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Effects of ocean acidification on hatch size and larval growth of walleye pollock (*Theragra chalcogramma*)

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Rising atmospheric concentrations of CO_2 are predicted to decrease the pH of high-latitude oceans by 0.3–0.5 units by 2100. Because of their limited capacity for ion exchange, embryos and larvae of marine fishes are predicted to be more sensitive to elevated CO_2 than juveniles and adults. Eggs and larvae of walleye pollock (*Theragra chalcogramma*) were incubated across a broad range of CO_2 levels (280–2100 μatm) to evaluate sensitivity in this critical resource species. Slightly elevated CO_2 levels (~450 μatm) resulted in earlier hatching times, but differences among egg batches were greater than those observed across CO_2 treatments. Egg batches differed significantly in size-at-hatch metrics, but we observed no consistent effect of CO_2 level. In three independent experiments, walleye pollock were reared at ambient and elevated CO_2 levels through the early larval stage (to ~30 days post-hatch). Across trials, there were only minor effects of CO_2 level on size and growth rate, but fish in the ambient treatments tended to be slightly smaller than fish reared at elevated CO_2 levels. These results suggest that growth potential of early life stages of walleye pollock is resilient with respect to the direct physiological effects of ocean acidification.

Keywords: climate change, early life history, fishes, gadids, growth rate, hatching, hypercapnia.

Introduction

Climate variation is known to drive productivity in many marine fishery species and some analyses suggest that long-term temperature increases will result in diminished fishery production (Mueter *et al.*, 2011; Cheung *et al.*, 2012). There is a significant concern that ocean acidification could further diminish the productivity of some species (Cooley and Doney, 2009; Denman *et al.*, 2011). Ocean acidification is occurring throughout the world's oceans due to the release of terrestrially sequestered CO_2 into the atmosphere and the subsequent diffusion of approximately one-third of that anthropogenically released CO_2 into the ocean (Feely *et al.*, 2004; Sabine *et al.*, 2004; Orr *et al.*, 2005). The dissolution of CO_2 into ocean waters results in a decrease in pH and reduces the availability of carbonate ions. Regional patterns of oceanic CO_2 concentrations and pH are driven by local flow fields and patterns of primary production (McElhany and Busch, 2012), with high-latitude seas predicted to be most affected by the combination of increasing temperatures and acidification (Fabry *et al.*, 2009; Mathis *et al.*, 2011). The Arctic Ocean is projected to experience pH declines of up to 0.45 units during the next century, with large regions becoming consistently undersaturated with respect to aragonite (Yamamoto-Kawai *et al.*, 2009; Steinacher *et al.*, 2009).

Experimental evidence is accumulating that elevated CO_2 ("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). The magnitude and nature of ocean acidification effects will vary among species and guilds but have been examined in only a small number of species to date (Ries *et al.*, 2009; Kroeker *et al.*, 2010). In general, fishes are expected to be more resilient to the direct effects of ocean acidification than calcifying marine invertebrates (Pörtner *et al.*, 2004; Melzner *et al.*, 2009). However, embryonic

and larval stages of fishes may be vulnerable to decreasing environmental pH due to their high surface-to-volume ratio and lack of specialized mechanisms for acid-base regulation (Kikkawa *et al.*, 2003; Ishimatsu *et al.*, 2008). Studies have documented reduced survival (Baumann *et al.*, 2012), increased incidence of morphological deformities (Frommel *et al.*, 2012), and sensory and cognitive impairment (Munday *et al.*, 2009b; Devine *et al.*, 2011) in larvae reared at elevated CO_2 levels. Because mortality rates among early life stages are high and tend to select against smaller individuals in the population, slight changes in early growth can have persistent effects (Hurst *et al.*, 2010a) and influence recruitment rates to natural populations (Houde, 1987). Additional work is needed to study the range of responses to projected ocean acidification in fishes, especially in the temperate and boreal marine species that support much of the world's fishery production.

Walleye pollock (*Theragra chalcogramma*) supports the largest single-species fishery in the US, with annual harvests averaging over 1.1 million metric tons over the past decade. In addition to their economic importance, walleye pollock are critical prey for a number of other fishery species, marine mammals, and seabirds (Livingston, 1993). Walleye pollock spawn at depth (usually >50 m) in late winter. Eggs and pre-flexion larvae drift at depth (~200 m in the Gulf of Alaska, Brodeur and Wilson, 1996) or rise to the upper 50 m (Smart *et al.*, 2012). It has been suggested that species-specific sensitivity to ocean acidification may be related to patterns of CO_2 variation in the habitats inhabited at different life stages (Munday *et al.*, 2011b). Midwater pelagic areas are more physiochemically stable than shallow coastal waters which can exhibit significant natural, short-term variation in CO_2 levels (Hofmann *et al.*, 2011; McElhany and Busch, 2012). If walleye pollock early life stages are adapted to these stable pelagic waters they may be more sensitive to projected increases in ocean CO_2 levels than other North Pacific fishery species with demersal spawning or shallow subtidal nursery areas (Munday *et al.*, 2011b).

In this study, we examined the direct effects of projected levels of ocean acidification on the eggs and larvae of walleye pollock in a series of laboratory experiments. A companion study examined effects of elevated CO_2 levels on juvenile walleye pollock (Hurst *et al.*, 2012). Because of the direct linkage to population productivity, the experiments presented here focus on determining the effects of elevated CO_2 levels on size-at-hatch and early larval growth rates. In multiple independent experiments, walleye pollock eggs and larvae were reared across a range of CO_2 levels to determine stage-specific sensitivity to environmental hypercapnia. Treatments were selected to reflect ambient conditions and conditions predicted to occur in high latitude seas in the next century (400–600 μatm increase; IPCC, 2007). A high CO_2 treatment (~2,100 μatm) was included as a conservative test of physiological sensitivity to elevated environmental CO_2 (Riebesell *et al.*, 2010).

Methods

Rearing system

A flow-through system was developed for the rearing of marine fish eggs, larvae, and juveniles under controlled temperature and pH conditions (additional details and schematic in Hurst *et al.*, 2012). Ambient temperature and chilled seawater were mixed to achieve 8°C in two conditioning tanks. Injection of CO_2 into one of these conditioning tanks was regulated with a pH probe (Ag/AgCl electrode, Aqua Medic) to achieve the high CO_2 treatment (pH ~7.4). The second tank was maintained at the ambient CO_2 level. Water from these conditioning tanks was pumped to two header tanks for the high CO_2 and ambient treatments, respectively. Two additional header tanks received a continuous supply of both ambient and high CO_2 seawater in fixed ratios to establish two treatments with intermediate levels of elevated CO_2 (medium and low CO_2 treatments). Each header tank gravity-fed four 100-L rearing tanks at a rate of 500 mL/min. Benchtop meters (VWR Symphony meter SB80PD) recorded the pH of outflow from one rearing tank in each treatment at 30 min intervals to monitor general functioning of the system. Carbonate conditions during experiments were described from chemical analysis of "bottle samples" drawn from each treatment header tank 2-3 times per week. Water samples were poisoned with $HgCl_2$ and shipped to the Ocean Acidification Research Center at the University of Alaska at Fairbanks. Samples were analysed 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, pCO_2, and carbonate mineral saturation states (Ω) of the water using the program developed by Lewis and Wallace (1998; Table 1). Water samples collected during trial 2 of the larval experiments were lost in shipping between labs. Therefore, for this period we calculated pH and CO_2 levels in rearing water based on the observed relationships between benchtop meters and chemical analysis of bottle samples from adjacent experimental periods. The consistency in carbonate conditions (e.g. mean coefficient of variation in TA of 0.3%) across egg incubations and larval trials suggests that this approach effectively captures the general pattern of conditions across experimental treatments in trial 2.

