Conservation Physiology

Volume 13 • 2025 10.1093/conphys/coaf083

SEB SOCIETY FOR EXPERIMENTAL BIOLOGY

Research Article

Ontogenetic and environmental responses in metabolic enzyme activity of Pacific Arctic larval gadids

Emily Slesinger[1,*], **Erik V. Thuesen**[2] **and Thomas P. Hurst**[1]

[1]Alaska Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, Hatfield Marine Science Center 2030 SE Marine Science Dr, Newport, OR 97365 USA
[2]Evergreen State College, Laboratory I, Olympia, WA 98505 USA

***Corresponding author:** Alaska Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, Hatfield Marine Science Center 2030 SE Marine Science Dr, Newport OR 97365, USA. Email: emily.slesinger@noaa.gov

Warming in high-latitude marine ecosystems is leading to the borealization of Arctic communities. Species-specific responses to temperature provide insight into potential co-occurrence or competitive advantage between Arctic and boreal species. Ocean acidification may also lead to unique species-specific responses. At the Pacific–Arctic interface, larval distributions of the boreal Pacific cod (*Gadus macrocephalus*) are increasingly overlapping with those of Arctic cod (*Boreogadus saida*). We assessed larval metabolic capacities by measuring metabolic enzyme activities of citrate synthase (CS; aerobic metabolism), lactate dehydrogenase (LDH; anaerobic metabolism), and β-hydroxyacyl CoA dehydrogenase (HOAD; fatty acid metabolism). Throughout early development, Pacific cod enzyme activities, including glycolytic capacity, were higher, and fatty acid metabolism lower than Arctic cod enzyme activities. These responses may reflect a more active larval lifestyle of Pacific cod. Separately, larvae were reared in multiple temperatures (Pacific cod: 3, 6, 10°C; Arctic cod 1.8, 5, 7.3°C) and pCO_2 levels (ambient = ∼350 μatm; high = ∼1500 μatm). At the cold temperature, Pacific cod enzyme activities were higher than at the control temperature, indicating they were acclimating but less cold adapted than Arctic cod. Arctic cod HOAD activity and LDH:CS ratio were elevated under warmer temperatures suggesting increased energy demand. Elevated pCO_2 levels only affected larvae at their control temperature and resulted in decreased Pacific cod HOAD activity and increased Arctic cod CS and HOAD activities. This indicates differing sensitivities to ocean acidification between the species. Overall, Pacific cod may continue to be constrained in their northern habitat by cold temperatures, but under slight warming to optimal growing temperatures, Pacific cod will have competitive advantage over Arctic cod.

Lay Summary

We measured metabolic enzyme activities in a boreal and an Arctic fish to assess degree of distribution overlap and competitive advantage. Larval development and sensitivity to environmental stressors differed for the two species, driven by differing metabolic strategies. Under slight warming, Pacific cod may have competitive advantage over Arctic cod.

Key words: *Boreogadus saida: Gadus macrocephalus*, metabolic enzymes, ocean acidification, ocean warming

Abbreviations: CS citrate synthase; DPH days post hatch; DW dry weight; HOAD β-hydroxyacyl CoA dehydrogenase; LDH lactate dehydrogenase; SL standard length; WW wet weight

Editor: Frank Seebacher

Received 21 May 2025; Revised 18 September 2025; Accepted 26 September 2025

Cite as: Slesinger E, Thuesen EV, Hurst TP (2025) Ontogenetic and environmental responses in metabolic enzyme activity of Pacific Arctic larval gadids. *Conserv Physiol* 13(1): coaf083; doi:10.1093/conphys/coaf083.

Introduction

High-latitude regions are experiencing rapid marine environmental change (Frey *et al.*, 2015). These regions are warming at a rate that is substantially faster than the global average (Hermann *et al.*, 2016; Danielson *et al.*, 2020; Huntington *et al.*, 2020), which has also led to declines in sea ice duration and extent (Overland and Wang, 2025). In the Arctic ecosystem, warming and loss of sea ice has led to "borealization" of fish communities (Fossheim *et al.*, 2015; Mueter *et al.*, 2021). Changes in species assemblages have been documented at the arctic interfaces in the Atlantic (Renaud *et al.*, 2012; Fossheim *et al.*, 2015) and Pacific oceans (Rand and Logerwell, 2011), including between the Bering Sea (North Pacific) and the Chukchi Sea (Arctic). In this region, northward adult movement of boreal species can be a response to warming in the southern edges of their distribution and/or opening of the northern habitat that was historically too cold (Stevenson and Lauth, 2019; Eisner *et al.*, 2020). For boreal larvae, the northward movement can occur through adults spawning in new regions (Spies *et al.*, 2020) and/or changes in the direction and strength of currents that advect larvae northward (Woodgate and Peralta-Ferriz, 2021). Temperatures in the northern regions can promote larval survival and growth of boreal species (Axler *et al.*, 2023) and also dictate the degree of overlap with Arctic species based on their upper thermal tolerances (Eriksen *et al.*, 2015).

Ocean acidification, the decline in pH due to the diffusion of excess atmospheric carbon dioxide (CO_2) into the ocean (Caldeira and Wickett, 2005), is a co-occurring environmental stressor in high-latitude ecosystems. The effects of ocean acidification are amplified in these regions due to the cold temperatures and addition of fresh water from sea ice melt (Fabry *et al.*, 2009; Mathis *et al.*, 2011; Mortenson *et al.*, 2020). Ocean acidification can negatively affect fish populations through changes in growth, survival and energy demand (Gobler *et al.*, 2018; Baumann *et al.*, 2022). However, the response to ocean acidification appears to be species-specific as fishery species have also exhibited neutral or positive reactions to ocean acidification (Hurst *et al.*, 2012; Perry *et al.*, 2015; Long *et al.*, 2024). Additionally, when assessed in a multistressor framework with temperature, ocean acidification can lead to unique organismal responses based on the prevailing temperature (Sswat *et al.*, 2018; Slesinger *et al.*, 2024), necessitating the exploration of species-specific responses to environmental change within a multi-stressor framework.

At the Pacific–Arctic interface of the Bering Sea and Chukchi Sea, two species of interest are Pacific cod (*Gadus macrocephalus*), a boreal species, and Arctic cod (*Boreogadus saida*), an Arctic species. Pacific cod supports the second largest groundfish fishery in Alaska (Fissel *et al.*, 2017), while Arctic cod are a key forage fish species found throughout the Arctic Ocean (Steiner *et al.*, 2019; Geoffroy *et al.*, 2023). These species historically did not co-occur, yet after recent warming events, the presence of Pacific cod in the Chukchi Sea at multiple life stages was documented (Axler *et al.*, 2023; Cooper *et al.*, 2023). The early life histories of Pacific cod and Arctic cod are relatively similar, as both species' larvae hatch in late spring and are pelagic, surface-dwelling, feeding upon small zooplankton prey (Michaud *et al.*, 1996; Farley *et al.*, 2016; Kono *et al.*, 2016; Logerwell *et al.*, 2020; Correa *et al.*, 2024). In the laboratory, larvae of Pacific cod and Arctic cod were capable of growing and developing in overlapping temperature ranges (Laurel *et al.*, 2008; Koenker *et al.*, 2018b), had similar estimated optimal temperatures for growth (5°C Arctic cod; 6°C Pacific cod), and demonstrated sensitivity to high temperatures seen by elevated mortality rates (7.3°C Arctic cod; 10°C Pacific cod; Slesinger *et al.*, 2024, 2025). Larval Pacific cod and Arctic cod also responded similarly to ocean acidification. Body condition (measured by morphometric indices) was negatively affected by ocean acidification at each species' optimal temperature for growth, but neither species experienced worsened growth, mortality or body condition when ocean acidification was combined with warming (Slesinger *et al.*, 2024, 2025).

While broad organismal responses such as mortality and growth are useful, species-specific physiological responses may expose underlying and more sensitive processes that provide complementary insight into differences in larval Pacific cod and Arctic cod development and function at varying temperatures and pCO_2 levels. Common methods to assess the response of metabolism and energy usage to environmental stressors include respirometry (Schwieterman *et al.*, 2019) and blood chemistry analyses (Sala-Rabanal *et al.*, 2003). While insightful, these methods can be challenging, and sometimes impossible, for very early life stages of fish. Metabolic enzyme activities can also be used as markers for various

components of energy metabolism and have been useful in identifying differences in growth strategies (Nathanailides, 1996; Overnell and Batty, 2000; Churova *et al.*, 2017) and tolerances to environmental stressors (Guderley and Blier, 1988; Feidantsis *et al.*, 2020; Schleger *et al.*, 2021) in a variety of marine fishes, including early life stages (Illing *et al.*, 2020; Kunzmann and Diemel, 2020). Citrate synthase (CS) is the first enzyme in the citric acid cycle and commonly used as a proxy for aerobic metabolism and mitochondrial density (Schnurr *et al.*, 2014; Brijs *et al.*, 2017). Lactate dehydrogenase (LDH) catalyzes the reaction between pyruvate and lactate and is used as a proxy for anaerobic metabolism capacity (Goolish, 1991). β-hydroxyacyl CoA dehydrogenase (HOAD) is an important enzyme in fatty acid oxidation, serving as an indicator of fatty acid metabolism (Hochachka *et al.*, 1982). These three enzymes are also useful to measure together, as their ratios can provide additional insight into the relative capacities of anaerobic to aerobic metabolism (LDH:CS) and fatty acid contribution to aerobic metabolism (HOAD:CS) (Pimentel *et al.*, 2020).

Metabolic enzyme activity levels change with ontogeny as larvae undergo rapid growth and major morphological changes. As larvae increase in size, their mass-specific metabolic rates can decrease, leading to lower mitochondrial demand and thus lower CS activity (Wieser and Fortner, 1986; Catalán *et al.*, 2007). At the same time, the fastest growing tissue in larval fish is white muscle, with increasing larval glycolytic capacity reflected in an increase in LDH activity (Segner and Verreth, 1995; Churova *et al.*, 2017). As such, as larvae grow, CS activity typically remains below the activity levels of LDH (Kunzmann and Diemel, 2020), while the LDH:CS ratio increases (Clarke *et al.*, 1992; Desrosiers *et al.*, 2008). Metabolic enzyme activities can be standardized relative to protein or to fish wet weight (when whole body homogenates are used), providing differing insight into ontogenetic changes. These distinctions are important to consider because protein content itself can change as the larvae develop. For example, larval red drum (*Sciaenops ocellatus*) protein concentration increased with age, which drove an overall increase in weight-specific LDH activity, yet protein-specific LDH activity exhibited a more complex pattern, indicating the overall ontogenetic increase in glycolytic capacity was driven by growth (Clarke *et al.*, 1992). Finally, enzyme activities may reflect the species-specific activity levels or swimming modes used during the early life stages (Hinterleitner *et al.*, 1987). For this reason, assessing metabolic enzyme activities throughout ontogeny can provide insight into differing metabolic strategies and associated activity levels of the larvae.

Metabolic enzyme activities are also sensitive to temperature and pCO_2 level, which can provide insight into differences in species responses to environmental stressors. With respect to ocean warming, metabolic enzyme activity levels can increase under high temperatures, seen as a response to increasing energy demand (Li *et al.*, 2015; Feidantsis *et al.*, 2020). Metabolic enzyme activity levels are also useful when comparing cold tolerance between Arctic and boreal fish species. The decelerating effect of temperature on enzyme kinetics can lead to an imbalance between ATP requirements and the ability of the organism to supply ATP (Overnell and Batty, 2000). Positive temperature compensation (i.e. elevated enzyme activity at cold temperatures) can aid in overcoming this limitation through the increase in enzyme concentrations under cold temperatures (Guderley, 1990; Lucassen *et al.*, 2003; McClelland *et al.*, 2006). Fish that exhibit no positive compensation at cold temperatures are suggested to be cold-adapted (Thibault *et al.*, 1997; Pörtner *et al.*, 2000) with enzyme activities that are adequate for meeting energy demands at the lowest occupied temperatures (Schleger *et al.*, 2021). The response of metabolic enzyme activities to ocean acidification remains more nuanced, with some enzyme activity responses reflecting metabolic suppression (Pimentel *et al.*, 2020) or increases in energy demands (Strobel *et al.*, 2013).

We investigated the influence of ontogeny and environmental stressors (i.e. climate impacts) on larval Pacific cod and Arctic cod biochemical responses through two independent experiments performed with each species (four experiments in total). In the *Ontogeny Experiments*, we explored the effect of growth on metabolic enzyme activity rates through the first ∼2 months of life in each species. We hypothesized that (i) aerobic enzymes would decrease while anaerobic capacity would increase with age for both species. In the *Climate Impacts Experiments*, we investigated the responses of metabolic enzyme activities to the combined effects of temperature and pCO_2. With respect to temperature, we hypothesized (ii) Pacific cod would exhibit signs of cold acclimation (i.e. elevated enzyme activities at a cold temperature) while Arctic cod exhibited signs of cold adaptation (i.e. no change in enzyme activities at a cold temperature), and that (iii) Arctic cod would experience elevated enzyme activity levels under ocean warming due to elevated energy demand. Due to the observed organismal level effects of ocean acidification at the control temperature for both species (Slesinger *et al.*, 2024, 2025), we hypothesized (iv) the response of metabolic enzyme activities under ocean acidification would be greatest when acclimated to the optimal temperature.

