation with the late-migrating phenotype, changes in its frequencies can be used to infer genetic changes in migration time. Genetic monitoring of this late-migrating marker locus (LMML) demonstrated that a selective event, first evident in brood year 1989, had reduced the abundance of the late-migrating subpopulation and that these demographic changes have persisted over the subsequent 13 generations. These results provided compelling evidence that the shifting phenology of Auke Creek pink salmon has a genetic basis (Kovach et al. 2012).

Here we investigate the genetic basis of change in phenology in Auke Creek pink salmon and its potential link to climate change by synthesizing previously detailed observations of genetically marked adults with newly detailed observations of emigrating fry. The primary questions that these analyses addressed were as follows: (1) Have fry exhibited changes at the LMML that parallel those observed in adults? (2) Is there evidence that natural selection underlies the genetic changes? (3) Is low fitness of late-migrating fish from brood year 1989 the result of poor reproductive success or low marine survival? (4) Is there evidence that the selection event is linked to environmental change?

Methods

Field and laboratory methods

Study site

Auke Creek, a 323 m outlet of Auke Lake that drains into Auke Bay, Alaska, is a migratory corridor and spawning habitat of pink salmon that has produced returns of adults that ranged from 1500 to 28 000 fish over the past 40 years. The US National Marine Fisheries Service operates Auke Creek Research Station, a permanent fish-counting weir and experimental salmon hatchery located at the head of tidewater, which enables complete censuses of spawning adults and emigrating fry.

Genetic marking of late-migration time

A selective mating experiment was conducted in 1979 with the goal of incorporating a putatively neutral genetic marker into the late-migrating subpopulation of the odd-year population in Auke Creek (Lane et al. 1990). During the spawning migration of 1979, skeletal muscle samples were collected from 3906 pink salmon that entered Auke Creek after 15 September and, therefore, represented the latest migrants that year. Each sample was subsequently genotyped by electrophoretically screening the malate dehydrogenase (*MDH-B1,2**) isolocus pair (Allendorf and Thorgaard 1984). Adults (n = 407) that had an *MDH-B1,2*70* allele but not an *MDH-B1,2*130* allele were artificially spawned, and the embryos were incubated in the Auke Creek Hatchery. Genetically marked fry (n = 178 219) were released into Auke Creek between 7 April and 7 May 1980 to coincide with the peak emigration of wild fry. Interbreeding between experimentally marked adults and wild adults in the 1981 spawning season produced changes in the allele frequencies of *MDH-B1,2** in the late-migrating subpopulation. Specifically, the frequency of the *70** allele increased from 0.027 in 1979 to 0.134 in 1983, and the frequency of the *130** allele decreased from 0.022 in 1979 to 0.010 in 1983. Monitoring of subsequent spawning migrations from 1983 to 1989 revealed that the allele frequencies at *MDH-B1,2** were stable and differed between samples of early- and late-migrating fish, thereby demonstrating that the experimental manipulations effectively marked genetic material that is correlated with migration time (Gharrett et al. 2013).

Census and genetic data

During each spring from 1984 to 1992, daily counts were made of all pink salmon fry that passed the Auke Creek weir during their downstream migration. Daily samples of fry (typically 10–20 fish) were collected for genotype determination. Genotyping

was accomplished by using starch gel protein electrophoresis to resolve allozyme banding patterns at the LMML (*MDH-B1,2**).

Auxiliary environmental data

The Auke Bay Laboratories Climatological Series is a data set consisting of environmental measurements recorded at the Auke Bay Marine Station since February 1963. These data include measurements of sea-surface temperatures of Auke Bay. Interannual variation in sea-surface temperature is a primary determinant of marine survival of fry (Manhard et al. 2017). Hence, these environmental data may provide evidence of an environmental basis change in migration time.

Statistical methods

Graphical and statistical comparisons of temporal patterns

Because the *MDH-B1,2*130* allele underwent relatively small manipulative changes and exhibited allele frequencies close to zero, it provided little power for detecting genetic changes. Consequently, the statistical methods that we used to characterize the temporal patterns of the LMML included only the *MDH-B1,2*70* allele, which we refer to as the late-migrating marker allele (LMMA). Temporal patterns of the LMMA frequencies were monitored during five odd-year fry emigration periods (1984–1992). Graphical comparisons of 5-day running averages of frequencies of the LMMA were made among brood years to describe interannual changes in genetic differences between subpopulations. A two-sample test of equality of proportions was used to determine whether these subpopulations differed at the LMMA. Only data from the calendar dates of the first and last 100 fish sampled during each migration period were included in the test.