Parental broodstock

Eggs for these experiments were produced over three spawning seasons by a captive broodstock of walleye pollock at the AFSC laboratory in Newport, Oregon. Fish for the broodstock were collected as early-juveniles (~10-20 mm total length) from coastal waters of Puget Sound at Port Townsend, Washington (48.135°N 122.760°W) and reared in the laboratory (see Hurst *et al.*, 2012 for additional details). A group of 40 age 3–6 walleye pollock (estimated 1:1 sex ratio) were held in 6-m tanks under a seasonally varying photoperiod. Temperatures in the spawner tanks were maintained at 7–9°C during the summer and reduced to 2–6°C during the spawning season. Although we did not monitor pH or CO_2 levels in the adult broodstock tanks regularly, measurements of ambient seawater during experiments presented here and in Hurst *et al.* (2012) demonstrate strong seasonal variation. During the summer, coastal upwelling brings CO_2–rich water to the surface (Gruber *et al.*, 2012) with ambient CO_2 levels reaching more than 700 μatm during especially strong upwelling events. However, estimated CO_2 levels during the winter/spring spawning season were in the range 250–350 μatm.

Egg incubation experiments

For experiments in 2010, a subset of female ($n = 11$) fish was captured from the tank and injected with luteinizing hormone at a dose of 0.25 mg·kg^{-1} in order to stimulate egg ripening and

Table 1. Conditions during experimental exposures of walleye pollock eggs and larvae to projected ocean acidification.

Experiment Treatment	Temp. (°C)	DIC (μmol kg^{-1})	TA (μmol kg^{-1})	pH (seawater scale)	pCO_2 (μatm)	$\Omega_{Aragonite}$
Eggs[a]						
Ambient	8.1 ± 0.4	2027.9 ± 19.1	2205.3 ± 5.2	8.13 ± 0.04	310 ± 36	2.01 ± 0.18
Low CO_2	8.2 ± 0.5	2094.1 ± 19.7	2212.0 ± 3.6	7.97 ± 0.06	475 ± 77	1.47 ± 0.18
Medium CO_2	8.1 ± 0.4	2165.4 ± 20.9	2214.7 ± 3.5	7.75 ± 0.07	828 ± 147	0.93 ± 0.13
High CO_2	8.1 ± 0.3	2272.0 ± 15.1	2219.5 ± 6.2	7.40 ± 0.04	1933 ± 204	0.42 ± 0.04
Larvae: trial 1						
Ambient	8.3 ± 0.5	2014.4 ± 17.3	2204.1 ± 5.9	8.16 ± 0.05	287 ± 34	2.12 ± 0.2
Low CO_2	8.4 ± 0.6	2088.3 ± 24.4	2210.8 ± 5.8	7.99 ± 0.07	457 ± 78	1.51 ± 0.2
Medium CO_2	8.5 ± 0.7	2164.3 ± 20.1	2216.5 ± 5.6	7.76 ± 0.08	805 ± 161	0.95 ± 0.14
High CO_2	8.3 ± 0.5	2257.4 ± 21.4	2216.2 ± 6.3	7.43 ± 0.07	1773 ± 259	0.46 ± 0.07
Larvae: trial 2[b]						
Ambient	8.6 ± 0.3	–	–	8.15 ± 0.01	293 ± 6	–
Low CO_2	8.6 ± 0.3	–	–	8.02 ± 0.01	411 ± 7	–
Medium CO_2	8.4 ± 0.3	–	–	7.70 ± 0.01	910 ± 19	–
High CO_2	8.5 ± 0.3	–	–	7.41 ± 0.01	1812 ± 34	–
Larvae: trial 3						
Ambient	8.2 ± 0.7	2025.0 ± 17.4	2210.2 ± 4.9	8.14 ± 0.05	297 ± 39	2.04 ± 0.17
Low CO_2	8.1 ± 0.8	2081.6 ± 14.9	2209.6 ± 15.3	8.01 ± 0.07	426 ± 71	1.52 ± 0.21
Medium CO_2	8.1 ± 0.9	2178.9 ± 12.6	2209.1 ± 4.9	7.68 ± 0.04	941 ± 94	0.78 ± 0.07
High CO_2	8.2 ± 0.8	2262.5 ± 10.3	2212.3 ± 2.6	7.4 ± 0.03	1858 ± 125	0.42 ± 0.03

Carbonate system parameters (dissolved inorganic carbon, DIC; total alkalinity, TA) were measured 2–3 times per week and used to calculate pH, pCO_2 and $\Omega_{Aragonite}$. Carbonate system parameters were estimated assuming a salinity of 30 during egg incubations and larval trial 1, and measured salinities (mean = 29.7) during larval trials 2 and 3. [a]Conditions are the averages of the mean and standard deviation in conditions observed during each of the five egg incubation periods. Response metrics are plotted against batch-specific CO_2 levels in Figures 1 and 2. [b]Water samples during trial 2 were lost. Therefore pH and CO_2 levels were based on observed relationships between benchtop meter readings of pH and calculations of pH and CO_2 based on DIC and TA measurements from adjacent time periods.

release. Following injection, fish were returned to the tank and allowed to spawn naturally. For 2011 experiments, fish were allowed to spawn naturally without hormone induction. Walleye pollock eggs float; they were collected from the surface outflow of the tank. Batches of eggs were collected from the spawning tanks periodically through the spawning period. Although specific parentage of collected eggs was not known, we selected spawning dates with high egg production (reflecting spawning by multiple females) in an attempt to maximize genetic diversity among incubated eggs. Eggs were collected and incubated from a total of five spawning events ("egg batches") for these experiments: 2 batches in 2010 and 3 batches in 2011.

From each egg batch, 3–4 replicate samples of 1.5–2 mL of eggs (approximately 300–400 eggs) were incubated in each of the four CO_2 treatments. Temperatures were maintained at 8°C during the incubation period. The design of the incubation beakers differed between the two years of experiments, but results were consistent across years. In 2010, the eggs were incubated in 100 ml beakers with mesh bottoms to allow water exchange with the pH-controlled tank. Beakers were suspended in the tank and were open to ambient air on the top. Gently raising and lowering each beaker twice daily stirred the eggs and provided further water exchange with the tank. In 2011, eggs were incubated in 800 ml jars with mesh screen over one end. These jars were completely submerged in the pH-controlled tank to eliminate the possibility of deviation from treatment conditions due to water contact with overlying ambient air. Egg beakers were gently shaken each day and 75% water changes were made 3 and 5 DPF prior to the initiation of hatch.

During the egg hatching cycle (beginning at 8 DPF), incubation beakers were checked each morning between 0800 and 1000 for the presence of newly hatched larvae and 50% of water in the incubation beaker was exchanged with tank water. On days when newly hatched larvae were present, all newly hatched larvae were counted and removed from the beakers with a pipette or weak siphon tube. At the end of the hatching cycle (at least 16 DPF) unhatched eggs remaining in each beaker were counted. The mean date of hatching was calculated for each replicate incubation beaker. Standard deviation in date of hatch reflected the degree of synchronicity or dispersion of hatching times within each replicate.

