Resiliency of juvenile walleye pollock to projected levels of ocean acidification

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ABSTRACT: As atmospheric concentrations of CO2 rise, the pH of high-latitude oceans is predicted to decrease by 0.3 to 0.5 units by 2100. Several biological consequences of ocean acidification across this pH range have already been documented in invertebrates and tropical marine fishes. However, little work has been done examining potential responses of the temperate and boreal marine fish species that support major fisheries. In 2 experiments, we examined the growth responses of juvenile walleye pollock Theragra chalcogramma at ambient and 3 elevated CO2 levels. In a short-term experiment with yearlings, CO2 treatment had no significant effect on growth or condition after 6 wk of rearing. Elevated CO2 levels (>450 µatm) increased the rate of otolith deposition, but did not affect otolith elemental composition. In a second experiment, growth in length of sub-yearlings over 12 wk at 8°C was 7.2% faster in the 2 higher CO2 treatments (>1200 µatm) than in the lower CO2 treatments (<900 µatm). Growth of sub-yearlings measured during 11 subsequent weeks of rearing at 2.5°C did not differ among CO2 treatments. There was no effect of CO2 treatment on condition factor following either phase of the experiment. Sub-yearling consumption rates were not directly affected by CO2 treatment, confirming that growth at elevated CO2 levels is not maintained through compensatory feeding. While not exhaustive of potential interactive environmental factors, these experiments demonstrate a general resiliency of growth energetics in juvenile walleye pollock to the direct effects of CO2 changes predicted for the Gulf of Alaska and Bering Sea in the next century.

KEYWORDS: Ocean acidification · Hypercapnia · Growth rate · Consumption · Otolith · Temperature

INTRODUCTION

Ocean acidification is a global phenomenon caused by the release of terrestrially sequestered CO2 into the atmosphere through the burning of fossil fuels and changes in land use practices. Approximately one-third of anthropogenically released CO2 has dissolved into the ocean (Feely et al. 2004, Sabine et al. 2004, Orr et al. 2005). The dissolution of CO2 into ocean waters results in a decrease of pH and reduces the availability of carbonate ions. High-latitude ecosystems are predicted to be most impacted by on-
going ocean acidification due to the high solubility of CO$_2$ at low temperatures as well as these systems’ unique circulation patterns (Byrne et al. 2010) and biogeochemical processes (Fabry et al. 2009, Mathis et al. 2011a,b). High-latitude oceans are projected to experience pH declines of up to 0.45 units during the next century, causing large regions to be consistently undersaturated with respect to aragonite (Yamamoto-Kawai et al. 2009, Steinacher et al. 2009). These high-latitude regions, including the Bering Sea and Gulf of Alaska, are highly productive ecosystems that support important commercial seafood resources and will likely be impacted to some degree by ocean acidification (Cooley & Doney 2009).

Experimental evidence is accumulating that elevated CO$_2$ concentrations (‘environmental hypercapnia’) and depressed pH can have a variety of effects on the growth and development of marine organisms (Fabry et al. 2008, Kroeker et al. 2010), but both the magnitude and direction of these effects will likely vary among species, trophic groups, and ontogenetic stages (Ries et al. 2009, Kroeker et al. 2010). It has been suggested that their high metabolic capacity and ability to maintain intra- and extracellular acid–base status will allow most marine fishes to physiologically cope with projected levels of ocean acidification (Pörtner et al. 2004, Melzner et al. 2009b). While studies on the growth rates of juvenile fishes generally support this perspective, there are few empirical examples of CO$_2$ responses of marine fishes relevant to the issue of ocean acidification (see review by Ishimatsu et al. 2008). Conversely, recent studies have demonstrated that the embryonic and larval stages of fishes may be more vulnerable to the effects of ocean acidification (Baumann et al. 2012, Frommel et al. 2012). Other experiments have demonstrated effects of elevated CO$_2$ on otolith growth (Checkley et al. 2009, Munday et al. 2011b), and it is unknown whether these effects are restricted to the larval stage. Furthermore, elemental incorporation in biogenic carbonates is influenced by pH (Al-Horani et al. 2003, Gaetani & Cohen 2006). Therefore, one would expect that changes in ambient pH could alter rates of elemental incorporation into the calcium carbonate matrix of fish otoliths. It is not yet clear what, if any, effect reduced pH will have on elemental incorporation in fish otoliths and how varied the response is among species. Additional empirical work is needed to determine the range of responses and ontogenetic patterns of sensitivity of marine fishes to projected ocean acidification, especially among the temperate and boreal species that support much of the world’s fishery production.

Walleye pollock *Theragra chalcogramma* are abundant over shelf and slope areas of the North Pacific Ocean and Bering Sea and extend to the south through the coastal waters of British Columbia (Canada) and Puget Sound. In the USA, harvests have averaged more than 1.1 Mt over the past decade and represent the largest single-species fishery in the nation. In addition to their economic importance, walleye pollock are a critical prey species for numerous marine birds and mammals (Livingston 1993). The degree of exposure of a species to natural (non-anthropogenic), diurnally or seasonally elevated CO$_2$ levels has been suggested as a potential factor in determining species’ relative sensitivity to future ocean acidification conditions (Denman et al. 2011, Munday et al. 2011a). In late winter, walleye pollock spawn at depth (usually >50 m) and eggs drift at depth (~200 m in the Gulf of Alaska, Brodeur & Wilson 1996) or rise to the surface (Smart et al. 2012). Juveniles are pelagic, inhabiting surface and sub-surface waters over the continental shelf as well as shallow coastal waters (Brodeur & Wilson 1996). With their midwater spawning and pelagic larval and juvenile distributions, walleye pollock are exposed to relatively stable physicochemical environments. Such a life history may render them more sensitive to ocean acidification than other North Pacific resource species with demersal spawning or which inhabit shallow subtidal nursery areas (Munday et al. 2011a).

