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Growth patterns of larval walleye pollock *Gadus chalcogrammus* from core and peripheral habitat differ in response to temperature

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ABSTRACT

Walleye pollock (*Gadus chalcogrammus*) supports one of the world's largest fisheries and is a key species in North Pacific ecosystems. Its future as a sustainable fishery may be dependent on its ability to adapt to changing water temperatures under climate change. The largest global average increase in sea surface temperature has occurred in Alaska, and increasing temperatures are predicted. Here, we examined responses to a range of temperatures in the early life history of walleye pollock from a northern (core) and a southern (peripheral) part of their range using growth measurements and genetics (RADseq). Crosses were conducted to generate families with one female and three male adults from Shelikof Strait, AK and Puget Sound, WA. Offspring were reared to the late larval stage at three temperatures corresponding to low (1.5 °C), medium (5.8 °C), and high (~12 °C) temperature relative to natural conditions. Alaska pollock outperformed Puget Sound pollock at the lowest temperatures, hatching earlier with a higher growth rate than Puget Sound pollock. At medium temperatures, Puget Sound and Alaska pollock grew at the same rate but Alaska pollock hatched sooner. The response to high temperatures was the same in both groups, characterized by early hatching at a small size and high growth rates. Genetic analysis did not show differences among family groups that survived different temperature treatments. Our results demonstrate local adaptation, although a link between genotype and phenotype was not found.

1. Introduction

Species will move, adapt, or perish in response to climate change if their environment exceeds their thermal preference. Species movement has been shown to track the climate velocity, the rate and direction of temperature changes (Pinsky et al., 2013). Poleward movement at the leading and lagging edge is predicted in marine species into water that matches their thermal tolerance (Sunday et al., 2012). Adaptation to changing temperatures is more difficult to observe than movement, and it has generally been thought to be very slow. However, adaptation may occur on much shorter time scales, and such evolutionary responses may help species persist in stressful environments (Carvalho et al., 1996; Conover and Munch, 2002).

The potential and speed of adaptation directly impacts marine resource management, which is challenged by maintaining the sustainability of commercial species under climate change. Since 2014, the eastern Bering Sea (EBS) has exhibited anomalously warm temperatures (Alabia et al., 2018). Sea surface temperatures higher than ever recorded were observed in the Bering Sea and Gulf of Alaska in 2015, 2016, and 2019 (Walsh et al., 2018; Laurel and Rogers, 2020). The extent of the cold pool, water below 2 °C that remains along the EBS shelf during the summer following sea ice retreat, has recently shifted northward and was virtually absent in 2018 for the first time in the 37-year history of the National Marine Fisheries Service Bering Sea Survey (Stevenson and Lauth, 2019; Basyuk and Zuenko, 2020).

Adaptation to increasing temperatures may be highly relevant for the management of walleye pollock, *Gadus chalcogrammus*, which supports the largest fishery in Alaska (Fissel et al., 2012). There is evidence for genetic adaptation to temperature in this species; for example, genotype frequencies at the pantophysin (*Pan* I) gene are significantly correlated with mean surface temperatures in walleye pollock, as well as for Atlantic cod, and other gadid fishes (Pogson and Fevolden, 2003; Canino and Bentzen, 2004; Case et al., 2005). Population differentiation at *Pan* I in adult pollock was approximately one order of magnitude larger than estimated from 14 microsatellite loci (global F_{ST} = 0.038 vs. 0.004; O'Reilly et al., 2004; Canino et al., 2005), indicating that it may be

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subject to directional selection. In addition, higher fitness in more heterozygous individuals has been documented in other species, including salinity tolerance in the guppy *Poecilia reticulata* (Shikano et al., 1997), growth rate in the coot clam *Mulinia lateralis* (Gaffney et al., 1990), and in *Drosophila melanogaster* (Carson, 1961) and other animal species (Allendorf and Leary, 1986). Whether heterozygosity plays a role in thermal tolerance in walleye pollock is unknown.

Summer distributions of walleye pollock have shifted northward in the Bering Sea since 2010, likely in part due to rising temperatures (Stevenson and Lauth, 2019). National Marine Fisheries Service surveys estimated 11,000 t of pollock in 2010 and 1.3 million t in 2017 in the northern Bering Sea, a region that historically represented marginal habitat (Stevenson and Lauth, 2019). Shelikof Strait represents a spawning area for walleye pollock within the Gulf of Alaska, core habitat for this species. Temperatures associated with newly hatched larval pollock in Shelikof Strait were 5.5–7.0 °C (Kendall et al., 1987). However, pollock habitat extends much farther south, with a concomitant range in temperature. For example, Puget Sound in Washington State provides peripheral habitat with considerably warmer temperatures. Temperature readings from a buoy near the Admiralty Lighthouse approximately 2 nautical miles from Port Townsend, WA showed that yearly bottom temperatures ranged between 7 and 10 °C at 80 m depth. Surface waters in Puget Sound can be even warmer, up to 15 °C or higher, depending on the location in Puget Sound (PSEMP Marine Waters Workgroup. 2013). This wide range in temperature in a highly dispersive species suggests high adaptability, which is relevant to forecasting population trends and managing sustainable fisheries under climate change.

Given the ecological and economic importance of walleye pollock, understanding its response to climate change is important for the management of this stock. Walleye pollock early larval mortality is high and variable and is strongly influenced by temperature (Bailey, 2000). Specifically, we hypothesized that differences in larval performance under a range of temperatures from northern and southern populations would provide an indication for local adaptation, especially if supported by evidence for genetic selection during the rearing process. In many marine fish species, the population response to their environment is determined in the early life history when potential mortality is highest (Laurel et al., 2016). Here, we performed a common garden experiment in larval walleye pollock from Shelikof Strait, AK and Puget Sound, WA to examine growth differences and evidence for selection in fertilized eggs reared past first feeding and subjected to three different temperatures. Restriction-site associated DNA sequencing (RADseq) was performed on all parents and a subsample of offspring to test for evidence of selective mortality due to rearing temperature.