Materials and Methods

Ontogeny experiments

Arctic cod and Pacific cod larvae were obtained from adult broodstocks maintained at NOAA's Alaska Fisheries Science Centre laboratory in Newport, OR, USA. Pacific cod larval incubations were conducted in 2022 and Arctic cod larval incubations were conducted in 2023. During the spawning season, ripe and running females were strip-spawned and their eggs were fertilized with the milt from males from the same tank. For Pacific cod, fertilized embryos were obtained

from one female and one male, while for Arctic cod, 13 females and 9 males contributed to a mixed embryo pool collected in one day. Low parental diversity for Pacific cod was an artefact of available spawning adults in that given year. Rearing conditions for Arctic cod and Pacific cod early life stages were based on previous experiments and were set to mimic "optimal" conditions for both species. Embryos were incubated at 2°C for Arctic cod (Koenker *et al.*, 2018b) and 6°C for Pacific cod (Hurst *et al.*, 2019) until they hatched, and were then transferred to three 100-L larval rearing tanks that were maintained at a flow rate of 0.5 L min^{-1}. During larval incubation, the temperature remained at 6°C for Pacific cod (Hurst *et al.*, 2019) and Arctic cod larvae went through a step-wise increase from 2 to 5°C at ∼3 weeks post-hatch (Koenker *et al.*, 2018b). Larvae were fed a diet of live rotifers (*Branchionus plicatilis*) at a concentration of 5000 rotifers L^{-1} twice daily with the addition of green water (RotiGreen *Nanno*; Instant Algae, Reed Mariculture). Sampling occurred prior to the daily provisioning of rotifer prey.

Larval sampling began at 6 days post-hatch (DPH) and was repeated weekly to 55 DPH (Pacific cod) and 76 DPH (Arctic cod). The end-date for sampling was bounded by standardizing two months of growth for each species in a stable temperature while also remaining in the window where larvae were still morphologically and developmentally similar (i.e. presence of finfold for swimming, rotifer diet). At each sampling, 15 fish were randomly selected from the rearing tanks, anaesthetized with tricaine methanesulfonate (MS-222), and photographed underneath a dissecting microscope for analysis in ImageJ to obtain standard lengths (SL; mm). Afterwards, three groups of five fish were pooled, rinsed with ammonium formate, and dried to a constant weight at 60°C (DW; mg). Measures of SL and DW were used to calculate length-based and mass-specific growth rates, respectively. Length-based growth was calculated as the linear change in length over time. Mass-specific growth rate (SGR) was calculated through Eq. (1):

$$\mathrm{SGR} = 100\left(e^{g} - 1\right), \tag{1}$$

where g is the instantaneous growth coefficient, calculated through Eq. (2):

$$g = \frac{\ln\left(W_2\right) - \ln\left(W_1\right)}{t_2 - t_1}, \tag{2}$$

where W_2 and W_1 are the dry weights at the final (t_2) and initial (t_1) sampling time points, respectively.

At each sampling, a separate sample of larvae was removed from the rearing tanks for enzyme activity assays. Due to their small size, larvae from the same tank were pooled to generate composite samples of >20mg wet weight (WW; mg) for Pacific cod and >40 mg WW for Arctic cod. Composite sample weights differed for the two species based on preliminary analyses of minimum weight needed to produce sufficient homogenate based on a species-specific dilution factor (see section *Metabolic Enzyme Assays*). For each composite sample, the pooled larvae were weighed and placed into 2-ml cryovials that were immediately submerged into liquid nitrogen and stored at −80°C until the day of measuring enzymes. Enzyme assays were run within 8 months of sampling (see below for analytical details).

Climate impacts experiments

To explore the effects of temperature and pCO_2 levels on Pacific cod and Arctic cod, larvae of each species were incubated in separate full-factorial experiments consisting of three temperature treatments and two pCO_2 levels, comprising six experimental treatments in total (Table 1). Animal husbandry and control of treatment conditions for combined temperature and pCO_2 exposures are detailed in Slesinger *et al.* (2024) and Slesinger *et al.* (2025). Briefly, Pacific cod embryos were obtained from wild-caught animals collected northeast of Kodiak, AK, while Arctic cod embryos were obtained from the same broodstock as in the *Ontogeny Experiment*. For each species, cold, control, and warm temperature treatments were established based on previous experimental studies on Pacific cod (3, 6, 10°C; Laurel *et al.*, 2008) and Arctic cod (1.8, 5, 7.3°C; Koenker *et al.*, 2018b). Because of the differing thermal tolerances between the species, enzyme activities in the designated cold and warm treatments were compared to the species-specific control temperature. The samples for enzyme analysis were not available from the Pacific cod warm treatment due to high mortality at 10°C. Water temperatures for the respective treatments were maintained by mixing ambient and chilled water sources fed from the Yaquina Bay, OR. The pCO_2 levels were chosen to represent an ambient (∼350 μatm) and a high pCO_2 (∼1500 μatm) scenario (Table 1). The pCO_2 levels in the experiment were maintained by bubbling CO_2 gas into header tanks, which were automatically regulated by pH probes to maintain temperature-dependent targets. For each experimental treatment (6 total), there were four replicate tanks.

Larval sampling only occurred once for each *Climate Impacts* experiment and the sampling protocols were identical to those in the *Ontogeny Experiments*. Larvae were sampled for enzyme assays at a common size rather than a common age, because temperature strongly influences development and growth, and sampling at a common age could have obscured additional sensitivity to cold and warm temperature treatments (Catalán *et al.*, 2007; Kunzmann and Diemel, 2020). Sampling at a common age would have instead addressed ontogenetic costs associated with differences in development rate across temperature treatments and their potential interactions with elevated pCO_2—questions outside the scope of this study. Sample timing was determined from temperature-dependent growth curves for Pacific cod (Hurst *et al.*, 2010) and Arctic cod (Koenker *et al.*, 2018b), and Pacific and Arctic cod

Table 1: Experimental parameters for Pacific cod and Arctic cod in the climate impacts experiments

Species	Exp. dates	CO_2 level	Temp level	pH_{SWS}	pCO_2 (μatm)	Temp (°C)	Salinity (ppt)	DIC (mol kg^{-1})	TA (mol kg^{-1})
Pcod[a]	4 April–3 June 2022	Amb	3	8.12 ± 0.07	297.20 ± 65.51	3.03 ± 0.39	30.21 ± 1.11	1936.76 ± 76.24	2074.63 ± 65.73
			6	8.03 ± 0.11	394.28 ± 132.84	6.14 ± 0.29	29.84 ± 1.48	1943.38 ± 105.62	2063.08 ± 84.73
			10	8.00 ± 0.03	409.60 ± 36.75	10.22 ± 0.24	29.63 ± 0.67	1911.35 ± 22.43	2040.80 ± 21.72
		High	3	7.48 ± 0.09	1434.95 ± 304.70	3.10 ± 0.18	29.77 ± 1.30	2093.99 ± 76.91	2050.55 ± 68.72
			6	7.47 ± 0.03	1480.80 ± 126.37	6.04 ± 0.51	29.36 ± 1.31	2078.14 ± 81.47	2042.54 ± 84.58
			10	7.40 ± 0.06	1780.01 ± 245.48	10.20 ± 0.37	29.63 ± 0.67	2076.27 ± 24.75	2038.04 ± 22.15
Acod[b]	5 April–17 May 2023	Amb	1.8	8.11 ± 0.04	300.98 ± 36.75	1.67 ± 0.13	30.25 ± 1.11	1937.31 ± 58.04	2064.72 ± 58.71
			5	8.08 ± 0.04	328.66 ± 33.48	5.04 ± 0.14	30.25 ± 1.11	1932.92 ± 56.30	2068.61 ± 57.59
			7.3	8.06 ± 0.03	348.79 ± 26.04	7.3 ± 0.25	30.11 ± 1.11	1924.29 ± 55.89	2063.99 ± 60.81
		High	1.8	7.46 ± 0.04	1494.78 ± 125.05	1.68 ± 0.14	30.25 ± 1.11	2133.61 ± 58.13	2079.39 ± 60.00
			5	7.47 ± 0.05	1509.59 ± 157.36	5.02 ± 0.17	30.16 ± 1.13	2111.75 ± 55.94	2072.14 ± 59.83
			7.3	7.52 ± 0.15	1401.09 ± 323.08	7.32 ± 0.37	30.42 ± 1.11	2101.31 ± 59.82	2086.26 ± 56.29

Values are presented as mean ± SD. Pcod = Pacific cod (*Gadus macrocephalus*); Acod = Arctic cod (*Boreogadus saida*); DIC = dissolved inorganic carbon; TA = total alkalinity.
Slesinger et al. (2024)
Slesinger et al. (2025)

were sampled at 6.6 ± 0.1 and 9.6 ± 0.3 mm, respectively (Supplementary Table S1). Although larvae of each species were sampled at a common size, Arctic cod were sampled at a larger size than Pacific cod due to interspecific differences in hatch size, growth rates, and the pre-determined experimental parameters that larvae experience at least two weeks of exposure within their respective treatments. Because these size differences precluded a direct comparison of absolute enzyme activity levels between species, analyses focused on relative changes in enzyme activity compared to species-specific controls across the range of temperature and pCO_2 treatments.

Metabolic enzyme assays

For each experiment, we measured the activities of citrate synthase (CS), lactate dehydrogenase (LDH), and β-hydroxyacyl CoA dehydrogenase (HOAD). For each composite sample, frozen pooled larvae were re-weighed to obtain a final WW and then homogenized on ice using a 7-ml Tenbroeck tissue grinder in 10 mM Tris HCl buffer (pH 7.2). The buffer volume was adjusted to the WW for each sample to obtain a dilution of 20:1 for Pacific cod and 10:1 for Arctic cod. Differing dilution factors were based on the relative species-specific activity rates of the enzymes. The homogenates were centrifuged at 10 000× g at 4°C for 10 min in a refrigerated microcentrifuge (IEC Micromax RF, Thermo Electron Corporation), and the supernatant was used for both the enzyme assays and for assessing protein concentrations.

Assays for CS and LDH were slightly modified from procedures in Thuesen *et al.* (1998) and HOAD assay procedures were slightly modified from Singer and Ballantyne (1989). Maximum activities (V_{max}) of CS, LDH and HOAD were measured spectrophotometrically (Genesys 150 UV–Vis, ThermoFisher) at 20°C in 1-ml cuvettes under non-limiting conditions. Preliminary checks confirmed that the 20°C assay temperature had no effect on enzyme function. All assays were run in triplicate and the intra-assay variation (CV) was <10% for each enzyme. CS activity was assayed in 50 mM Imidazole buffer (pH 7.8), 1.5 mM $MgCl_2$, 0.1 mM DTNB and 0.1 mM acetyl-CoA. Once the homogenate was added, background activity was measured, and then the reaction was initiated by adding 25 μl oxaloacetic acid for a final concentration of 1 mM. Activity was measured for 5 min at 412 nm, using an extinction coefficient of 13.6 OD μmol^{-1} ml^{-1} to calculate activity rates. LDH and HOAD activities were measured at 340 nm for 2 min, and activity rates were calculated using an extinction coefficient of 6.22 OD μmol^{-1} ml^{-1}. LDH was assayed in 80 mM Imidazole buffer (pH 7.2), 100 mM KCl, 5 mM pyruvate and 0.15 mM NADH. HOAD was assayed in 50 mM Imidazole buffer (pH 7.4), 100 mM KCl, 0.15 mM NADH, and 0.1 mM acetoacetyl CoA. Protein concentrations (mg ml^{-1}) were determined spectrophotometrically (SpectraMax Plus 384, Molecular Devices) at 37°C using a BCA kit (ThermoFisher). Enzyme activities were expressed as units (U, μmol of substrate converted to product

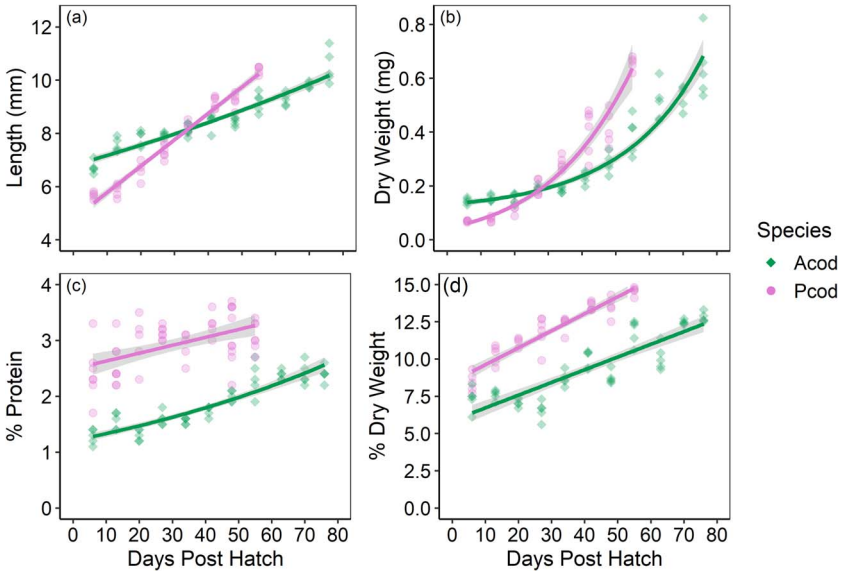

Figure 1: Growth metrics for Arctic cod (Acod: *B. saida*) and Pacific cod (Pcod: *G. macrocephalus*) throughout ontogeny. Modelled line and shaded area (Acod: green; Pcod: pink) represent the mean and confidence interval for each measurement with a linear model for length (**a**), % protein (**c**), and % dry weight (**d**), and a quadratic model for dry weight (**b**). Raw data are shown as points (Acod: diamonds; Pcod: circles). Sample size per sampling day is an *n* of 5 for Arctic cod length, dry weight, % protein, and % dry weight, 4–6 for Pacific cod for length, dry weight, % dry weight, and 5–10 for Pacific cod % protein.

per minute) and relative to protein concentration (U mg protein^{-1}) and body mass (U mg WW^{-1}). In addition, enzyme ratios LDH:CS and HOAD:CS were calculated to explore the relative contribution of anaerobic to aerobic metabolism and of fatty acid metabolism towards aerobic metabolism, respectively.