In the present study, we examined the direct effects of projected ocean acidification on juvenile walleye pollock. To provide an ontogenetic perspective on CO$_2$-sensitivity among early life history stages, a companion study examined the effects of elevated CO$_2$ levels on egg and larval walleye pollock (T. Hurst unpubl.). Because of the direct linkage of growth energetics to population productivity, these experiments were focused on determining the growth, feeding, condition, and survival responses of juvenile walleye pollock to elevated CO$_2$ levels. In one experiment, ‘yearling’ (age-1) walleye pollock were reared for 6 wk to evaluate short-term responses in growth and condition, and the potential for hypercapnia-induced changes in otolith accretion and elemental composition. In a second experiment, ‘sub-yearling’ (age-0) walleye pollock were reared under elevated CO$_2$ conditions for 28 wk to describe the cumulative effects of prolonged exposure to elevated CO$_2$ levels. The second experiment included seasonally-reflective warm and cold phases and evaluated the possibility that growth rates are maintained in the face of elevated metabolic costs through compensatory feeding. Treatments were selected to
reflect ambient conditions and conditions predicted to occur in high latitude seas in the next century (400 to 600 µatm increase). A high CO₂ treatment (>1200 µatm increase) was included to evaluate physiological sensitivity over a broader range of conditions than those predicted for ocean acidification.

MATERIALS AND METHODS

Rearing system

A system was developed for the rearing of marine fish eggs, larvae, and juveniles under controlled temperatures and CO₂ levels (Fig. 1). A pH probe (Ag/AgCl electrode) in the conditioning tank was used to regulate the injection of CO₂ to achieve the highest CO₂ treatment. When pH was above the target condition, a solenoid valve opened, introducing CO₂ into the conditioning tank through a fluid-gas membrane contactor. The CO₂-conditioned water was then pumped to a series of 3 header tanks where it was mixed in fixed proportions with ambient seawater to achieve the 3 CO₂ treatments. An additional header tank received only ambient seawater. Water from the elevated header tank for each treatment gravity-fed 4 (16 tanks total) 100 l treatment tanks. Water temperatures were controlled by mixing ambient temperature water with chilled seawater in the conditioning tank prior to CO₂ injection or pumping to elevated header tanks. The outflow from 1 treatment tank in each treatment was diverted past a benchtop meter (VWR SympHony meter SB80PD) with pH and temperature probes for monitoring. Temperature and pH were recorded every 15 to 30 min throughout the experiment. Monitoring pH probes were calibrated approximately weekly using NBS calibration standards of pH 4.0, 7.0, and 10.0.

To describe the carbonate parameters of water in the experiments, water samples were taken 1 to 2 times per week from each treatment. Seawater samples were drawn into pre-cleaned 300 ml Pyrex bottles, treated with HgCl₂ to halt biological activity, sealed, and then sent to the analytical laboratory at the University of Alaska at Fairbanks. These water samples were analyzed for dissolved inorganic carbon (DIC) and total alkalinity (TA) using a VINDTA 3C (Versatile Instrument for the Determination of dissolved inorganic carbon and Total Alkalinity) coupled to a UIC 5014 coulometer. These data were used to calculate the pH, pCO₂, and carbonate mineral saturation states (Ω) of the water using the program developed by Lewis & Wallace (1998).

Experiments were conducted with 4 treatments (ambient, low, medium, and high CO₂). Targets for the manipulated CO₂ treatments were 0.1, 0.3, and 0.7 pH units below the average ambient condition (~8.05). Measured conditions are presented in Table 1. The main departure from targeted conditions occurred during the warm phase of the sub-yearling experiment when local upwelling of deep waters resulted in periodic increases in ambient CO₂ concentration to >700 µatm. During these periods, the pH targets of the manipulated CO₂ treatments were adjusted to maintain separation between treatments, resulting in higher CO₂ levels across all treatments (Table 1).

Yearling growth experiment

Walleye pollock were captured at age-0, 10 to 20 mm total length (Lₜ), from nearshore waters of Puget Sound at Port Townsend, Washington (USA), with a lighted lift net suspended from a dock. Fish were held for at least 24 h in ambient seawater prior to shipment to the Alaska Fisheries Science Center’s laboratory in Newport, Oregon. Fish were reared in groups at 8 to 9°C for 18 mo prior to use in the experi-
Table 1. *Theragra chalcogramma*. Conditions during experiments exposing early life stages of walleye pollock to projected ocean acidification (mean ± SD). Carbonate system parameters (dissolved inorganic carbon, DIC; total alkalinity, TA) were measured 2 to 3 times wk−1 and used to calculate pH, pCO2, and $\Omega_{\text{Aragonite}}$. Seasonal upwelling caused periodic elevation in ambient CO2 during the warm phase of the experiment. Target conditions in other treatments were adjusted to maintain differences between treatments