Overall LMMA frequency

Interannual patterns in the overall (population-wide) frequency of the LMMA in the odd-year population can provide insight into whether allele frequency changes are most likely caused by increased gene flow between subpopulations or reduced fitness of late-migrating fish. Gene flow between subpopulations within the stream should have caused the frequency of the LMMA to decline within the late-migrating subpopulation, but remain stable throughout the population. Conversely, reduced fitness of late-migrating fish should have caused a reduction in the population-wide frequency of the LMMA. Estimating the overall frequency of the LMMA and its statistical certainty is complicated by the variation of daily return numbers and unequal sample representation throughout the migratory periods. These issues were addressed with a parametric bootstrap algorithm (Kovach et al. 2012) that resampled alleles at the LMML (1000 iterations) in running 5-day pools of genetic samples collected during each fry migration. The algorithm divided the migration distribution each year into n 5-day periods, beginning on the first date in which genetic samples were collected. Within each ith period, the maximum likelihood frequency of the LMMA (f_i) was estimated from the genetic samples that were collected during that period. Each iteration of the algorithm began with a random draw of alleles (x_i) from a binomial distribution:

$$x_i \sim \mathrm{Bin}(f_i, a_i) \quad (1)$$

where a_i was the number of alleles sampled within a period (two times the number of genetically sampled fish). In each year, the overall frequency of the LMMA (f) was estimated as

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

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

Stock separation algorithm

A stock separation algorithm (Pella and Milner 1987), which used allele frequencies of the LMML in collections of early- and late-migrating adults from 1983 as a baseline, estimated the number of migrant adults and fry that belonged to each subpopulation. The baseline consisted of 564 adults collected on 10 and 18 August and 161 adults collected on 21 September, which represented the unmarked early-migrating subpopulation and the marked late-migrating subpopulation, respectively. Statistical replication of the stock separation algorithm was accomplished with a nonparametric bootstrap simulation that resampled alleles (20 000 iterations) in both the baseline and in running 5-day pools of fish. On each iteration of the bootstrap simulation, an expectation-maximizing algorithm estimated the proportion of fish that belonged to each subpopulation by comparing running 5-day averages of allele frequencies of genetic samples with allele frequencies of the baseline. The estimated proportion of fish from each subpopulation was then multiplied by the number of fish observed on that date to produce daily estimates of the number of early- and late-migrating fish. This process was repeated on each day of the migration period. Daily estimates were used to plot the annual migration distributions of each subpopulation. The bootstrap simulation provided point estimates and 95% confidence intervals for the annual abundance of adults and fry from each subpopulation, as well as two metrics of fitness: the number of fry produced per adult (freshwater productivity) and the number of returned adults per emigrant fry (marine survival). Comparisons of freshwater productivity and marine survival between subpopulations and among brood years provided insight into the role of natural selection in promoting genetic changes in Auke Creek.

Environmental conditions

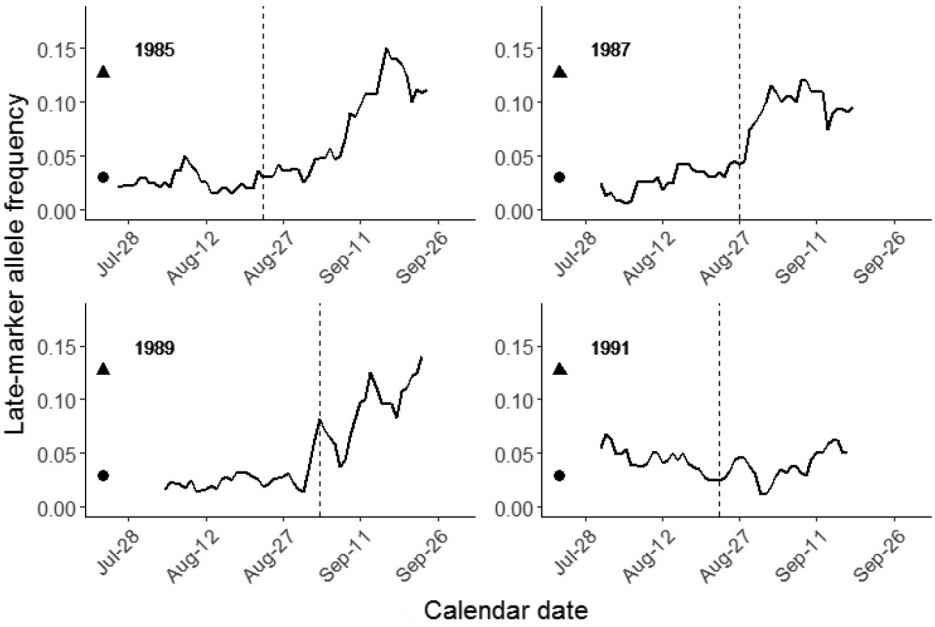
Graphical depictions of sea-surface temperatures experienced by fry shortly after entering the ocean were used to qualitatively explore potential environmental drivers of natural selection. The incipient marine residence period is critical to the survival of juvenile salmon (Beamish and Mahnken 2001; Farley et al. 2007; Manhard et al. 2017). Observations of tagged fry in Auke Bay have demonstrated that the duration of this period varies seasonally and interannually (Mortensen et al. 2000). Given our uncertainty of the duration of this critical period, we focused on environmental conditions experienced by fry during the first few days in Auke Bay, when they are most susceptible to mortality from predation and starvation. Demographic plots of the number of early- and late-migrating fry residing in Auke Bay on each date were made with estimates from the stock separation algorithm and included only fish that had emigrated within the past 7 days. Thermal-gradient plots of sea-surface temperatures, which were superimposed over the demographic plots, depicted conditions encountered by fry during their first week in the marine environment.