Data analysis

All analyses were conducted in R (Version 4.3.0, R Core Team, 2023). For the *Ontogeny Experiments*, the change in enzyme activities with age was modelled as a linear, quadratic, or cubic function based on the best-fit model determined through AICc scores (Burnham and Anderson, 2004). For the *Climate Impacts Experiments*, the effect of temperature, pCO_2, and their interaction was determined through Type-III ANOVA, and *post hoc* Tukey's honestly significant difference tests were used to identify pairwise differences. Effect sizes were determined from the ANOVA tests. Because experiments were run independently for each species, we did not statistically explore the difference in enzyme activities between the species and instead focused on comparing the differences in patterns among the species. Data residuals were tested for normality and homogeneity, and significance was taken at an alpha level of 0.05.

Ethics statement

This research was carried out in accordance with all applicable institutional and national guidelines. Pacific cod broodstock were collected under Alaska Department of Fish and Game Permit #CF-18-021 and Pacific cod embryos were collected under Alaska Department of Fish and Game Permit #P-22-005. Arctic cod adult broodstock were collected under Alaska Department of Fish and Game Permits #CF-12-088 and #CF-13-080. This research was conducted at NOAA's Alaska Fisheries Science Centre Laboratory in Newport, Oregon. NOAA National Marine Fisheries Service does not have an Institutional Animal Care and Use Committee (IACUC) approval process for research on fishes. All work followed American Fisheries Society policies on the Guidelines for Use of Fishes in Research (American Fisheries Society: https://fisheries.org/docs/policy_useoffishes.pdf) and the AVMA Guidelines on Euthanasia (American Veterinary Medical Association: https://olaw.nih.gov/sites/default/files/Euthanasia2007.pdf).

Results

Ontogeny experiments

At first sampling (6 DPH), Arctic cod were ~1 mm longer and twice as heavy as Pacific cod, but % protein (mg protein mg•WW^{-1}) was twice as high and % DW (mg DW•mg WW^{-1}) was 15% higher in Pacific cod than in Arctic cod (Fig. 1; Table 2). As the fish aged, Pacific cod grew almost twice as fast as Arctic cod larvae as seen in the length-based and mass-specific growth rates (Fig. 1; Table 2). Both species increased in % DW and % protein (Fig. 1) with age at similar rates but Pacific cod were higher in both metrics throughout their development. At the end of sampling for Pacific cod (55

Table 2: Parameter estimates for growth estimates across ontogeny for Arctic cod (*B. saida*) and Pacific cod (*G. macrocephalus*)

Species	Measure	Initial measure (6 DPH)	Δ (measure day^{-1})	Parameter estimates Measure = $y_o + a$DPH + bDPH^2			Model R^2
				y_o	a	b	
Arctic cod	Length (mm)[a]	6.72	0.050	0.833 ± 0.005	$0.002 \pm 1.1 \times 10^{-4}$		0.90
	Dry weight (mg)[a]	0.14	0.005	-0.878 ± 0.027	0.003 ± 0.002	$8.5 \times 10^{-5} \pm 1.8 \times 10^{-5}$	0.94
	% Protein[a]	1.28	0.021	0.081 ± 0.013	$0.004 \pm 2.7 \times 10^{-4}$		0.83
	% Dry weight	7.34	0.092	5.865 ± 0.306	0.085 ± 0.007		0.76
Pacific cod	Length (mm)	5.67	0.096	4.789 ± 0.104	0.099 ± 0.003		0.97
	Dry weight (mg)[a]	0.07	0.012	-1.363 ± 0.044	0.025 ± 0.003	$-6.8 \times 10^{-5} \pm 5.3 \times 10^{-5}$	0.95
	% Protein	2.41	0.014	2.488 ± 0.111	0.014 ± 0.003		0.28
	% Dry weight	8.44	0.125	8.475 ± 0.235	0.114 ± 0.007		0.88

Values are presented as mean ± SD from the parameter estimates from an n of 5 for Arctic cod length, dry weight, % protein, and % dry weight, 4–6 for Pacific cod for length, dry weight, % dry weight, and 5–10 for Pacific cod % protein. The amount of corresponding parameter estimates reflects the type of model fit (linear [a], quadratic [a,b]). Δ is the change in the specific measure from 6 to 55 DPH for both species. DPH = days post hatch. % Protein = mg protein mg WW^{-1}. % Dry weight = mg dry weight mg WW^{-1}. All model fits were significant ($P < 0.05$).
Data were $\log_{10}$ transformed and parameter estimates are on $\log_{10}$ scale.

DPH), they were longer, heavier, and higher in % protein and dry weight when compared to Arctic cod at 55 DPH.

Throughout ontogeny, Pacific cod had higher enzyme activity rates than Arctic cod when expressed as protein-specific (per mg protein) and weight-specific (per mg WW) rates, and the ontogenetic patterns in enzyme activities differed between the two species (Table 3, Fig. 2). Pacific cod CS and HOAD protein-specific enzyme activity decreased with age while protein-specific LDH activity increased with age. Arctic cod protein-specific enzyme activities all initially increased with age until ∼35 DPH when activity levels plateaued (CS, LDH) or the increase slowed (HOAD) (Fig. 2a,c,e). The weight-specific enzyme activities also differed between the two species (Fig. 2b,d,f) due to ontogenetic changes in protein concentrations. For Pacific cod, weight-specific CS and HOAD activities were constant throughout ontogeny while LDH activity increased (Table 3). For Arctic cod, weight-specific activity of each enzyme increased with age as a result of the plateau in protein-specific enzyme activity coinciding with an ontogenetic shift in higher protein concentration. For both species, the LDH:CS ratio increased with ontogeny (Table 3; Fig. 3). The HOAD:CS ratio did not change with age for Pacific cod but increased for Arctic cod (Table 3; Fig. 3).

Climate impacts experiments

Rearing temperature had a significant effect on protein-specific enzyme activities in Pacific cod (CS: $F_{1,29} = 18.961$, $P < 0.001$; LDH: $F_{1,29} = 6.813$, $P < 0.05$; HOAD: $F_{1,29} = 34.087$, $P < 0.001$; Table 4) where each protein-specific enzyme activity was higher at 3°C than at 6°C (Fig. 4). There was no effect of rearing temperature on Pacific cod weight-specific enzyme activities (CS: $F_{1,29} = 0.684$; LDH: $F_{1,29} = 2.702$; HOAD: $F_{1,29} = 0.043$; all $P > 0.05$; Table 4; Fig. 5), which was likely driven by the small increase in % protein at higher rearing temperatures (Supplementary Table S1). There was no significant effect of rearing temperature on Pacific cod LDH:CS ($F_{1,29} = 3.925$, $P > 0.05$; Table 4; Fig. 6) and HOAD:CS ($F_{1,29} = 1.156$, $P > 0.05$; Table 4; Fig. 6).

For Arctic cod, temperature significantly affected protein-specific CS and HOAD activities (CS: $F_{2,66} = 14.155$, $P < 0.001$; HOAD: $F_{2,66} = P < 0.05$; Table 4) primarily driven by lower CS activity and higher HOAD activity at 7.3°C than at 1.8 and 5°C (Fig. 4). Protein-specific LDH activity was not significantly affected by temperature ($F_{2,66} = 0.445$, $P > 0.05$; Table 4). Arctic cod % protein increased with temperature (Supplementary Table S1), which led to differing responses between protein- and weight-specific enzyme activities (a pattern similar to that in Pacific cod). Arctic cod weight-specific LDH and HOAD activities were significantly affected by temperature (LDH: $F_{2,66} = 7.604$, $P < 0.05$; HOAD: $F_{2,66} = 15.167$, $P < 0.001$; Table 4) with higher enzyme activities at 7.3°C than at 1.8 and 5°C (Fig. 5). Both the LDH:CS and HOAD:CS ratios were significantly affected by temperature (LDH:CS: $F_{2,66} = 10.978$; HOAD:CS: $F_{2,66} = 46.636$, all $P < 0.001$; Table 4) where both ratios were significantly higher at 7.3°C than at 1.8 and 5°C (Fig. 6).

Compared to the pervasive effect of temperature, the effect of pCO_2 was less pronounced as reflected in the effect sizes (Table 4) and was only significant at the control temperature for both species. In Pacific cod, only protein-specific HOAD activity was affected by elevated pCO_2

Table 3: Parameter estimates for the change in enzyme activities across ontogeny for Arctic cod (*B. saida*) and Pacific cod (*G. macrocephalus*)

Species	Units	Enzyme	△ (activity day^{-1})	Parameter estimates Measure = $y_o + a\text{DPH} + b\text{DPH}^2 + c\text{DPH}^3$				Model R^2
				y_o	a	b	c	
Arctic cod	U mg protein^{-1}	CS	0.005	0.62 ± 0.04	0.030 ± 0.005	$-6.6 \times 10^{-4} \pm 1.3 \times 10^{-4}$	$4.3 \times 10^{-6} \pm 1.0 \times 10^{-6}$	0.60
		LDH	0.047	4.07 ± 0.26	0.131 ± 0.015	$-0.001 \pm 1.7 \times 10^{-4}$		0.72
		HOAD	0.011	0.95 ± 0.09	0.042 ± 0.009	$-8.0 \times 10^{-4} \pm 2.5 \times 10^{-4}$	$5.3 \times 10^{-6} \pm 2.0 \times 10^{-6}$	0.74
	U mg WW^{-1}	CS	0.000	0.01 ± 0.00	$2.0 \times 10^{-4} \pm 1.1 \times 10^{-5}$			0.86
		LDH	0.002	0.06 ± 0.01	$6.5 \times 10^{-4} \pm 9.0 \times 10^{-4}$	$5.0 \times 10^{-5} \pm 2.5 \times 10^{-5}$	$-5.1 \times 10^{-7} \pm 2.0 \times 10^{-7}$	0.92
		HOAD	0.000	0.01 ± 0.00	$4.3 \times 10^{-4} \pm 1.7 \times 10^{-5}$			0.92
	Ratio	LDH:CS	0.018	5.62 ± 0.24	0.056 ± 0.014	$-4.1 \times 10^{-4} \pm 1.6 \times 10^{-4}$		0.52
		HOAD:CS	0.003	1.45 ± 0.03	0.006 ± 0.001			0.59
Pacific cod	U mg protein^{-1}	CS	−0.007	2.61 ± 0.04	-0.009 ± 0.001			0.53
		LDH	0.044	12.65 ± 0.17	0.039 ± 0.005			0.54
		HOAD	−0.003	2.40 ± 0.11	-0.008 ± 0.008	$-1.9 \times 10^{-5} \pm 1.2 \times 10^{-4}$		0.47
	U mg WW^{-1}	CS	0.000	0.06 ± 0.01	$9.5 \times 10^{-4} \pm 4.0 \times 10^{-4}$	$-1.4 \times 10^{-5} \pm 6.4 \times 10^{-6}$		0.10[a]
		LDH	0.003	0.31 ± 0.01	$0.003 \pm 4.1 \times 10^{-4}$			0.51
		HOAD	0.000	0.06 ± 0.00	$2.5 \times 10^{-5} \pm 9.0 \times 10^{-5}$			0.00[a]
	Ratio	LDH:CS	0.038	4.77 ± 0.10	0.039 ± 0.003			0.80
		HOAD:CS	0.002	0.89 ± 0.00	$6.0 \times 10^{-4} \pm 5.4 \times 10^{-4}$			0.03[a]

△ is the change in activity level from 6 to 55 DPH for both species. Values are presented as mean ± SD from the parameter estimates from an n of ∼five composite samples per DPH. Models were chosen based on AICc scores, and the amount of corresponding parameter estimates reflect the best fit model (linear [a], quadratic [a,b], cubic [a,b,c]). DPH = days post hatch. CS = citrate synthase. LDH = lactate dehydrogenase. HOAD = β-hydroxyacyl CoA dehydrogenase. WW = wet weight.

