Received: 19 July 2024 | Revised: 6 December 2024 | Accepted: 30 April 2025

DOI: 10.1111/jfb.70082

REGULAR ARTICLE

JOURNAL OF FISH BIOLOGY fsbi

Larval Arctic cod (*Boreogadus saida*) exhibit stronger developmental and physiological responses to temperature than to elevated pCO_2

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Funding information
Ocean Acidification Program, Grant/Award Number: 20903

Abstract

High-latitude ecosystems are simultaneously warming and acidifying under ongoing climate change. Arctic cod (*Boreogadus saida*) are a key species in the Arctic Ocean and have demonstrated sensitivity to ocean warming and acidification as adults and embryos, but their larval sensitivity to the combined stressors is unknown. In a laboratory multi-stressor experiment, larval Arctic cod were exposed to a combination of three temperatures (1.8, 5 and 7.3°C) and two carbon dioxide (pCO_2) levels (ambient: 330 μatm, high: 1470 μatm) from hatching to 6-weeks of growth. Mortality rates were highest at 7.3°C (5% day^{-1}); however, both growth and morphometric-based condition were also highest at this temperature. When these metrics were assessed via a mortality: growth (M:G) ratio, 5°C appeared to be an optimal temperature for net population biomass, as faster growth at 7.3°C did not fully compensate for higher mortality. In contrast, although morphometric-based condition was lowest at 1.8°C, lipid-based condition was highest, which may reflect prioritization of lipid storage at cold temperatures. The capacity of larval Arctic cod to acclimate to a range of temperatures was exhibited by two lipid-based indicators of membrane fluidity, including a ratio of unsaturated to saturated fatty acids and a ratio of polar lipids to sterols. The effects of elevated pCO_2 were subtle, as well as temperature- and metric dependent. When exposed to elevated pCO_2 levels, Arctic cod at 1.8°C exhibited signs of lipid dysregulation, suggesting potential interference with membrane acclimation; larvae at 5°C were in lower morphometric-based condition; and larvae at 7.3°C had higher activity eicosanoid substrates, indicating possible physiological stress. Overall, Arctic cod physiological response to temperature variation was more pronounced than their response to elevated pCO_2. Future projections of pCO_2 effects on Arctic cod health in a warming ecosystem will need to consider the complexity of temperature-dependence and the specificity of multiple physiological responses.

KEYWORDS
Arctic cod, *Boreogadus saida*, early life history, lipid composition, ocean acidification, ocean warming

1 | INTRODUCTION

Arctic cod (*Boreogadus saida*) are a circumpolar gadid (Geoffroy et al., 2023; Logerwell et al., 2015; Matarese et al., 1989) and are considered a key species, accounting for a high proportion of energy transfer from lower trophic levels to large marine predators, including whales, seals, larger fish species and seabirds (Hop & Gjøsæter, 2013; Steiner et al., 2019). Within their circumpolar habitat, climate change is leading to rapid alterations in the physical and chemical properties of the ocean. Arctic ocean warming (OW) has been measurable for decades, but since the 2010s this process has accelerated and now results in persistent above-average (+ 2°C) summer and fall temperatures (i.e., Chukchi Sea; Danielson et al., 2020; Huntington et al., 2020). Future OW has the potential to raise Arctic Ocean temperatures >10°C under continuous increases in anthropogenic CO_2 [Shared Socioeconomic Pathway (SSP)5–8.5; Cai et al., 2021]. Ocean acidification (OA), the increase in partial pressure of carbon dioxide (pCO_2) and decrease in pH, is also occurring in the Arctic (Mortenson et al., 2020; Pilcher et al., 2022), partially driven by cold temperatures, sea ice loss and the intrusion of fresh water from sea ice melt (Mathis et al., 2011; Qi et al., 2022). Future OA may lead to increases in pCO_2 levels in the Arctic ocean above 1400 μatm under SSP5–8.5, an almost 1000 μatm increase from present-day levels (Terhaar et al., 2021). Understanding how these combined stressors affect Arctic cod is imperative for projecting future changes in the Arctic ecosystem (Geoffroy et al., 2023).

Arctic cod are well adapted to their high-latitude environment and are associated with sea ice throughout their early life (Geoffroy et al., 2023). During the late winter, Arctic cod spawn under ice (Ponomarenko, 1968) and historically the eggs hatch after ~60 days of development (−1.8°C; Laurel et al., 2018). The timing of hatch is associated with spring ice break-up and formation of polynyas, as well as the onset of phytoplankton blooms (Graham & Hop, 1995; Logerwell et al., 2020). As Arctic cod age, young fish (age 1–2) also associate with pack ice (David et al., 2016) and form an important component of the sympagic food web (Kohlbach et al., 2017). Adults are semi-demersal and eventually move onto the shelf away from sea ice during the summer but will return to the ice for spawning (Geoffroy et al., 2023). As such, the earliest life stages (eggs, larvae) of Arctic cod have stronger association with sea ice and exhibit unique adaptations to their high-latitude environment when compared to more sub-Arctic gadids. For example, in a comparative larval growth study, Arctic cod were more resilient to starvation at water temperatures below 5°C than walleye pollock (*Gadus chalcogrammus*) (Koenker, Copeman, et al., 2018a). Therefore, Arctic cod larvae may be adapted to survive variable spring production following ice break-up. Young Arctic cod also overwinter in the Arctic and prepare by storing energy in the form of lipids (Copeman, Stowell, et al., 2022b; Nahrgang et al., 2010). As such, Arctic cod tend to be more lipid-rich than associated gadids that do not overwinter in the Arctic, including walleye pollock, Pacific cod (*Gadus macrocephalus*) and saffron cod (*Eleginus gracilis*) (Copeman et al., 2017; Copeman, Salant, et al., 2022a).

Early life stages of fish tend to be more sensitive to environmental stressors than juvenile or adult stages due to their small body sizes, limited energy reserves and developing physiological capacities (Esbaugh, 2018; Melzner et al., 2009). Arctic cod adults can acclimate to warmer temperatures (>9°C) in the laboratory as seen in measures of heart rates (temperatures for maximum heartrate and arrhythmic heartbeat; Drost et al., 2014; Drost, Fisher, et al., 2016a; Drost, Lo, et al., 2016b), yet field studies have revealed negative effects of warming on lipid storage (Copeman, Salant, et al., 2022a). The early life stages of Arctic cod are more thermally sensitive than the adults (Geoffroy et al., 2023). Optimal temperature for development and growth of Arctic cod shifts from <3.5°C at the egg stage (Laurel et al., 2018) to ~5°C at the larval stage (Koenker, Copeman, et al., 2018a; Koenker, Laurel, et al., 2018b). Compared to other co-occurring gadids, Arctic cod are more sensitive to high-temperature stress but have competitive growth and survival advantage at sub-zero temperatures (Laurel et al., 2016; Laurel et al., 2018). As such, under OW, Arctic cod may be less competitive with co-occurring fishes and become less capable of predator evasion (Marsh & Mueter, 2020).

The effects of temperature can increase the sensitivity of Arctic cod to OA at certain life stages. The response of adult and juvenile Arctic cod to combined OW and OA has been variable, with some studies reporting temperature-dependent sensitivity to OA (Kunz et al., 2016; Kunz et al., 2018; Schmidt et al., 2017), whereas other studies show no impact of OA across all experimentally tested temperatures (Leo et al., 2017; Leo et al., 2020). There is less information on how OW and OA affect the earlier life stages (eggs, larvae) of Arctic cod, with just one published study showing that embryonic metabolic rates exhibited temperature-dependent responses to OA (Dahlke et al., 2018). The direct effects of OA and the combined impacts of OW and OA in a multi-stressor framework are unknown for larval Arctic cod.

Controlled laboratory experiments can be used to investigate how fish respond to multiple stressors. Experimental measurements on the study organism can range from higher-level whole-animal metrics to finer-scale physiological responses, which together can provide an integrative assessment on the effects of OW and OA. At the higher-level, mortality and growth rates provide a broad organismal response and can be used to track changes in biomass within a population (Suthers et al., 2021). Condition factors can be used as a proxy for the energetic status of the animal (Amara et al., 2007). At a finer-scale, lipid biochemistry can help inform how the physiology of the animal is regulated or changes in response to OW and OA (Ericson et al., 2019). The major lipid classes in marine fish include triacylglycerols (TAG), sterols (ST) and polar lipids (PL). Both ST and PL are incorporated in cell membranes, whereas TAG is an energy storage lipid class (Parrish et al., 2000). Fatty acids (FAs) are present in multiple acyl lipid classes and can be broadly grouped by the number of carbons in the chain and the number and position of their double bonds (Parrish, 2013). These groupings include saturated FAs (SFAs) with no double bonds, monounsaturated fatty acids (MUFAs) with a single double bond and polyunsaturated fatty acids (PUFAs)

comprising two or more double bonds. Lipid-based condition metrics, such as the TAG:ST ratio, assess the balance between energy storage (TAG) to structural membrane lipids (ST) (Fraser, 1989). Different lipid ratios, including a lipid class-based (PL:ST) and fatty acid-based [(MUFA + PUFA):SFA] ratio, can indicate membrane fluidity in the tissue. Following the homeoviscous elasticity hypothesis (Sinensky, 1974) of thermal acclimation, both of these ratios are expected to decrease with increasing temperature (Ericson et al., 2019). Specific PUFAs, 20:4n-6 [arachidonic acid (AA)], 20:5n-3 [eicosapentaenoic acid (EPA)] and their ratio (AA:EPA), can inform changes in immune function (Calder, 2010; Ericson et al., 2019), where higher relative proportions of AA and/or AA:EPA ratio may indicate increased physiological stress.