Results

Monitoring of the LMMA

The temporal characteristics of the LMMA in adults were described previously (Kovach et al. 2012), and we briefly present those results here so that comparisons can be made between adults and fry. In each generation from 1985 to 1989, the LMMA frequency increased substantially after the midpoint of the migration (Fig. 1), and there was a significant difference ($P \ll 0.001$) in allele frequencies between samples of early- and late-migrating fish. Beginning in the adult migration of 1991, there was a marked decline in the magnitude of the LMMA frequency increase over the migration period and the LMMA frequencies of samples of early- and late-migrating adults were nearly identical ($P = 0.820$).

Fig. 1. Five-day running averages of the frequency of the late-migrating marker allele (*MDH-B1,2*70*) in adult pink salmon sampled in each odd-year migration from 1985 to 1991. Median migration dates are indicated by a dashed line, and the 1983 late-migrating marker allele frequencies are depicted for early-migrating (circles) and late-migrating (triangles) fish.



Fry exhibited patterns in the LMMA that were similar to those observed in adults. During each generation from 1984 to 1990, there was a rapid increase in the LMMA frequency near the midpoint of the migration (Fig. 2). In each of these generations, the LMMA frequency was approximately 0.08–0.10 higher in fry sampled from the late-migrating segment than in the early-migrating segment, which was a significant difference ($P \ll 0.001$). A substantial decline in the LMMA frequency was first evident in migrating fry in spring of 1992. The LMMA frequency was only 0.02 higher in fry sampled from the late-migrating segment that year, which was not a significant difference ($P = 0.456$).

The bootstrap analysis demonstrated that the overall frequency of the LMMA declined substantially in adults that returned to Auke Creek in 1991 after being relatively stable during the previous three generations (Fig. 3). Similarly, the overall frequency of the LMMA declined substantially in fry that emigrated in 1992 relative to the previous four generations, in which the overall frequency was relatively stable. These patterns indicated that the LMMA frequency was reduced by a selective event following the fry emigration of 1990.

Patterns in population demography

The stock separation algorithm produced estimates of early- and late-migrating population components in fry. During the first four generations (1984–1990), late-migrating fry accounted for an estimated 51%–68% of the total fry abundance. However, beginning in the migration of 1992, the proportion of late-migrating fry declined to only 12%. Bootstrap replication estimated marine survival and freshwater productivity in the early- and late-migrating subpopulations from 1983 to 1989 (Fig. 4). In each brood year, marine survival of late-migrating fry was significantly lower than in early-migrating fry. This discrepancy was particularly pronounced in fry that emigrated in 1990, in which the survival rate of late migrants was only 1.4%. This survival rate was the lowest observed over the monitoring period and was less than one-tenth of the survival rate of early migrants from the same brood year. By contrast, freshwater productivity of late-migrating adults was significantly higher than that of early-migrating adults in each generation from 1985 to 1989 (Fig. 4). The migration of 1991 was distinct in that the mean estimate of freshwater productivity was similar between early- and late-migrating adults. However, aspects of the sampling design produced a high standard error for the estimated freshwater productivity of late migrants that brood year.

Environmental conditions

The estimated fry migration distributions indicated that early-migrating fry entered Auke Bay between 7 and 15 days earlier than late-migrating fry and, consequently, encountered cooler sea-surface temperatures each year, although the extent of this thermal discrepancy varied from year to year (Fig. 5). The migrations of 1986 and 1990 are particularly noteworthy because of the contrasting temperatures experienced by early- and late-migrating fry in those years. In 1986, sea-surface temperatures remained relatively cool throughout April, and early-migrating fry experienced a mean temperature of 5.4 °C during their first week in Auke Bay, whereas late-migrating fry began to enter Auke Bay in peak numbers during a warming event in the beginning of May and experienced a mean temperature of 8.8 °C. In 1990, an early warming period that began on 15 April and coincided with the peak emigration of early-migrating fry provided a mean temperature of 7.0 °C, which was only slightly cooler than that experienced by late-migrating fry (7.7 °C) during their first week of marine residency. The migrations of 1986 and 1990 respectively marked the largest and smallest thermal discrepancies encountered between runs over the monitoring period.