2. Methods

Walleye pollock in spawning condition were collected from two regions, Shelikof Strait, Alaska and Puget Sound, Washington, U.S.A. (Fig. 1). One female and three males were spawned from each sampling location resulting in three half-sib families per region, sired by Males 10, 11, and 12 from Puget Sound and Males 7, 8, and 9 from Alaska. Walleye pollock from Puget Sound were collected near Port Townsend, WA (48° 11.7′ N, 122° 50.50’ W) and spawned March 31, 2012 (Table 1). Pollock from Alaska were taken onboard a National Marine Fisheries Service (NMFS) Midwater Assessment Conservation Engineering (MACE) Research Cruise from Shelikof Strait, AK in spawning condition (57° 58.75′ N, 154° 25.79′ W) and spawned on board March 25, 2012. Samples are hereafter referred to as the “Alaska” and “Puget Sound” groups.

Fertilized eggs were transported to the Alaska Fisheries Science Center, Seattle, WA, and reared in separate tanks by region, with three temperature treatments for each region, for a total of six 50-gallon tanks. Roughly equal numbers of fertilized eggs from each family were used in each temperature treatment in a common garden format, approximately

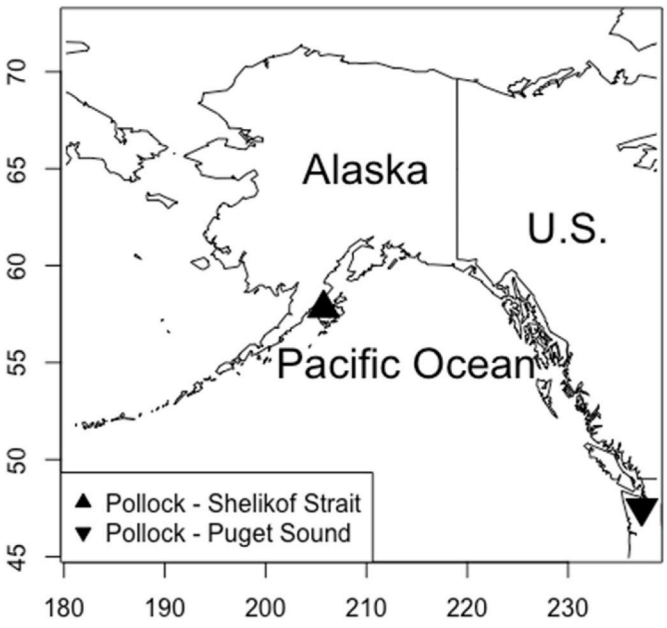

Fig. 1. Map showing sample locations for walleye pollock collected from Shelikof Strait, AK, and Puget Sound, WA.

Table 1
Average temperature after equilibration for samples from each region, as well as the day (post spawning) on which 50% hatch, first feeding, and final harvest occurred for Alaska and Puget Sound samples.

Region	Shelikof Strait, AK			Puget Sound, WA		
Temperature	1.5 °C	5.8 °C	11.8 °C	1.5 °C	5.8 °C	11.9 °C
50% Hatch	22	17	14	27	19	15.0
First feeding	36	22	22	39	24	17.0
Harvest	60	40	25	55	47	23

380–500 larvae per tank (Table 2). Temperatures were selected based on in situ conditions for walleye pollock: low (1.5 °C), medium (5.8 °C), and high (~11.9 °C) relative to in situ conditions. Tank temperatures were 5 °C at day zero for all tanks, and reached equilibrium for the Alaska families on day 18, 11, and 16 for low, medium, and high temperatures, respectively, and on day 19, 12, and 14, respectively, for the Puget Sound families (Fig. 2). Hatch success is highest (~80%) from 1.5 to 6 °C, then declines to approximately 20–40% by 12 °C (Laurel et al., 2018). Therefore, experimental conditions represented an extreme low, a medium, and an extreme high temperature, within the range of those experienced in situ. Tanks were exposed to a 14/10 light/dark cycle, and larvae in all tanks were fed rotifers to satiation daily, starting with the day of first feeding (Table 1). Rotifers (*Brachionus plicatilis*) were added to the tanks at a density of 1–5 individuals/ml and rotifer density was maintained at that density throughout the experiment. Tanks were checked daily for mortalities, which were removed, and water was

Table 2
Total number of offspring (n) used in the final dataset, sired by Males 10, 11, and 12 and a single female from Puget Sound, WA, and Males 7, 8, and 9 and a single female from Alaska. Roughly equal numbers sired by each male were reared in each temperature treatment, referred to as “Initial number in each tank”. “Number sired in dataset” refers to the number each male sired represented in the final set of larvae that were sequenced and used in the final dataset.

	Shelikof Strait (n = 95)			Puget Sound (n = 98)		
Male	7	8	9	10	11	12
Initial number in each tank	450	250	450	500	500	500
Number sired in dataset	8	50	34	26	56	14

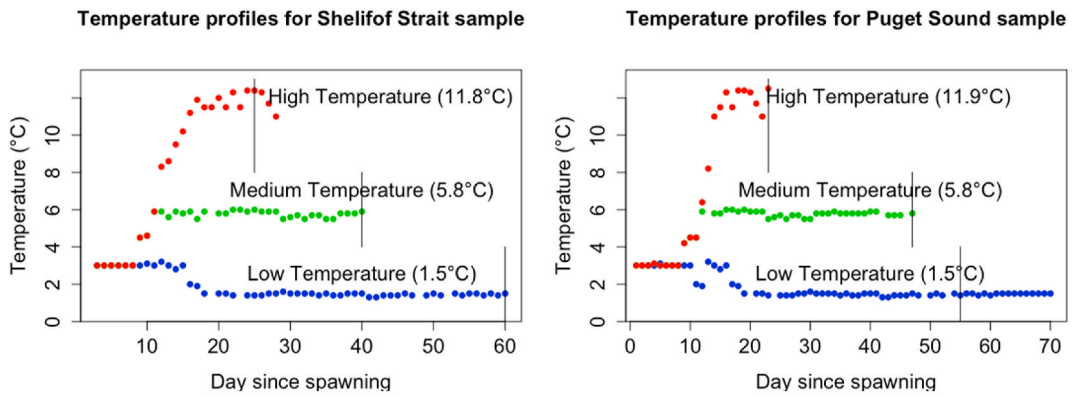

Fig. 2. Daily temperature readings for larval sample treatment from Shelikof Strait, AK, and Puget Sound, WA. The date of the final sample used in sequencing is demarcated as a vertical line for each temperature treatment.

cleaned regularly. The three temperature treatments were on average 1.5 °C, 5.8 °C, and 11.9 °C for the Puget Sound families, and 1.5 °C, 5.8 °C, and 11.8 °C for the Alaska families from the day they reached equilibrium until the termination of the experiment, which occurred after the first feeding (Table 1).