The goal of this study was to examine the combined effects of OW and OA on larval Arctic cod development and physiology over a 6-week period after hatch. Arctic cod larvae were reared in a cross of three temperatures (1.8, 5 and 7.3°C) and two pCO_2 levels (ambient: ~330 µatm; high: ~1470 µatm). These values were chosen to represent current and future Arctic Ocean temperatures (Cai et al., 2021) and pCO_2 levels (Jiang et al., 2023; Terhaar et al., 2021) by 2100. We measured whole-animal responses (mortality, growth and condition), as well as a series of lipid and FA endpoints, reflecting potential changes in energy, stress and cellular structure. Based on previous studies of Arctic cod and related species, we expected that the effects of OA would be dependent on thermal conditions.

2 | MATERIALS AND METHODS

2.1 | Experimental animals and husbandry

In 2012 and 2013, juvenile Arctic cod were collected from the Beaufort Sea (Prudhoe Bay, AK) and maintained as broodstock within the National Marine Fisheries Service's Alaska Fisheries Science Center laboratory in Newport, Oregon. Adults were fed twice weekly with chopped capelin and a laboratory-formulated diet consisting of herring, squid, krill, commercial fish pellets, amino acids and vitamin supplements. Rearing conditions mimicked seasonal temperatures (2–5°C) and photoperiods (9–16 h of daylight) that fish would experience in the wild. The pH seasonally varied and was ~8 during the prespawning and spawning periods leading up to egg collection. In February 2023, the laboratory-reared F1-generation adults were spawned to provide larvae for the experiment. The eggs of each female ($n = 8$) were fertilized with the milt of three randomly collected males (total $n = 9$) from the same rearing tank. Fertilized embryos were reared in static 38-L tanks maintained at 2°C and a salinity of 33 that were refreshed with daily water changes of ~30% total volume. At the completion of the hatch cycle, hatched larvae were transferred to the flow-through experimental system (see below) at a density of ~50 larvae L^{-1}. The experimental tanks were 40-L conical upwelling tanks with a flow rate of 800 mL min^{-1} and airstones for mixing. Larvae were fed a diet of live rotifers (*Branchionus plicatilis*) and green water (RotiGreen *Nanno*; Instant Algae, Reed Mariculture) at a frequency of thrice daily and a density of 5000 rotifers L^{-1}. Photoperiod was maintained at a 12:12-h schedule. Temperature and pH were controlled throughout the experiment (see *Experimental System*), whereas salinity and dissolved oxygen were monitored. Larvae were incubated in their respective temperature and pCO_2 treatments for 6 weeks and sampled throughout this time period. At each sampling time point either for use in morphometric measurements or lipid analyses, larvae were anaesthetized with tricaine methanesulfonate (MS-222) at a concentration of 5–10 ppm, including sodium bicarbonate as a buffer.

2.2 | Experimental system

Larvae were exposed to one of six treatments representing a full cross of three temperatures (1.8, 5, 7.3°C) and two pCO_2 levels (ambient: ~330 µatm; high: ~1470 µatm). Treatment water was made in six header tanks that each provided water to four replicate experimental tanks (24 experimental tanks in total). Temperature treatment targets were achieved by mixing two water sources, an ambient and a chilled water line, both sourced from Yaquina Bay. A Durafet III pH probe (Honeywell) in each of the high pCO_2 treatment header tanks regulated the injection of CO_2 based on achieving temperature-dependent pH targets (see Slesinger et al., 2024). Each header tank was aerated to maintain mixing within the tank.

Temperature and pH were recorded daily from each experimental tank. Twice weekly, water samples were collected from an experimental tank from each treatment and were fixed with mercuric chloride ($HgCl_2$). These samples were analysed for characterization of the carbonate system at the University of Alaska Fairbanks Ocean Acidification Research Center. Total alkalinity (TA) and dissolved inorganic carbon (DIC) were measured using an Automated InfraRed Inorganic Carbon Analyser (AIRICA) and Versatile Instrument for the Determination of Total dissolved inorganic carbon and Alkalinity (VINDTA 3C), which were calibrated using Certified Reference Materials (CRMs) from the Dickson Laboratory at the Scripps Institution of Oceanography (Batch 199), with a mean deviation from CRM values of 2.25 for DIC and 1.01 for TA. Temperature, salinity, TA and DIC values were used to calculate pCO_2, and a derived pH of the water using the SeaCarb package in R (Gattuso et al., 2023), with dissociation constants from Waters et al. (2014). Water chemistry of the experimental system is presented in Table 1.

2.3 | Larval data collection

2.3.1 | Morphometric and mortality measurements

At 2-week intervals, a random subset of 15 larvae was removed from each tank to obtain morphometric measurements. Larvae were photographed using a dissecting microscope, and images were processed in ImageJ to measure standard length (SL; mm) and myotome height at

TABLE 1 Seawater treatment conditions and carbonate chemistry for each treatment.

pCO_2 treatment	Temperature treatment	Temperature (°C)	pH	pCO_2 (µatm)	DIC (µmol/kg)	TA (µmol/kg)
Ambient	1.8	1.67 ± 0.13	8.11 ± 0.04	300.98 ± 36.75	1937.31 ± 58.04	2064.72 ± 58.71
	5	5.04 ± 0.14	8.08 ± 0.04	328.66 ± 33.48	1932.92 ± 56.30	2068.61 ± 57.59
	7.3	7.30 ± 0.25	8.06 ± 0.03	348.79 ± 26.04	1924.29 ± 55.89	2063.99 ± 60.81
High	1.8	1.68 ± 0.14	7.46 ± 0.04	1494.78 ± 125.05	2133.61 ± 58.13	2079.39 ± 60.00
	5	5.02 ± 0.17	7.47 ± 0.05	1509.59 ± 157.36	2111.75 ± 55.94	2072.14 ± 59.83
	7.3	7.32 ± 0.37	7.52 ± 0.15	1401.09 ± 323.08	2101.31 ± 59.82	2086.26 ± 56.29

Note: Values are presented as mean ± standard deviation (SD). Temperature and salinity were measured daily, and dissolved inorganic carbon (DIC) and total alkalinity (TA) were measured from preserved bottle samples that were collected twice weekly. pH_{SWS} and pCO_2 were calculated from temperature, salinity, DIC and TA.

the anus (MH; mm). The 15 larvae were then pooled into groups of three, rinsed with ammonium formate and dried at 60°C to a constant weight (DW; mg). For each experimental tank, the average SL and DW were used to calculate length-based (G_L; mm day^{-1}) and mass-specific growth rates (G_M; % mass day^{-1}), respectively. G_L was defined as the difference in length over a specific time period. G_M was calculated using Equation (1):

$$\mathrm{SGR} = 100(e^{g} - 1) \quad (1)$$

where g is the instantaneous growth coefficient, calculated using Equation (2):

$$g = \frac{\ln(W_2) - \ln(W_1)}{t_2 - t_1} \quad (2)$$

where W_2 and W_1 are the dry weights at the final (t_2) and initial (t_1) time points, respectively. Both G_L and G_M were calculated for an early [0–14 days post-hatching (DPH)] and later (14–42 DPH) stage of growth.

Morphometric-based condition metrics were calculated based on the residuals of the relationship between SL and MH for body depth-based condition (K_{MH}), and SL and DW for dry weight-based condition (K_{DW}). K_{MH} was defined as the difference between the expected to measured MH at a specific SL based on a cubic fit. K_{DW} was defined as the quotient of the expected and measured DW for a specific SL based on a quadratic fit.

At the end of the experiment, remaining larvae were counted and removed from the tanks to provide an estimate for mortality rates (Equation 3) across the 6-week span.

$$\text{Mortality rate} = \frac{\ln(N_2) - \ln(N_1)}{t_2 - t_1} \quad (3)$$

Here, N_1 is the estimated number of larvae initially stocked in a tank, N_2 is the number of larvae at the end of the experiment and t_1 and t_2 are DPH for each count of larvae, respectively. One treatment tank at 7.3°C and high pCO_2 was terminated at 33 DPH due to unusually high mortality and was not included in the analysis.