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Temperature (°C)</th>
<th>DIC (µmol kg$^{-1}$)</th>
<th>TA (µmol kg$^{-1}$)</th>
<th>pH (seawater scale)</th>
<th>pCO2 (µatm)</th>
<th>$\Omega_{\text{Aragonite}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Yearlings</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Ambient</td>
<td>8.7 ± 0.3</td>
<td>2072.2 ± 14.7</td>
<td>2209.3 ± 6.5</td>
<td>8.02 ± 0.04</td>
<td>414 ± 45</td>
<td>1.64 ± 0.13</td>
</tr>
<tr>
<td>Low</td>
<td>8.9 ± 0.3</td>
<td>2091.9 ± 11.7</td>
<td>2210.5 ± 4.7</td>
<td>7.97 ± 0.04</td>
<td>478 ± 50</td>
<td>1.47 ± 0.11</td>
</tr>
<tr>
<td>Medium</td>
<td>8.8 ± 0.3</td>
<td>2159.2 ± 21.9</td>
<td>2213.3 ± 6.0</td>
<td>7.76 ± 0.08</td>
<td>815 ± 167</td>
<td>0.97 ± 0.16</td>
</tr>
<tr>
<td>High</td>
<td>8.7 ± 0.3</td>
<td>2262.3 ± 13.9</td>
<td>2221.0 ± 5.9</td>
<td>7.43 ± 0.05</td>
<td>1805 ± 212</td>
<td>0.46 ± 0.05</td>
</tr>
<tr>
<td><strong>Sub-yearlings: warm phase</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ambient</td>
<td>8.3 ± 0.7</td>
<td>2116.9 ± 45.0</td>
<td>2211.5 ± 5.9</td>
<td>7.89 ± 0.13</td>
<td>596 ± 178</td>
<td>1.28 ± 0.36</td>
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<tr>
<td>Low</td>
<td>8.2 ± 0.7</td>
<td>2161.9 ± 25.7</td>
<td>2211.1 ± 7.7</td>
<td>7.74 ± 0.07</td>
<td>828 ± 144</td>
<td>0.92 ± 0.16</td>
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<tr>
<td>Medium</td>
<td>8.2 ± 0.7</td>
<td>2216.0 ± 25.4</td>
<td>2214.8 ± 5.0</td>
<td>7.57 ± 0.09</td>
<td>1285 ± 321</td>
<td>0.62 ± 0.11</td>
</tr>
<tr>
<td>High</td>
<td>8.3 ± 0.8</td>
<td>2334.6 ± 20.0</td>
<td>2223.8 ± 3.9</td>
<td>7.23 ± 0.05</td>
<td>2894 ± 343</td>
<td>0.29 ± 0.03</td>
</tr>
<tr>
<td><strong>Sub-yearlings: cold phase</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ambient</td>
<td>2.4 ± 0.5</td>
<td>2002.9 ± 27.9</td>
<td>2199.8 ± 8.0</td>
<td>8.24 ± 0.06</td>
<td>225 ± 35</td>
<td>2.13 ± 0.26</td>
</tr>
<tr>
<td>Low</td>
<td>2.4 ± 0.6</td>
<td>2088.2 ± 52.0</td>
<td>2209.1 ± 6.5</td>
<td>8.05 ± 0.13</td>
<td>386 ± 112</td>
<td>1.46 ± 0.45</td>
</tr>
<tr>
<td>Medium</td>
<td>2.4 ± 0.5</td>
<td>2161.6 ± 31.3</td>
<td>2216.6 ± 3.4</td>
<td>7.85 ± 0.10</td>
<td>643 ± 169</td>
<td>0.94 ± 0.21</td>
</tr>
<tr>
<td>High</td>
<td>2.4 ± 0.5</td>
<td>2722.3 ± 23.8</td>
<td>2224.2 ± 5.6</td>
<td>7.48 ± 0.08</td>
<td>1543 ± 293</td>
<td>0.42 ± 0.09</td>
</tr>
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</table>

Initial size of fish used in the experiment was 209.3 ± 11.9 (SD) mm $L_T$ and 72.0 ± 12.4 g wet weight. There were no significant differences in initial size among the treatments (length $F_{3,43} = 1.960$, $p = 0.134$; weight $F_{3,43} = 0.963$, $p = 0.419$). Throughout the experiment, fish were fed gel food to satiation (cessation of feeding) once a day. After 6 wk of rearing, all fish were captured, weighed, measured, and sacrificed. Otoliths were removed and preserved for analysis of increment width and elemental composition. Livers were removed and weighed ($M_I$ to 0.01 g) for calculation of the hepatosomatic index ($I_{HI}$), reflecting variation in lipid storage and nutritional condition.

Growth rates in length ($M_L$ to 0.01 mm d$^{-1}$) and mass ($M_M$ to 0.01 g d$^{-1}$) of each fish were determined based on change in size between initial and final measurements. Although fish were not individually marked, size variation within each tank allowed easy identification of individuals. A whole body condition index ($I_C$) was calculated using the residual weight method (Blackwell et al. 2000). Individual fish condition was expressed as the deviation from the relationship between log$_{10}(M_M)$ and log$_{10}(L_T)$ based on all experimental fish. Similarly, $I_{HI}$ expressed individual deviation from the relationship between log$_{10}(M_I)$ and log$_{10}(L_T)$. For statistical analyses, fish metrics were pooled across replicate tanks, as preliminary analyses indicated no significant differences among tanks within CO2 treatments (all $p > 0.05$). Growth rates and condition indices were analyzed across CO2 treatments with a 1-way ANOVA.

**Yearling otolith analysis**

Otoliths were stored dry prior to analysis. Otoliths were ultrasonically cleaned in NANOpure® (18 megaohm) water for 15 min, dried under Class 100 clean conditions to prevent contamination, and stored dry in acid-washed plastic trays. The left otolith from each fish was embedded in resin (Polytranspar™), sectioned on the transverse plane using an IsoMet® low-speed diamond blade saw (BUEHLER®, and polished with lapping film and Al2O3 powder (0.3 µm). Right otoliths were used when the left otolith was missing or broken. Polished otoliths were photographed under a com-
pound microscope at 1000x magnification. Image-Pro® was used to identify and measure the widths (µm) of daily growth increments at the edge of the otolith along the ventral edge (corresponding to the experimental period). However, as observed in other fish in laboratory culture, daily increments were indistinct in many fish and could not be unambiguously read. We were able to unambiguously measure a series of at least 10 consecutive increments in 20 fish across treatments.