Larvae were collected in groups of 50 at regular intervals throughout the experiment, dependent on growth and mortality rates, to be preserved in 100% non-denatured ethanol. Mortality increased with temperature, so the experiment was continued until most larvae in the high temperature treatment had died. The last sample from each region and temperature treatment was used for DNA sequencing. Length prior to preservation was measured for a subsample of 10 fish during each collection using an optical micrometer (Table A1).

A linear model of length (mm) as a function of day after hatch with an interaction term for region was used to test whether the slope (growth rate) and intercept (size at hatch) differed among regions. Intercepts were allowed to vary under the assumption that differential growth occurs with temperature prior to hatching (Laurel et al., 2018). Linear models were implemented in R software (R Core Team, 2012) for each temperature treatment using a linear mixed-effect model in the R package *lme4*. The model was run for each temperature treatment:

$$\text{Length}_T = \beta_0 + \beta_1 X_{1T} + \beta_2 X_{2T} + \beta_3 X_{1T} X_{2T} + \varepsilon,$$

where X_{1T} is the number of days after hatch and X_{2T} is region, Alaska or Puget Sound. An AMOVA was run on each model to test for significance of each predictor. The model was run for each temperature treatment, and systematically reduced to the simplest model in which all terms were significant.

DNA sequencing (restriction associated DNA sequencing, RADseq) was performed on offspring and parents from each region to examine genetic differences among samples from different regions and to assign parentage. DNA was extracted from whole larvae after removal of stomachs to avoid contamination. Extractions were performed using a DNeasy Blood and Tissue Kit (Qiagen, Inc., Valencia, CA) in a 96 well format. RAD libraries were prepared according to Drinan et al. (2018), using 300 ng DNA per sample. Pollock larvae were sequenced in three libraries, and grouped into three pools. There were 60 individuals in Pool 1, 65 in Pool 2, and 84 in Pool 3, for a total of 209 larvae sequenced. Pools 1 and 3 were sequenced on an Illumina HiSeq 2500 Sequencer in a single direction at 150 bp (Illumina, Inc., San Diego, CA), and Pool 2 was sequenced on an Illumina HiSeq 4000 paired-end sequencing 150 bp. All sequencing was performed at the University of Oregon Genomics and Cell Characterization Core Facility (GC3F).

Raw reads were filtered and demultiplexed using the Stacks v. 1.44 (Catchen et al., 2013) component program process_radtags with flags (-r -c –q), which rescued barcodes and RAD-Tags, removed any read with an uncalled base, and discarded reads with low quality scores (<90% probability of being correct). All reads were trimmed to 125 bp, based on sequence quality scores using FastQC High Throughput Sequence QC Report Version 0.11.5 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc). Reads were aligned to the gadMor2 Atlantic cod genome as a conservative method for filtering SNP markers using bowtie legacy 1.2 software v. 2.3.4.1 (Langmead and Salzberg, 2012), with options (*-v* 3, *-k* 2), where *-v* is the number of mismatches permitted regardless of quality, *-k* is the number of valid alignments to report. The Atlantic cod genome was selected because it is a close relative of the walleye pollock, even more closely related than Pacific cod *Gadus macrocephalus* (Árnason and Halldórsdóttir, 2019). The Stacks pipeline was run with the stack depth set to at least 5 (*–m* 5), biallelic SNPs with minor allele frequency <0.05 were removed, and the minimum number of populations a locus must be present in to process and the minimum percentage of individuals were both set to 0.75 (-p 0.75, -r 0.75), as the Alaska and Puget Sound families were considered separate populations. Stacks processing parameters (*-m* 5 in pstacks and *–m* 5 in populations) were used to effectively combine datasets run on different platforms, given typically lower stack depth from the HiSeq 2500. Default parameter options included *-g* in cstacks, *–M* 2, which was selected to avoid large, repetitive sequences and paralogs (Mastretta-Yanes et al., 2015), where *–M* is the maximum distance in nucleotides allowed between stacks. The first SNP per RAD-Tag was retained for further analysis, and individuals with >30% missing data were removed.

Parentage was analyzed on the final dataset which included 99 larvae from Puget Sound and 95 larvae from Alaska using the R package *apparent* using default parameters for significance testing (Melo and Hale, 2019). This package calculates the Gower Dissimilarity metric between offspring and potential parents and uses a Dixon test to identify a threshold that separates true parent offspring pairs from random associations (Gower, 1971). Individuals with the MaxIdent lower than the default value of 0.1 were removed. Two datasets were assembled: one for Alaska offspring and the other for Puget Sound offspring. All parents were included in each dataset (2 mothers and 6 males) as a negative control. Individuals with fewer than 300 useable loci were removed. PLINK software v1.90b5.3 (Purcell et al., 2007) was used to identify and remove genotypes that were not possible given Mendelian inheritance and parentage assignments, then remove all loci with >10% missing genotypes.