[a]Non-significant model fit ($P > 0.05$)

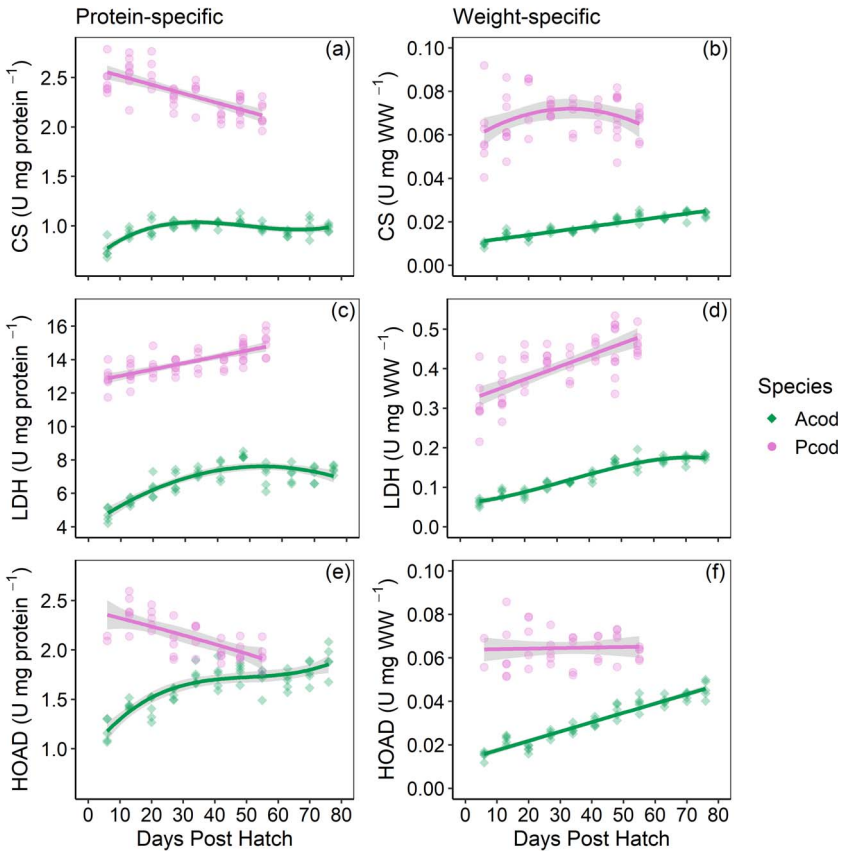

Figure 2: Change in protein (**a**,**c**,**e**) and wet-weight specific (**b**,**d**,**f**) enzyme activities throughout ontogeny for Arctic cod (Acod: *B. saida*) and Pacific cod (Pcod: *G. macrocephalus*). Modelled line and shaded area (Acod: green; Pcod: pink) represent the mean and confidence interval for the best fit model for each enzyme and species (parameters available in Table 3). Raw data are shown as points (Acod: diamonds; Pcod: circles). Sample size per sampling day is 5 for Arctic cod and 5–10 for Pacific cod. Enzyme assays were conducted at 20°C. CS = citrate synthase. LDH = lactate dehydrogenase. HOAD = β-hydroxyacyl CoA dehydrogenase. WW = wet weight.

($F_{1,29} = 4.592$, $P < 0.05$; Table 4), where activity levels were lower at high pCO_2 than at ambient pCO_2 at 6°C (Fig. 4). There was not a significant effect of elevated pCO_2 on the weight-specific enzyme activities (CS: $F_{1,29} = 0.001$; LDH: $F_{1,29} = 0.220$; HOAD: $F_{1,29} = 1.413$; all $P > 0.05$; Table 4) nor the LDH:CS ratio ($F_{1,29} = 2.134$, $P > 0.05$; Table 4). The HOAD:CS ratio was also not significantly affected by elevated pCO_2 ($F_{1,29} = 4.127$, $P = 0.051$; Table 4), but the *post hoc* test indicated a significant decrease in HOAD:CS ratio at high pCO_2 compared to ambient pCO_2 at 6°C (Fig. 6). There was no significant effect of elevated pCO_2 on Arctic cod protein-specific enzyme activities (CS: $F_{1,66} = 3.627$; LDH: $F_{1,66} = 2.11$; HOAD: $F_{1,66} = 0.745$; all $P > 0.05$; Table 4) and weight-specific enzyme activities (CS: $F_{1,66} = 0.262$; LDH: $F_{1,66} = 0.390$; HOAD: $F_{1,66} = 0.037$; all $P > 0.05$; Table 4). However, there was a significant temperature × pCO_2 interaction for protein-specific CS activity ($F_{2,66} = 3.452$; $P < 0.05$; Table 4) and weight-specific HOAD activity ($F_{1,66} = 3.365$; $P < 0.05$; Table 4). For protein- and weight-specific CS and HOAD activities, the *post hoc* tests indicated a significant increase in each enzyme activity under elevated pCO_2 when compared to ambient pCO_2 at 5°C (Figs 4 and 5). Both LDH:CS and HOAD:CS ratios were not significantly affected by elevated pCO_2 (LDH:CS: $F_{1,66} = 0.059$; HOAD:CS: $F_{1,66} = 0.181$; all $P > 0.05$; Table 4) or the temperature × pCO_2 interaction (LDH:CS: $F_{1,66} = 1.303$; HOAD:CS: $F_{1,66} = 0.058$; all $P > 0.05$; Table 4).

Discussion

We examined biochemical responses of two species throughout ontogeny and in response to environmental stressors to provide insight into competitive dynamics between species with increasing overlap of their larval ranges. High larval metabolic enzyme activities in Pacific cod supported their faster growth rates than the growth rates of Arctic cod. The changes in specific enzyme activities throughout ontogeny pointed to differences in energy metabolism pathways between the two species, such that Pacific cod larval metabolism appeared to be fuelled more by carbohydrates while Arctic cod larval metabolism was fuelled more by

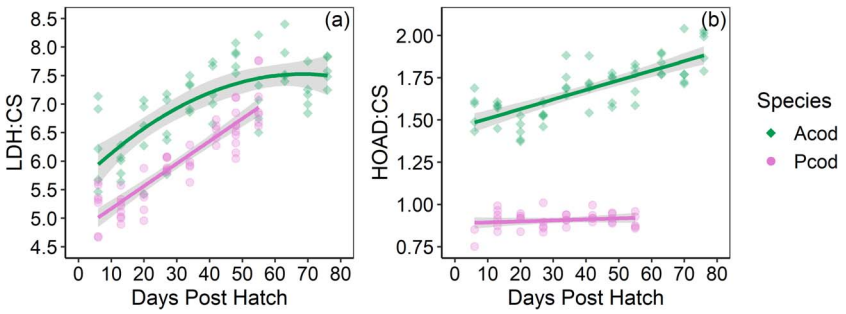

Figure 3: Change in enzyme ratios LDH:CS (**a**) and HOAD:CS (**b**) throughout ontogeny for Arctic cod (Acod: *B. saida*) and Pacific cod (Pcod: *G. macrocephalus*). Modelled line and shaded area (Acod: green; Pcod: pink) represent the mean and CI for the best fit model for each enzyme and species (parameters available in Table 3). Raw data are shown as points (Acod: diamonds; Pcod: circles). Sample size per sampling day is 5 for Arctic cod and 5–10 for Pacific cod. Enzyme ratios were calculated from protein-specific enzyme activities. Enzyme assays were conducted at 20°C. CS = citrate synthase. LDH = lactate dehydrogenase. HOAD = β-hydroxyacyl CoA dehydrogenase.

lipids. Pacific cod and Arctic cod also differed in their responses to rearing temperature and pCO_2 levels. Pacific cod biochemical responses were indicative of a species that can acclimate, but is not fully adapted, to cold temperature, in contrast with Arctic cod, which demonstrated cold-water adaptation. Arctic cod enzyme activities increased with warm temperatures, suggesting increased metabolism and energy demand. Both species exhibited only minor responses to elevated pCO_2, which primarily manifested in a change in HOAD activity. Interestingly, these effects only occurred at the control temperature for both species.

Influence of ontogeny on metabolic enzyme activities

Pacific cod larval enzymatic responses followed expectations based on previous studies focused on larval fish metabolic enzymes, where anaerobic capacity increased faster than aerobic capacity throughout early development (Clarke *et al.*, 1992; Desrosiers *et al.*, 2008; Kunzmann and Diemel, 2020). Increases in fish body size can lead to a decrease in CS activity through a decrease in mass-specific metabolic rates (Catalán *et al.*, 2007) and an increase in LDH activity predominantly through growth of white muscle tissue (Churova *et al.*, 2017). In addition, for active larvae, an increase in glycolytic capacity can support quick bursts of energy required for swimming (Somero and Childress, 1990; Hickey and Clements, 2003). While Pacific cod larval metabolic enzymes follow this pattern, it is intriguing that the same response was not seen in Arctic cod. This difference between the two species may represent differing swimming/activity strategies tailored to their native larval environments. Pacific cod eggs are demersal, but after hatch, the larvae rise to the surface where they are active in their pelagic environment (Mecklenburg *et al.*, 2002; Hurst *et al.*, 2009). Arctic cod eggs incubate underneath the sea ice and the larvae hatch in spring as the ice breaks up (Kohlbach *et al.*, 2017; Steiner *et al.*, 2019). Ice melt can stabilize the water column leading to less turbulence, where Arctic cod larvae may not rely on quick energy production. Recently, the Northern Bering Sea and Chukchi Sea ice extents have been dramatically reduced, leading to more open water days (Stabeno and Bell, 2019; Baker *et al.*, 2020), which could pose additional energetic constraints on Arctic cod and benefit Pacific cod, which may be adapted to higher energy environments.

While Arctic cod anaerobic capacity (LDH) remained higher than aerobic (CS, HOAD) in absolute values, the pattern of metabolic enzyme activities with age differed substantially from Pacific cod. Each Arctic cod metabolic enzyme activity increased after hatch before plateauing at 35 DPH (~8 mm). A period of unchanging enzyme activity during larval development has been observed in other fish (Hinterleitner *et al.*, 1987; Segner and Verreth, 1995). For Arctic charr (*Salvelinus alpinus*), the plateau in enzyme activity was suggested to be a precursor for another rapid period of growth and potentially related to the increasing activity levels of digestive enzymes (Lemieux *et al.*, 2003). In each of these examples, as well as in Arctic cod larvae, the plateau in enzyme activity occurred when enzyme activities were expressed relative to protein. When expressed relative to WW, Arctic cod enzyme activity did not plateau and instead increased with age, which appeared to be driven by the ontogenetic changes in protein concentration. This suggests that the proportions of enzymes in the total enzyme pool did not change but by increasing protein concentration, the *size* of the total enzyme pool continued to increase, raising the activity levels of enzymes within the entire larval body. These periods of developmental stasis, where the larval body grows yet the proportions of specific tissues remains constant, likely precede the next important developmental timepoint (Forstner *et al.*, 1983). Pairing more frequent sampling, biochemical responses, and histological data could help uncover important developmental changes in body composition in larval Arctic cod.

Another major distinction between Pacific cod and Arctic cod physiology was the differing fatty acid usage in the two species. As Pacific cod larvae grew, HOAD:CS remained

Table 4: Analysis of variance (Type III) of enzyme activities and enzyme ratios for Arctic cod (*B. saida*) and Pacific cod (*G. macrocephalus*)

			Temperature			CO_2			Temperature × CO_2		
Species	Units	Enzyme	*F*	*P*	Effect size	*F*	*P*	Effect size	*F*	*P*	Effect size
Arctic cod (*n* = 71)	U mg protein^{-1}	CS	14.155	<**0.001**	0.27	3.627	0.061	0.04	3.452	**0.038**	0.06
		LDH	0.445	0.643	0.00	2.111	0.151	0.02	0.040	0.961	0.00
		HOAD	7.554	**0.001**	0.15	0.745	0.391	0.00	2.030	0.139	0.03
	U mg WW^{-1}	CS	0.614	0.544	0.00	0.262	0.610	0.00	2.890	0.063	0.05
		LDH	7.604	**0.001**	0.15	0.390	0.534	0.00	1.352	0.266	0.01
		HOAD	15.167	<**0.001**	0.28	0.037	0.848	0.00	3.365	**0.041**	0.06
	Ratio	LDH:CS	10.978	<**0.001**	0.22	0.059	0.809	0.00	1.303	0.279	0.01
		HOAD:CS	46.636	<**0.001**	0.56	0.181	0.672	0.00	0.058	0.944	0.00
Pacific cod (*n* = 33)	U mg protein^{-1}	CS	18.961	<**0.001**	0.35	0.221	0.642	0.00	0.055	0.816	0.00
		LDH	6.813	**0.014**	0.15	0.191	0.665	0.00	0.074	0.787	0.00
		HOAD	34.087	<**0.001**	0.50	4.592	**0.041**	0.10	1.633	0.211	0.02
	U mg WW^{-1}	CS	0.684	0.415	0.00	0.001	0.980	0.00	0.370	0.548	0.00
		LDH	2.702	0.111	0.05	0.220	0.643	0.00	0.126	0.725	0.00
		HOAD	0.043	0.838	0.00	1.413	0.244	0.01	0.009	0.924	0.00
	Ratio	LDH:CS	3.925	0.057	0.08	2.134	0.155	0.03	0.720	0.403	0.00
		HOAD:CS	1.156	0.291	0.00	4.127	0.051	0.09	1.493	0.232	0.01

Values in **bold** indicate a significant difference ($P < 0.05$). CS = citrate synthase. LDH = lactate dehydrogenase. HOAD = β-hydroxyacyl CoA dehydrogenase. WW = wet weight