In order to account for change in hatch size through the hatch cycle (Porter and Bailey, 2007a), newly hatched larvae were sampled and measured daily throughout the hatching period. Each day during the hatch cycle, up to 20 newly hatched larvae from each beaker were digitally photographed under a dissecting microscope. An image analysis system was used to measure standard length (SL), myotome height at the anus (MH), eye diameter (ED), and yolk area (YA) from the photographs (Laurel *et al.*, 2008). Larval condition index (K) was calculated as the deviation from a best-fit relationship between $\log_{10}$(MH) and $\log_{10}$(SL). Mean values of each metric in each replicate beaker were calculated by weighting individual fish measurements by the fraction of hatching occurring each day and the fraction of each day's hatch subsampled for measurement. The mean value in each replicate beaker was used as the level of observation in these analyses. Hatching success, time-to-hatch, and size-at-hatch metrics (SL, K, and YA) were analysed with 2-way ANOVA with CO_2 treatment (ambient, low, medium, and high CO_2) and egg batch as fixed and random main effects, respectively.

Larval growth experiments

Three independent larval rearing experiments were conducted with offspring from natural spawning events of broodstock walleye pollock. Walleye pollock larvae have been successfully reared in the laboratory (Porter and Bailey, 2007b; Colton and

Table 2. Summary of experimental conditions in three trials examining growth responses of larval walleye pollock to elevated CO_2 levels. See Table 1 for water chemistry conditions.

Trial	Year	Spawn dates	Egg incubation conditions	Light level ($\mu E/m^2s$)	Initial stocking density	Growth measured (DPH)
1	2011	22–24 March	treatment CO_2	0.7 (uniform)	4000 eggs	5–33
2	2012	19–21 March	ambient CO_2	6.7 (half-shaded)	1100 larvae	7–35
3	2012	5–7 May	treatment CO_2	6.7 (half-shaded)	6000 eggs	10–38

Hurst, 2010) but techniques for large-scale rearing of larval walleye pollock have yet to be thoroughly established. For these experiments, we modified procedures used in experimental studies of related Atlantic cod (*Gadus morhua*, Brown *et al.*, 2003) and Pacific cod (*Gadus macrocephalus*, Hurst *et al.*, 2010b; Laurel *et al.*, 2011) based on the observations of Porter and Bailey (2007b) with walleye pollock. Three independent larval growth trials were conducted with slightly differing experimental protocols (Table 2). For each experiment eggs were collected over a 3-day period from natural broodstock spawning. For trials 1 and 3, approximately 4000 and 6000 eggs, respectively, were incubated in each of three replicate tanks maintained at the four CO_2 treatment levels and reared in these tanks through hatching and the early larval period. All unhatched eggs were removed from the rearing tanks 4 days after the end of the hatching cycle. For trial 2, all eggs were incubated together at ambient CO_2 levels in two 400-L upwelling tanks. At the first feeding stage, 7 days post-hatch (DPH; based on estimated date of peak hatching), 1100 larvae were transferred to three replicate rearing tanks maintained at each of the four CO_2 treatment levels. These stocking densities are within the range commonly used in experimental culture of gadids (e.g. Monk *et al.*, 2006; Laurel *et al.*, 2011) and below levels that might induce density-dependent effects on growth rates (Baskerville-Bridges and Kling, 2000). Larvae were reared in black, 100-l tanks with weak upwelling circulation maintained by light aeration and positioning the in-flow (500 ml/min) at the bottom center of the tank. Light was provided by overhead fluorescent bulbs on a 12:12 h light:dark photoperiod. For trial 1, light levels averaged 0.7 $\mu E/m^2s$ at the water surface; for trials 2 and 3, light levels were 6.7 $\mu E/m^2s$ with horizontal variation in light levels created by an opaque cover on one half of each tank. Prey was introduced beginning 4–5 days after the estimated date of peak hatching. Larvae were reared on a combination of rotifers (*Brachionus plicatilis*) enriched with Algamac 3050 (Aquafauna, Hawthorne, CA) and microparticulate dry food (Otohime A, Marubeni Nisshin Feed Co., Tokyo). Rotifers were supplied at densities of 4 prey·ml^{-1} twice daily beginning approximately 4 DPH and dry food was provided 2–3 times per day beginning approximately 14 DPH.

A sample of 15–20 fish was drawn from each replicate tank at weekly intervals (10-d intervals in trial 1) from the first feeding stage until fish reached 33–38 DPH. Samples were not available from some tanks during trial 1 due to low survival rates. Sampled larvae were digitally photographed under a dissecting microscope and measurements were made of SL and MH as above and used to calculate larval condition index. Mean size of fish in each replicate tank was used as the level of observation in statistical analyses. Within each experiment, size metrics (SL and K) were analysed with 2-way ANOVA with CO_2 level and sampling date as main effects. Post-hoc LSD tests were used to identify differences among treatments on specific sampling dates. In addition, length measurements were used to calculate growth rates in each replicate tank throughout the experiment; these data were analysed across trials with trial number and CO_2 treatment as main effects. Due to potential variation in hatch success among replicates in trials 1 and 3 and initial mortality associated with capture and transport of larvae in trial 2, we were unable to calculate precise estimates of survival. However, the number of fish remaining in each replicate at the end of each trial is presented as a general reflection of relative differences in survival rates among CO_2 treatments.

Results

Egg incubation experiments

Hatching success was highly variable among incubation beakers, ranging from 9–99%, and may have been related to variation in fertilization success during natural tank spawning events or damage during egg capture. There was a significant effect of batch on hatching success ($F_{[4,12]} = 13.090$, $p < 0.001$) with egg batches 1 and 3 having lower hatch success than the other batches. There was no significant main effect of incubation CO_2 ($F_{[3,12]} = 0.411$, $p = 0.748$) or interaction between CO_2 treatment and batch on hatch success ($F_{[12,48]} = 1.441$, $p = 0.181$).

Hatching of walleye pollock eggs occurred between 5 and 14 DPF, with most eggs hatching between 8 and 11 DPF. There was a significant effect of incubation CO_2 on the mean time-to-hatch ($F_{[3,12]} = 5.330$, $p = 0.014$), with the low CO_2 treatment having the shortest time-to-hatch. However, the magnitude of the CO_2 effect (0.18 day s.d. in CO_2 treatment means, pooled across egg batches) was less than the difference between egg batches (0.51 day s.d. in egg batch means, pooled across CO_2 treatments, $F_{[4,12]} = 33.647$, $p < 0.001$; Figure 1). There was no significant interaction between CO_2 treatment and egg batch on time-to-hatch ($F_{[12,48]} = 1.36$, $p = 0.220$).

Across all batches and CO_2 treatments there was a significant negative correlation between time-to-hatch and hatch time variation (standard deviation in hatch time within each incubation replicate; $n = 68$, $r = -0.65$). There was a significant interaction between CO_2 treatment and egg batch on variation in time-to-hatch ($F_{[12,48]} = 2.98$, $p = 0.004$) caused by high synchrony and late hatch of fish in the ambient and high CO_2 treatments in egg batch 5 (Figure 1). There was also a significant main effect of egg batch on hatch time variation ($F_{[4,12]} = 20.310$, $p < 0.001$) with dispersed hatching in batch 4 and synchronized hatching in batch 1. There was no significant main effect of CO_2 level on hatch time variation ($F_{[3,12]} = 0.752$, $p = 0.542$).