Basic statistics were also calculated for data from each region and temperature treatment to look for any selective differences that may have arisen. F_{IS} was examined to determine whether there was evidence for balancing selection or heterosis, and was expected to be most notable in the high temperature treatment because it was subject to the highest mortality rates. Heterosis, also known as hybrid vigor, is the improved function of any biological quality in a hybrid offspring (Hanot et al., 2019), so we quantified heterozygosity for each unique family and temperature treatment. Rarefied allelic richness was calculated using

the R package *hierfstat* (Goudet, 2005). The R package *diveRsity* was used to calculate heterozygosity and homozygosity (Keenan et al., 2013). Individuals with anomalously high heterozygosity were removed if they appeared to be low F_{IS} outliers (<first quantile-1.5* the IQR, interquartile range), as this was indicative of contamination (mixture of more than a single individual). We also removed high F_{IS} outliers, as anomalously low heterozygosity could be an indication of poor DNA quality. F_{IS} (Nei, 1987) was then calculated across groups of individuals with the same parentage and temperature using the basic.stats command in *hierfstat* to examine whether there were differences among families or trends by temperature. Genic tests for differentiation were performed among family groups reared in different temperatures using the R package *Genepop* (Rousset, 2008) using dememorization = 10,000, batches = 100, iterations = 5000.

We measured 130 larvae from the Alaska group: 50 at low temperatures, 50 at medium temperature, and 30 at high temperature. There were 150 larvae measured for the Puget Sound group: 70 at low temperature, 50 medium, and 30 at high temperature. The final samples (used for genetic analysis) were taken from Puget Sound and Alaska groups on day 23 and 25 from the high temperature treatment, day 47 and 40 from the medium temperature treatment, and 55 and 60 from the low temperature treatment, respectively (Table 1). The high temperature treatment was terminated before the medium and low tanks, as hatch occurred sooner, and growth and mortality were higher, although mortality was not quantified (Table 1, Fig. 2). We sequenced 95 larvae from the Alaska group and 98 from the Puget Sound group, as well as the single dam and three sires from each region.

3. Results

3.1. Growth

Linear model predictors included day post-hatch, region, and the interaction term between the two. Significant predictors differed by temperature, with all predictors significant for low temperature samples, day post-hatch and region significant for medium temperature samples, and only day post-hatch for the high temperature samples (Table 3). The effect of region on growth was significant at 1.5 °C ($p = 0.007$), as well as an interaction term between day post-hatch and region ($p < 0.000$). The effect of region on growth was also significant at 5.8 °C ($p = 0.000$), but not in the high ($p = 0.221$) temperature treatments (Table 3, Fig. 3). Growth rate was significantly higher at 1.5 °C in the Alaska group than in the Puget Sound group (Fig. 4), and the size at hatch decreased with temperature (Fig. 4). Overall, fastest growth occurred in the medium treatments and did not differ between regions.

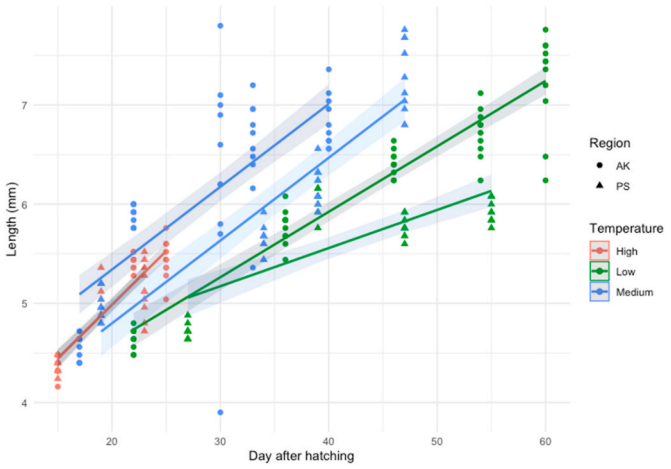

Fig. 3. Length (mm) of samples taken throughout the experiment for low (1.5 °C), medium (5.8 °C) and high (11.8 °C AK, 11.9 °C PS) temperature treatments, where AK represents families from Shelikof Strait, and PS represents Puget Sound families. Lines represent modeled of length vs. day after hatching, as specified in Table 3. The last time point sampled was sequenced for all six treatment/region combinations.

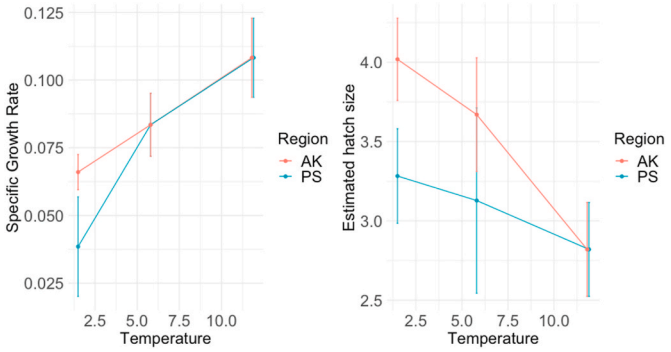

Fig. 4. Left panel: growth rate (slope of length (mm) vs. day after hatching) for the Shelikof Strait, AK family (red) and the Puget Sound, WA family (blue). Right panel: estimated hatch size (estimated size at day of hatch) in different temperatures by region. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 3
Analysis of variance (ANOVA) of linear models fit to length (mm) as a function of day after hatch, separately by temperature. Region and an interaction term between day after hatch and region were included in the final model if significant. Results shown include estimates for each coefficient, standard error, degrees of freedom (df), sum of squares (Sum Sq.), the value of the F-statistic, and Pr(>F), the probability of getting the observed F value or higher, under the null hypothesis in which the response has no effect.

Temp.	Model	Estimate	Std. Err.	Pr(>|t|)	df	Sum Sq.	F-val.	Pr(>F)
Low	Intercept	3.283	0.152	<0.000***				
1.5 °C	Day post-hatch	0.066	0.003	<0.000***	1	46.52	447.84	***
	Region	0.736	0.268	0.007**	1	4.36	42.00	***
	Day post-hatch:Region	−0.028	0.006	<0.000***	1	2.15	20.67	***
	Residuals		0.322		87	9.04		
Med.	Intercept	3.671	0.183	<0.000***				
5.8 °C	Day post-hatch	0.083	0.006	<0.000***	1	46.43	175.17	***
	Region	−0.542	0.115	0.000***	1	5.853	22.08	***
	Residuals		0.515		88	23.325		
High	Intercept	2.820	0.151	<0.000***				
>11 °C	Day post-hatch	0.108	0.007	<0.000***	1	10.133	210.77	***
	Residuals		0.219		57	2.740		

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.