Discussion

During the first 4 years of genetic monitoring, the patterns of the LMMA in fry paralleled those observed in adults in a previous study (Kovach et al. 2012). Both life history stages exhibited a pronounced increase in the LMMA frequency beginning near the midpoint of the migration, and fish sampled from the early- and late-migrating segments displayed significantly different allele frequencies, thereby demonstrating considerable genetic structure (Figs. 1, 2). The presence of parallel seasonal patterns in adults and fry demonstrates that the phenology of these two life history stages is tightly coupled and indicates that natural selection upon one stage would likely produce an evolutionary response in the

Fig. 2. Five-day running averages of the frequency of the late-migrating marker allele (*MDH-B1,2*70*) in pink salmon fry sampled in each odd-year migration from 1984 to 1992. Median migration dates are indicated by a dashed line.

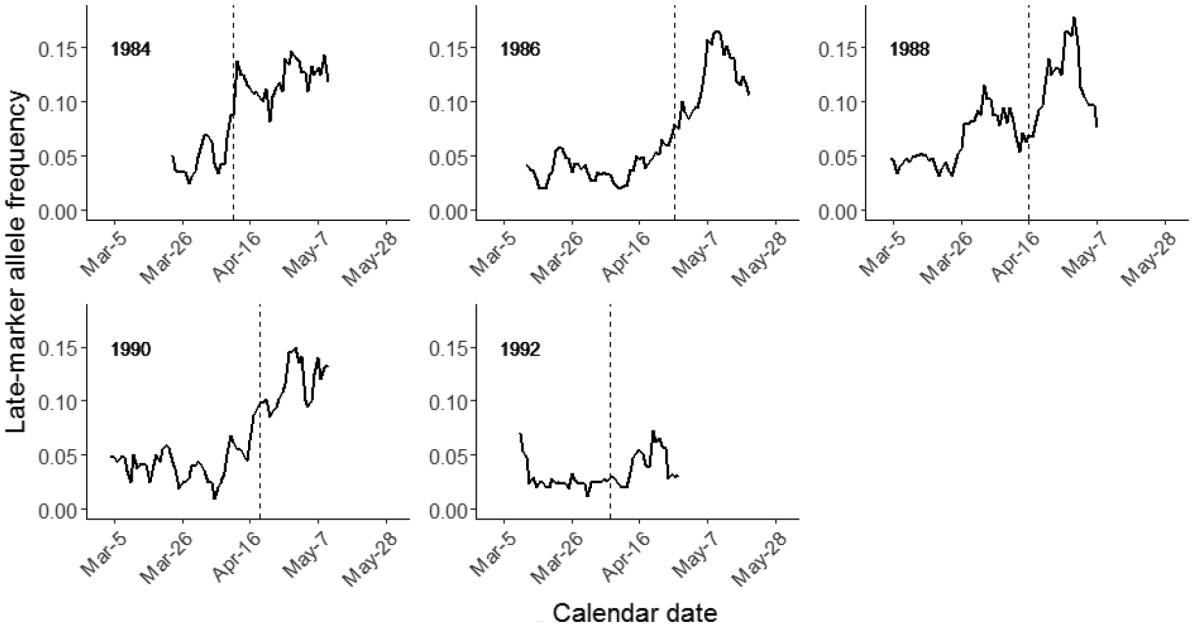
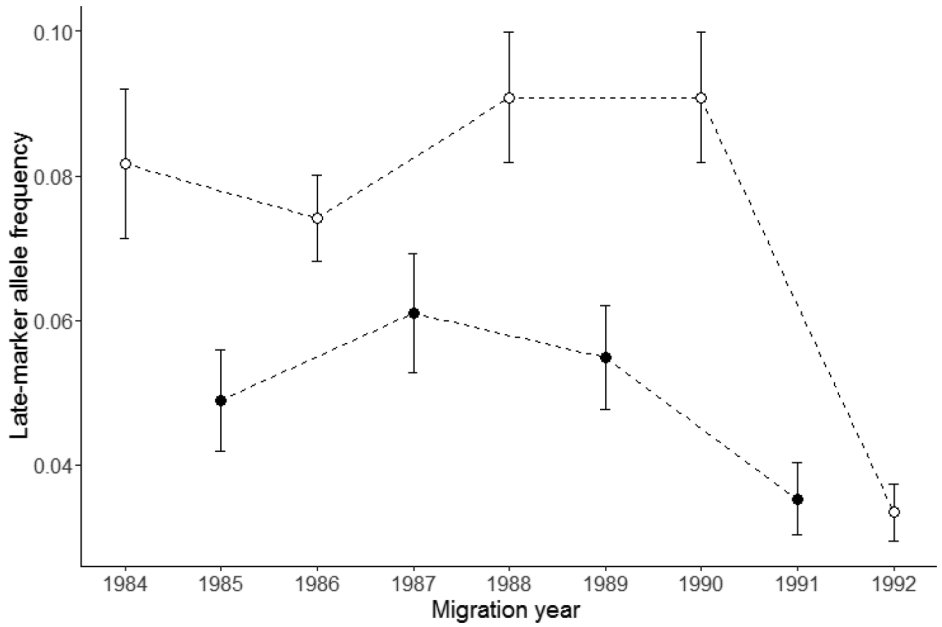