2.3.2 | Lipid measurements

Larvae were sampled for lipid measurements at a common size (~9.5 mm SL), with approximately 31 fish sampled per experimental tank to provide a composite measurement. Larvae were removed from the tanks, anaesthetized and then pipetted onto pre-combusted glass fibre filters (Whatman GF/C, 47 mm). Filters were immediately submerged in 2 mL of chloroform under a layer of nitrogen and stored at −20°C until extraction, within 4 months of sampling. In total, there was a sample size of four per experimental treatment, with lipid extracts ranging in strength from 598 to 1118 µg of total lipid.

Lipids were extracted using the methods described by Parrish (1987), based on a modified Folch procedure (Folch et al., 1956). Total lipids and lipid classes were analysed by thin-layer chromatography with flame ionization detection (TLC-FID) on a MARK VI Iatroscan (Iatron Laboratories) as described by Lu et al. (2008) and Copeman et al. (2017). Briefly, lipid extracts were micropipetted onto duplicate silica-gel-coated Chromarods and developed in a three-stage solvent development system, which resulted in the separation of four identifiable lipid classes [TAGs, free fatty acids (FFA), STs and PLs]. After the last development, rods were scanned using Peak Simple software (version 4.95, SRI Inc.). The signal, detected in millivolts, was quantified using lipid class-specific calibration curves. Standards for FFA, ST and PL were purchased from Sigma (St. Louis, MO, USA), and a gadid-specific liver-based TAG standard was purified using column chromatography (from adult *B. saida* liver) following methods from Ohman (1997).

Following lipid class analyses, samples were analysed for FA composition. An internal standard (23:0 methyl ester) was added at ~10% of the total FA to all samples. Lipid extracts were then derivatized into their FA methyl esters (FAMEs) using sulfuric acid-catalysed transesterification (Budge et al., 2006). Resulting FAMEs were analysed on an HP 7890 GC-FID equipped with an autosampler and a DB wax+ GC column (Agilent Technologies, Inc., Santa Clara, USA) that was 30 m in length, with an internal diameter of 0.25 mm and film thickness of 0.25 µm. The oven temperature started at 65°C for 0.5 min, ramped up to 195°C (40°C min^{-1}), held for 15 min and then increased again (2°C min^{-1}) to a final temperature of 220°C. This final temperature was held for an additional minute. The carrier gas was hydrogen,

flowing at a rate of 2 mL min^{-1}. The injector temperature was set at 250°C and remained constant throughout the 32-min run time. Peaks were identified using retention times based on standards purchased from Supelco (37-component FAME, BAME, PUFA 1, PUFA 3) and in consultation with retention index maps performed under similar chromatographic conditions (Wasta & Mjøs, 2013). Column function was checked by comparing chromatographic peak areas to empirical response areas using a quantitative FA mixed standard, GLC 487 (Nu-Chek Prep). Chromatograms were integrated using Chem Station (version A.01.02, Agilent). Arctic cod larval samples were expressed both in absolute (lipid per DW mg g^{-1}) and relative (% of total lipids or total FA) amounts. FAs were also classified based on their carbon structure into SFAs, MUFAs and PUFAs.

2.4 | Data analysis

Mortality rates, growth rates (G_L and G_M), condition factors (K_{MH}, K_{DW}, total lipids, TAG:ST), lipid-based indicators for membrane acclimation [PL:ST, (MUFA + PUFA):SFA] and immune system responses (AA, EPA, AA:EPA), were all analysed using type III two-way analysis of variance (ANOVA) to test the effects of temperature (factor; three levels), pCO_2 treatment (factor; two levels) and their interaction. Experimental tank was the level of observation ($n = 4$ per treatment) for all response variables. Post hoc Tukey's honest significance tests were used to identify significant pair-wise comparisons, and effect sizes were calculated to provide relative importance of individual predictor variables on each response variable. Morphometric-based condition indices were available for each measurement time period, whereas the lipid-based condition indices were available only on the day of lipid sampling. Therefore, to compare both condition indices, the data for morphometric-based condition were subset to match the sampling day for lipid-based condition indices (28 DPH for 5 and 7.3°C; 42 DPH for 1.8°C). In addition, a generalized additive model (GAM) was individually fit to each treatment for a visual expression of the changes in SL, MH and DW throughout the experiment. All analyses were performed in R (version 4.3.0, R Core Team, 2023). Prior to analysis, data were tested for normality and homogeneity, and significance was taken at an alpha level of 0.05.

2.5 | Ethics statement

This research was carried out in accordance with all applicable institutional and national guidelines. Arctic cod adults were collected under Alaska Department of Fish and Game Permits #CF-12-088 and #CF-13-080 in 2012 and 2013. This research was conducted at NOAA's Alaska Fisheries Science Center's 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).

3 | RESULTS

3.1 | Mortality and growth rates

Mortality rates significantly increased with increasing temperature ($p < 0.001$; Table 3; Figure 1). By the end of the 6-week experiment, ~35% of larvae survived at 1.8°C compared to only 10% survival at 7.3°C. There was no significant effect of pCO_2 level on mortality rates ($p > 0.05$; Table 3).

Both length- and mass-specific growth rates (G_L and G_M) at each stage of growth (early: 0–14 DPH; later: 14–42 DPH) were significantly affected by temperature ($p < 0.001$; Tables 2 and 3). From 0 to 14 DPH, larvae at the coldest treatment (1.8°C) grew significantly slower than those at 5 and 7.3°C (Tukey's post hoc; $p < 0.05$; Figure 2), and from 14 to 42 DPH, growth increased significantly with each incremental temperature treatment (Tukey's post hoc; $p < 0.01$; Figure 2). There were minor pCO_2 effects on growth seen only at the later stage. G_M was lower at high pCO_2 than at ambient pCO_2 in the 5°C treatment (Tukey's post hoc; $p < 0.05$; Figure 2), but the overall effect of pCO_2 was non-significant ($p = 0.063$). By the end of the experiment, fish in 5°C and reared at high pCO_2 had ~14% less mass than those at ambient pCO_2. The GAMs on size-at-age metrics (SL, MH and DW) reflected additional trends in the temperature- and pCO_2-based responses of growth not captured in discrete growth calculations. For example, the GAMs highlighted a divergence in growth

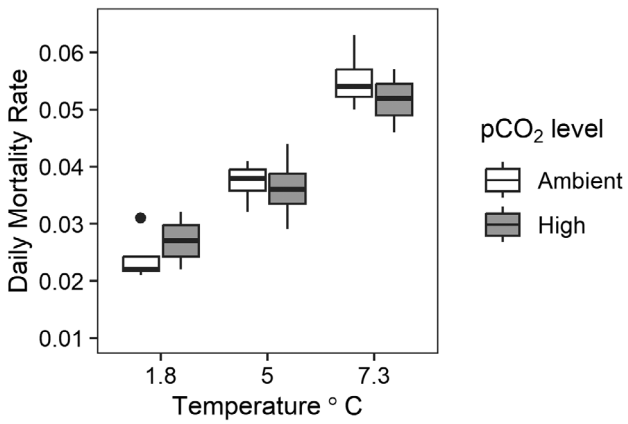

FIGURE 1 The effects of temperature and pCO_2 level on larval daily mortality rates of Arctic cod. Data are presented as boxplots, representing the first and third quantiles (lower and upper bounds of box), outliers (black dots) and median (black line) of the data, with ambient (white) and high (grey) pCO_2 levels designated separately. Statistically significant differences are shown with different letters (upper case = ambient pCO_2; lower case = high pCO_2) between temperature treatments. Significance was taken at an alpha level of 0.05 from the type III analysis of variance (ANOVA) tests (Table 3). Sample size for each treatment is four replicate tanks ($n = 15$ per replicate tank).

TABLE 2 Whole-animal responses for each treatment and sampling week.