3.2. Genetic analysis

Initial Stacks output contained 22,092 SNPs. Following removal of loci with Mendelian errors, loci with >10% missing data, and individuals with >30% missing data, there were 16,066 loci and 99 individuals (95 offspring, 4 parents) in the Alaska dataset and 97.2% genotyping rate, and there were 16,550 loci and 102 individuals (98 offspring, 4 parents) 97.2% genotyping rate in the Puget Sound dataset. There were 13,412 SNPs shared between the Puget Sound and the Alaska datasets, indicative of differences in their genomes. The final dataset included 98 offspring from Puget Sound crosses (36 reared at low temperature, 34 reared at medium temperature, and 28 at high temperature) and 95 individuals from Alaska (25 reared at low temperature, 35 reared at medium temperature, and 35 at high temperature). The number of individuals from each family was not evenly represented across temperature treatments, as parentage was determined only after larvae were sequenced. Insufficient data were collected to suggest differential mortality by family or other causes of unequal family representation.

Parentage analysis assigned all offspring to the correct sire with significance <0.01 (only one dam was present). No negative controls were indicated as a result. Three individuals from Alaska and four from Puget Sound did not pass the threshold (MaxIdent 0.1) and were removed, generally as a result of low genotyping rates, and these individuals were removed from the dataset (Table A2). The number of individuals sequenced per family and temperature treatment ranged from one to 28 (Table 4)

Mean F_{IS} was consistently negative because families of full siblings have more heterozygotes than expected in a large population in Hardy-Weinberg equilibrium (Table 3). Mean F_{IS} and observed heterozygosity by family and region did not show any significant trends. Rarefied allelic richness ranged from 1.198 to 1.558 and was relatively consistent within families. Observed heterozygosity ranged from 0.244 to 0.285 and observed gene diversity ranged from 0.191 to 0.220. Genic tests for differentiation were universally not significant (p-value = 1.000) among family groups genotyped following completion of the experiment.

4. Discussion

How walleye pollock will respond to increasing ocean temperatures due to climate change is an important question for the future of the fishery and the ecosystem. Here we attempted to delineate patterns of larval growth in walleye pollock from a northern core and a southern peripheral population. We also attempted to examine whether there were observable genetic differences among larvae surviving different temperature treatments using thousands of SNP loci. We examined larval growth, as the larval stage is typically associated with the highest natural mortality rates (Laurel et al., 2016).

A significant finding was that larval rates of growth differed by region within the common garden framework. Alaska pollock grew more quickly, as measured by a combination of hatch date, growth rate, and length achieved, in lower temperatures than Puget Sound pollock (Table 3). Walleye pollock larvae from Alaska family groups hatched sooner and grew significantly faster than Puget Sound larvae at 1.5 °C (Figs. 3 and 4). At medium temperatures (5.8 °C), growth rate did not differ among larvae from the two regions, but the Alaska group hatched earlier and at a larger size. Finally, at high temperatures, growth rates for Puget Sound and Alaska larvae were indistinguishable. Larvae hatched earlier and at the smallest sizes in the high temperature treatment than in any other temperature and grew more quickly than in other treatments, but did not achieve the sizes associated with lower temperatures. Laurel et al. (2016) also found that size at hatch of juvenile walleye pollock from Puget Sound decreased with temperature. Overall, growth rate increased with temperature, and size at hatching generally decreased (Fig. 4), consistent with previous results (Laurel et al., 2016, 2018). The range of growth rates from previous research was consistent with the growth rates observed in this experiment (Kendall et al., 1987). Temperature has been noted in other studies as having a strong effect on growth in pollock; temperature and prey quality accounted for 66% of bioenergetic and otolith growth variation in juveniles (Mazur et al., 2007). Ocean temperature has been found to influence larval feeding rates, and larval and juvenile growth rates and survival (Bailey et al., 1996a; Porter et al., 2005; Dougherty et al., 2007). Similar to the present project Otterlei et al. (1999) studied two populations of Atlantic cod, collected in the south (coastal cod) and the north (northeast Artic cod). The two components did not show temperature adaptation by latitude, although the southern component grew faster.

Increasing growth rate observed in our experiment was accompanied by daily feeding to satiation, but marine fish likely experience different conditions in situ. For example, Barbeaux et al. (2020) found that increased metabolism by Pacific cod associated with the marine heat wave of 2014–2016 was accompanied by high mortality rates, possibly as a result of consumption not meeting metabolic demand. Whether a similar high mortality event could occur in walleye pollock early life history stages is unknown. Recruitment to the fishery at age 3 is highly variable and has been correlated with temperature and food availability during early life history (Bailey et al., 1996b; A'Mar et al., 2009).

Results did not show significant trends in genetic diversity or heterozygosity related to temperature treatment (Fig., Table 4). Tests for genic differentiation also did not show any significant differences among

Table 4
Region (PS=Puget Sound, WA; AK = Alaska), temperature treatment, paternity, number of fish (n), F_{IS} (Weir and Cockerham 1984, WC), F_{IS} (Nei 1987), observed heterozygosities (H_O), observed gene diversities (H_S), and allelic richness, or rarefied allele counts. F_{IS} estimates for parents were calculated among parents from each region.