Fig. 3. Annual bootstrap estimates (20 000 iterations) of the population-wide frequency of the late-migrating marker allele (*MDH-B1,2*70*) in migrating adults (solid circles) and fry (open circles). Errors bars are the 95% confidence intervals of each estimate.



other. While the fingerprint of the LMMA was strong in fry that emigrated in spring of 1990 (brood year 1989), the adult migration of that generation in 1991 was the first instance in which a substantial mid-season increase in the LMMA frequency and significant allelic differences between samples of early- and late-migrating fish were not observed. Further, similar changes in the characteristics of the LMMA were evident in the fry emigration of the following spring (1992), wherein samples of early- and late-migrating fry did not exhibit significant differences in the LMMA frequency. Hence, it appears that this population experienced genetic changes in phenology at two different life history stages, which were first evident following the oceanic phase of brood year 1989. Subsequent observations of this population in 1993, 2001, and 2011, which also failed to demonstrate a fingerprint of the LMMA in late-migrating fish, suggest that these changes have persisted over multiple generations (Kovach et al. 2012).

Analyses of genetic data at the LMMA were used to investigate causative agents of genetic change, which may include gene flow between subpopulations, gene flow from nearby populations, and genetic drift. We evaluated the plausibility gene flow between subpopulations as a causal agent by iteratively simulating the overall frequency of the LMMA and observed that the overall frequency was stable in fry during the first 4 years of genetic monitoring, before undergoing a substantial, population-wide decline in 1992. This pattern, when considered alongside the similar pattern observed in adults (Fig. 3), demonstrates that gene flow between subpopulations was unlikely to have caused the changes. Gene flow among populations was quantified by analyzing LMMA

Fig. 4. Bootstrap estimates (20 000 iterations) of marine survival and freshwater productivity (fry produced per adult) over four brood years in the early-migrating (open circles) and late-migrating (solid circles) subpopulations. Error bars are the 95% confidence intervals of each estimate. Shading emphasizes the comparison of subpopulations within each brood year.