The size-at-hatch metrics of SL, K, and YA were significantly correlated with one another at both the individual fish level ($n = 4361$, $p < 0.001$) and among beaker means across the experiment ($n = 68$, $p < 0.01$). Eye diameter was correlated with the other metrics (SL, L, YA) at the individual level ($p < 0.01$) but not among beaker means ($p > 0.15$). There were no consistent

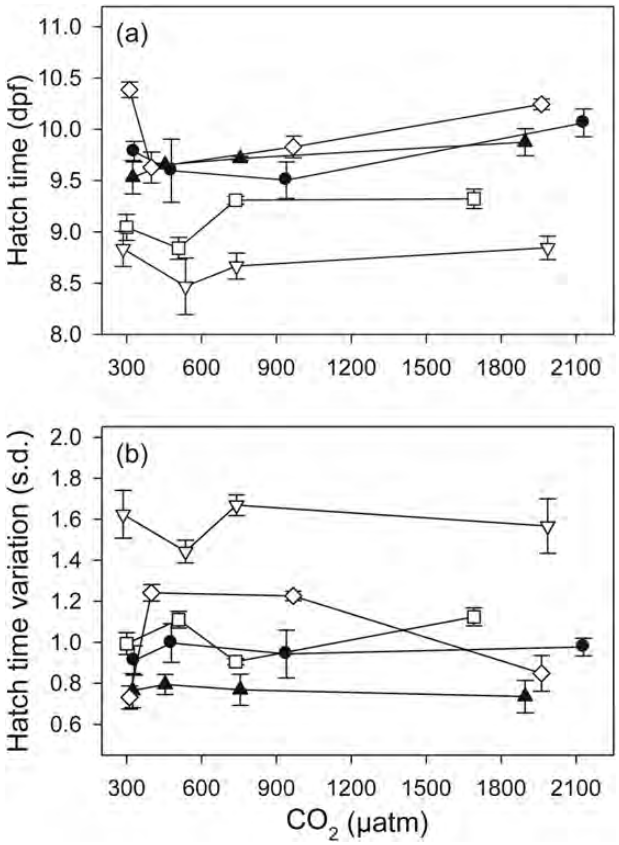

Figure 1. Time-to-hatch (a) and variation in hatch time (b) in walleye pollock embryos as a function of incubation CO_2 level. Points are the mean values ($\pm$ s.e.) of 3–4 replicate incubation beakers in each CO_2 treatment. Symbols represent different batches of incubated eggs collected from natural spawning activity of a laboratory broodstock. Batch 1: triangles, Batch 2: circles, Batch 3: squares, Batch 4: inverted triangles, Batch 5: diamonds; filled symbols 2010 experiments, open symbols 2011 experiments.

effects of incubation CO_2 level on any of the size-at-hatch metrics across the five egg batches (Figure 2, Table 3). There were significant interactions between egg batch and CO_2 level ($F_{[12,47]} > 3.10$, $p < 0.01$) on mean SL, YA, and ED but not on K ($F_{[12,47]} = 1.260$, $p = 0.273$) of walleye pollock at hatch. There was no significant main effect of CO_2 level on any of the size-at-hatch characteristics ($F_{[3,12]} < 1.20$, $p > 0.20$) but all metrics varied significantly between egg batches ($F_{[4,12]} > 8.0$, $p < 0.01$), with larvae from egg batch 2 being smaller at hatch than the other batches. In addition, the magnitude of differences between egg batches was much greater than that observed between CO_2 treatments (range of means pooled across batches was 4.9–7.6 times the range of means pooled across CO_2 treatments). Further, the general pattern of non-significant responses to CO_2 treatment are not due to low power associated with using replicate means as the level of observation; similar results were obtained in tests pooled across replicate beakers using all individual fish measurements as the level of observation.

Larval growth experiments

Using the number of fish remaining in each tank at the end of each experiment as a reflection of relative survival rates (Figure 3), there was no clear effect of CO_2 treatment on larval survival. Two

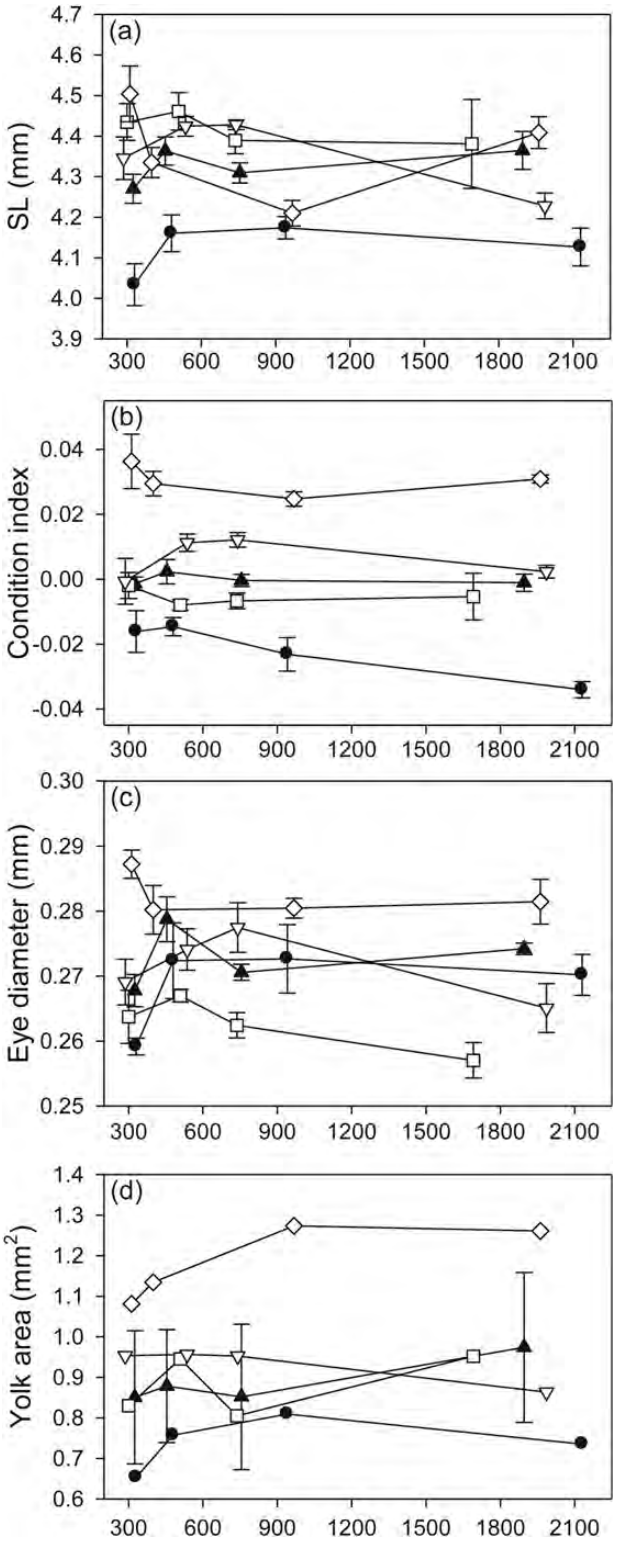

Figure 2. Size-at-hatch metrics of walleye pollock embryos as a function of incubation CO_2 level. Points are the mean values ($\pm$ s.e.) of 3–4 replicate incubation beakers in each CO_2 treatment. Symbols represent different batches of incubated eggs collected from natural spawning activity of a laboratory broodstock. Batch 1: triangles, Batch 2: circles, Batch 3: squares, Batch 4: inverted triangles, Batch 5: diamonds; filled symbols 2010 experiments, open symbols 2011 experiments.

Table 3. Analysis of variance of hatching metrics of five batches of walleye pollock eggs incubated across a range of CO_2 levels.

Measure	Factor	d.f.	F	*p*
Time-to-hatch				
	Egg batch	4	33.647	<0.001
	CO_2 level	3	5.330	0.014
	CO_2 × batch	12	1.357	0.220
	Error	48		
SL at hatch				
	Egg batch	4	5.700	0.008
	CO_2 level	3	0.451	0.721
	CO_2 × batch	12	3.726	<0.001
	Error	48		
Condition at hatch				
	Egg batch	4	48.199	<0.001
	CO_2 level	3	1.001	0.425
	CO_2 × batch	12	1.786	0.077
	Error	48		
Eye diameter at hatch				
	Egg batch	4	8.276	0.002
	CO_2 level	3	1.346	0.305
	CO_2 × batch	12	2.038	0.041
	Error	48		
Yolk area at hatch				
	Egg batch	4	27.346	<0.001
	CO_2 level	3	1.895	0.184
	CO_2 × batch	12	3.219	0.002
	Error	48		

Response variables are the mean values measured in 3–4 replicate incubation beakers for each batch in each CO_2 treatment.