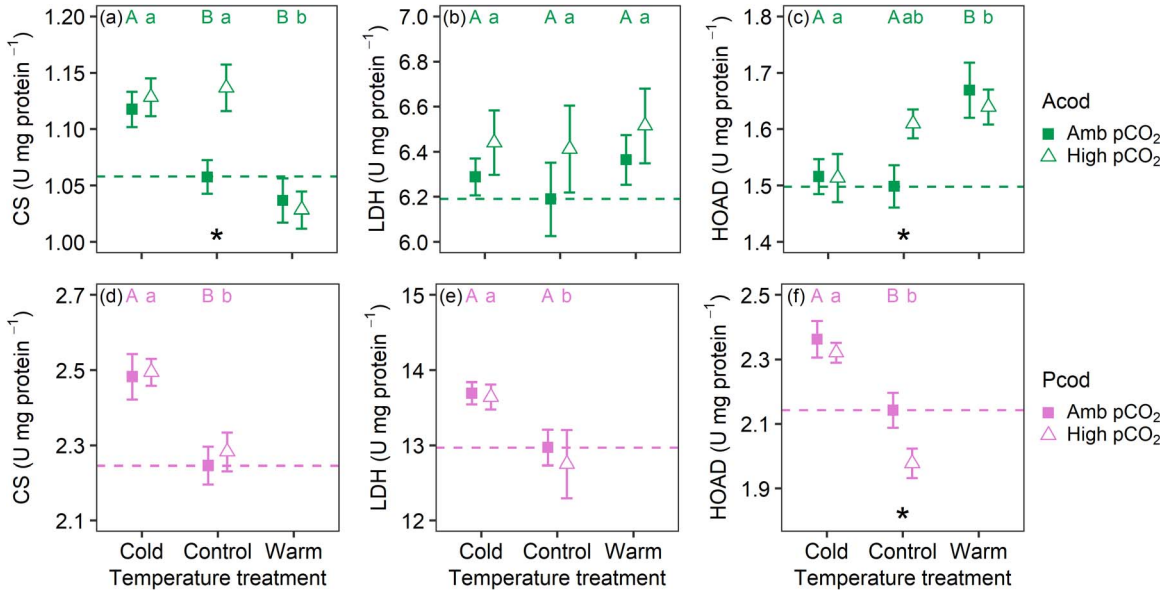

Figure 4: The effects of temperature and pCO_2 level on Arctic cod (Acod: *B. saida*; green; $n = 71$; top row) and Pacific cod (Pcod: *G. macrocephalus*; pink; $n = 33$; bottom row) protein-specific CS (**a** and **d**), LDH (**b** and **e**), and HOAD (**c** and **f**) activity. Values are presented as mean ± SE (high pCO_2 = open triangle; ambient pCO_2 = closed square). Temperature treatments were identified as cold (1.8°C = Arctic cod; 3°C Pacific cod), control (5°C = Arctic cod; 6°C = Pacific cod), and warm (7.3°C = Arctic cod; no data available for Pacific cod). Dashed line represents the mean of the control (ambient pCO_2 and control temperature), where values above and below indicate an upregulation or downregulation of that specific enzyme, respectively. Significant differences between temperature treatments within a specific pCO_2 treatment are depicted by different letters at the top (upper case: ambient pCO_2; lower case: high CO_2). The ∗ indicates a significant effect of pCO_2 at the specific temperature treatment. Full ANOVA results can be found in Table 4. Enzyme assays were conducted at 20°C. CS = citrate synthase. LDH = lactate dehydrogenase. HOAD = β-hydroxyacyl CoA dehydrogenase.

constant; in contrast, Arctic cod HOAD:CS increased and was substantially higher than in Pacific cod. This suggests that the fatty acid contribution towards aerobic metabolism is an important energy pathway for growing Arctic cod (Hochachka, 1994; Egginton *et al.*, 2000), while Pacific cod may rely more on carbohydrate metabolism (Tong *et al.*, 2017). A driver of this difference may relate to fundamental differences in lipid content between the two species. At hatch, Arctic cod have larger yolk sacs than Pacific cod (Laurel *et al.*, 2008, 2018), and throughout larval and juvenile stages, Arctic cod generally are higher in lipid content than Pacific cod (Copeman *et al.*, 2017; Koenker *et al.*, 2018a). Increased fatty acid metabolism in Arctic cod may be facilitated by a larger pool of fats as an energy source and/or a general propensity for fatty acid uptake (Egginton *et al.*, 2000). A metabolism fuelled largely by lipids may also explain the lower glycolytic capacity of Arctic cod when compared to Pacific cod (Crockett and Sidell, 1990).

Altogether, these data provide insight into which species may have competitive advantage in overlapping ranges. With respect to similar growing temperatures (5°C Arctic cod; 6°C Pacific cod), Pacific cod grow faster than Arctic cod, which could result in lower mortality risk from size-selective predators (Anderson, 1988). Based on metabolic enzyme activities, Pacific cod metabolic strategy may be more carbohydrate-fuelled and dependent on anaerobic metabolism than Arctic cod, which appear to rely on aerobic metabolism and lipids as an important fuel source. Glucose initiates glycolysis, which produces pyruvate that can either be used in anaerobic metabolism or oxidized into acetyl-CoA to enter the citric acid cycle. Lipids (as fatty acids) are fuel substrate for aerobic metabolism as beta oxidation bypasses glycolysis, incorporating fatty acids into the citric acid cycle after oxidation into acetyl-CoA. A difference between these two strategies is the relative speed at which glucose and lipids can be used as it relates to the speed of glycolysis versus oxidative phosphorylation. Pacific cod may be able to generate ATP rapidly, but at the cost of using up available energy sources quickly. As such, under similar growing temperatures, prey availability may determine which species has competitive advantage. High food scenarios could support the fast growth and high metabolic enzyme activities in Pacific cod. However, under low food scenarios, Arctic cod may have competitive advantage, with slower growth, lower enzyme activity levels, and larger lipid energy stores. In a previous study, the competitive advantage of Arctic cod larvae over walleye pollock (*Gadus chalcogrammus*), a congener of Pacific cod, was apparent at ≤5°C with Arctic cod surviving longer in the absence of food (Laurel *et al.*, 2018). Follow-up studies

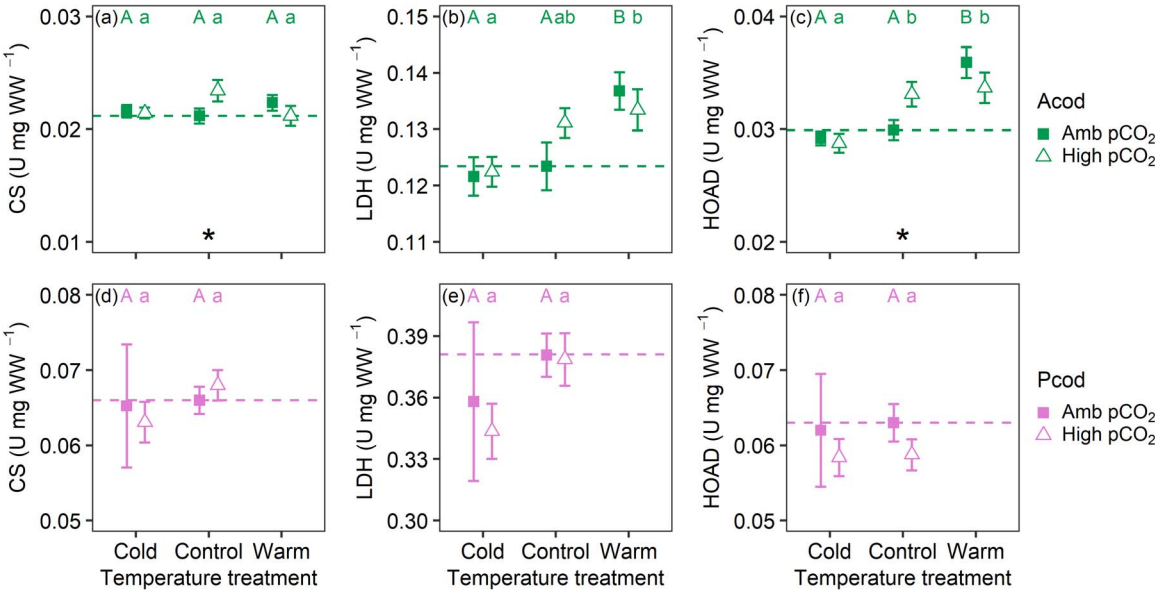

Figure 5: The effects of temperature and pCO_2 level on Arctic cod (Acod: *B. saida*; green; $n = 71$; top row) and Pacific cod (Pcod: *G. macrocephalus*; pink; $n = 33$; bottom row) weight-specific CS (**a**, **d**), LDH (**b**, **e**), and HOAD (**c**, **f**) activity. Values are presented as mean ± SE (high pCO_2 = open triangle; ambient pCO_2 = closed square). Temperature treatments were identified as cold (1.8°C = Arctic cod; 3°C Pacific cod), control (5°C = Arctic cod; 6°C = Pacific cod), and warm (7.3°C = Arctic cod; no data available for Pacific cod). Dashed line represents the mean of the control (ambient pCO_2 and control temperature), where values above and below indicate an upregulation or downregulation of that specific enzyme, respectively. Significant differences between temperature treatments within a specific pCO_2 treatment are depicted by different letters at the top (upper case: ambient pCO_2; lower case: high CO_2). The ∗ indicates a significant effect of pCO_2 at the specific temperature treatment. Full ANOVA results can be found in Table 4. Enzyme assays were conducted at 20°C. CS = citrate synthase. LDH = lactate dehydrogenase. HOAD = β-hydroxyacyl CoA dehydrogenase. WW = wet-weight.

could focus on directly testing the hypothesis that Pacific cod have competitive growth advantage over Arctic cod at similar incubation temperatures and high prey densities.

Biochemical responses to temperature and pCO_2

In previous studies, Pacific cod and Arctic cod grew faster at warmer temperatures and mortality was highest under ocean warming conditions (Laurel *et al.*, 2008; Koenker *et al.*, 2018b). The effects of elevated pCO_2 were inconsistent among larval traits and across temperature treatments but for both species, sensitivity to ocean acidification was most apparent at the optimal (control) temperature for growth (Slesinger *et al.*, 2024, 2025). These results were seen in organismal responses to combined environmental stressors, but a comparison of how the finer physiological processes responded to temperature and pCO_2 has not been explored.

A potential limitation in the degree to which Pacific cod larvae can survive in the same habitat as Arctic cod is based on cold water tolerance. Pacific cod protein-specific enzyme activities all increased at the coldest temperature (3°C), while Arctic cod did not exhibit positive temperature compensation at their coldest temperature (1.8°C) in LDH and HOAD expressed relative to protein. A positive temperature compensation in enzyme activity at cold temperatures has been suggested as evidence for cold acclimation (Guderley, 1990; Lucassen *et al.*, 2003; McClelland *et al.*, 2006), but not cold-adaptation (Thibault *et al.*, 1997; Pörtner *et al.*, 2000), because increases in enzyme concentrations can counteract slowing enzyme function in temperatures that are too cold (Overnell and Batty, 2000). However, this argument for metabolic cold adaptation remains debated. Some studies have used positive temperature compensation at cold temperatures as evidence for metabolic cold adaptation when comparing polar and temperate species (Crockett and Sidell, 1990; Kawall *et al.*, 2002; Dymowska *et al.*, 2012). In these studies, the comparison in enzyme activity was measured across the same acclimation temperatures between two species of similar life histories. In our study, Pacific cod enzyme activity levels were always elevated relative to those measured in Arctic cod. This trend was apparent throughout development (*Ontogeny Experiments*), and it may likely reflect differences in activity levels and life histories between the two species (see above; DeVries and Eastman, 1981). As such, we suggest Pacific cod have the capacity to acclimate, but are not fully adapted, to cold temperatures, and future studies would benefit by identifying if there is a trade-off associated with acclimation to colder temperatures.