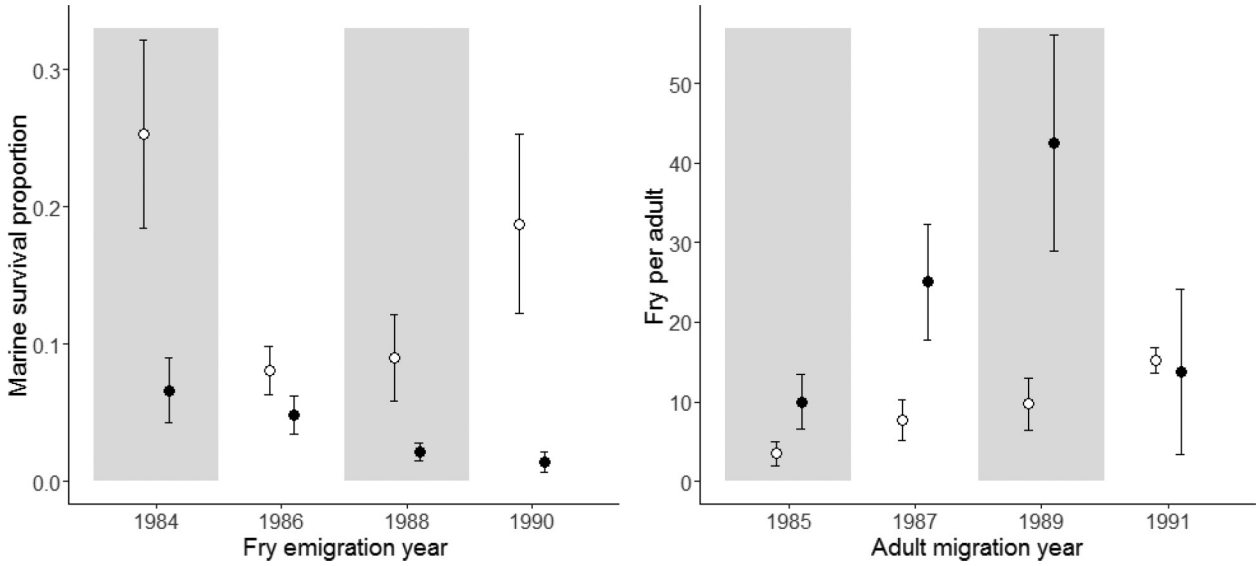
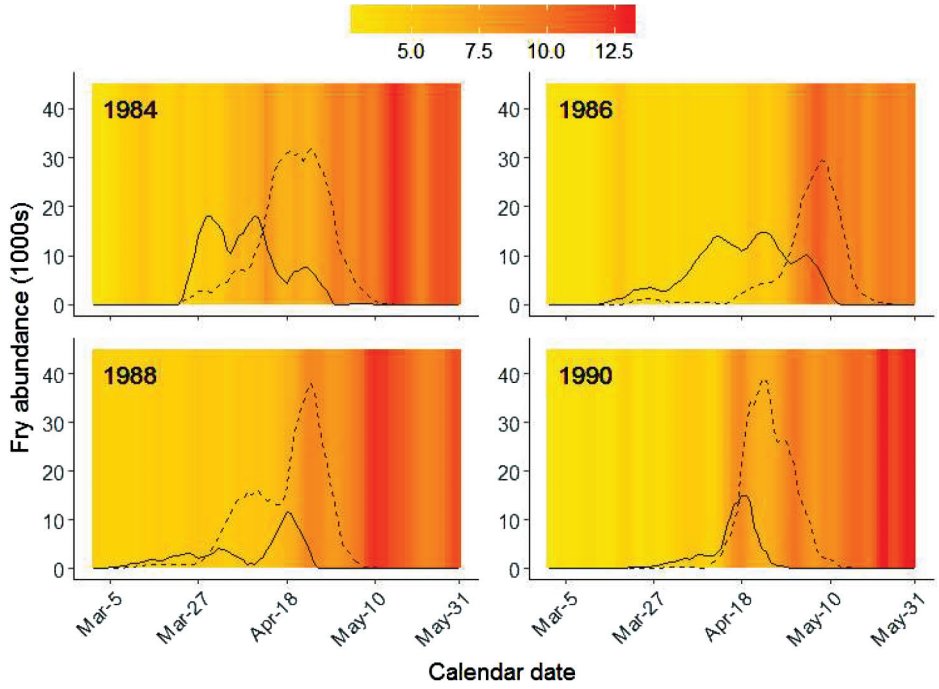
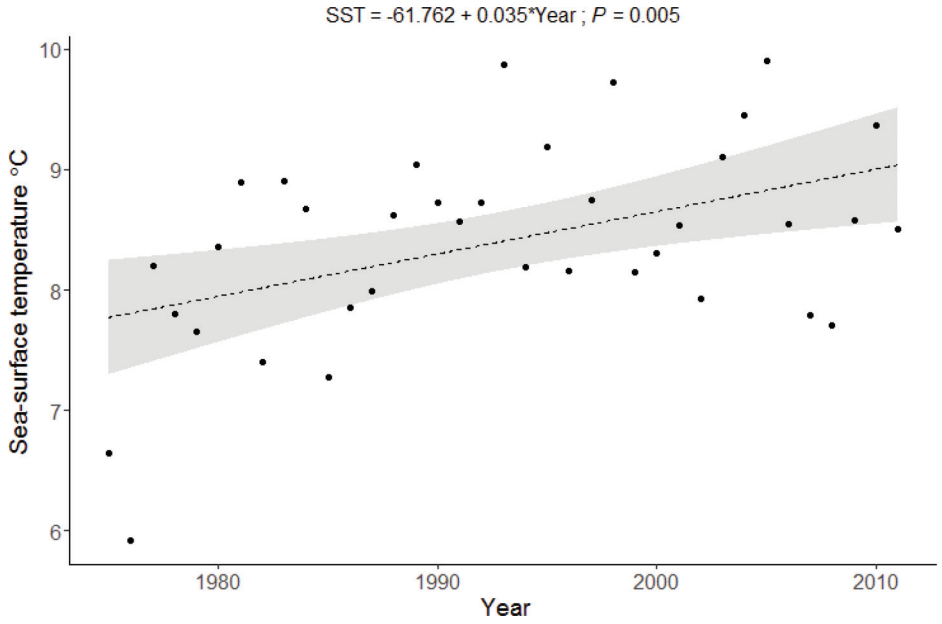