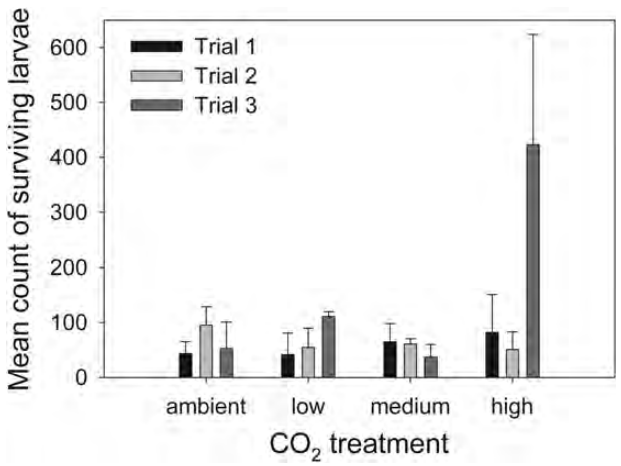

Figure 3. Mean number of larvae remaining at the end of each experiment (±s.e.) of three replicate tanks in each CO_2 treatment.

replicates of the high CO_2 treatment in trial 3 had more than twice the number of remaining fish than in all other tanks during that trial, but the trend toward high survival at high CO_2 was not observed in the other two trials. Variation in fish density did not appear to impose density-dependent constraints, as the number of fish remaining at the end of the trial was not negatively correlated (in any trial or pooled across trials) with tank-specific growth rates or final condition (all $r > -0.40$).

In general, there were only minor effects of CO_2 treatment on body sizes, but on the last sampling date of each trial, fish in the ambient treatment were smaller than fish in the elevated CO_2 treatments (but not significantly so; Figure 3). Further, while there was a trend toward lower growth rates in the ambient CO_2 treatments, growth rates were more variable among the three trials than they were among the four CO_2 treatments (Figure 4).

There were no significant interactions between CO_2 level and sampling date on walleye pollock SL in any of the three experimental trials ($p > 0.45$; Table 4). In trial 1, fish in the medium CO_2 treatment were smaller than fish in most other treatments

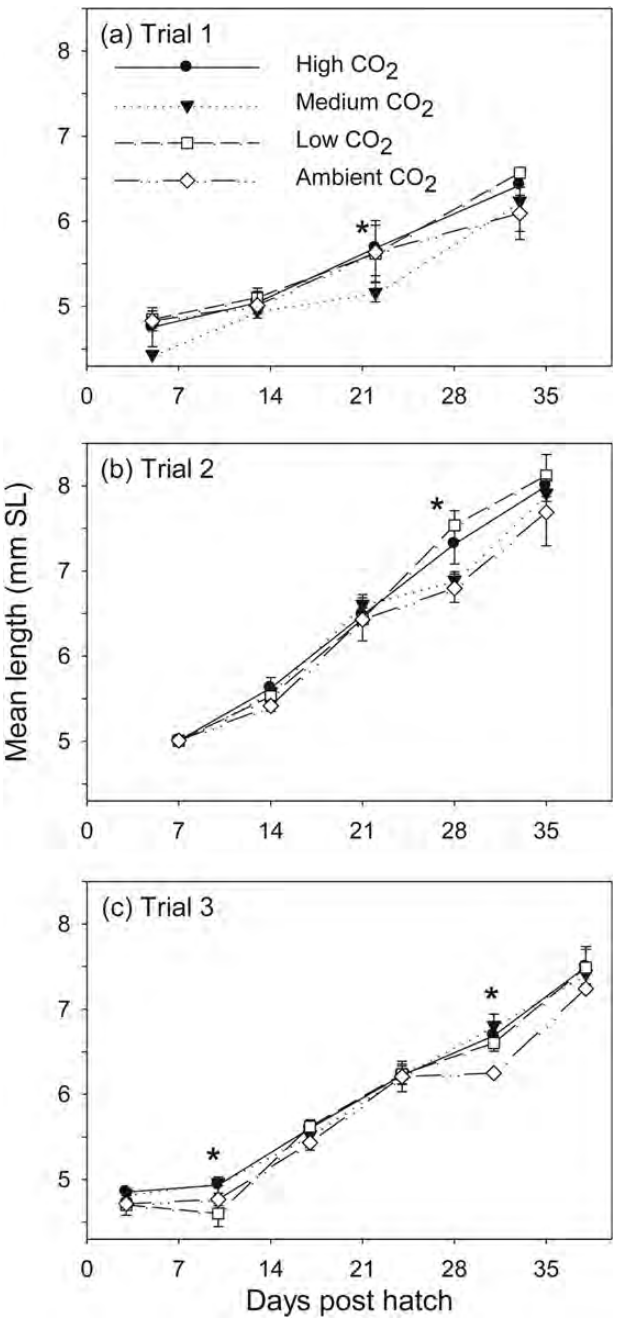

Figure 4. Growth of larval walleye pollock as a function of CO_2 levels in three replicate trials. Points are the mean values (±s.e.) of three replicate tanks in each CO_2 treatment. In trials 1 and 3, eggs were incubated and at the treatment CO_2 level in larval rearing tanks; in trial 2, eggs were incubated at ambient CO_2 levels and larvae were transferred to CO_2-controlled larval rearing tanks at 7 DPH.

Table 4. Analysis of variance of size and condition of walleye pollock larvae reared across a range of CO_2 levels.

		Standard length		Condition	
Factor	**d.f.**	**F**	***p***	**F**	***p***
Trial 1					
CO_2 level	3	0.199	0.006	0.19	0.900
Day	3	4.063	<0.001	30.05	<0.001
CO_2 × day	9	0.039	0.4778	0.75	0.661
Error	28				
Trial 2					
CO_2 level	3	2.40	0.086	1.07	0.374
Day[a]	3	122.06	<0.001	4.23	0.013
CO_2 × day	9	0.089	0.546	1.69	0.132
Error	32				
Trial 3					
CO_2 level	3	3.69	0.018	0.34	0.397
Day	5	305.76	<0.001	3.84	<0.001
CO_2 × day	12	0.91	0.555	1.12	0.189
Error	47				

Response variables are the tank mean value measured on each sampling date in each of three replicate rearing tanks in each experiment, except that four tanks in trial 1 and one tank in trial 3 had insufficient survival to measure sizes on the final sampling date. [a]Data for the first sampling date (3 DPH) in trial 2 was excluded from analyses because fish were incubated together at ambient CO_2 levels until this period. See Methods.

throughout the experiment (Figure 5), resulting in a significant difference between CO_2 treatments. Conversely, fish in the ambient CO_2 treatment were similar in size to those in other treatments at the start of the experiment, but were smaller at the end of the experiment. There were no significant differences in overall growth rates between CO_2 treatments from 5–33 DPH ($F_{[3,5]} = 1.197$; $p = 0.400$).

Growth rates in trial 2 were consistently higher across CO_2 treatments than those observed in the other trials (Figure 4). Body lengths differed between treatments at 28 DPH, and fish in the ambient treatment were slightly, but not significantly, smaller than fish in the other treatments at 35 DPH. Overall, there was no significant effect of CO_2 treatment on body size or growth rates from 7–35 DPH ($F_{[3,8]} = 0.894$; $p = 0.086$).