Otolith elemental composition (Mg, Ca, Mn, Sr, and Ba) was quantified using a VG PQ ExCell inductively coupled plasma mass spectrometer with a New Wave DUV193 excimer laser at Oregon State University’s WM Keck Collaboratory for Plasma Spectrometry. Background levels of all analytes were measured before ablation and subtracted from measurements during ablation. Analytes were measured along a transect along the ventral edge that was parallel to growth increments. A pre-ablation was completed with a 100 µm spot size at 1 Hz and 100 µm s⁻¹. For data collection, the laser was set at a 50 µm spot size, 6 Hz, and translated across the sample at 5 µm s⁻¹. Normalized ion ratios (e.g. Mg:Ca) were converted to concentration based on measurements of National Institute of Standards and Technology (NIST) 612 standard glass and are presented as molar ratios (Miller 2009). The mean percent relative standard deviations (%RSD) for NIST 612 standard glass were: 24Mg = 4.3, 43Ca = 3.0, 54Mn = 3.8, 86Sr = 6.7, and 138Ba = 4.1. A calcium carbonate standard (USGS MACS-1) was used to assess accuracy of Mn, Sr, and Ba, which were all within 10% of reported values.

We collected data on water chemistry to confirm that elemental concentrations of rearing waters were not altered by the process of CO₂ level manipulation and to calculate elemental partition coefficients to more accurately determine whether there were detectable changes in the rates of elemental incorporation among CO₂ treatments (Morse & Bender 1990). Weekly water samples were collected from each CO₂ treatment throughout the experiment. Water samples were analyzed with a Leeman-Teledyne inductively coupled plasma-optical emission spectrometer according to the methods of DiMaria et al. (2010). Partition coefficients (D) were then calculated for Mg, Mn, Sr, and Ba using individual otolith elemental ratios (e.g. Mg:Caotolith) and mean tank water ratios (Mg:Cawater). Otolith elemental ratios and partition coefficients were analyzed with a 1-way ANOVA across CO₂ treatments.

Sub-yearling growth and feeding experiment

Age-0 walleye pollock were captured from near-shore nursery grounds and transported to and reared in the laboratory as described above. Six weeks after capture, fish were removed from the rearing tanks, measured (LT to 1.0 mm), and weighed (MW to 0.01 g), and 10 fish were introduced into each of 12 experimental treatment tanks held at ambient pH. Prior to stocking, fish were loosely sorted by size in order to minimize the potential for intra-cohort cannibalism frequently observed in larval and juvenile gadids (Folkvord & Otterå 1993). As a result, there were significant differences in initial size among replicates within treatments (length and mass p < 0.01), but not between treatments (both p > 0.9). This variation in size was small compared to the amount of growth occurring over the experiment; therefore, the 3 tanks are considered treatment replicates in these analyses (with initial mean size included as a covariate where necessary). Initial mean ± SD sizes of fish used in the experiment were 47.7 ± 5.1 mm and 0.63 ± 0.25 g. During the first 4 d after measurement and stocking (tanks at ambient pH), several fish died and were replaced with remaining fish from the holding tanks. On Days 4 to 6 of the experiment, pH levels of the experimental tanks were adjusted to treatment targets. Fish that died later in the experiment (n = 7) were not replaced and were excluded from growth rate calculations.

Fish were reared for 12 wk at 8°C (‘warm phase’). During this phase, fish were fed to apparent satiation once a day with thawed krill (3 times wk⁻¹) or gel food (4 times wk⁻¹). Tank temperatures were checked twice each day and maintained at 8°C. At the end of the warm phase, water temperatures were lowered to a target temperature of 2.5°C over a period of 7 d. Fish were reared at 2.5°C for an additional 15 wk (‘cold phase’), for a cumulative experimental exposure of 28 wk. During the cold phase, feeding was reduced to 3 times wk⁻¹ (thawed krill once per week; gel food twice per week). Data on growth and consumption (see below) during the first 4 wk of the cold phase were not included to allow for thermal acclimation.

Growth rates were measured by weighing and measuring all fish in the experiment at 14 d intervals during the warm phase of the experiment and at 21 d intervals during the cold phase. Mean growth rate of fish in each replicate tank was used as the level of observation in statistical analyses. Tank means were calculated from individual growth trajectories of fish within the tank. Although growth rates of individual fish within each replicate
tank were not used in the analyses, the calculation of individual trajectories provided an additional check on patterns of growth variation among replicates and treatments. Because fish were too small to mark individually, we assumed that size rank was maintained within each replicate tank during the experiment. During each phase of the experiment, the relationship between ln-transformed mass and measurement time was approximately linear, and $g_M \text{ d}^{-1}$ was determined by regressing the measurements of ln(mass) against measurement date for each fish during the 2 phases of the experiment (warm and cold). $g_L \text{ mm d}^{-1}$ was determined by regressing length against date.

Due to the underlying allometry of growth rates among small fishes, there was a significant negative relationship between mean initial mass and tank mean $g_M$ during the warm phase of the experiment (test of initial mass as a covariate, $p = 0.002$), but this effect was consistent across CO2 treatments (homogeneity of slopes, $p = 0.312$). Therefore, tank mean $g_M$ among CO2 treatments was tested using analysis of covariance (ANCOVA) with tank mean initial mass included as a covariate. $g_L$ was similarly tested with mean initial length included as a covariate. During the cold phase of the experiment, $g_M$ and $g_L$ were not correlated with mass at the start of the cold phase (both $p > 0.35$). Therefore, differences among pH treatments were tested with 1-way ANOVA of tank mean growth rate. Similar results were obtained when analyses were conducted with all individual fish growth rates pooled across replicates for each CO2 treatment.