Age	°C	pCO_2 level	Length (mm)	Myotome height (mm)	Dry weight (mg)	K_{MH}	K_{DW}
2 weeks	1.8	Ambient	7.72 ± 0.05	0.389 ± 0.005	0.172 ± 0.003	0.004 ± 0.003	0.100 ± 0.013
		High	7.52 ± 0.06	0.377 ± 0.005	0.165 ± 0.003	0.004 ± 0.003	1.021 ± 0.011
	5	Ambient	8.07 ± 0.07	0.425 ± 0.006	0.197 ± 0.005	0.012 ± 0.003	0.998 ± 0.013
		High	8.09 ± 0.07	0.414 ± 0.007	0.193 ± 0.007	0.000 ± 0.004	0.968 ± 0.016
	7.3	Ambient	7.99 ± 0.07	0.421 ± 0.007	0.196 ± 0.008	0.015 ± 0.003	1.014 ± 0.014
		High	8.12 ± 0.07	0.426 ± 0.007	0.206 ± 0.010	0.010 ± 0.003	1.016 ± 0.022
4 weeks	1.8	Ambient	9.10 ± 0.05	0.486 ± 0.005	0.285 ± 0.007	−0.016 ± 0.004	0.925 ± 0.030
		High	9.02 ± 0.06	0.479 ± 0.005	0.270 ± 0.008	−0.014 ± 0.004	0.907 ± 0.019
	5	Ambient	9.45 ± 0.10	0.546 ± 0.011	0.376 ± 0.016	0.006 ± 0.006	1.041 ± 0.024
		High	9.38 ± 0.08	0.514 ± 0.010	0.328 ± 0.012	−0.018 ± 0.005	0.935 ± 0.014
	7.3	Ambient	9.75 ± 0.11	0.580 ± 0.013	0.427 ± 0.024	0.006 ± 0.005	1.027 ± 0.014
		High	10.05 ± 0.09	0.618 ± 0.011	0.476 ± 0.024	0.013 ± 0.006	1.015 ± 0.020
6 weeks	1.8	Ambient	9.94 ± 0.10	0.568 ± 0.010	0.405 ± 0.017	−0.026 ± 0.006	0.913 ± 0.030
		High	9.84 ± 0.09	0.551 ± 0.009	0.382 ± 0.009	−0.031 ± 0.004	0.896 ± 0.017
	5	Ambient	11.37 ± 0.14	0.794 ± 0.018	0.828 ± 0.032	0.023 ± 0.006	1.078 ± 0.036
		High	11.05 ± 0.13	0.735 ± 0.019	0.715 ± 0.029	0.006 ± 0.009	1.040 ± 0.032
	7.3	Ambient	11.83 ± 0.16	0.846 ± 0.021	0.930 ± 0.033	0.013 ± 0.006	1.041 ± 0.036
		High	12.01 ± 0.20	0.862 ± 0.026	0.996 ± 0.066	0.004 ± 0.008	1.033 ± 0.015

Note: Values are presented as mean ± standard error (SE). Sample size was 60 fish for length, myotome height and K_{MH}, and 12 fish for dry weight and K_{DW}.
Abbreviations: K_{DW} = dry weight-based condition; K_{MH} = body depth-based condition.

TABLE 3 Results of analysis of variance (ANOVA) tests on the effects of temperature, pCO_2 and the temperature × pCO_2 interaction on larval Arctic cod.

	Temperature (2 *df*)			pCO_2 (1 *df*)			Temperature*pCO_2 (2 *df*)		
	F	*p*	Effect size	*F*	*p*	Effect size	*F*	*p*	Effect size
Survival and growth									
Mortality	56.426	**<0.001**	0.83	0.062	0.807	0.00	0.802	0.465	0.00
G_L (0–14)	18.910	**<0.001**	0.60	0.071	0.793	0.00	2.071	0.155	0.08
G_M (0–14)	15.109	**<0.001**	0.54	0.034	0.856	0.00	1.033	0.376	0.00
G_L (14–42)	85.860	**<0.001**	0.88	0.593	0.452	0.00	2.042	0.160	0.08
G_M (14–42)	223.593	**<0.001**	0.95	3.947	0.063	0.11	1.752	0.203	0.06
Condition factors									
K_{MH}	18.625	**<0.001**	0.61	2.339	0.145	0.06	3.263	0.063	0.16
K_{DW}	12.919	**<0.001**	0.51	6.289	**0.023**	0.19	2.892	0.083	0.14
Total lipid	1.756	0.203	0.06	0.031	0.864	0.00	0.350	0.710	0.00
TAG:ST	20.595	**<0.001**	0.63	1.237	0.282	0.01	2.149	0.147	0.09
Membrane fluidity									
PL:ST	13.051	**<0.001**	0.51	0.713	0.410	0.00	0.519	0.604	0.00
(MUFA+PUFA):SFA	25.039	**<0.001**	0.68	3.245	0.089	0.10	7.335	**0.005**	0.36
Immune response									
% 20:4n-6 (AA)	25.270	**<0.001**	0.69	12.048	**0.003**	0.33	3.850	**0.042**	0.20
% 20:5n-3 (EPA)	41.161	**<0.001**	0.78	0.879	0.362	0.00	3.729	**0.045**	0.19
AA:EPA	100.600	**<0.001**	0.90	1.170	0.295	0.00	1.387	0.277	0.03

Note: Significant *p*-values are presented in bold. Effect sizes are also shown to indicate relative influence of a predictor on the specific response variable.
Abbreviations: G_L, length-based growth; G_M, mass-specific growth; K_{DW}, dry weight-based condition; K_{MH}, body depth-based condition; MUFA, monounsaturated fatty acid; PL, polar lipids; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; ST, sterols; TAG:ST, triacylglycerol to sterol ratio.

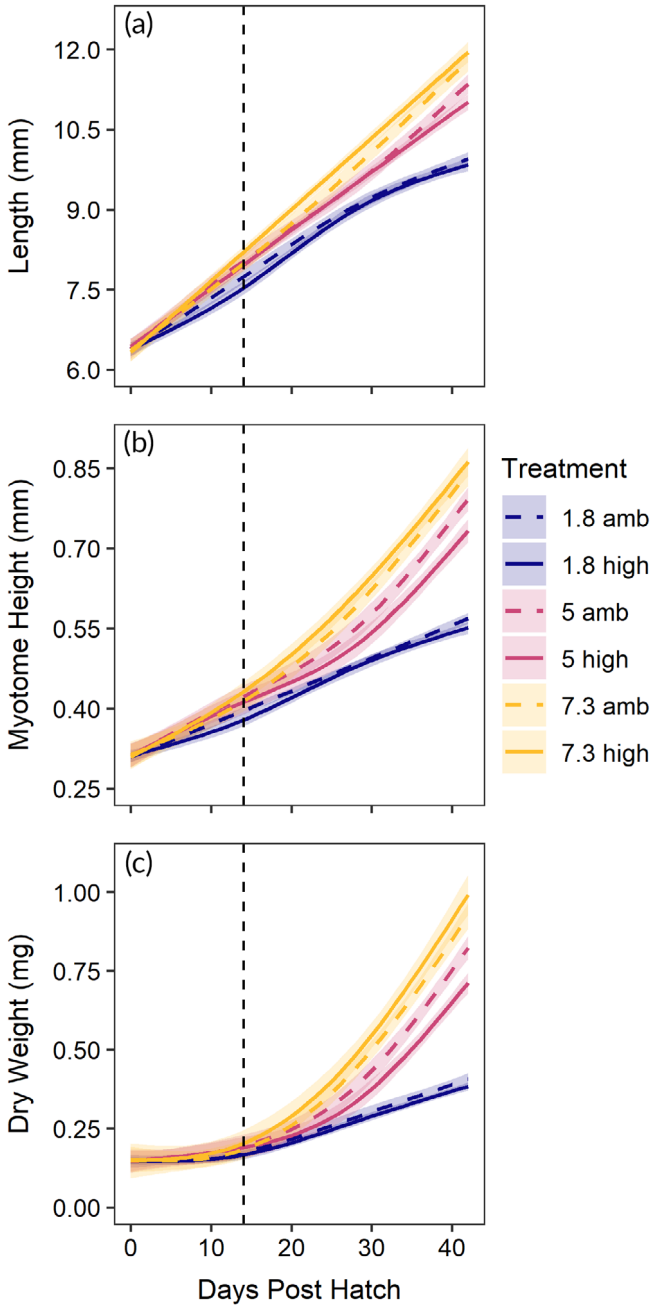

FIGURE 2 The trend in (a) standard length (SL), (b) myotome height (MH) and (c) dry weight (DW) of larval Arctic cod age is depicted for each temperature (purple = 1.8°C; pink = 5°C; yellow = 7.3°C) and pCO_2 level (dashed lines = ambient; solid lines = high), using independently generated generalized additive models. Data are presented as the model fit (line) and error (2 × the standard error; shaded background). The dashed line indicates the division between early (0–14 DPH) and late (14–42 DPH) growth.

rates of larvae at ~30 DPH in 1.8°C compared to those reared at other temperatures, as well as an intensifying pCO_2 effect with fish age at 5°C, where larvae reared at high pCO_2 became increasingly lighter than their conspecifics at ambient pCO_2 (Figure 2).