In trial 3, there was a significant effect of CO_2 treatment ($p = 0.018$), as smaller body sizes were observed among fish in the ambient treatment on several sampling dates. However, when body sizes were used to calculate growth rates from 10–38 DPH, there was no significant difference between CO_2 treatments ($F_{[3,8]} = 1.685$; $p = 0.247$).

Results for the larval condition showed similar patterns across trials and experimental CO_2 treatments. In each trial, there was no significant effect of CO_2 level or interactive effect between CO_2 level and sampling date on K (Table 4). As observed for SL, larval conditions were highest in trial 2 (Figure 4).

Discussion

There is significant concern that ocean acidification, caused by the dissolution of anthropogenically released CO_2 into the ocean will disrupt the productivity and functioning of high-latitude marine ecosystems, which support a number of the world's largest capture fisheries (Fabry *et al.*, 2009; Denman *et al.*, 2011). In fact recent experimental work suggests significant negative effects of elevated CO_2 levels on the survival of red king crab (*Paralithodes camtschaticus*) larvae (Long *et al.*, 2013). In a series

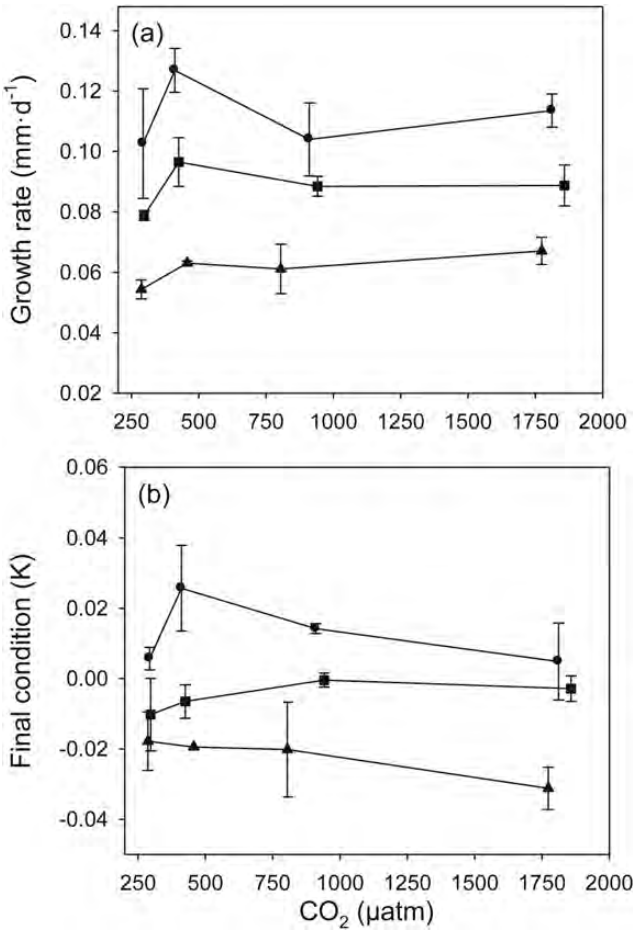

Figure 5. Growth rates and condition factor of larval walleye pollock as a function of CO_2 levels in three replicate trials. Points are the mean values (± s.e.) of three replicate tanks in each CO_2 treatment. Symbols: trial 1: triangles, trial 2: circles; trial 3: squares. Growth rates were calculated over the period of 5–33 DPH, 7–35 DPH, and 10–38 DPH for trials 1, 2, and 3, respectively. Condition levels were calculated as deviations from a best-fit relationship between $\log_{10}$(MH) and $\log_{10}$(SL)

of laboratory experiments, we found that growth of walleye pollock embryos and larvae exhibited only minor responses to elevated environmental CO_2. These results held across a wide range of CO_2 levels, including levels well beyond those predicted for the Bering Sea and Gulf of Alaska over the next century, and are similar to the resiliency observed in larger walleye pollock (Hurst *et al.*, 2012). These results suggest that other effects of ocean acidification, such as sensory impairment and changes in production of lower trophic level marine calcifiers, will play a larger role in determining the population productivity of walleye pollock than the direct effects of elevated CO_2 levels on their physiological capacity for growth.

Because they have both an internal skeleton composed primarily of calcium phosphate and an advanced physiological acid-base and gas exchange regulation system, marine fishes generally appear to be less sensitive to the effects of ocean acidification than those invertebrates that precipitate external skeletons of calcium carbonate (Ishimatsu *et al.*, 2008; Melzner *et al.*, 2009). Most experimental work to date has supported the conclusion that juvenile and

adult fishes are generally resilient to the effects of ocean acidification, at least in terms of growth and energetics (Foss *et al.*, 2003; Hurst *et al.*, 2012). However, it has been suggested that the embryonic and larval stages, which have less well-developed ion-exchange systems may be more vulnerable to negative effects of elevated CO_2 levels in the environment (Ishimatsu *et al.*, 2008).

Experimental work has demonstrated negative effects of high CO_2 on the eggs and larvae of some marine fishes (Munday *et al.*, 2009a; Baumann *et al.*, 2012; Frommel *et al.*, 2012), but not others (Munday *et al.*, 2009c and 2011a; Frank and Clemmesen, 2011). In the experiments presented here, exposure of walleye pollock to elevated CO_2 levels during the egg stage had only minor effects on hatching characteristics. We found that time-to-hatch was slightly shorter (< 1 day difference) in the low CO_2 treatment than the other treatments and that higher levels of CO_2 did not result in smaller sizes at hatch as observed in inland silversides (*Menidia beryllina*; Baumann *et al.*, 2012). Across experimental trials with walleye pollock larvae, there was a (non-significant) trend toward larger body sizes among fish reared at elevated CO_2 levels. This trend toward faster growth rates among larvae reared at elevated CO_2 levels has also been observed in experiments with larval Atlantic cod (Frommel *et al.*, 2012) and orange clownfish (*Amphiprion percula*, Munday *et al.*, 2009c). It is also consistent with the increased growth observed in juvenile walleye pollock at elevated CO_2 levels (Hurst *et al.*, 2012). Interestingly, in the experiment with larval Atlantic cod, the high CO_2 levels that resulted in elevated growth and lipid storage also appeared to induce severe tissue damage (Frommel *et al.*, 2012). However, the complete loss of an experimental treatment in that experiment suggests the possibility that other aspects of the mesocosm environment may have contributed to the observed differences between CO_2 treatments. Similarly, the fact that overall growth rates differed between our three larval trials suggests that a suite of other factors can affect the outcomes of specific experiments with pelagic larvae, including egg and prey quality and characteristics of the foraging environment such as light and turbulence regimes (Dower *et al.*, 1997). Therefore, caution should be applied when inferring the potential sensitivity of individual species to ocean acidification from limited or unreplicated observations.