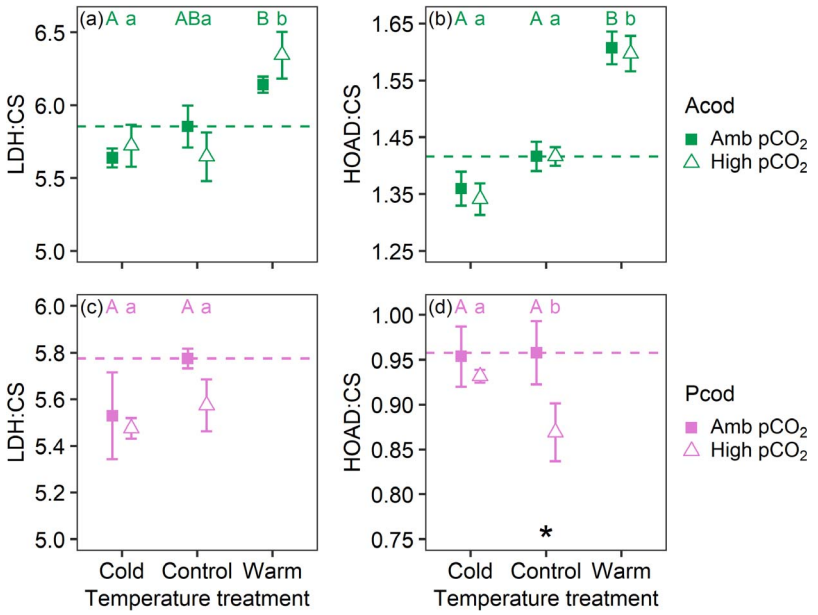

Figure 6: The effects of temperature and pCO_2 level on Arctic cod (Acod: *B. saida*; green; $n = 71$; top row) and Pacific cod (Pcod: *G. macrocephalus*; pink; $n = 33$; bottom row) LDH:CS ratio (**a** and **c**) and HOAD:CS ratio (**b** and **d**). Values are presented as mean ± SE (high pCO_2 = open triangle; ambient pCO_2 = closed square). Temperature treatments were identified as cold (1.8°C = Arctic cod; 3°C Pacific cod), control (5°C = Arctic cod; 6°C = Pacific cod), and warm (7.3°C = Arctic cod; no data available for Pacific cod). Dashed line represents the mean of the control (ambient pCO_2 and control temperature), where values above and below indicate an upregulation or downregulation of that specific enzyme, respectively. Significant differences between temperature treatments within a specific pCO_2 treatment are depicted by different letters at the top (upper case: ambient pCO_2; lower case: high CO_2). The ∗ indicates a significant effect of pCO_2 at the specific temperature treatment. Enzyme ratios were calculated from protein-specific enzyme activities. Full ANOVA results can be found in Table 4. Enzyme assays were conducted at 20°C. CS = citrate synthase. LDH = lactate dehydrogenase. HOAD = β-hydroxyacyl CoA dehydrogenase.

For both species, these experiments included incubation at a warm rearing temperature to represent future ocean warming, but an enzymatic comparison was unavailable due to high mortality rates in Pacific cod larvae (Slesinger *et al.*, 2024). Arctic cod larvae also had higher mortality rates at the warm temperature treatment (Slesinger *et al.*, 2025), but were abundant enough for enzyme analysis. Enzyme activities in warm-reared Arctic cod indicate higher fatty acid oxidation to fuel aerobic metabolism (HOAD, HOAD:CS) and greater contribution from anaerobic metabolism (LDH:CS) (Table 4; Figs 5 and 6). Under ocean warming conditions, larval medaka (*Oryzias latipes*) enzyme activities were upregulated, potentially to support elevated energy demand (Li *et al.*, 2015), while for some marine tropical fishes (*Acanthochromis polyacanthus*, *Amphiprion melanopus*, *Lates calcarifer*), CS and LDH activities remained constant (Illing *et al.*, 2020). For larval Arctic cod, there was less evidence that elevated energy demands were met through increased LDH activities, as the increased LDH:CS ratio was largely driven by the decrease in CS. However, Arctic cod did demonstrate substantial increases in protein- and weight-specific HOAD activities indicating elevated fatty acid usage to fuel aerobic metabolism. Other species, including gilthead seabream (*Sparus aratus*) and rainbow trout (*Oncorhynchus mykiss*), have also exhibited higher rates of beta oxidation under high-temperature stress (Cordiner and Egginton, 1997; Feidantsis *et al.*, 2020). While higher fatty acid oxidation may help meet energy demands, increased lipid metabolism can result in detrimental effects of exposure to higher concentrations of reactive oxygen species (Abele and Puntarulo, 2004). However, if Arctic cod are predisposed to naturally higher rates of fatty acid metabolism, they may already have existing antioxidant strategies to cope with increased reactive oxygen species. Future assessments of gene expression could help elucidate additional physiological pathways and processes utilized by Arctic cod to meet their increased energy demands at warm temperatures, as has been done for Pacific cod larvae in response to ocean warming (Spencer *et al.*, 2025).

Ocean acidification can disrupt the acid–base balance in fish (Burggren and Bautista, 2019), which can lead to a multitude of downstream physiological responses. Pacific cod and Arctic cod larvae demonstrated some sensitivity to elevated pCO_2 levels, but interestingly, only at the control temperature. The control temperatures for experiments were set to the optimal growing temperature for each species based on previous studies (Hurst *et al.*, 2010; Koenker *et al.*, 2018b). These were also the only temperatures where negative effects of elevated pCO_2 on whole-animal condition indices were observed (Slesinger *et al.*, 2024, 2025). However, in this study, the type

of physiological response to elevated pCO_2 at the control temperature differed between the two species, which may provide insight into differing biochemical sensitivities and responses to ocean acidification (Esbaugh, 2018). For Pacific cod exposed to elevated pCO_2 levels, fatty acid metabolism (HOAD, HOAD:CS) was reduced. In Hurst *et al.* (2019), Pacific cod larvae exposed to ocean acidification conditions exhibited signs of lipid dysregulation, and other gadids have shown similar lipid sensitivities to ocean acidification (Frommel *et al.*, 2011; Hurst *et al.*, 2021; Spencer *et al.*, 2025). Lipid dysregulation in Pacific cod larvae may lead to fewer fatty acids available as fuel substrate, which would limit fatty acid oxidation as an energy pathway (Egginton *et al.*, 2000). For Arctic cod, there was significant upregulation of CS and HOAD under elevated pCO_2 conditions, but because both were elevated, there was no change in the HOAD:CS ratio. We suggest that elevated pCO_2 may have led to an increase in metabolic demand, and an upregulation of enzymes important for maintaining ATP production (as was seen under ocean warming). An increase in aerobic enzyme activities to match higher energy demand under elevated pCO_2 has also been demonstrated in Antarctic nototheniids (Strobel *et al.*, 2013; Feidantsis *et al.*, 2015). Collectively, the different responses in the fatty acid metabolism between Pacific cod and Arctic cod may be fundamentally driven by their different physiology throughout development as demonstrated in the *Ontogeny Experiments*.

While Pacific cod and Arctic cod enzyme activities differed under high pCO_2 exposure, they were similar in that HOAD activity was the most sensitive. These results differ from other studies focused on the effects of ocean acidification on biochemical responses of marine fish. Under elevated pCO_2 levels, sand smelt (*Atherina presbyter*) larval LDH activity was upregulated indicating an increase in anaerobic metabolism to match elevated energy demands (Silva *et al.*, 2016). For gilthead seabream, an elevation of anaerobic metabolism under ocean acidification conditions was identified in adult fish tissues (Michaelidis *et al.*, 2006; Ruiz-Jarabo *et al.*, 2021) and in whole-animal homogenates of larvae (Pimentel *et al.*, 2020). However, these fish inhabit warmer waters than the fish in our study. Other cold-water species, such as the Antarctic fishes *Pagothenia borhgrevinki* and *Trematomus newnesi*, did not exhibit elevated LDH activity under ocean acidification conditions (Enzor *et al.*, 2017). This limited evidence suggests that the physiological mechanisms of regulating acid–base balance under ocean acidification may be sensitive to a species' thermal environment.

Conclusions

Metabolic enzyme activities were used to assess changes in energy metabolism in two gadid species throughout ontogeny and when exposed to environmental stressors. Throughout ontogeny, Pacific cod larval metabolic enzyme activity levels and trends differed from those measured in Arctic cod, which was likely driven by the differing activity levels of the larval fish species. This has important implications in the wild where under slight warming to optimal growing temperatures (5–6°C), Pacific cod may have competitive advantage over Arctic cod if prey is abundant. When exposed to differing temperatures and pCO_2 levels, the response of metabolic enzyme activities also differed between the two cod species. Pacific cod were less adapted to cold temperature than Arctic cod, which suggests Pacific cod may continue to be constrained in their northern habitat by cold temperatures. Under warming, Arctic cod upregulated enzymes associated with aerobic metabolism which suggests an increase to match higher energy demands (high Pacific cod mortality at high temperatures precluded a similar comparison). Finally, both cod species' fatty acid metabolism was affected by elevated pCO_2, but the response differed such that Pacific cod demonstrated potential signs of lipid dysregulation, while Arctic cod showed signs of elevated metabolic demand. This suggests the effects of ocean acidification, while still acting on the fatty acid metabolism, could lead to differing outcomes for the two species. Overall, in a controlled laboratory setting, exploring the change in metabolic enzyme activities in the two larval cod species provides insight into assessing the potential effect of large-scale environmental change in the Pacific–Arctic interface and increased frequency of the co-occurrence of both species.

Acknowledgements

We would like to thank Mary Beth Rew Hicks, Paul Iseri, Mara Spencer, Jessica Andrade, Samantha Mundorff and Carlissa Salant for assistance with animal husbandry; Bjorn Illing, Brad Seibel and Erica Ombres for helpful discussions on enzyme assays; and Joseph Dietrich for equipment time and support. We also are grateful to Brad Seibel and Laura Spencer for providing feedback on a previous version of the manuscript, as well as two anonymous reviewers for their constructive edits. Reference to trade names does not imply endorsement by the National Marine Fisheries Service. The findings and conclusions in this paper are those of the authors and do not necessarily represent the views of the National Marine Fisheries Service.

Author Contributions

E.S. and T.P.H. conceptualized and planned the research; E.S. generated the data with guidance from E.V.T.; E.S. analyzed the data and wrote the initial manuscript; and all authors edited and reviewed the final manuscript.

Conflict of Interest

None declared

Funding

This project was funded by an NOAA's Ocean Acidification Program Grant #20903 to T.P.H.

Data availability

Data are available upon request.

Supplementary material

Supplementary Material is available at *Conservation Physiology* online.

References

Abele D, Puntarulo S (2004) Formation of reactive species and induction of antioxidant defence systems in polar and temperate marine invertebrates and fish. *Comp Biochem Physiol A Mol Integr Physiol* 138: 405–415. https://doi.org/10.1016/j.cbpb.2004.05.013.

Anderson JT (1988) A review of size dependent survival during prerecruit stages of fishes in relation to recruitment. *J Northwest Atl Fish Sci* 8: 55–66. https://doi.org/10.2960/J.v8.a6.

Axler KE, Goldstein ED, Nielsen JM, Deary AL, Duffy-Anderson JT (2023) Shifts in the composition and distribution of Pacific Arctic larval fish assemblages in response to rapid ecosystem change. *Glob Chang Biol* 29: 4212–4233. https://doi.org/10.1111/gcb.16721.

Baker MR, Kivva KK, Pisareva MN, Watson JT, Selivanova J (2020) Shifts in the physical environment in the Pacific Arctic and implications for ecological timing and conditions. *Deep Sea Res* 177: 104802. https://doi.org/10.1016/j.dsr2.2020.104802.

Baumann H, Jones LF, Murray CS, Siedlecki SA, Alexander M, Cross EL (2022) Impaired hatching exacerbates the high CO_2 sensitivity of embryonic sand lance *Ammodytes dubius*. *Mar Ecol Prog Ser* 687: 147–162. https://doi.org/10.3354/meps14010.

Brijs J, Sandblom E, Sundh H, Grans A, Hinchcliffe J, Ekstrom A, Sundell K, Olsson C, Axelsson M, Pichaud N (2017) Increased mitochondrial coupling and anaerobic capacity minimizes aerobic costs of trout in the sea. *Sci Rep* 7: 45778. https://doi.org/10.1038/srep45778.

Burggren W, Bautista N (2019) Invited review: development of acid-base regulation in vertebrates. *Comp Biochem Physiol A Mol Integr Physiol* 236: 110518. https://doi.org/10.1016/j.cbpa.2019.06.018.

Burnham K, Anderson D (2004) Multimodel inference: understanding AIC and BIC in modelselection. *Sociol Method Res* 33: 261–304. https://doi.org/10.1177/0049124104268644.

Caldeira K, Wickett ME (2005) Ocean model predictions of chemistry changes from carbon dioxide emissions to the atmosphere and ocean. *J Geophys Res Oceans* 110: C09S04. https://doi.org/10.1029/2004JC002671.

Catalán IA, Berdalet E, Olivar MP, Roldán C (2007) Response of muscle-based biochemical condition indices to short-term variations in food availability in post-flexion reared sea bass *Dicentrarchus labrax* (L.) larvae. *J Fish Biol* 70: 391–405. https://doi.org/10.1111/j.1095-8649.2007.01307.x.

Churova MV, Meshcheryakova OV, Veselov AE, Efremov DA, Nemova NN (2017) Activity of metabolic enzymes and muscle-specific gene expression in parr and smolts Atlantic salmon *Salmo salar* L. of different age groups. *Fish Physiol Biochem* 43: 1117–1130. https://doi.org/10.1007/s10695-017-0357-0.

Clarke ME, Calvi C, Domeier M, Edmonds M, Walsh PJ (1992) Effects of nutrition and temperature on metabolic enzyme activities in larval and juvenile red drum, *Sciaenops ocellatus*, and lane snapper, *Lutjanus synagris*. *Mar Biol* 112: 31–36. https://doi.org/10.1007/BF00349724.

Cooper DW, Cieciel K, Copeman LA, Emelin PO, Logerwell E, Ferm N, Lamb J, Levine R, Axler K, Woodgate RA *et al.* (2023) Pacific cod or tikhookeanskaya treska (*Gadus macrocephalus*) in the Chukchi Sea during recent warm years: distribution by life stage and age-0 diet and condition. *Deep Sea Res II* 208: 105241. https://doi.org/10.1016/j.dsr2.2022.105241.