3.2 | Condition indices

The morphometric-based condition indices (K_{MH} and K_{DW}) significantly increased with increasing temperature ($p < 0.001$; Tables 2 and 3; Figure 3a,b). Both K_{MH} and K_{DW} responded to elevated pCO_2, but K_{DW} was more sensitive to high pCO_2, reflected in the effect sizes for pCO_2 level ($K_{MH} = 0.06$ vs. $K_{DW} = 0.19$; Table 3). For K_{MH}, there was a non-significant interaction between temperature and pCO_2 ($p = 0.063$), but the post hoc test indicated lower K_{MH} under elevated pCO_2 than ambient pCO_2 at 5°C. For K_{DW}, there was a significant effect of pCO_2 ($p < 0.05$) where DW-based condition was lower under high pCO_2 when compared to ambient CO_2. Altogether, these data reflect that fish similar in length were skinnier and lighter when reared under elevated pCO_2 conditions, which was most evident at 5°C.

The lipid-based condition indices (total lipid; TAG:ST) were also affected by temperature and pCO_2, but they did not increase with increasing temperature as seen in the morphometric-based condition indices. Total lipid was not significantly affected by temperature, pCO_2 or their interaction ($p > 0.05$), although there was a general trend of decreasing lipid content with increasing temperature (Figure 3c). The TAG:ST ratio was significantly affected by temperature ($p < 0.001$) and was highest at 1.8°C (Figure 3d), indicating that fish reared at the coldest temperature were in higher lipid-based condition than those in the warmer temperature. There was no significant pCO_2 or temperature × pCO_2 interaction effect on TAG:ST ($p > 0.05$; Table 3).

3.3 | Lipid-based indicators

The ratios PL:ST and (MUFA + PUFA):SFA were analysed as proxies for temperature acclimation via changes in membrane fluidity. Both ratios significantly decreased with increasing temperature ($p < 0.001$; Table 3; Figure 4a,b) as predicted. There was no pCO_2 effect on PL:ST, but the (MUFA + PUFA):SFA ratio was significantly affected by the interaction between temperature and pCO_2 ($p < 0.01$; Table 3), which was driven by the higher (MUFA + PUFA):SFA ratio at 1.8°C under ambient pCO_2 conditions compared to that under high pCO_2 (Tukey's post hoc: $p < 0.01$; Figure 4a). This change in (MUFA + PUFA):SFA ratio was a result of increased SFA and decreased PUFA under high pCO_2 (Table 4).

The percentage composition of two specific fatty acids (AA, EPA) and their ratio (AA:EPA) were assessed as indicators of immune system responses. All metrics were significantly affected by temperature ($p < 0.01$; Table 3), but the temperature-dependent change in their values differed for each of the three measures (Figure 5). Relative composition of AA was lowest at 5°C, EPA decreased with increasing temperature and the AA:EPA ratio increased with increasing temperature. The level of AA was also significantly affected by pCO_2 ($p < 0.01$) and the interaction between temperature and pCO_2 ($p < 0.05$). AA was elevated under high pCO_2 conditions, particularly at 7.3°C (Figure 5a). The interaction of temperature and pCO_2 also had a significant effect on EPA (temperature × pCO_2: $p < 0.05$), in which EPA was elevated under high pCO_2 conditions at 7.3°C

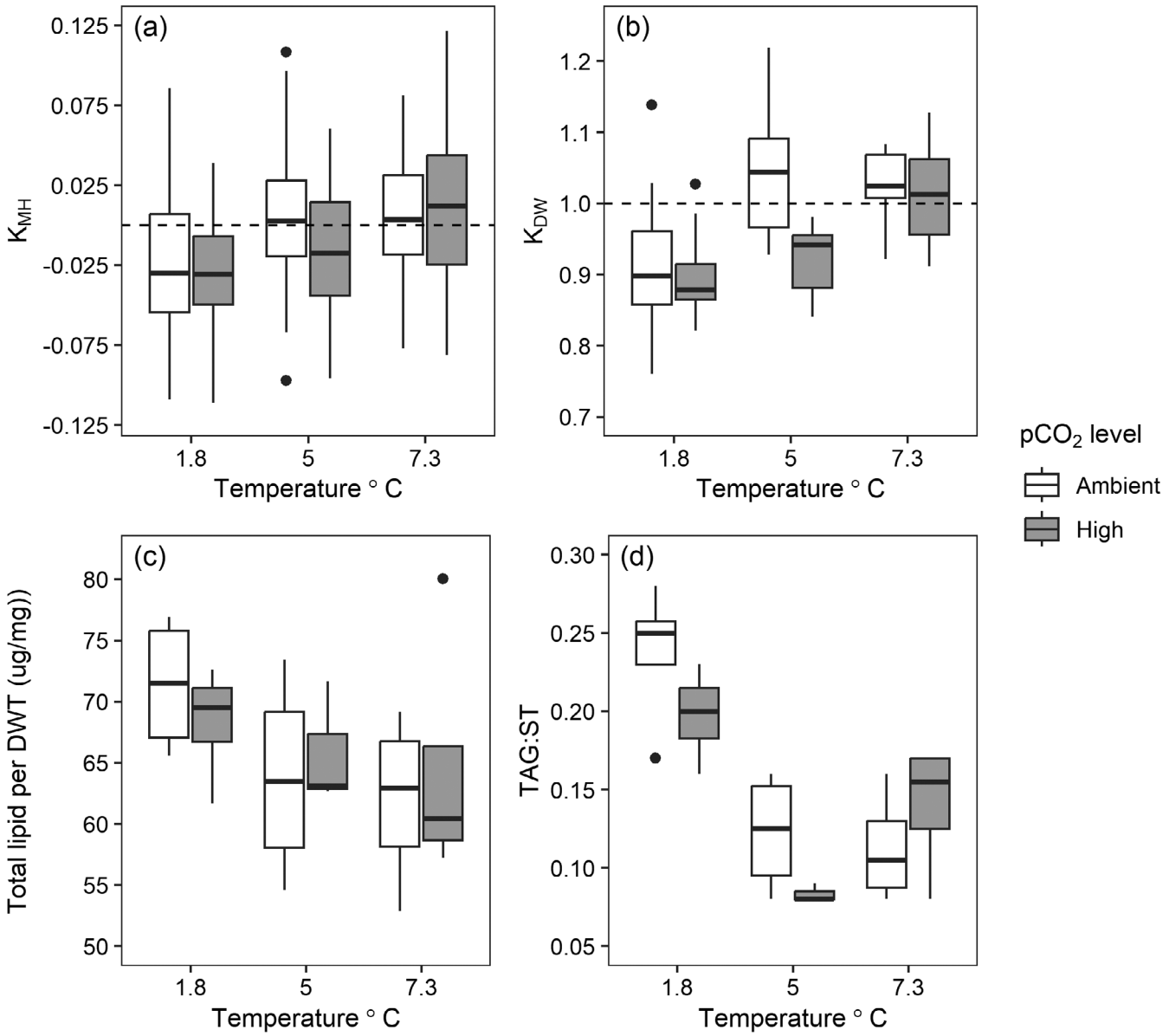

FIGURE 3 The effects of temperature and pCO_2 level on the (a, b) morphometric-based (body depth: K_{MH}; dry weight: K_{DW}) and (c, d) lipid-based condition indices of larval Arctic cod. Data are presented as boxplots, representing the first and third quantiles (lower and upper bounds of box), outliers (black dots) and median (black line) of the data, with ambient (white) and high (grey) pCO_2 levels designated separately. Statistically significant differences are shown with different letters (upper case = ambient pCO_2; lower case = high pCO_2) between temperature treatments and a "*" between pCO_2 treatments within the same temperature treatment. Significance was taken at an alpha level of 0.05 from the type III analysis of variance (ANOVA) tests (Table 3). Sample size for each treatment is four replicate tanks (a, b: $N = 15$ per replicate tank; c, d: $N = 31$ per replicate tank). To compare condition indices at a common size, fish reared at 5 and 7.3°C were sampled at 28 DPH, and those reared at 1.8°C were sampled at 42 DPH. K_{MH}, body-depth-based condition; K_{DW}, dry weight-based condition; TAG:ST, triacylglycerol to sterol ratio.

(Figure 5b). The AA:EPA ratio was not significantly affected by pCO_2 or by the temperature × pCO_2 interaction ($p > 0.05$). All measured FAs at > %1 are presented in Table 4.