$I_C$ was calculated at the end of each phase of the experiment using the residual weight method, as described above. Separate relationships between $\log_{10}(M_V)$ and $\log_{10}(L_I)$ were derived for each phase of the experiment, and individual fish condition was expressed as the deviation from the phase-specific regressions. The effect of CO2 treatment on $I_C$ was evaluated with a 1-way ANOVA of mean $I_C$ in each replicate tank.

Consumption rates of fish in each experimental tank were measured once per week throughout the growth experiment (except during the acclimation period at the beginning of the cold phase). Feeding schedule and diet schedules were maintained throughout the experiment to minimize daily variation in consumption rates. Pre-weighed meals of gel food were created for each experimental tank. Food was slowly offered to fish in the tank until they stopped feeding (‘apparent satiation’). Food remaining in the meal (unoffered) when the tank reached satiation was weighed. Because of variation in particle size and rapid disintegration of soft foods, we could not estimate the amount of uneaten food remaining in the tank. However, this was minimized by reducing the rate of food addition as fish fed less vigorously. Meals were kept frozen until 15 min prior to feeding and were covered throughout the trial to minimize desiccation of unused food (<1% based on replicate, unused meals prepared during each feeding trial). Consumption rates of the 3 tanks in each pH treatment were measured simultaneously, and CO2 treatments were fed in a randomized order each week. Total consumption in each tank (g) was converted to weight-specific consumption rate ($C$, g g$^{-1}$) based on total fish mass in each tank. Cumulative fish mass in each tank was based on direct measures 3 d prior to feeding, or interpolated between measurements. Finally, consumption rates during the cold phase of the experiment were converted to daily rates (g g$^{-1}$ d$^{-1}$) to account for the reduced meal frequency (3 times wk$^{-1}$).

$C$ decreased as fish size increased during the warm phase of the experiment (test of mean size as a covariate, $p < 0.01$), but this effect was consistent across CO2 treatments (homogeneity of slopes, $p = 0.928$). Interestingly, during the cold phase of the experiment, the opposite pattern was observed with consumption rates increasing with increasing body size (test of mean size as a covariate, $p < 0.01$). Therefore, weekly measurements of tank consumption rates were corrected to a standard mean fish mass (2 g in warm phase; 6 g in cold phase) and averaged across each phase of the experiment for each replicate tank. Size-corrected tank mean consumption rates were tested across CO2 treatments with 1-way ANOVA.

**RESULTS**

**Yearling growth and condition**

No mortalities were associated with CO2 treatments (1 fish jumped from the tank). Averaged across all treatments, fish increased in length by 28.85 ± 8.25 mm (SD) and increased in mass by 25.60 ± 7.84 g (36.02 ± 11.22 % over initial mass). There was no significant difference in growth rates across CO2 treatments ($g_L$: $F_{3,42} = 0.214$, $p = 0.886$; $g_M$: $F_{3,42} = 0.129$, $p = 0.942$; Fig. 2). There was also no significant difference among CO2 treatments in $I_C$ ($F_{3,42} = 0.777$, $p = 0.514$) or $I_H$ ($F_{3,42} = 1.255$, $p = 0.302$) measured at the end of the experiment.
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Mean otolith increment width (MIW) averaged 3.57 (± 0.891 SD) and ranged from 1.81 to 4.80 µm d−1 across all treatments. There was a significant effect of CO2 treatment on MIW (F3,16 = 7.59, p = 0.002). Post hoc comparisons indicated that MIW in the ambient CO2 treatment was lower than in all other treatments (Fig. 3), which did not vary significantly. Elemental composition of the water (Mg:Ca, Mn:Ca, Sr:Ca, and Ba:Ca) did not vary among treatments (all p > 0.59). There was no significant effect of CO2 treatment on otolith elemental composition (Table 2; all F3,43 < 1.90, all p > 0.10) or elemental partition coefficients (all F3,43 < 2.10, all p > 0.11) for any of the elemental ratios.

Sub-yearling experiment: warm phase

After the initial handling and acclimation period, there were only 5 mortalities among the 120 fish used in the experiment, and these were not clustered in any specific treatment or tank. During the warm phase of the experiment, gL and gM averaged 0.43 ± 0.05 (SD) mm d−1 and 0.022 ± 0.002 d−1, respectively, with faster fish growth at the 2 higher CO2 treat-