Fig. 5. Sea-surface temperatures experienced by pink salmon fry from the early-migrating (solid line) and late-migrating (dashed line) subpopulations during their first week of marine residency. Assignment to subpopulations was made with a stock separation algorithm that used allele frequencies at the late-migrating marker locus. Three-day running averages of Auke Bay sea-surface temperatures (°C) are depicted by a gradation scheme. [Colour online.]



frequencies from nearby pink salmon populations in an island–continent model. The model estimated that the migration rate (m) necessary to produce the observed genetic changes ranged from 0.69 to 0.85 (Kovach et al. 2012), which was substantially higher than estimates of straying rates of Auke Creek pink salmon (m = 0.02–0.04; Mortensen et al. 2002) and gene flow between proximate populations and Auke Creek (m = 0.0015; Gharrett et al. 2001), thereby demonstrating that interpopulation gene flow was inadequate to produce the observed changes. Genetic drift was addressed by quantifying expected changes from genetic drift with estimates of the effective population size (N_e). Analyses of genotype data over 23 microsatellite loci estimated values of N_e in the odd-year population that ranged between 788 and 6005 adults (Kovach et al. 2013*a*), which indicated that we could expect a change of 0.003–0.007 in the LMMA frequency from genetic drift. The observed change between the adult migrations of 1989 and 1991 was substantially higher than this range (ΔLMMA = 0.020). Observations of another putatively neutral control locus, which exhibited minimal change during the monitoring period, provided additional evidence that genetic drift was not strong enough to cause the observed genetic changes (Kovach et al. 2012). Collectively, these results demonstrate that genetic change from gene flow and genetic drift was

Fig. 6. Temporal trend in spring sea-surface temperature of Auke Bay (1975–2011). Sea-surface temperatures were averaged from 1 April to 31 May. The shaded area depicts the 95% confidence interval.



likely minimal, leaving natural selection as the most likely causative agent.

Iterative simulations of the early- and late-migrating subpopulations with a stock separation algorithm provided evidence that the primary cause of genetic change was a natural selection event in brood year 1989, which resulted from relatively low marine survival of late-migrating fish that emigrated in 1990. The relative fitness of these two subpopulations appears to be balanced by life history trade-offs; late-migrating adults generally produced more fry per adult, but late-migrating fry consistently exhibited lower marine survival rates during the monitoring period (Fig. 4). Higher freshwater productivity of late-migrating adults can be attributed to mortality of early-spawned embryos from redd superimposition by late-migrating adults (Fukushima et al. 1998), whereas lower marine survival of late-migrating fry can be attributed to their comparatively smaller body size and, by extension, higher predator vulnerability during the incipient marine residence period (Mortensen et al. 2000). Although these trade-offs appear to balance fitness in most years, brood year 1989 was unique in that the marine survival rate of late-migrating fry was less than one-tenth that of early-migrating fry; in comparison, the marine survival rate of late-migrating fry ranged between one-fourth and one-half that of early-migrating fry in the other years. Since there was little evidence of a compensating increase in freshwater productivity of late-migrating adults from brood year 1989, this marine survival disparity was likely sufficient to convey a lifetime fitness advantage to the early-migrating subpopulation. Further demographic declines were evident in the late-migrating subpopulation in the following brood year (1991), in which late-migrating adults apparently did not have their characteristic freshwater productivity advantage. This decline in relative freshwater fitness may be attributed to increased overlap of spawning activity between early- and late-migrating adults that year and resulting competition for limited spawning habitat.

The incipient marine residence period is the primary determinant of lifetime fitness of Auke Creek pink salmon (Manhard et al. 2017), and consequently, this stage has the potential to drive natural selection. Because their later ocean entry time confers a shorter growth period prior to seasonal increases in nearshore predator abundance, late-migrating fry are smaller, and therefore more vulnerable to size-selective predation (Parker 1971; Hargreaves and LeBrasseur 1986), than early-migrating fry during this stage (Mortensen et al. 2000). Additionally, late migrants may be at a disadvantage in competing with larger early migrants for food. The extent of this size disparity is governed by physical conditions that modulate the potential for growth in the nearshore environment. In Auke Bay, temperature is the primary factor controlling growth of fry because of its effects on their physiology and because it drives the abundance and size composition of their planktonic prey (Bienfang and Ziemann 1995). Although their consistently higher survival indicates that early-migrating fry benefit from being on the leading edge of the migration, low temperatures and sparse prey abundance in Auke Bay limit the growth potential of early migrants in many years (Mortensen et al. 2000). Hence, the balancing of the benefit of leading the migration with the cost of poor growth conditions largely determines the relative marine fitness of these subpopulations and is likely an outcome of seasonal temperature patterns. Comparisons of vernal sea-surface temperatures during the fry emigrations of 1986 and 1990 supported this (Fig. 5). In 1986, sea-surface temperatures remained relatively cool as early-migrating fry were entering the ocean, and substantial vernal warming did not occur until late-migrating fry began emigrating in large numbers. In contrast, an early vernal warming event in 1990 likely provided favorable growth conditions for early-migrating fry. Consistent with these temperature patterns, early-migrating fry exhibited their smallest marine survival advantage in 1986 and their largest advantage in 1990.