4 | DISCUSSION

Arctic cod have previously demonstrated sensitivity to OW and OA, but only one published study has focused on the combined effects at an early stage of development (embryos; Dahlke et al., 2018). Expanding this research into feeding stages of larvae revealed a number of patterns consistent with earlier studies (e.g., Laurel et al., 2016; Dahlke et al., 2018; Koenker, Copeman, et al., 2018a; Koenker, Laurel, et al., 2018b). Arctic cod larvae were sensitive to OW, indicated by elevated mortality rates at 7.3°C compared to 5°C. Optimal temperature for larval development may be near 5°C based on combined growth and mortality rates. Condition metrics suggested potential size-energy trade-offs in larval Arctic cod incubated at 5 and 7.3°C in

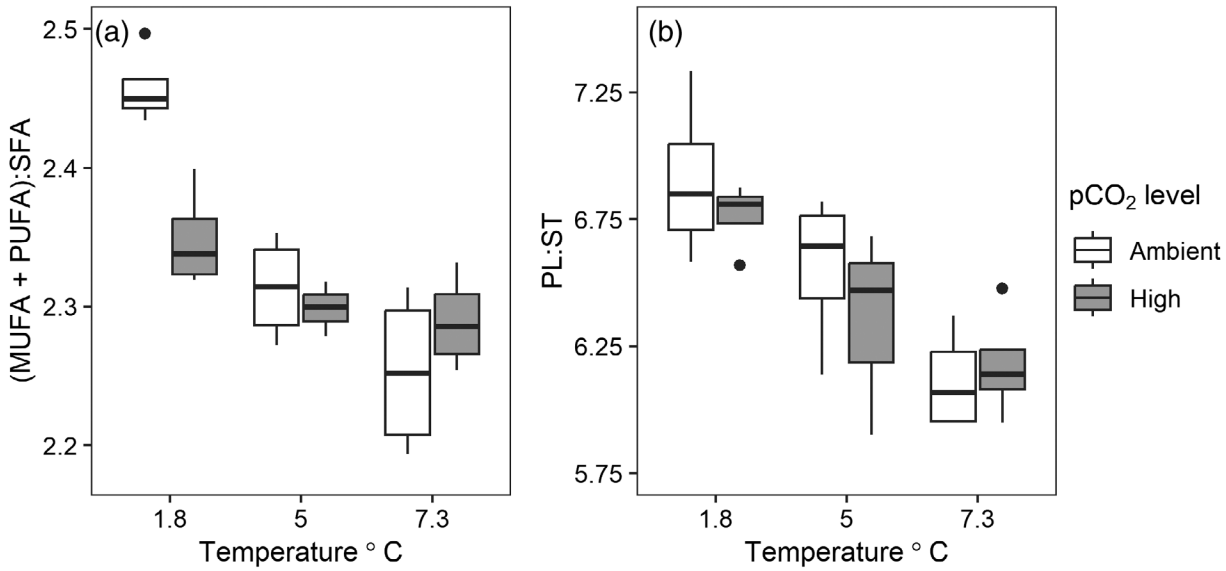

FIGURE 4 The effects of temperature and pCO_2 on proxies of membrane fluidity, (a) (MUFA + PUFA):SFA and (b) PL:ST ratios of larval Arctic cod. Data are presented as boxplots, representing the first and third quantiles (lower and upper bounds of box), outliers (black dots) and median (black line) of the data, with ambient (white) and high (grey) pCO_2 levels designated separately. Statistically significant differences are shown with different letters (upper case = ambient pCO_2; lower case = high pCO_2) between temperature treatments and a '*' between pCO_2 treatments within the same temperature treatment. Significance was taken at an alpha level of 0.05 from the type III analysis of variance (ANOVA) tests (Table 3). Sample size for each treatment is four replicate tanks ($n = 31$ per replicate tank). SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; PL, polar lipids; ST, sterols.

high morphometric-based condition but lower lipid-based condition compared to fish incubated at 1.8°C. Although these results may suggest Arctic cod larvae can acclimate to a range of temperatures, changes in energy density under OW may have downstream consequences for the larvae (overwintering capabilities) and their predators (nutritional quality). With respect to elevated pCO_2 conditions, larval Arctic cod demonstrated temperature-dependent sensitivity to OA. Additionally, the pCO_2 effects were consistently smaller than those from temperature, which was reflected in the effect sizes from each measured response variable (temperature 0.06–0.95 vs. CO_2 0–0.33; Table 3). The pattern of a stronger temperature response compared to that under elevated pCO_2 is consistent across several species of marine fish larvae, including Pacific cod (Slesinger et al., 2024), European sea bass (*Dicentrarchus labrax*) (Cominassi et al., 2019) and Pacific herring (*Clupea pallasii*) (Villalobos et al., 2020). We discuss the role of temperature in a single- and multi-stressor framework below.

4.1 | Mortality and growth

Larval Arctic cod grew progressively faster with increasing temperature, a trend that intensified with age. This result slightly contrasts with that in Koenker, Laurel, et al. (2018b), where Arctic cod feeding stage larvae (0–35 DPH) increasingly grew faster up to 5°C and then the rate of growth slowed at temperatures > 5°C. The larger tank sizes and increased feeding frequency used in this experiment may have permitted faster growth among Arctic cod at higher temperatures. Although growth rates were high at 7.3°C, mortality rates were also significantly higher at 7.3°C than at 5 and 1.8°C. The mortality to growth (M:G) ratio, calculated as daily mortality rates (% dead fish day^{-1}) divided by mass-specific growth rates (% mg day^{-1}), is a suggested integrated index of productivity that balances increased biomass from growth with decreased biomass from mortality (Secor & Houde, 1995; Suthers et al., 2021). Although this metric is typically applied in field studies, the M:G ratio provides a comprehensive indicator that can also be applied in experimental studies. The M:G ratio for 1.8 and 5°C was ~0.75, whereas the ratio increased to ~0.97 at 7.3°C, indicating that growth at 7.3°C was not fast enough to overcompensate the rapid loss in biomass due to mortality. As such, 5°C appears to be an optimal temperature for development when accounting for both growth and mortality. Other studies have also proposed 5 to 6°C as an optimal temperature for Arctic cod at multiple life stages (except for eggs; Laurel et al., 2018), including larvae (Koenker, Copeman, et al., 2018a; Koenker, Laurel, et al., 2018b), juveniles (Laurel et al., 2016) and adults (Drost et al., 2014; Drost, Lo, et al., 2016b). Although Arctic cod can grow in sub-zero temperatures (Koenker, Copeman, et al., 2018a), their propensity for faster growth at slightly warmer temperatures (5°C) may reflect that nursery areas sometimes occur at thermal-salinity fronts in the Arctic, which tend to be warmer than under the ice (Laurel et al., 2016). Slightly elevated temperatures may also benefit larvae in the near term and/or in certain portions of the Arctic, but coastal regions within the southern Arctic extent (e.g., Chukchi Sea, Prudhoe Bay, AK, USA) may warm beyond optimal temperatures for growth (Craig et al., 1982; Deary et al., 2021).

There was no significant effect of elevated pCO_2 on mortality or growth, although there was a near-significant trend of lower weight and myotome height under elevated pCO_2 conditions at 5°C as fish

TABLE 4 Lipid classes and fatty acids (FAs) of larval Arctic cod under various temperature and pCO_2 treatments.