<table>
<thead>
<tr>
<th>CO2 Treatment</th>
<th>Low</th>
<th>Medium</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>pCO2 (µatm)</td>
<td>478</td>
<td>815</td>
<td>1805</td>
</tr>
<tr>
<td>n</td>
<td>12</td>
<td>11</td>
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<th>Elemental ratios</th>
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<th>Low (478 µatm)</th>
<th>Medium (815 µatm)</th>
<th>High (1805 µatm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 12</td>
<td>n = 12</td>
<td>n = 11</td>
<td>n = 12</td>
</tr>
<tr>
<td>Mg:Ca&lt;sub&gt;otolith&lt;/sub&gt;</td>
<td>0.216 ± 0.036</td>
<td>0.203 ± 0.028</td>
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<td>0.216 ± 0.044</td>
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<tr>
<td>Sr:Ca&lt;sub&gt;otolith&lt;/sub&gt;</td>
<td>1.284 ± 0.124</td>
<td>1.273 ± 0.123</td>
<td>1.393 ± 0.201</td>
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<tr>
<td>Ba:Ca&lt;sub&gt;otolith&lt;/sub&gt;</td>
<td>1.051 ± 0.239</td>
<td>1.004 ± 0.364</td>
<td>1.339 ± 0.455</td>
<td>1.000 ± 0.208</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Partition coefficients</th>
<th>Low (478 µatm)</th>
<th>Medium (815 µatm)</th>
<th>High (1805 µatm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D&lt;sub&gt;mg&lt;/sub&gt; (×1000)</td>
<td>0.042 ± 0.007</td>
<td>0.039 ± 0.006</td>
<td>0.039 ± 0.005</td>
</tr>
<tr>
<td>D&lt;sub&gt;mn&lt;/sub&gt;</td>
<td>0.596 ± 0.351</td>
<td>0.560 ± 0.196</td>
<td>0.574 ± 0.246</td>
</tr>
<tr>
<td>D&lt;sub&gt;sr&lt;/sub&gt;</td>
<td>0.151 ± 0.015</td>
<td>0.149 ± 0.014</td>
<td>0.164 ± 0.024</td>
</tr>
<tr>
<td>D&lt;sub&gt;ba&lt;/sub&gt;</td>
<td>0.352 ± 0.080</td>
<td>0.346 ± 0.125</td>
<td>0.430 ± 0.146</td>
</tr>
</tbody>
</table>

Fig. 2. Theragra chalcogramma. Growth and condition of yearling walleye pollock reared under elevated CO2 levels. Points are the means (± SD) of fish pooled across 3 replicate tanks in each treatment. In the bottom panel, filled circles are wet mass condition factor (I<sub>C</sub>); open circles are hepato somatic index (I<sub>H</sub>); overlapping points are offset for clarity.

Fig. 3. Theragra chalcogramma. Mean otolith increment width of yearling walleye pollock reared under elevated CO2 levels. Points are the means (± SD) of fish pooled across 3 replicate tanks in each treatment.
ments (Fig. 4). Taking into account variation in mean size at the beginning of the experiment (treated as a covariate), there was a significant effect of CO2 treatment on tank mean growth rates (ANCOVA, \( g_L: F_{3,7} = 10.217, p = 0.006; g_M: F_{3,7} = 292.4, p < 0.001 \)). There was no significant interaction between CO2 treatment and initial size on \( g_L \) or \( g_M \) (homogeneity of slopes among treatments, both \( p > 0.30 \)). Post-hoc LSD tests indicated that growth rates in the 2 higher CO2 treatments were significantly greater than those in both of the lower CO2 treatments. In the 2 higher CO2 treatments, mean growth rates in length and mass, respectively, averaged 7.2 and 2.3% greater than in the 2 lower CO2 treatments.

There was no effect of CO2 treatment on wet weight condition factor following 12 wk of rearing. Mean \( I_C \) did not differ among pH treatments (ANOVA, \( F_{3,8} = 0.483, p = 0.703 \)). Tank mean \( I_C \) was not correlated with either \( g_L \) (\( r = 0.005, p = 0.987 \)) or \( g_M \) (\( r = -0.156, p = 0.625 \)).

\( C \) for each tank measured throughout the warm phase of the experiment did not differ significantly across CO2 treatments (Fig. 5; ANOVA, \( F_{3,8} = 1.281, p = 0.345 \)). However, across all CO2 treatments, \( C \) tended to be higher in tanks with higher mean growth rates (\( g_L: r = 0.519, p = 0.084; g_M: r = 0.574, p = 0.051 \)), suggesting that higher growth rates in the higher CO2 treatments were accomplished via higher consumption rates.

**Sub-yearling experiment: cold phase**

Growth rates during the cold phase were lower than during the warm phase of the experiment, with \( g_L \) and \( g_M \) averaging 0.146 ± 0.031 (SD) mm d\(^{-1}\) and 0.007 ± 0.002 d\(^{-1}\), respectively. Although there was a trend toward faster growth in the higher CO2 treatments, this result was not significant (Fig. 4; ANOVA, \( g_L: F_{3,8} = 2.707, p = 0.116; g_M: F_{3,8} = 0.8, p = 0.511 \)). There was no effect of CO2 treatment on \( I_C \) at the end of the cold phase. Mean \( I_C \) did not differ among CO2 treatments (ANOVA, \( F_{3,8} = 0.167, p = 0.915 \)). Tank mean \( I_C \) was not correlated with growth rates (\( g_L: r = -0.172, p = 0.593; g_M: r = -0.150, p = 0.641 \)).

Differences in body size among CO2 treatments generated by differences in growth rates during the warm phase of the experiment carried over to the end
of the cold phase. There were slight differences in size among CO2 treatments following 29 wk of rearing (warm and cold phases combined), during which fish had more than doubled in length and increased 10-fold in mass (ANCOVA tank mean final size with tank mean initial size as covariate, length: F3,7 = 3.97, p = 0.061; mass: F3,7 = 4.47, p = 0.047). The mean size of fish in the medium CO2 treatment tanks (corrected for variation in initial sizes) was 3.47 mm and 0.61 g greater than the fish in the ambient CO2 treatment.

C for each tank measured throughout the cold phase of the experiment did not differ significantly across CO2 treatments, but there was a trend toward higher C in the ambient treatment (ANOVA, F3,8 = 3.34, p = 0.077). Across CO2 treatments, C during the cold phase was not correlated with either increases in length (r = −0.294, p = 0.354) or mass (r = −0.376, p = 0.229).