Region	Temperature	Paternity	n	F_{IS} (Nei)	H_O	H_S	Allelic Richness
PS	Low (1.5 °C)	M10	16	−0.321	0.252	0.191	1.475
PS	Medium (5.8 °C)	M10	1	–	0.258	–	1.258
PS	High (11.9 °C)	M10	9	−0.320	0.258	0.195	1.450
PS	Low (1.5 °C)	M11	13	−0.289	0.281	0.218	1.551
PS	Medium (5.8 °C)	M11	28	−0.293	0.281	0.217	1.554
PS	High (11.9 °C)	M11	15	−0.304	0.286	0.220	1.554
PS	Low (1.5 °C)	M12	6	−0.302	0.251	0.193	1.198
PS	Medium (5.8 °C)	M12	3	−0.294	0.251	0.194	1.207
PS	High (11.9 °C)	M12	5	−0.341	0.249	0.186	1.242
AK	Low (1.5 °C)	M7	2	−0.339	0.244	0.182	1.208
AK	High (11.8 °C)	M7	6	−0.322	0.244	0.185	1.191
AK	Low (1.5 °C)	M8	22	−0.289	0.284	0.220	1.560
AK	Medium (5.8 °C)	M8	17	−0.303	0.285	0.219	1.558
AK	High (11.8 °C)	M8	11	−0.300	0.283	0.218	1.545
AK	Medium (5.8 °C)	M9	17	−0.311	0.261	0.199	1.497
AK	High (11.8 °C)	M9	17	−0.292	0.256	0.198	1.497

allele frequencies in larvae from the same family that completed the experiment under different temperature conditions. There were no other significant genetic results that could be attributed to differential adaptation based on the genetic data. While we controlled for environment though identical rearing conditions, phenotype may reflect genotype or epigenetics (Barros and Offenbacher, 2009). Detailed understanding of epigenetics may be an important step in understanding response to climate change (Bernatchez, 2016). Further, examination of the set of genes responsible for the response to temperature in walleye pollock, such as Pan I, may provide more informative results. While RADseq methodology allowed for a large portion of the genome to be screened, much of the genome was missed using this technique. Other tools, such as RNA transcriptomics may be better suited for examining genomic responses to temperature (Oomen and Hutchings, 2017). Future work is required that investigates genotypes on a more focused, functionally significant part of the genome, such as Pan I, as in Otterå et al. (2020).

While the experimental design was sufficient for measurement of larval growth through time, it led to low sample sizes in certain family groups for the genetic portion of the experiment. Common garden rearing was an important aspect of the experiment, but it did not allow a means for equal representation by family group. Whether the growth rates observed in our experiment were inherited traits is not clear because the experimental design did not control for non-heritable differences (e.g. maternal effects) passed to offspring. Future experiments should use multiple females in order to distinguish between population differences and individual genotypes, as it is well known that female age and body size affect larval growth and survival (e.g. Berkeley et al., 2004). Optimally, future studies would include replicate tanks for each temperature. Whether low growth rates indicate lower fitness or an adaptation to survive under increased temperatures is unknown, and could be the topic of future investigation. In the current study, sufficient prey was provided of suitable size; therefore, the results should be considered in the context of prey availability under climate change. Furthermore, counts of natural mortality and proportion of larvae that hatched successfully would have contributed to our understanding of thermal stress.

Understanding thermal sensitivity and growth of walleye pollock in different parts of their range may help inform their response to climate change. This study identified regional differences in the growth of larval walleye pollock in response to temperature. While the genetic aspect of the study did not find evidence for changes to heterozygosity or allelic richness, differences in growth indicate that walleye pollock from different regions have adapted to regional thermal conditions at some point throughout their evolutionary history. In the south, Puget Sound larvae appeared to have somewhat higher thermal tolerance to warmer water (5.8 °C), and Alaska pollock grew more quickly and hatched earlier in colder water (1.5 °C). However, neither group appeared to grow as well in warm conditions (~12 °C), which may portend a decrease in abundance if temperatures increase significantly at the southern parts of their range.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dsr2.2022.105083.

References

Alabia, I.D., García Molinos, J., Saitoh, S.I., Hirawake, T., Hirata, T., Mueter, F.J., 2018. Distribution shifts of marine taxa in the Pacific Arctic under contemporary climate changes. Divers. Distrib. 24 (11), 1583–1597. https://doi.org/10.1111/ddi.12788.

Allendorf, F.W., Leary, R.F., 1986. Heterozygosity and fitness in natural populations of animals. In: Chapter 4 in: Conservation Biology: the Science of Scarcity and Diversity, 57, pp. 58–72.

A'Mar, Z.T., Punt, A.E., Dorn, M.W., 2009. The evaluation of two management strategies for the Gulf of Alaska walleye pollock fishery under climate change. ICES J. Mar. Sci. 66, 1614–1632. https://doi.org/10.1093/icesjms/fsp044.

Árnason, E., Halldórsdóttir, K., 2019. Codweb: whole-genome sequencing uncovers extensive reticulations fueling adaptation among Atlantic, Arctic, and Pacific gadids. Sci. Adv. 5 (3), 8788. https://doi.org/10.1126/sciadv.aat8788.

Bailey, K.M., Brown, A.L., Yoklavich, M.M., Mier, K.L., 1996a. Interannual variability in growth of larval and juvenile walleye pollock *Theragra chalcogramma* in the western Gulf of Alaska, 1983-91. Fish. Oceanogr. 5, 137–147. https://doi.org/10.1111/j.1365-2419.1996.tb00088.x.

Bailey, K.M., Picquelle, S.J., Spring, S.M., 1996b. Mortality of larval walleye pollock *Theragra chalcogramma* in the western Gulf of Alaska, 1988-91. Fish. Oceanogr. 5, 124–136. https://doi.org/10.1111/j.1365-2419.1996.tb00087.x.

Bailey, K.M., 2000. Shifting control of recruitment of walleye pollock *Theragra chalcogramma* after a major climatic and ecosystem change. Mar. Ecol. Prog. Ser. 198, 215–224. https://doi.org/10.3354/meps198215.

Barbeaux, S.J., Holsman, K., Zador, S., 2020. Marine heatwave stress test of ecosystem-based fisheries management in the Gulf of Alaska Pacific cod fishery. Front. Mar. Sci. 703 https://doi.org/10.3389/fmars.2020.00703.

Barros, S.P., Offenbacher, S., 2009. Epigenetics: connecting environment and genotype to phenotype and disease. J. Dent. Res. 88 (5), 400–408. https://doi.org/10.1177/0022034509335868.

Basyuk, E., Zuenko, Y., 2020. Extreme oceanographic conditions in the northwestern Bering Sea in 2017–2018. Deep-Sea Res. II. 181, 104909 https://doi.org/10.1016/j.dsr2.2020.104909.