	1.8°C		5°C		7.3°C	
	Ambient pCO_2	High pCO_2	Ambient pCO_2	High pCO_2	Ambient CO_2	High pCO_2
n samples	4	4	4	3	4	4
Total lipids (μg/mg)	71.35 ± 2.81	68.33 ± 2.37	63.73 ± 4.22	65.80 ± 2.93	61.97 ± 3.59	64.54 ± 5.25
% Polar lipids	83.69 ± 0.27	83.62 ± 0.12	84.10 ± 0.43	84.31 ± 0.29	83.51 ± 0.50	82.99 ± 0.32
% Sterols	12.14 ± 0.26	12.37 ± 0.13	12.84 ± 0.26	13.31 ± 0.45	13.67 ± 0.14	13.45 ± 0.23
% Triacylglycerols	2.86 ± 0.25	2.43 ± 0.17	1.57 ± 0.22	1.10 ± 0.04	1.55 ± 0.26	1.90 ± 0.28
% Free FAs	1.31 ± 0.07	1.58 ± 0.09	1.50 ± 0.11	1.29 ± 0.16	1.27 ± 0.15	1.66 ± 0.10
% 14:0	1.19 ± 0.07	1.22 ± 0.04	1.19 ± 0.09	1.03 ± 0.10	1.26 ± 0.04	1.12 ± 0.07
% 16:0	20.65 ± 0.14	21.07 ± 0.08	20.82 ± 0.01	20.98 ± 0.08	20.72 ± 0.16	20.65 ± 0.09
% 18:0	5.67 ± 0.04	6.02 ± 0.04	6.55 ± 0.08	6.70 ± 0.11	7.14 ± 0.13	6.95 ± 0.08
∑SFA[a]	28.93 ± 0.11	29.87 ± 0.16	30.18 ± 0.17	30.32 ± 0.10	30.75 ± 0.28	30.41 ± 0.16
% 16:1n-7	3.14 ± 0.11	2.92 ± 0.09	2.60 ± 0.04	2.45 ± 0.03	2.42 ± 0.05	2.43 ± 0.04
% 18:1n-9	7.54 ± 0.05	7.69 ± 0.10	8.05 ± 0.05	8.23 ± 0.04	8.35 ± 0.09	8.12 ± 0.07
% 18:1n-7	3.75 ± 0.03	3.79 ± 0.04	3.86 ± 0.03	3.89 ± 0.03	3.72 ± 0.03	3.62 ± 0.02
% 20:1n-9	1.46 ± 0.02	1.52 ± 0.02	1.34 ± 0.01	1.40 ± 0.02	1.24 ± 0.03	1.19 ± 0.01
∑MUFA[b]	17.75 ± 0.02	18.03 ± 0.13	18.12 ± 0.07	18.25 ± 0.18	18.00 ± 0.16	17.71 ± 0.09
% 18:2n-6	3.94 ± 0.07	3.68 ± 0.08	3.63 ± 0.14	3.24 ± 0.09	3.62 ± 0.12	3.68 ± 0.07
% 20:2n-6	1.10 ± 0.01	1.04 ± 0.01	0.93 ± 0.04	0.86 ± 0.01	0.85 ± 0.02	0.85 ± 0.01
% 20:4n-6	4.57 ± 0.01	4.59 ± 0.03	4.40 ± 0.03	4.46 ± 0.01	4.57 ± 0.04	4.75 ± 0.03
% 20:5n-3	10.01 ± 0.07	9.81 ± 0.10	9.21 ± 0.06	9.28 ± 0.13	8.70 ± 0.16	9.11 ± 0.14
% 22:5n-6	2.33 ± 0.05	2.33 ± 0.05	2.28 ± 0.06	2.21 ± 0.02	2.51 ± 0.07	2.63 ± 0.03
% 22:5n-3	5.75 ± 0.06	5.37 ± 0.08	5.26 ± 0.13	4.93 ± 0.02	5.20 ± 0.09	5.41 ± 0.08
% 22:6n-3	21.70 ± 0.24	21.61 ± 0.18	22.34 ± 0.41	22.96 ± 0.08	22.11 ± 0.19	21.75 ± 0.23
∑PUFA[c]	53.33 ± 0.13	52.10 ± 0.28	51.70 ± 0.17	51.44 ± 0.24	51.25 ± 0.41	51.89 ± 0.21

Abbreviations: MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.
[a]∑SFA also contained <1% of i15:0, ai15:0, 15:0, i16:0, ai16:0, i17:0, ai17:0, 17:0, 22:0, 24:0 and not used in analysis.
[b]∑MUFA also contained <1% of 14:1, 16:1n-11, 16:1n-9, 16:1n-5, 18:1n-11, 18:1n-6, 20:1n-11, 22:1n-11, 22:1n-9, 22:1n-7, 24:1n-9 and not used in analysis.
[c]∑PUFA also contained <1% of 16:2n-4, 16:3n-4, 16:4n-3, 16:4n-1, 18:2, 18:2n-4, 18:3n-6, 18:3n-4, 18:3n-3, 18:4n-3, 20:3n-6, 20:3n-3, 20:4n-3, 21:5n-3, 22:4n-6 and not used in analysis.

aged (Figure 2). This result may suggest that, at 5°C, the effects of elevated pCO_2 may appear under prolonged exposure. As such, slightly older early life stages may exhibit greater sensitivity to OA rather than the earliest life stages.

4.2 | Morphometric and lipid-based condition

Larval Arctic cod morphometric based-condition (K_{MH}; K_{DW}) increased with increasing temperature, which was a similar response to temperature as seen in both growth metrics. However, the lipid-based condition indices (total lipid; TAG:ST) were higher at the coldest temperature, which suggests differing temperature-dependent energy storage strategies. TAG is preferentially metabolized during periods of starvation or stress, whereas ST are structural membrane-associated lipids that can reflect organism size (Amara et al., 2007; Lochmann et al., 1995). As such, the TAG:ST ratio can represent the relative amounts of lipids incorporated in energy storage versus structural membranes (Suthers, 1998) and has been previously demonstrated as a reliable condition index in Arctic cod juveniles (Copeman et al., 2017). The disagreement between the morphometric- and lipid-based condition metrics in our study was driven by smaller, lighter and lipid-rich larvae at the coldest temperature compared to larger, heavier and lipid-poor larvae in the warmer temperatures. The DW of fish primarily comprises lipid and protein (Love, 1970). Therefore, higher protein content must account for the increase in DW in the

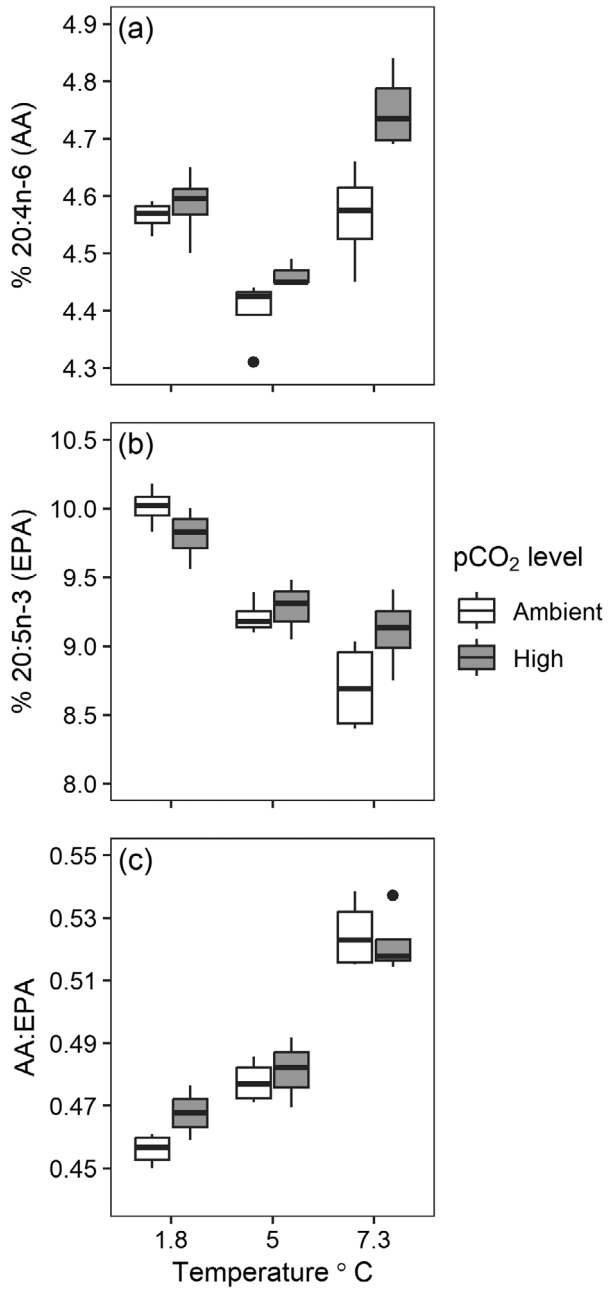

FIGURE 5 The effects of temperature and pCO_2 on immune response-associated essential fatty acids: (a) arachidonic acid (AA) (20:4n-6), (b) eicosapentaenoic acid (EPA) (20:5n-3) and (c) their ratio AA:EPA. Data are presented as boxplots, representing the first and third quantiles (lower and upper bounds of box), outliers (black dots) and median (black line) of the data, with ambient (white) and high (grey) pCO_2 levels designated separately. Statistically significant differences are shown with different letters (upper case = ambient pCO_2; lower case = high pCO_2) between temperature treatments and a '*' between pCO_2 treatments within the same temperature treatment. Significance was taken at an alpha level of 0.05 from the type III analysis of variance (ANOVA) tests (Table 3). Sample size for each treatment is four replicate tanks ($n = 31$ per replicate tank).

larvae held at warmer temperatures. In a warming Arctic, the growth strategies seen in larvae at warmer temperatures in this experiment may reflect future changes in body condition. Although faster growth of young fish reduces size-dependent mortality (Miller et al., 1998), reduced lipid storage may lower resilience in a patchy food environment and could lessen juvenile overwintering survival (Copeman, Stowell, et al., 2022b; Hurst, 2007; Pepin et al., 2015; Post & Parkinson, 2001; Sokolova et al., 2012). Additionally, lipid-poor Arctic cod could negatively impact predator populations that depend on them as a primary food source (Geoffroy et al., 2023).