**DISCUSSION**

There is significant concern that ocean acidification, caused by the dissolution of anthropogenically released CO2 into the ocean, will cause major disruptions to the productivity and functioning of high-latitude marine ecosystems (Cooley & Doney 2009, Fabry et al. 2009). Increased CO2 and decreased pH is known to influence a variety of physiological and behavioral processes in a wide range of marine organisms (Fabry et al. 2008, Munday et al. 2009b), but as of yet there is little understanding of relative sensitivities among species within broad taxonomic guilds. In laboratory experiments, we found juvenile walleye pollock to be resilient to the direct effects of elevated environmental CO2; growth rates of yearlings and sub-yearlings were not negatively affected by elevated levels of environmental CO2, even under prolonged exposures. This resiliency was apparent under seasonally warm and cold conditions, and walleye pollock appeared resilient to conditions well beyond the range of CO2 and pH predicted for the North Pacific Ocean and Bering Sea over the next century. While the conclusions drawn here are derived from experiments with juveniles, a similar resiliency was observed in companion experiments with egg and larval stages of walleye pollock (T. Hurst unpubl.). However, ocean acidification has been shown to induce other physiological and behavioral responses that have yet to be evaluated in this and other North Pacific resource species.

We conducted 2 independent experiments to evaluate different aspects of potential responses of juvenile walleye pollock to elevated CO2 levels. A short-term (6 wk), acute exposure experiment with yearling walleye pollock was conducted first to evaluate general sensitivity of the species to elevated CO2 and examine otolith deposition responses. Concern has been expressed that the resiliency observed in fishes to simulated ocean acidification may be limited in scope and that most ocean acidification experiments have been of insufficient duration to capture potential cumulative effects of chronic exposures to elevated CO2 conditions (Riebesell et al. 2010, but see Melzner et al. 2009a). To examine the potential for longer-term effects, a second experiment was conducted with smaller sub-yearlings. In addition to simply extending the duration of exposure, the long-term experiment included seasonally-reflective warm and cold phases. Most ocean acidification experiments have been conducted on the warmer end of the species’ thermal range (but see Walther et al. 2011), in some cases testing organisms at temperatures near or above current exposure limits (e.g. Munday et al. 2009a). However, it is important to recognize that arctic and subarctic fishes, and species living in highly seasonal environments, will continue to be exposed to low winter temperatures. It has been hypothesized that ocean acidification may restrict the ‘thermal window’ of fishes by reducing physiological performance at both high and low temperatures (Pörtner 2010). Low temperatures are known to reduce the effectiveness of ion balance at both the cellular and organismal level and depress feeding ability (Ibarz et al. 2010). The degree to which low temperature responses interact with, or exacerbate the effects of, other physiochemical stressors is largely unknown (Hurst 2007). For walleye pollock, it was important to determine whether the effects of ocean acidification are more pronounced at the upper or lower end of the thermal range, as potential interactions with low temperature stress would disproportionately affect the high-latitude populations in the Gulf of Alaska and Bering Sea regions which support major commercial fisheries. Additional experiments evaluated responses of eggs and larvae, which may be expected to be more sensitive to the effects of ocean acidification (Ishimatsu et al. 2008).

**Growth energetics**

Survival and growth rates of sub-yearling and yearling walleye pollock were not negatively affected by exposure to elevated levels of environmental CO2 and reduced pH. This resiliency appears to
apply across a broad range of CO$_2$ levels and does not appear to be compromised by the physiological constraints imposed by low temperatures. The results observed here for walleye pollock are generally consistent with hypothesized (Michaelidis et al. 2007, Melzner et al. 2009b) and observed responses in other juvenile fishes (Ishimatsu et al. 2008). In intensive aquaculture settings, negative growth effects were not seen in juvenile Atlantic cod Gadus morhua at pH as low as 7.1 (Foss et al. 2006) and were only seen in juvenile spotted wolfish Anarhichas minor at extreme pH levels (<6.5; Foss et al. 2003). In fact, during the warm phase of our sub-yearling experiment, growth rates were slightly (but significantly) higher in the higher CO$_2$ treatments, a pattern also observed in several other studies (Munday et al. 2009c, Frommel et al. 2012).

While it is notable that we did not see negative effects of elevated CO$_2$ on somatic growth rates of walleye pollock, measurement of growth rates does not provide a complete picture of potential energetic effects of ocean acidification (Cohen & Holcomb 2009, Nowicki et al. 2012). Fish have the capacity to increase feeding in response to energetic stress. While most studies of compensatory feeding have examined responses to reduced energy stores following periods of food deprivation (Ali et al. 2003), other studies have documented elevated feeding rates in response to increased metabolic costs (Hurst & Conover 2001). In the experiment with sub-yearling walleye pollock, measurement of consumption rates allowed us to confirm that fish were not maintaining growth rates in the face of increased metabolic demands through compensatory feeding. Similar conclusions were reached in experiments where spotted wolfish and Pacific cod Gadus macrocephalus exhibited similar growth efficiencies across the range of CO$_2$ concentrations used here (Foss et al. 2003, T. Hurst unpubl.). Further, there was no effect of rearing CO$_2$ on condition factor of sub-yearling or yearling walleye pollock, suggesting that significant amounts of energy were not being diverted from accumulation of reserves in order to maintain adequate growth (Hurst et al. 2005). The metabolic costs of swimming activity represent the final piece of the energetic budget that could be adjusted to compensate for elevated metabolic costs. Although activity levels were not explicitly measured in these experiments, routine observations did not suggest overall differences in activity levels of fish in the different CO$_2$ treatments. While several experiments have demonstrated some behavioral consequences of ocean acidification (Munday et al. 2009b, Dixson et al. 2010), there have been no documented cases of compensatory reductions in swimming activity (Nowicki et al. 2012, Maneja et al. in press) or reduced maximum swimming capacity (Melzner et al. 2009a, Munday et al. 2009a) of fish reared under elevated environmentally relevant CO$_2$ levels. For these species, if exposure to environmental hypercapnia does in fact induce an ongoing metabolic expense, the magnitude of that expenditure appears to be negligible in the context of the overall energy budget.