It is generally recognized that there is an optimal window for ocean entry in many salmonid populations (Cury and Roy 1989) and that the ability of fry to synchronize their emigration with this window is a critical component of fitness. Our results provide evidence of a mechanism by which adaptation of earlier migration time can occur under an advancing vernal warming period and associated optimal window. Furthermore, although we do not have information on trends in zooplankton phenology in Auke Bay, trends toward earlier onsets of spring zooplankton blooms have been observed in other systems (Edwards and Richardson 2004; Thackeray et al. 2010), and an earlier plankton bloom would reinforce a shift in the optimal window. Because migration times of fry and adults are connected through the heritability of this trait, selection against late-migrating fry would be expected to produce earlier average migration times in adults. Therefore, our

hypothesis provides a unifying explanation for the coinciding trends toward earlier migrations of adults and fry (Kovach et al. 2013*b*) and warmer vernal sea-surface temperatures of Auke Bay (Fig. 6) that have been observed in this system over the past four decades. An advance in the optimal growth window, without a corresponding plastic response, would be expected to cause directional selection against late-migration time, thereby reducing phenotypic variation in this trait. Hence, this hypothesis is also consistent with the declines in phenotypic variation of migration time that have been observed in adults and fry in the even- and odd-year populations at Auke Creek (Kovach et al. 2013*b*). Importantly, our study is limited to an observation of natural selection in a single generation, whereas the long-term trend in median migration time spans 17 generations at Auke Creek. The rapid genetic change observed in this population suggests that, in some cases, phenotypic change from climate-induced selection may be extreme and episodic, rather than gradual. However, because the long-term census data reveal a nearly continuous shift in median migration time (rather than rapid truncation in one generation), plasticity must also be a substantial component of the pattern.

Long-term abundance data collected from populations of pink, coho, and chum salmon in rapidly warming Southeast Alaska demonstrated that 16 out of 19 observed populations are migrating into fresh water earlier than they historically did (Kovach et al. 2015). Stream temperature is an important factor governing freshwater entry in salmonids, which require sufficiently cool water temperatures for developing embryos (generally <14 °C; Velsen 1987). The median migration times of these three species generally occur after stream temperatures have peaked, suggesting that selection or plasticity should favor later stream entry times that enable adults to avoid increasingly warm temperatures. However, the regional temporal pattern of earlier phenology in these species contrasts such a response of adults to warming stream temperatures, suggesting that these phenological changes may instead reflect an adaptive response of fry to an earlier optimal window for oceanic entry. Moreover, Kovach et al. (2015) observed that the largest changes in median migration time were correlated with decreases in the duration of the migration, indicative of a loss of phenotypic variation resulting from natural selection against late-migrating fish. Collectively, these regional patterns of phenological and environmental change are consistent with an adaptive response of multiple salmonid species to warming oceanic conditions, as we have hypothesized for Auke Creek pink salmon.

We have presented an example of contemporary evolution of phenology in a salmonid population that meets all the criteria necessary to demonstrate adaptive genetic change (Hansen et al. 2012). Despite apparently strong natural selection against late-migrating fish and resulting demographic changes, the odd-year population has exhibited sustained levels of adult recruitment over the past 17 generations. Similarly, the even-year population, which has exhibited parallel changes in phenology, has also sustained its recruitment levels over the same period (Kovach et al. 2013*b*). This indicates that genetically based variation in phenology has supported sustained productivity in Auke Creek during rapid climate change, an observation that underscores the importance of life history variation to the resilience of fish populations (Hilborn et al. 2003; Greene et al. 2010). In populations where this adaptive variation does not exist, the limited scope of phenotypic plasticity may provide the only means of shifting phenology in response to climate change. Collectively, our results emphasize the importance of tailoring fisheries management programs to recognize and conserve seasonal genetic variation in the face of inexorable, yet unpredictable, climate change.

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