In order to predict the ecosystem consequences of ocean acidification, it is critical to evaluate the potential effects of elevated CO_2 levels in relation to other natural and anthropogenic influences and to consider the potential for species to acclimate or adapt to changing environmental conditions. For example, numerous studies have explored the effect of incubation temperature on hatching characteristics of marine gadids (Galloway *et al.*, 1998; Laurel *et al.*, 2008). In our experiments, we did not specifically attempt to evaluate the potential for genetic variation in CO_2 sensitivity using a controlled breeding design (e.g. Miller *et al.*, 2012). Rather, we attempted to evaluate likely population-level responses by capturing potential genetic and non-genetic variation in sensitivity to ocean acidification with replicated experiments conducted over multiple years. In walleye pollock the observed effects of elevated CO_2 levels on time-to-hatch were small (but statistically significant) compared with the variation observed among the five incubated egg batches, and the variation generated by a 2°C difference in incubation temperature (Blood, 2002). While there was a significant interaction between CO_2 level and egg batch on some size-at-hatch characteristics, the lack of a consistent response of all hatching characteristics to CO_2 level across the five incubated egg batches suggests that the effects are minor in comparison with other known influences on size-at-hatch. Again, incubation temperature is known to induce significant variation in size-at-hatch in walleye pollock and other gadids (Canino, 1994; Laurel *et al.*, 2008). Further, differences in broodstock rearing protocols suggest an explanation for some of the variation observed between egg batches. For the 2011 spawning season, the laboratory broodstock was divided and maintained at two different temperatures (2.5 and 6°C). High condition and YA were observed in fish from egg batch 5 from parents reared at the low temperature (Figure 2), suggesting that parental thermal experience may have a greater effect on hatch characteristics than exposure to even significantly elevated levels of environmental CO_2.

Recent work has demonstrated that sensitivity of early life stages to elevated CO_2 levels may be affected by the parental environment. These effects can be observed as population-specific differences in sensitivity to ocean acidification (Parker *et al.*, 2011) and as non-genetic parent–offspring acclimation ("adaptive transgenerational plasticity"). In experiments with both oysters and tropical fish, experiments have shown that exposure of the parents to elevated CO_2 levels can mediate the negative effects of CO_2 on their offspring (Miller *et al.*, 2012; Parker et al 2012). For both egg and larval experiments, we used the progeny from natural spawning activity of a laboratory-maintained broodstock that had previously been exposed to elevated CO_2 levels, potentially preconditioning offspring for resiliency to ocean acidification. The broodstock was established with fish captured from Puget Sound, WA, USA, where some areas currently experience pH levels below 7.7 due to reduced mixing rates and natural or anthropogenically enhanced microbial respiration (Feely *et al.*, 2010). Ambient CO_2 levels in our laboratory system are subject to significant natural variation associated with upwelling/downwelling events in the California Current system. While CO_2 levels during the winter spawning season average 250–350 μatm, CO_2 is significantly higher during summer upwelling events when ambient CO_2 levels in our laboratory can exceed 700 μatm (Gruber *et al.*, 2012; Hurst *et al.*, 2012). Hence, it is possible that adaptation of the Puget Sound population or seasonal exposure of our broodstock to low pH may have preconditioned experimental offspring for resiliency to high CO_2 levels. However, we believe that the results presented here are applicable for evaluating the potential consequences of ocean acidification for the Gulf of Alaska and Bering Sea populations, as those populations are also exposed to pH levels in the 7.7–7.8 range (Mathis *et al.*, in press), and those populations will experience further ocean acidification in the form of a gradual increase in CO_2 level to which the population may be able to acclimate or adapt.

While it is expected that species will differ in sensitivity to elevated CO_2 (Ries *et al.*, 2009), the ecological and evolutionary bases for such variation are not yet clear. Munday *et al.*, (2011b) noted that otolith size was affected in two reef fish species with pelagic offspring, but not in a demersal, sheltering species, which might be routinely exposed to elevated CO_2. Such observations have lead to the expectation that the range of CO_2 variation in a species' natural environment may determine their sensitivity to projected levels of ocean acidification. Based on this perspective (and their economic and ecosystem importance), walleye pollock was selected for focused study as their offshore–pelagic distribution would suggest a greater sensitivity to ocean acidification than other Alaskan fishery species inhabiting shallow coastal waters with high natural variation in CO_2 levels (*sensu* Munday *et al.*, 2011a). However, the general resilience

observed in early life stages of walleye pollock, and the sensitivity observed in the inland silverside (Baumann *et al.*, 2012), suggest that other factors must be involved in determining species-specific sensitivity to ocean acidification. Alternatively, Pane and Barry (2007) suggested that sensitivity to ocean acidification might be greatest in sedentary organisms residing in deep waters due to their lower metabolic capacity. In high-latitude seas, this perspective suggests that species such as snailfishes and some commercially valuable flatfishes should be more closely considered. Clearly, identifying those factors associated with relative sensitivity to ocean acidification will require additional experimentation across a wide range of fish species with contrasting life histories and habitats.

It is important to recognize that the conclusion drawn from these experiments—that larval walleye pollock were not negatively affected by projected ocean acidification—is specific to the fishes' growth responses to elevated CO_2 levels. It is possible that other biotic or abiotic stressors could be exacerbated by elevated CO_2. For example, the metabolic effects of ocean acidification may cause a narrowing of the "thermal window" (Pörtner 2008, 2010) and recent work on reef species has demonstrated that elevated CO_2 levels can disrupt behavioural responsiveness to auditory and olfactory stimuli (Dixson *et al.*, 2010; Simpson *et al.*, 2011). In addition, several studies have shown that exposure to elevated CO_2 levels results in hypercalcification of fish otoliths (Checkley *et al.*, 2009; Munday *et al.*, 2011b; Hurst *et al.*, 2012). These effects, shown to reduce survival in natural field settings (Devine *et al.*, 2011), may be related to CO_2-induced ionic disruption of the GABA-A neuroreceptor (Nilsson *et al.*, 2012). We do not yet know if such sensory or behavioural disruption occurs in walleye pollock (Maneja *et al.*, 2012).

Isolated from other potential stressors, ocean acidification did not appear to negatively affect size or condition of early larval walleye pollock. We did not see the reduced sizes or qualitative measures of survival observed in several other marine species (Baumann *et al.*, 2012; Frommel *et al.*, 2012). Rather, we observed a slight trend toward higher growth rates at high CO_2 levels, as seen in several other studies (Munday *et al.*, 2009c; Hurst *et al.*, 2012). Further, even the most extreme effects of high CO_2 observed throughout these experiments were more modest than those induced by environmentally relevant variation in temperature (Canino, 1994; Hurst *et al.*, 2010b) or prey availability (Laurel *et al.*, 2011). This suggests that the growth dynamics of early life stages of walleye pollock are resilient to projected levels of ocean acidification and that other aspects of long-term climate variation are likely to play a more important role in population productivity changes (Munday *et al.*, 2011a; Hunt *et al.*, 2011; Mueter *et al.*, 2011). However, it remains possible that ocean acidification may significantly affect populations of walleye pollock through other mechanistic pathways including CO_2-induced sensory impairment or changes to the productivity of lower trophic levels. Additional research is required to evaluate these potential effects on walleye pollock and other critical fishery resource species.

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References

Baskerville-Bridges, B., and Kling, L. J. 2000. Larval culture of Atlantic cod (*Gadus morhua*) at high stocking densities. Aquaculture, 181: 61–69.

Baumann, H., Talmage, S. C., and Gobler, C. J. 2012. Reduced early life growth and survival in a fish in direct response to increased carbon dioxide. Nature Climate Change, 2: 38–41.

Blood, D. M. 2002. Low-temperature incubation of walleye pollock (*Theragra chalcogramma*) eggs from the southeast Bering Sea shelf and Shelikof Strait, Gulf of Alaska. Deep-Sea Research Part II: Topical Studies in Oceanography, 49: 6095–6108.

Brodeur, R. D., and Wilson, M. T. 1996. A review of the distribution, ecology, and population dynamics of age-0 walleye pollock in the Gulf of Alaska. Fisheries Oceanography, 5(Supp.1): 148–166.

Brown, J. A., Minkoff, G., and Puvanendran, V. 2003. Larviculture of Atlantic cod (*Gadus morhua*): progress, protocols and problems. Aquaculture, 227: 357–372.