Otolith responses

Having an internal skeleton composed primarily of calcium phosphate, marine fishes are generally assumed to be less sensitive to the effects of ocean acidification than invertebrates which precipitate external skeletons of calcium carbonate (Cooley & Doney 2009, Kroeker et al. 2010). Juvenile and adult fishes have highly developed systems for acid–base regulation and gas exchange. The physiological responses to environmental hypercapnia in fishes are well described and include an active increase in extracellular HCO$_3^-$, which minimizes variance in blood pH (Melzner et al. 2009b). This increase in internal buffering was observed in yearling walleye pollock (E. R. Fernandez unpubl.) and has been suggested as the driver of changes in otolith calcification rates observed in some species under ocean acidification (Checkley et al. 2009, Munday et al. 2011b). Despite limited sample sizes, analysis of daily increment widths in otoliths of yearling walleye pollock demonstrated that the ocean acidification effects on otoliths are not limited to larval stages. However, as yearling walleye pollock in this experiment were reared under elevated CO$_2$ levels for 6 wk, it is unclear whether such an increased deposition rate response would persist under prolonged exposure to elevated CO$_2$. The ultimate consequences of increased deposition rates (and larger otolith sizes) for otolith function in hearing and orientation in temperate fishes are still unknown. Reduced (or reversed) responses to auditory cues were observed in clownfish Amphiprion percula (Simpson et al. 2011), a species in which there were no apparent differences in otolith size or symmetry in response to environmental hypercapnia (Munday et al. 2011b). Alternatively, the altered behavioral response to auditory cues in clownfish may be related to an alteration of the activity of the GABA-A receptor, as this receptor has also been linked to ocean acidification-induced behavioral responsiveness to olfactory stimuli (Dixson et al. 2011).
2010, Nilsson et al. 2012). Alteration of Cl− and/or HCO3− gradients over neuronal membranes, which result from acid-balance compensation for increased blood and tissue CO2, appear to stimulate an excitatory neuronal response rather than the normal inhibitory response, thus resulting in abnormal behaviors (Nilsson et al. 2012). Interestingly, whereas the physiological responses to hypercapnia of elevated blood HCO3− may have altered neureceptor function and precipitation rate of the otolith matrix, there was no concurrent change in the elemental composition of otoliths of walleye pollock or clownfish (Munday et al. 2011b).

**Population exposure history**

A significant unknown in predicting the consequences of ocean acidification for marine communities is the potential for acclimation or evolutionary adaptation to new climate conditions (but see Parker et al. 2011, Miller et al. in press). In interpreting the results from laboratory exposures to elevated CO2 for predicting species’ responses to ocean acidification, it is critical to consider life history and natural patterns of environmental variation experienced by the species or population (Hofmann et al. 2011, McElhaney & Busch in press). For example, marine species or populations that naturally experience episodic (diurnal or seasonal) or chronic exposures (near seafloor CO2 vents or seeps) to high CO2 may be less sensitive to increases attributable to anthropogenic CO2 releases (Munday et al. 2009a, Hofmann et al. 2010). In this study, we found that juvenile walleye pollock captured from Puget Sound were resilient to even prolonged exposure to markedly elevated CO2 levels. Some deep areas of Puget Sound currently experience pH levels below 7.7 due to reduced mixing rates and natural or anthropogenically enhanced microbial respiration (Feely et al. 2010). Prior exposure of Puget Sound walleye pollock to elevated CO2 levels may have preconditioned the population (via either acclimation or adaptation), contributing to the observed resiliency to the effects of high CO2 (Miller et al. 2012). However, seasonal upwelling and respiration of exported organic matter over the shelf can also create low pH conditions in the 7.7 to 7.8 range in summer and fall in the Gulf of Alaska and the Bering Sea (Mathis et al. in press). Further, Puget Sound walleye pollock were resilient to CO2 levels far exceeding those anticipated for either region over the next 100 yr. Finally, Bering Sea and Gulf of Alaska walleye pollock populations would be expected to undergo similar adaptation or acclimation to long-term changes in environmental CO2 (Hofmann et al. 2010). Hence, it is likely that the general resiliency observed for Puget Sound walleye pollock applies equally to Alaskan populations facing ocean acidification.

**CONCLUSIONS**

Ocean acidification is predicted to have significant effects on high-latitude ecosystems (Fabry et al. 2009). Several recent studies have documented negative direct effects of elevated CO2 on developing fish eggs and larvae (Baumann et al. 2012, Frommel et al. 2012). However, ocean acidification did not appear to negatively affect the growth energetics of juvenile walleye pollock. Throughout our experiments, there was no evidence that exposure to elevated CO2 reduced growth or condition, or required elevated consumption rates to offset increased metabolic costs. In fact, as has been seen in other studies (Munday et al. 2009c, Frommel et al. 2012), a trend toward higher growth rates in treatments with higher CO2 levels among sub-yearlings was observed. Even in this case, the magnitude of the CO2 effect was smaller than those induced in marine gadids by environmentally relevant variation in temperature (Hurst et al. 2010) or prey availability (Laurel et al. 2011). Hence, the results presented here, and parallel work on eggs and larval stages (T. Hurst unpubl.) suggest that production of walleye pollock appears more resilient to the direct effects of ocean acidification than other aspects of long-term climate variation (Munday et al. 2009a, Hunt et al. 2011, Mueter et al. 2011). Additional work should be focused on the potential consequences of ocean acidification-induced sensory impairment and the indirect consequences of food web alterations.

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