Copeman LA, Laurel BJ, Spencer M, Sremba A (2017) Temperature impacts on lipid allocation among juvenile gadid species at the Pacific Arctic-boreal interface: an experimental laboratory approach. *Mar Ecol Prog Ser* 566: 183–198. https://doi.org/10.3354/meps12040.

Cordiner S, Egginton S (1997) Effects of seasonal temperature acclimatization on muscle metabolism in rainbow trout, *Oncorhyncus mykiss*. *Fish Physiol Biochem* 16: 333–343. https://doi.org/10.1023/A:1007732003452.

Correa GM, Hurst TP, Stockhausen WT, Ciannelli L, Kristiansen T, Pilcher DJ (2024) Modeling the larval growth and survival of Pacific cod (*Gadus macrocephalus*) in the eastern Bering Sea. *Prog Oceanogr* 225: 103282. https://doi.org/10.1016/j.pocean.2024.103282.

Crockett EL, Sidell BD (1990) Some pathways of energy metabolism are cold adapted in Antarctic fishes. *Physiol Zool* 63: 472–488. https://doi.org/10.1086/physzool.63.3.30156223.

Danielson SL, Ahkinga O, Ashjian C, Basyuk E, Cooper LW, Eisner L, Farley E, Iken KB, Grebmeier JM, Juranek L *et al.* (2020) Manifestation and consequences of warming and altered heat fluxes over the Bering and Chukchi Sea continental shelves. *Deep-Sea Res II Top Stud Oceanogr* 177: 104781. https://doi.org/10.1016/j.dsr2.2020.104781.

Desrosiers V, Le Francois NR, Tveiten H, Andreassen I, Blier PU (2008) Ontogenesis of catabolic and energy metabolism capacities during the embryonic development of spotted wolffish (*Anarhichas minor*). *Comp Biochem Physiol B Biochem Mol Biol* 150: 200–206. https://doi.org/10.1016/j.cbpb.2008.03.001.

DeVries AL, Eastman JT (1981) Physiology and ecology of notothenioid fishes of the Ross Sea. *J Roy Soc New Zealand* 11: 329–340. https://doi.org/10.1080/03036758.1981.10423324.

Dymowska AK, Manfredi R, Rosenthal JJC, Seibel BA (2012) Temperature compensation of aerobic capacity and performance in the Antarc-

tic pteropod, *Clione antarctica*, compared with its northern congener. *C limacina J Exp Biol* 215: 3370–3378. https://doi.org/10.1242/jeb.070607.

Egginton S, Cordiner S, Skilbeck C (2000) Thermal compensation of peripheral oxygen transport in skeletal muscle of seasonally acclimatized trout. *Am J Physiol Integr Comp Physiol* 279: R375–R388. https://doi.org/10.1152/ajpregu.2000.279.2.R375.

Eisner LB, Zuenko YI, Basyuk EO, Britt LL, Duffy-Anderson JT, Kotwicki S, Ladd C, Cheng W (2020) Environmental impacts on walleye Pollock (*Gadus chalcogrammus*) distribution across the Bering Sea shelf. *Deep Sea Res II* 181-182: 104881. https://doi.org/10.1016/j.dsr2.2020.104881.

Enzor LA, Hunter EM, Place SP (2017) The effects of elevated temperature and ocean acidification on the metabolic pathways of notothenioid fish. *Conserv Physiol* 5: cox019. https://doi.org/10.1093/conphys/cox019.

Eriksen E, Ingvaldsen RB, Nedreaas K, Prozorkevich D (2015) The effect of recent warming on polar cod and beaked redfish juveniles in the Barents Sea. *Reg Stud Mar Sci* 2: 105–112. https://doi.org/10.1016/j.rsma.2015.09.001.

Esbaugh AJ (2018) Physiological implications of ocean acidification for marine fish: emerging patterns and new insights. *J Comp Physiol B* 188: 1–13. https://doi.org/10.1007/s00360-017-1105-6.

Fabry VJ, McClintock JB, Mathis JT, Grebmeier JM (2009) Ocean acidification at high latitudes: the bellweather. *Oceanogr* 22: 160–171. https://doi.org/10.5670/oceanog.2009.105.

Farley EV, Heintz RA, Andrews AG, Hurst TP (2016) Size, diet, and condition of age-0 Pacific cod (*Gadus macrocephalus*) during warm and cool climate states in the eastern Bering Sea. *Deep-Sea Res II Top Stud Oceanogr* 134: 247–254. https://doi.org/10.1016/j.dsr2.2014.12.011.

Feidantsis K, Pörtner HO, Antonopoulou E, Michaelidis B (2015) Synergistic effects of acute warming and low pH on cellular stress responses of the gilthead seabream *Sparus aurata*. *J Comp Physiol B* 185: 185–205. https://doi.org/10.1007/s00360-014-0875-3.

Feidantsis K, Pörtner HO, Giantsis IA, Michaelidis B (2020) Advances in understanding the impacts of global warming on marine fishes farmed offshore: *Sparus aurata* as a case study. *J Fish Biol* 98: 1509–1523. https://doi.org/10.1111/jfb.14611.

Fissel B, Dalton M, Garber-Yonts G, Haynie A, Kasperski S, Lee J, Lew D, Lavoie A, Seung C, Sparks K, *et al.* (2017) Stockfish assessment and fishery evaluation report for the groundfish fisheries of the Gulf of Alaska and Bering Sea/Aleutian Islands Area: Economic Status of the Groundfish Fisheries off Alaska, 2017. North Pacific Fishery Management Council, 1007 West 3rd Ave., Suite 400 L92 Building, 4th floor, Anchorage, Alaska 99501.

Forstner H, Hinterleitner S, Mahr K, Wieser W (1983) Towards a better definition of "metamorphosis" in *Coregonus* sp.: biochemical, histological, and physiological data. *Can J Fish Aquat Sci* 40: 1224–1232. https://doi.org/10.1139/f83-139.

Fossheim M, Primicerio R, Johannesen E, Ingvaldsen RB, Aschan MM, Dolgov AV (2015) Recent warming leads to a rapid borealization of fish communities in the Arctic. *Nat Clim Change* 5: 673–677. https://doi.org/10.1038/nclimate2647.

Frey KE, Moore GWK, Cooper LW, Grebmeier JM (2015) Divergent patterns of recent sea ice cover across the Bering, Chukchi, and Beaufort seas of the Pacific Arctic region. *Prog Oceanogr* 136: 32–49. https://doi.org/10.1016/j.pocean.2015.05.009.

Frommel AY, Maneja R, Lowe D, Malzahn AM, Geffen AJ, Folkvord A, Piatkowski U, Reusch TBH, Clemmesen C (2011) Severe tissue damage in Atlantic cod larvae under increasing ocean acidification. *Nat Clim Change* 2: 42–46. https://doi.org/10.1038/nclimate1324.

Geoffroy M, Bouchard C, Flores H, Robert D, Gjøsæter H, Hoover C, Hop H, Hussey NE, Nahrgang J, Steiner N *et al.* (2023) The circumpolar impacts of climate change and anthropogenic stressors on Arctic cod (*Boreogadus saida*) and its ecosystem. *Elem Sci Anth* 11: 1. https://doi.org/10.1525/elementa.2022.00097.

Gobler CJ, Merlo LR, Morrell BK, Griffith AW (2018) Temperature, acidification, and food supply interact to negatively affect the growth and survival of the forage fish, *Menidia beryllina* (inland silverside), and *Cyprinodon variegatus* (Sheepshead minnow). *Front Mar Sci* 5: 86. https://doi.org/10.3389/fmars.2018.00086.

Goolish EM (1991) Aerobic and anaerobic scaling in fish. *Biol Rev* 66: 33–56. https://doi.org/10.1111/j.1469-185X.1991.tb01134.x.

Guderley H (1990) Functional significance of metabolic responses to thermal acclimation in fish muscle. *Am J Physiol Integr Comp Physiol* 259: R245–R252. https://doi.org/10.1152/ajpregu.1990.259.2.R245.

Guderley H, Blier P (1988) Thermal acclimation in fish: conservative and labile properties of swimming muscle. *Can J Zool* 66: 1105–1115. https://doi.org/10.1139/z88-162.

Hermann AJ, Gibson GA, Bond NA, Curchitser EN, Hedstrom K, Cheng W, Wang M, Cokelet ED, Stabeno PJ, Aydin K (2016) Projected future biophysical states of the Bering Sea. *Deep-Sea Res II Top Stud Oceanogr* 134: 30–47. https://doi.org/10.1016/j.dsr2.2015.11.001.

Hickey AJ, Clements KD (2003) Key metabolic enzymes and muscle structure in triplefin fishes (Tripterygiidae): a phylogenetic comparison. *J Comp Physiol B* 173: 113–123. https://doi.org/10.1007/s00360-002-0313-9.

Hinterleitner S, Platzer U, Wieser W (1987) Development of the activities of oxidative, glycolytic and muscle enzymes during early larval life in three families of freshwater fish. *J Fish Biol* 30: 315–326. https://doi.org/10.1111/j.1095-8649.1987.tb05755.x.

Hochachka PW (1994) *Muscles as molecular and metabolic machines*. CRC, Boca Raton

Hochachka PW, Stanley C, Merkt J, Sumar-Kalinowski J (1982) Metabolic meaning of elevated levels of oxidative enzymes in high altitude adapted animals: an interpretive hypothesis. *Respir Physiol* 52: 303–313. https://doi.org/10.1016/0034-5687(83)90087-7.

Huntington HP, Danielson SL, Wiese FK, Baker M, Boveng P, Citta JJ, De Robertis A, Dickson DMS, Farley E, George JC *et al.* (2020) Evidence suggests potential transformation of the Pacific Arctic ecosystem

is underway. *Nat Clim Change* 10: 342–348. https://doi.org/10.1038/s41558-020-0695-2.

Hurst TP, Cooper DW, Scheingross JS, Seale EM, Laurel BJ, Spencer ML (2009) Effects of ontogeny, temperature, and light on vertical movements of larval Pacific cod (*Gadus macrocephalus*). *Fish Oceanogr* 18: 301–311. https://doi.org/10.1111/j.1365-2419.2009.00512.x.

Hurst TP, Copeman LA, Andrade JF, Stowell MA, Al-Samarrie CE, Sanders JL, Kent ML (2021) Expanding evaluation of ocean acidification responses in a marine gadid: elevated CO_2 impacts development, but not size of larval walleye Pollock. *Mar Biol* 168: 119. https://doi.org/10.1007/s00227-021-03924-w.

Hurst TP, Copeman LA, Haines SA, Meredith SD, Daniels K, Hubbard KM (2019) Elevated CO_2 alters behavior, growth, and lipid composition of Pacific cod larvae. *Mar Environ Res* 145: 52–65. https://doi.org/10.1016/j.marenvres.2019.02.004.

Hurst TP, Fernandez ER, Mathis JT, Miller JA, Stinson CM, Ahgeak EF (2012) Resiliency of juvenile walleye Pollock to projected levels of ocean acidification. *Aquat Biol* 17: 247–259. https://doi.org/10.3354/ab00483.

Hurst TP, Laurel BJ, Ciannelli L (2010) Ontogenetic patterns and temperature-dependent growth rates in early life stages of Pacific cod (*Gadus macrocephalus*). *Fish Bull* 108: 382–392.

Illing B, Downie AT, Beghin M, Rummer JL (2020) Critical thermal maxima of early life stages of three tropical fishes: effects of rearing temperature and experimental heating rate. *J Therm Biol* 90: 102582. https://doi.org/10.1016/j.jtherbio.2020.102582.

Kawall HG, Torres JJ, Sidell BD, Somero GN (2002) Metabolic cold adaptation in Antarctic fishes: evidence from enzymatic activities of brain. *Mar Biol* 140: 279–286. https://doi.org/10.1007/s002270100695.

Koenker BL, Copeman LA, Laurel BJ, Robert D (2018a) Impacts of temperature and food availability on the condition of larval Arctic cod (*Boreogadus saida*) and walleye Pollock (*Gadus chalcogrammus*). *ICES J Mar Sci* 75: 2370–2385. https://doi.org/10.1093/icesjms/fsy052.

Koenker BL, Laurel BJ, Copeman LA, Ciannelli L, Robert D (2018b) Effects of temperature and food availability on the survival and growth of larval Arctic cod (*Boreogadus saida*) and walleye Pollock (*Gadus chalcogrammus*). *ICES J Mar Sci* 75: 2386–2402. https://doi.org/10.1093/icesjms/fsy062.

Kohlbach D, Schaafsma FL, Graeve M, Lebreton B, Lange BA, David C, Vortkamp M, Flores H (2017) Strong linkage of polar cod (*Boreogadus saida*) to sea ice algae-produced carbon: evidence from stomach content, fatty acid and stable isotope analyses. *Prog Oceanogr* 152: 62–74. https://doi.org/10.1016/j.pocean.2017.02.003.

Kono Y, Sasaki H, Kurihara Y, Fujiwara A, Yamamoto J, Sakurai Y (2016) Distribution pattern of polar cod (*Boreogadus saida*) larvae and larval fish assemblages in relation to oceanographic parameters in the northern Bering Sea and Chukchi Sea. *Polar Biol* 39: 1039–1048. https://doi.org/10.1007/s00300-016-1961-7.