Berkeley, S.A., Chapman, C., Sogard, S.M., 2004. Maternal age as a determinant of larval growth and survival in a marine fish, *Sebastes melanops*. Ecology 85 (5), 1258–1264. https://doi.org/10.1890/03-0706.

Bernatchez, L., 2016. On the maintenance of genetic variation and adaptation to environmental change: considerations from population genomics in fishes. J. Fish. Biol. 89 (6), 2519–2556. https://doi.org/10.1111/jfb.13145.

Canino, M.F., Bentzen, P., 2004. Evidence for positive selection at the pantophysin (Pan I) locus in walleye pollock, *Theragra chalcogramma*. Mol. Biol. Evol. 21, 1391–1400. https://doi.org/10.1093/molbev/msh141.

Canino, M.F., O'Reilly, P.T., Hauser, L., Bentzen, P., 2005. Genetic differentiation in walleye pollock (*Theragra chalcogramma*) in response to selection at the pantophysin (PanI) locus. Can. J. Fish. Aquat. Sci. 62, 2519–2529. https://doi.org/10.1139/f05-155.

Carson, H.L., 1961. Heterosis and fitness in experimental populations of *Drosophila melanogaster*. Evolution 15 (4), 496–509. https://doi.org/10.1111/j.1558-5646.1961.tb03180.x.

Carvalho, G.R., Shaw, P.W., Hauser, L., Seghers, B.H., Magurran, A.E., 1996. Artificial introductions, evolutionary change and population differentiation in Trinidadian guppies (*Poecilia reticulata*: Poeciliidae). Biol. J. Linn. Soc. 57, 219–234. https://doi.org/10.1006/bijl.1996.0012.

Case, R.A.J., Hutchinson, W.F., Hauser, L., Van Oosterhout, C., Carvalho, G.R., 2005. Macro- and microgeographic variation in pantophysin (PanI) allele frequencies in NE Atlantic cod *Gadus morhua*. Mar. Ecol. Prog. Ser. 301, 267–278. https://doi.org/10.3354/meps301267.

Catchen, J., Hohenlohe, P.A., Bassham, S., Amores, A., Cresko, W.A., 2013. Stacks: an analysis tool set for population genomics. Mol. Ecol. 22, 3124–3140. https://doi.org/10.1111/mec.12354.

Conover, D.O., Munch, S.B., 2002. Sustaining fisheries yields over evolutionary time scales. Science 297, 94–96. https://doi.org/10.1126/science.1074085.

Dougherty, A.B., Bailey, K.M., Mier, K.L., 2007. Interannual differences in growth and hatch date distributions of age-0 year walleye pollock *Theragra chalcogramma* (Pallas) sampled from the Shumagin Islands region of the Gulf of Alaska, 1985-2001. J. Fish. Biol. 71, 763–780. https://doi.org/10.1111/j.1095-8649.2007.01543.x.

Drinan, D.P., Gruenthal, K.M., Canino, M.F., Lowry, D., Fisher, M.C., Hauser, L., 2018. Population assignment and local adaptation along an isolation-by-distance gradient

in Pacific cod (*Gadus macrocephalus*). Evol. Appl. 11 (8), 1448–1464. https://doi.org/10.1111/eva.12639.

Fissel, B., Dalton, M., Felthoven, R., Garber-Yonts, B., Haynie, A., Himes-Cornell, A., Kasperski, S., Lee, J., Lew, D., Pfeiffer, L., 2012. Stock assessment and fishery evaluation report for the groundfish fisheries off the Gulf of Alaska and Bering Sea/Aleutian Islands area: economic status of the groundfish fisheries off Alaska, 2011. N Pac. Fish. Manag. Council 1007. West Third, Suite 400, Anchorage, Alaska 99501.

Gaffney, P.M., Scott, T.M., Koehn, R.K., Diehl, W.J., 1990. Interrelationships of heterozygosity, growth rate and heterozygote deficiencies in the coot clam, Mulinia. Genetics 124 (3), 687–699. https://doi.org/10.1093/genetics/124.3.687.

Goudet, J., 2005. Hierfstat, a package for R to compute and test hierarchical F-statistics. Mol. Ecol. Notes 5 (1), 184–186. https://doi.org/10.1111/j.1471-8286.2004.00828.x.

Gower, J.C., 1971. A general coefficient of similarity and some of its properties. Biometrics 27 (4), 857–871. https://doi.org/10.2307/2528823.

Hanot, P., Herrel, A., Guintard, C., Cornette, R., Hanot P., 2019. Unravelling the hybrid vigor in domestic equids: the effect of hybridization on bone shape variation and covariation. BMC Evol. Biol. 19 (1), 1–13. https://doi.org/10.1186/s12862-019-1520-2.

Keenan, K., McGinnity, P., Cross, T.F., Crozier, W.W., Prodöhl, P.A., 2013. diveRsity: an R package for the estimation of population genetics parameters and their associated errors. Methods Ecol. Evol. 4 (8), 782–788. https://doi.org/10.1111/2041-210X.12067.

Kendall, A., Clarke, M.E., Yoklavich, M., Boehlert, G., 1987. Distribution, feeding, and growth of larval walleye pollock, *Theragra chalcogramma*, from Shelikof Strait, Gulf of Alaska. Fish. Bull. 85, 499–521.

Langmead, B., Salzberg, S.L., 2012. Fast gapped-read alignment with Bowtie 2. Nat. Methods 9 (4), 357. https://doi.org/10.1038/nmeth.1923.

Laurel, B.J., Spencer, M., Iseri, P., Copeman, L.A., 2016. Temperature-dependent growth and behavior of juvenile Arctic cod (Boreogadus saida) and co-occurring North Pacific gadids. Polar Biol. 39 (6), 1127–1135. https://doi.org/10.1007/s00300-015-1761-5.

Laurel, B.J., Copeman, L.A., Spencer, M., Iseri, P., 2018. Comparative effects of temperature on rates of development and survival of eggs and yolk-sac larvae of Arctic cod (*Boreogadus saida*) and walleye pollock (*Gadus chalcogrammus*). ICES J. Mar. Sci. 75 (7), 2403–2412. https://doi.org/10.1093/icesjms/fsy042.