The effects of OA were only significant for the morphometric-based condition factors at the optimal growing temperature, 5°C. Here, fish were in lower condition under elevated pCO_2, being skinnier and lighter with respect to their length. This effect intensified as the fish aged, which contrasts with other studies that have shown varying directionality of stage-specific responses to OA (e.g., Hurst et al., 2019; Slesinger et al., 2024). Although not statistically significant, the TAG:ST ratio was lowest in the high pCO_2 treatment at 5°C, which could indicate higher energy demand in these fish. Additionally, upregulated metabolic enzyme activity (citrate synthase and β-hydroxyacyl CoA-dehydrogenase) in fish at 5°C and elevated pCO_2 support the notion that metabolism was likely higher in these fish than those held at 5°C and ambient pCO_2 (E. Slesinger, unpublished data). These data as a whole suggest that under elevated pCO_2 conditions, available energy may have been diverted towards higher metabolic demands at the cost of growth and energy accumulation, an effect that has also been demonstrated in other organisms (Carter et al., 2013; Strahl et al., 2016). It is unexpected that this response only manifested in the 5°C treatment, which was also suggested to be near an optimal growing temperature for Arctic cod larvae (Koenker, Copeman, et al., 2018a; Koenker, Laurel, et al., 2018b). Although metabolic demands were likely higher at 7.3°C than at 5°C, there appeared to be no synergistic effect of combined OW and OA on condition. In Pacific cod larvae, OW increased the number of differentially expressed genes, yet there was an antagonistic effect on gene expression when OA was combined with OW (Spencer et al., in press). Perhaps similar mechanisms are occurring here for Arctic cod larvae that can be further explored through comparable molecular techniques.

4.3 | Larval lipid dynamics

Specific components of larval Arctic cod lipid composition, including lipid classes and FAs, were assessed as proxies to explore additional sensitivities to combined temperature and elevated pCO_2. As paralleled in the other broader morphometric and whole-animal responses, temperature was also a dominant driver of lipid dynamics, with smaller and temperature-specific influences of elevated pCO_2.

Organisms acclimating to a range of temperatures can undergo multiple physiological adjustments. Specifically at cold temperatures, membrane acclimation can be achieved by increasing unsaturated FA content relative to SFA (Cossins et al., 1977; Siliakus et al., 2017) and by increasing PL relative to ST (Ericson et al., 2019). In larval Arctic cod, both of these indicators of membrane fluidity increased with decreasing temperature (Figure 4), suggesting that they are capable of acclimating to a range of temperatures, at least at a biomolecular level. Interestingly, this cold-temperature acclimation response for (MUFA + PUFA):SFA was not apparent under high pCO_2 conditions. This

ratio was largely unchanged at high pCO_2 across the temperature treatments, which contrasted with the significant increase in (MUFA + PUFA):SFA with decreasing temperature at ambient pCO_2. Lipid dysregulation has also been demonstrated in young gadids under OA conditions in Atlantic cod (*Gadus morhua*) (Frommel et al., 2011), walleye pollock (Hurst et al., 2021) and Pacific cod (Hurst et al., 2019). Further investigation into the effects of OA on Arctic cod at cold temperatures representative of their current environment would help identify the mechanisms and potential consequences of OA as a single stressor.

Essential fatty acids (EFAs) for marine larval fish are long-chain (C_{20+22}) PUFAs that generally cannot be synthesized de novo at levels necessary to maintain normal physiological function (Takeuchi, 1997). Specific EFAs, such as EPA and AA, are precursors for eicosanoids, including prostaglandins and lipokines, which are part of the inflammatory response (Stanley-Samuelson, 1987). The eicosanoids derived from AA are considered more biologically active than those from EPA (Calder & Grimble, 2002) and are part of the pro-inflammatory response, in contrast to EPA-derived eicosanoids, which tend to be part of the anti-inflammatory response (Calder, 2010). A higher AA:EPA ratio can indicate higher physiological stress (Ericson et al., 2019) and has been associated with increased mortality rates or developmental issues in some (Copeman et al., 2002; Mommens et al., 2015; Samaee et al., 2009), but not all (Czesny et al., 1999), larval fish. Furthermore, a change in immune system function in response to combined OW and OA has been identified in other marine species, including Norway lobster (*Nephrops norvegicus*) (Hernroth et al., 2012) and Atlantic halibut (*Hippoglossus hippoglossus*) (Bresolin de Souza et al., 2016). In our study, AA, EPA and AA:EPA showed differing relationships to temperature: AA displayed a non-linear response to temperature with values lowest at 5°C; EPA decreased with increasing temperature; and the AA:EPA ratio increased with increasing temperature. Compared to those at 1.8 and 5°C, the AA:EPA ratio at 7.3°C was substantially higher, which could indicate an increase in the pro-inflammatory response. The effects of elevated pCO_2 also may have increased the pro-inflammatory immune response, shown by a significant elevation of AA in each temperature treatment and a considerably higher increase at 7.3°C. However, the AA:EPA ratio was not affected by elevated pCO_2. A potential discrepancy between the relative amounts of the FAs and their ratio may be the result of assessing percentage levels of each FA from the total lipid pool, rather than within specific lipid classes or by DW (mg) of tissue. A more targeted approach to assess the Arctic cod immune system response under combined stressors would be useful, as there appears to be some sensitivity in the larvae through the broad assessment of eicosanoidal compounds.

4.4 | Experimental considerations

In our experimental design, Arctic cod embryos were sourced from laboratory-reared adults and incubated with routine husbandry conditions before exposure to OW and OA treatments. F2 laboratory-spawned offspring were used because of the logistical constraints of collecting wild Arctic cod. However, the larvae in this experiment responded to temperature in a manner similar to F1 Arctic cod larvae produced by wild-caught parents (Koenker, Copeman, et al., 2018a; Koenker, Laurel, et al., 2018b). Additionally, larvae were exposed to experimental treatments after incubation at a common incubation temperature. Although initiating experimental treatments in the embryo stage and continuing into larval stages (Hurst et al., 2021) offers the opportunity to observe cumulative effects, this approach can introduce experimental issues such as lack of synchrony and varying densities across treatments (Slesinger et al., 2024). The stage-specific approach has been used previously (Dahlke et al., 2018; Villalobos et al., 2020) and has the benefit of identifying effects of environmental stressors based on stage-specific development. Finally, Arctic cod larvae were fed high densities of live food thrice daily, as nutritional stress was not a focus of our multi-stressor experiment. Feed frequency and nutritional quality can have pronounced effects on Arctic cod larvae (Koenker, Copeman, et al., 2018a; Koenker, Laurel, et al., 2018b). This effect is worth considering, as climate change is simultaneously affecting the food web, which could alter the availability and quality of prey for Arctic cod (Suprenand et al., 2018). Future experiments assessing Arctic cod response to elevated temperature and pCO_2 levels under food stress conditions would be valuable.

5 | CONCLUSION

As the Arctic environment continues to change, larval Arctic cod will experience new oceanic conditions during their sensitive early life history. Here, we found that 5°C appeared to represent an optimal temperature for development, in agreement with previous laboratory studies. However, when lipid-based condition metrics were considered, incubation at 1.8°C resulted in higher condition, suggesting that larvae prioritized lipid storage over growth at low temperatures, a potential adaptive strategy for surviving variable Arctic conditions. Although larval Arctic cod exhibited some responses to high temperatures that may suggest a capacity to acclimate to OW (i.e., changes in membrane fluidity proxies, faster growth, higher morphometric-based condition), signs of physiological stress were also evident, including elevated AA:EPA ratio and higher mortality rates. Our data suggest that when exposed to elevated pCO_2 levels, Arctic cod will face novel constraints beyond those elicited by temperature alone. This is notable because it suggests that Arctic cod populations could respond in multiple ways, depending on the relative rates of warming and acidification in the Arctic. This presents a series of challenges for predicting changes in the Arctic ecosystem.

AUTHOR CONTRIBUTIONS

E.S. B.J.L., and T.P.H. conceptualized and designed the experiment; E.S., M.B.R.H., P.J.I., and M.A.S. collected the data; E.S. analyzed the data with input from L.A.C.; E.S. led the writing of the manuscript with

contributions from L.A.C., B.J.L., and T.P.H. All authors reviewed and contributed to the writing of the final manuscript.

ACKNOWLEDGEMENTS

We would like to thank Michele Ottmar, Mara Spencer and Jessica Andrade for their assistance with animal husbandry. We are grateful to Nadja Steiner and Craig Norrie for their helpful comments on a previous version of this manuscript. We also would like to thank two anonymous reviewers for their constructive feedback during the review process. This project was funded by a NOAA's Ocean Acidification Program Grant: #20903 to Thomas P. Hurst.

FUNDING INFORMATION

This study was funded by NOAA's Ocean Acidification Program Grant: #20903 to Thomas P. Hurst.

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How to cite this article: Slesinger, E., Copeman, L. A., Laurel, B. J., Hicks, M. B. R., Iseri, P. J., Stowell, M. A., & Hurst, T. P. (2025). Larval Arctic cod (*Boreogadus saida*) exhibit stronger developmental and physiological responses to temperature than to elevated pCO_2. *Journal of Fish Biology*, 1–15. https://doi.org/10.1111/jfb.70082