Kunzmann A, Diemel VC (2020) The effect of spatial position and age within an egg-clutch on embryonic development and key metabolic enzymes in two clownfish species, *Amphiprion ocellaris* and *Amphiprion frenatus*. *PloS One* 15: e0226600. https://doi.org/10.1371/journal.pone.0226600.

Laurel BJ, Copeman LA, 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: 2403–2412. https://doi.org/10.1093/icesjms/fsy042.

Laurel BJ, Hurst TP, Copeman LA, Davis MW (2008) The role of temperature on the growth and survival of early and late hatching Pacific cod larvae (*Gadus macrocephalus*). *J Plankton Res* 30: 1051–1060. https://doi.org/10.1093/plankt/fbn057.

Lemieux H, Le Francois NR, Blier PU (2003) The early ontogeny of digestive and metabolic enzyme activities in two commercial strains of arctic charr (*Salvelinus alpinus* L.). *J Exp Zool A Comp Exp Biol* 299A: 151–160. https://doi.org/10.1002/jez.a.10298.

Li AJ, Leung PT, Bao VW, Lui GC, Leung KM (2015) Temperature-dependent physiological and biochemical responses of the marine medaka *Oryzias melastigma* with consideration of both low and high thermal extremes. *J Therm Biol* 54: 98–105. https://doi.org/10.1016/j.jtherbio.2014.09.011.

Logerwell EA, Busby M, Mier KL, Tabisola H, Duffy-Anderson J (2020) The effect of oceanographic variability on the distribution of larval fishes of the northern Bering and Chukchi seas. *Deep-Sea Res II Top Stud Oceanogr* 177: 104784. https://doi.org/10.1016/j.dsr2.2020.104784.

Long WC, Conrad AL, Gardner JL, Foy RJ (2024) Red king crab larval survival and development are resilient to ocean acidification. *J Exp Mar Biol Ecol* 577: 152028. https://doi.org/10.1016/j.jembe.2024.152028.

Lucassen M, Schmidt A, Eckerle LG, Pörtner HO (2003) Mitochondrial proliferation in the permanent vs temporary cold: enzyme activities and mRNA levels in Antarctic and temperate zoarcid fish. *Am J Physiol: Integr Comp Physiol* 285: R1410–R1420. https://doi.org/10.1152/ajpregu.00111.2003.

Mathis JT, Cross JN, Bates NR (2011) The role of ocean acidification in systemic carbonate mineral suppression in the Bering Sea. *Geophys Res Lett* 38: L19602. https://doi.org/10.1029/2011GL048884.

McClelland GB, Craig PM, Dhekney K, Dipardo S (2006) Temperature- and exercise-induced gene expression and metabolic enzyme changes in skeletal muscle of adult zebrafish (*Danio rerio*). *J Physiol* 577: 739–751. https://doi.org/10.1113/jphysiol.2006.119032.

Mecklenburg C, Mecklenburg T, Thorsteinson L (2002) *Fishes of Alaska*. American Fisheries Society, Bethesda, MD

Michaelidis B, Spring A, Pörtner HO (2006) Effects of long-term acclimation to environmental hypercapnia on extracellular acid–base status and metabolic capacity in Mediterranean fish *Sparus aurata*. *Mar Biol* 150: 1417–1429. https://doi.org/10.1007/s00227-006-0436-8.

Michaud J, Fortier L, Rowe P, Ramseier R (1996) Feeding success and survivorship of Arctic cod larvae, *Boreogadus saida*, in the northwest water polynya (Greenland Sea). *Fish Oceanogr* 5: 120–135. https://doi.org/10.1111/j.1365-2419.1996.tb00111.x.

Mortenson E, Steiner N, Monahan AH, Hayashida H, Sou T, Shao A (2020) Modeled impacts of sea ice exchange processes on Arctic Ocean carbon uptake and acidification (1980–2015). *J Geophysical Research: Oceans* 125:e2019JC015782.

Mueter FJ, Planque B, Hunt GLJ, Alabia ID, Hirawake T, Eisner L, Dalpadado P, Chierici M, Drinkwater KF, Harada N *et al.* (2021) Possible future scenarios in the gateways to the Arctic for subarctic and Arctic marine systems: II. Prey resources, food webs, fish and fisheries. *ICES J Mar Sci* 78: 3017–3045. https://doi.org/10.1093/icesjms/fsab122.

Nathanailides C (1996) Metabolic specialization of muscle during development in cold-water and warmwater fish species exposed to different thermal conditions. *Can J Fish Aquat Sci* 53: 2147–2155. https://doi.org/10.1139/f96-136.

Overland JE, Wang M (2025) Future climate change in the northern Bering Sea. *Internat J Clim* 45: e8697. https://doi.org/10.1002/joc.8697.

Overnell J, Batty RS (2000) Scaling of enzyme activity in larval herring and plaice: effects of temperature and individual growth rate on aerobic and anaerobic capacity. *J Fish Biol* 56: 577–589. https://doi.org/10.1111/j.1095-8649.2000.tb00756.x.

Perry DM, Redman DH, Widman JC Jr, Meseck S, King A, Pereira JJ (2015) Effect of ocean acidification on growth and otolith condition of juvenile scup, *Stenotomus chrysops*. *Ecol Evol* 5: 4187–4196. https://doi.org/10.1002/ece3.1678.

Pimentel MS, Faleiro F, Machado J, Pousao-Ferreira P, Rosa R (2020) Seabream larval physiology under ocean warming and ocean acidification. *Fishes* 5: 5010001.

Pörtner HO, van Dijk PLM, Hardewig I, Sommer A (2000) Levels of metabolic cold adaption: tradeoffs in eurythermal and stenothermal ectotherms. In W Davison, CS Williams, eds, *Antarctic ecosystems: models for wider ecological understanding*. Caxton Press, Christchurch, pp. 109–122.

R Core Team (2023) *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria (https://www.R-project.org./).

Rand KM, Logerwell EA (2011) The first demersal trawl survey of benthic fish and invertebrates in the Beaufort Sea since the late 1970s. *Polar Biol* 34: 475–488. https://doi.org/10.1007/s00300-010-0900-2.

Renaud PE, Berge J, Varpe Ø, Lønne OJ, Nahrgang J, Ottesen C, Hallanger I (2012) Is the poleward expansion by Atlantic cod and haddock threatening native polar cod, *Boreogadus saida*? *Polar Biol* 35: 401–412. https://doi.org/10.1007/s00300-011-1085-z.

Ruiz-Jarabo I, Gregorio SF, Alves A, Mancera JM, Fuentes J (2021) Ocean acidification compromises energy management in *Sparus aurata* (Pisces: teleostei). *Comp Biochem Physiol Part A: Mol Integrat Physiol* 256: 110911. https://doi.org/10.1016/j.cbpa.2021.110911.

Sala-Rabanal M, Sanchez J, Ibarz A, Fernandez-Borras J, Blasco J, Gallardo MA (2003) Effects of low temperatures and fasting on hematology and plasma composition of gilthead sea bream (*Sparus aurata*). *Fish Phys Biochem* 29: 105–115. https://doi.org/10.1023/B:FISH.0000035904.16686.b6.

Schleger IC, Pereira DMC, Resende AC, Romao S, Herrerias T, Neundorf AKA, Sloty AM, Guimaraes IM, Pedreiro de Souza MRD, Carster GP *et al.* (2021) Cold and warm waters: energy metabolism and antioxidant defenses of the freshwater fish *Astyanax lacustris* (Characiformes: Characidae) under thermal stress. *J Comp Physiol B* 192: 1–18.

Schnurr ME, Yin Y, Scott GR (2014) Temperature during embryonic development has persistent effects on metabolic enzymes in the muscle of zebrafish. *J Exp Biol* 217: 1370–1380. https://doi.org/10.1242/jeb.094037.

Schwieterman GD, Crear DP, Anderson BN, Lavoie DR, Sulikowski JA, Bushnell PG, Brill RW (2019) Combined effects of acute temperature change and elevated pCO_2 on the metabolic rates and hypoxia tolerance of clearnose skate (*Rostaraja eglanteria*), summer flounder (*Paralichthys dentatus*), and thorny skate (*Amblyraja radiata*). *Biol* 8: 56. https://doi.org/10.3390/biology8030056.

Segner H, Verreth J (1995) Metabolic enzyme activities in larvae of the African catfish, *Clarias gariepinus*: changes in relation to age and nutrition. *Fish Physiol Biochem* 14: 385–398. https://doi.org/10.1007/BF00003376.

Silva CS, Novais SC, Lemos MF, Mendes S, Oliveira AP, Goncalves EJ, Faria AM (2016) Effects of ocean acidification on the swimming ability, development and biochemical responses of sand smelt larvae. *Sci Total Environ* 563-564: 89–98. https://doi.org/10.1016/j.scitotenv.2016.04.091.

Singer TD, Ballantyne JS (1989) Absence of extraheptic lipid oxidation in a freshwater elasmobranch, the dwarf stingray *Potamotrygon magdalenae*: evidence from enzyme activities. *J Exp Zool* 251: 355–360. https://doi.org/10.1002/jez.1402510312.

Slesinger E, Copeman LA, Laurel BJ, Hicks MBR, Iseri PJ, Stowell MA, Hurst TP (2025) Larval Arctic cod (*Boreogadus saida*) exhibit stronger devleopmental and physiological respones to temperatre than to elevated pCO_2. *J Fish Biol* 1–15. https://doi.org/10.1111/jfb.70082.

Slesinger E, Mundorff S, Laurel BJ, Hurst TP (2024) The combined effects of ocean warming and ocean acidification on Pacific cod (*Gadus macrocephalus*) early life stages. *Mar Biol* 171: 121. https://doi.org/10.1007/s00227-024-04439-w.

Somero GN, Childress JJ (1990) Scaling of ATP-supplying enzymes, myofibrillar proteins and buffering capacity in fish muscle: relationship to locomotory habit. *J Exp Biol* 149: 319–333. https://doi.org/10.1242/jeb.149.1.319.

Spencer LH, Slesinger E, Spies I, Laurel BJ, Hurst TP (2025) Molecular indicators of warming and other climate stressors in larval Pacific cod, *Canadian J Fish Aquat Sci.* https://doi.org/10.1139/cjfas-2024-0264.

Spies I, Gruenthal KM, Drinan DP, Hollowed AB, Stevenson DE, Tarpey CM, Hauser L (2020) Genetic evidence of northward range expansion in the eastern Bering Sea stock of Pacific cod. *Evol Appl* 13: 362–375. https://doi.org/10.1111/eva.12874.

Sswat M, Stiasny MH, Jutfelt F, Riebesell U, Clemmesen C (2018) Growth performance and survival of larval Atlantic herring, under the combined effects of elevated temperatures and CO_2. *PloS One* 13: e0191947. https://doi.org/10.1371/journal.pone.0191947.

Stabeno PJ, Bell SW (2019) Extreme conditions in the Bering Sea (2017-2018): record-breaking low sea-ice extent. *Geophys Res Let* 46: 8952–8959. https://doi.org/10.1029/2019GL083816.

Steiner NS, Cheung WWL, Cisneros-Montemayor AM, Drost H, Hayashida H, Hoover C, Lam J, Sou T, Sumaila UR, Suprenand P *et al.* (2019) Impacts of the changing ocean-sea ice system on the key forage fish Arctic cod (*Boreogadus saida*) and subsistence fisheries in the Western Canadian Arctic—evaluating linked climate, ecosystem and economic (CEE) models. *Front Mar Sci* 6: 179. https://doi.org/10.3389/fmars.2019.00179.

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

Strobel A, Leo E, Pörtner HO, Mark FC (2013) Elevated temperature and pCO_2 shift metabolic pathways in differentially oxidative tissues of *Notothenia rossii*. *Comp Biochem Physiol B Biochem Mol Biol* 166: 48–57. https://doi.org/10.1016/j.cbpb.2013.06.006.

Thibault M, Blier PU, Guderley H (1997) Seasonal variation of muscle metabolic organization in rainbow trout (*Oncorhynchus mykiss*). *Fish Physiol Biochem* 16: 139–155. https://doi.org/10.1007/BF00004671.

Thuesen EV, Miller CB, Childress JJ (1998) Ecophysiological interpretation of oxygen consumption rates and enzymatic activities of deep-sea copepods. *Mar Ecol Prog Ser* 168: 95–107. https://doi.org/10.3354/meps168095.

Tong X, Yang L, Tang X, Yang X, Bao C, Wang J, Zhou Y, Tang M (2017) Patterns of catabolic capacities and energy metabolism in developing embryos and yolk-sac larvae of turbot (*Scophthalmus maximus* L.). *Aquac* 481: 177–183. https://doi.org/10.1016/j.aquaculture.2017.09.004.

Wieser W, Fortner H (1986) Effects of temperature and size on the routine rate of oxygen consumption on the relative scope for activity in larval cyprinids. *J Comp Physiol* 156: 791–796. https://doi.org/10.1007/BF00694252.

Woodgate RA, Peralta-Ferriz C (2021) Warming and freshening of the Pacific inflow to the Arctic from 1990-2019 implying dramatic shoaling in Pacific winter water ventilation of the Arctic water column. *Geophys Res Let* 48: e2021GL092528. https://doi.org/10.1029/2021GL092528.