Laurel, B.J., Rogers, L.A., 2020. Loss of spawning habitat and prerecruits of Pacific cod during a Gulf of Alaska heatwave. Can. J. Fish. Aquat. Sci. 77 (4), 644–650. https://doi.org/10.1139/cjfas-2019-0238.

Mastretta-Yanes, A., Arrigo, N., Alvarez, N., Jorgensen, T.H., Piñero, D., Emerson, B.C., 2015. Restriction site-associated DNA sequencing, genotyping error estimation and de novo assembly optimization for population genetic inference. Mol. Ecol. Resour. 15 (1), 28–41. https://doi.org/10.1111/1755-0998.12291.

Mazur, M.M., Wilson, M.T., Dougherty, A.B., Buchheister, A., Beauchamp, D.A., 2007. Temperature and prey quality effects on growth of juvenile walleye pollock *Theragra chalcogramma* (Pallas): a spatially explicit bioenergetics approach. J. Fish. Biol. 70 (3), 816–836. https://doi.org/10.1111/j.1095-8649.2007.01344.x.

Melo, A., Hale, I., 2019. 'apparent': a simple and flexible R package for accurate SNP-based parentage analysis in the absence of guiding information. BMC Bioinform. https://doi.org/10.1186/s12859-019-2662-3. https://doi.org/10.1186/s12859-019-2662-3.

Nei, M., 1987. Molecular Evolutionary Genetics. Columbia University Press.

Oomen, R.A., Hutchings, J.A., 2017. Transcriptomic responses to environmental change in fishes: insights from RNA sequencing. Facets 2 (2), 610–641. https://doi.org/10.1139/facets-2017-0015.

O'Reilly, P.T., Canino, M.F., Bailey, K.M., Bentzen, P., 2004. Inverse relationship between FST and microsatellite polymorphism in the marine fish, walleye pollock (*Theragra chalcogramma*): implications for resolving weak population structure. Mol. Ecol. 13, 1799–1814. https://doi.org/10.1111/j.1365-294X.2004.02214.x.

Otterå, H., Johansen, T., Folkvord, A., Dahle, G., Solvang Bingh, M.K., Westgaard, J.-I., Glover, K.A., 2020. The pantophysin gene and its relationship with survival in early life stages of Atlantic cod. R. Soc. Open Sci. 7, 191983 https://doi.org/10.1098/rsos.191983.

Otterlei, Erling, Nyhammer, Gunnar, Folkvord, Arild, Stefansson, Sigurd O., 1999. Temperature-and size-dependent growth of larval and early juvenile Atlantic cod (*Gadus morhua*): a comparative study of Norwegian coastal cod and northeast Arctic cod. Can. J. Fish. Aquat. Sci. 56 (11), 2099–2111. https://doi.org/10.1139/f99-168.

Pinsky, M.L., Worm, B., Fogarty, M.J., Sarmiento, J.L., Levin, S.A., 2013. Marine taxa track local climate velocities. Science 341 (6151), 1239–1242. https://doi.org/10.1139/f99-168.

Pogson, G.H., Fevolden, S.E., 2003. Natural selection and the genetic differentiation of coastal and Arctic populations of the Atlantic cod in northern Norway: a test involving nucleotide sequence variation at the pantophysin (PanI) locus. Mol. Ecol. 12, 63–74. https://doi.org/10.1046/j.1365-294X.2003.01713.x.

Porter, S.M., Ciannelli, L., Hillgruber, N., Bailey, K.M., Chan, K.S., Canino, M.F., Haldorson, L.J., 2005. Environmental factors influencing larval walleye pollock *Theragra chalcogramma* feeding in Alaskan waters. Mar. Ecol. Prog. Ser. 302, 207–217. https://doi.org/10.3354/meps302207.

PSEMP Marine Waters Workgroup, 2013. In: Moore, S.K., Stark, K., Bos, J., Williams, P., Newton, J., Dzinbal, K. (Eds.), Puget Sound Marine Waters: 2012 Overview. https://www.pmel.noaa.gov/pubs/PDF/sutt4354/sutt4354.pdf.

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A., Bender, D., Maller, J., Sklar, P., De Bakker, P.I., Daly, M.J., Sham, P.C., 2007. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am. J. Hum. Genet. 81 (3), 559–575. https://doi.org/10.1086/519795.

R Core Team, 2012. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Rousset, F., 2008. genepop'007: a complete re-implementation of the genepop software for Windows and Linux. Mol. Ecol. Resour. 8 (1), 103–106. https://doi.org/10.1111/j.1471-8286.2007.01931.x.

Shikano, T., Nakadate, M., Nakajima, M., Fujio, Y., 1997. Heterosis and maternal effects in salinity tolerance of the guppy *Poecilia reticulata*. Fish. Sci. 63 (6), 893–896. https://doi.org/10.2331/fishsci.63.893.

Stevenson, D.E., Lauth, R.R., 2019. Bottom trawl surveys in the northern Bering Sea indicate recent shifts in the distribution of marine species. Polar Biol. 42 (2), 407–421. https://doi.org/10.1007/s00300-018-2431-1.

Sunday, J.M., Bates, A.E., Dulvy, N.K., 2012. Thermal tolerance and the global redistribution of animals. Nat. Clim. Change 2 (9), 686. https://doi.org/10.1038/nclimate1539.

Walsh, J.E., Thoman, R.L., Bhatt, U.S., Bieniek, P.A., Brettschneider, B., Brubaker, M., Danielson, S., Lader, R., Fetterer, F., Holderied, K., Iken, K., 2018. The high latitude marine heat wave of 2016 and its impacts on Alaska. Bull. Am. Meteorol. Soc. 99 (1), S39–S43. https://doi.org/10.1175/BAMS-D-17-0105.1.

Weir, B.S., Cockerham, C.C., 1984. Estimating F-statistics for the analysis of population structure. Evolution 38 (6), 1358–1370. https://doi.org/10.2307/2408641.