Canino, M. F. 1994. Effects of temperature and food availability on growth and RNA/DNA ratios of walleye pollock *Theragra-chalcogramma* (Pallas) eggs and larvae. Journal of Experimental Marine Biology and Ecology, 175: 1–16.

Checkley, D. M., Dickson, A. G., Takahashi, M., Radich, J. A., Eisenkolb, N., and Asch, R. 2009. Elevated CO_2 enhances otolith growth in young fish. Science, 324: 1683–1683.

Cheung, W. W. L., Sarmiento, J. L., Dunne, J., Frölicher, T. L., Lam, V. W. Y., Deng Palomares, M. L., Watson, R., *et al.* 2012. Shrinking of fishes exacerbates impact of global ocean changes on marine ecosystems. Nature Climate Change, 3: 254–258.

Colton, A. R., and Hurst, T. P. 2010. Behavioral responses to light gradients, olfactory cues, and prey in larvae of two North Pacific gadids (*Gadus macrocephalus* and *Theragra chalcogramma*). Environmental Biology of Fishes, 88: 39–49.

Cooley, S. R., and Doney, S. C. 2009. Anticipating ocean acidification's economic consequences for commercial fisheries. Environmental Research Letters, 4: 1–8.

Denman, K., Christian, J. R., Steiner, N., Pörtner, H. O., and Nojiri, Y. 2011. Potential impacts of future ocean acidification on marine ecosystems and fisheries: current knowledge and recommendations for future research. ICES Journal of Marine Science, 68: 1019–1029.

Devine, B. M., Munday, P. L., and Jones, G. P. 2011. Homing ability of adult cardinalfish is affected by elevated carbon dioxide. Oecologia, 168: 269–276.

Dixson, D. L., Munday, P. L., and Jones, G. P. 2010. Ocean acidification disrupts the innate ability of fish to detect predator olfactory cues. Ecology Letters, 13: 68–75.

Dower, J. F., Miller, G. M., and Leggett, W. C. 1997. The role of microscale turbulence in the feeding ecology of larval fish. Advancements in Marine Biology, 31: 169–220.

Fabry, V. J., Seibel, B. A., Feely, R. A., and Orr, J. C. 2008. Impacts of ocean acidification on marine fauna and ecosystem processes. ICES Journal of Marine Science, 65: 414–432.

Fabry, V. J., McClintock, J. B., Mathis, J. T., and Grebmeier, J. M. 2009. Ocean acidification at high latitudes: the bellweather. Oceanography, 22: 160–171.

Feely, R. A., Alin, S. R., Newton, J., Sabine, C. L., Warner, M., Devol, A., Krembs, C., *et al.*, 2010. The combined effects of ocean acidification, mixing, and respiration on pH and carbonate saturation in an urbanized estuary. Estuarine, Coastal and Shelf Science, 88: 442–449.

Feely, R. A., Sabine, C. L., Lee, K., Berelson, W., Kleypas, J., Fabry, V. J., and Millero, F. J. 2004. Impact of anthropogenic CO_2 on the $CaCO_3$ system in the oceans. Science, 305: 362–366.

Foss, A., Rosnes, B. A., and Oiestad, V. 2003. Graded environmental hypercapnia in juvenile spotted wolffish (*Anarhichas minor* Olafsen): effects on growth, food conversion efficiency and nephrocalcinosis. Aquaculture, 220: 607–617.

Franke, A., and Clemmesen, C. 2011. Effect of ocean acidification on early life stages of Atlantic herring (*Clupea harengus* L.). Biogeosciences, 8: 3697–3707.

Frommel, A. Y., Maneja, R., Lowe, D., Malzahn, A. M., Geffen, A. J., Folkvord, A., Piatkowski, U., *et al.* 2012. Severe tissue damage in Atlantic cod larvae under increasing ocean acidification. Nature Climate Change, 2: 42–46.

Galloway, T. F., Kjorsvik, E., and Kryvi, H. 1998. Effect of temperature on viability and axial muscle development in embryos and yolk sac larvae of the Northeast Arctic cod (*Gadus morhua*). Marine Biology, 132: 559–567.

Gruber, N., Hauri, C., Lachkar, Z., Loher, D., Frolicher, T. L., and Plattner, G. K. 2012. Rapid progression of ocean acidification in the California Current system. Science, 337: 220–223.

Hofmann, G. E., Smith, J. E., Johnson, K. S., Send, U., Levin, L. A., Micheli, F., Paytan, A., *et al.* 2011. High-frequency dynamics of ocean pH: a multi-ecosystem comparison. Plos One, 6: e28983.

Houde, E. D. 1987. Fish early life dynamics and recruitment variability. American Fisheries Society Symposium, 2: 17–29.

Hunt, G. L., Coyle, K. O., Eisner, L. B., Farley, E. V., Heintz, R. A., Mueter, F., Napp, J. M., *et al.* 2011. Climate impacts on eastern Bering Sea foodwebs: a synthesis of new data and an assessment of the Oscillating Control Hypothesis. ICES Journal of Marine Science, 68: 1230–1243.

Hurst, T. P., Abookire, A. A., and Knoth, B. 2010a. Quantifying thermal effects on contemporary growth variability to predict responses to climate change in northern rock sole (*Lepidopsetta polyxystra*). Canadian Journal of Fisheries and Aquatic Sciences, 67: 97–107.

Hurst, T. P., Laurel, B. J., and Ciannelli, L. 2010b. Ontogenetic patterns and temperature-dependence of growth rate in early life stages of Pacific cod (*Gadus macrocephalus*). Fishery Bulletin, 108: 382–392.

Hurst, T. P., Fernandez, E. R., Mathis, J. T., Miller, J. A., Stinson, C. S., and Ahgeak, E. F. 2012. Resiliency of juvenile walleye pollock to projected levels of ocean acidification. Aquatic Biology, 17: 247–259.

IPCC. 2007. Climate Change 2007: Synthesis Report. Contribution of Working Groups I, II, and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, Geneva. 104 pp.

Ishimatsu, A., Hayashi, M., and Kikkawa, T. 2008. Fishes in high-CO_2, acidified oceans. Marine Ecology Progress Series, 373: 295–302.

Kikkawa, T., Ishimatsu, A., and Kita, J. 2003. Acute CO_2 tolerance during the early developmental stages of four marine teleosts. Environmental Toxicology, 18: 375–382.

Kroeker, K. J., Kordas, R. L., Crim, R. N., and Singh, G. G. 2010. Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms. Ecology Letters, 13: 1419–1434.

Laurel, B. J., Hurst, T. P., and Ciannelli, L. 2011. An experimental examination of temperature interactions in the match–mismatch hypothesis for Pacific cod larvae. Canadian Journal of Fisheries and Aquatic Sciences, 68: 51–61.

Laurel, B. J., Hurst, T. P., Copeman, L. A., and Davis, M. W. 2008. The role of temperature on the growth and survival of early and late hatching Pacific cod larvae (*Gadus macrocephalus*). Journal of Plankton Research, 30: 1051–1060.

Lewis, E., and Wallace, D. W. R. 1998. Program developed for CO_2 system calculations. Oak Ridge Tennessee, Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, US Department of Energy.

Livingston, P. A. 1993. Importance of predation by groundfish, marine mammals and birds on walleye pollock *Theragra chalcogramma* and Pacific herring *Clupea pallasi* in the eastern Bering Sea. Marine Ecology Progress Series, 102: 205–215.

Long, W. C., Swiney, K. M., and Foy, R. J. 2013. Effects of ocean acidification on the embryos and larvae of red king crab, *Paralithodes camtschaticus*. Marine Pollution Bulletin, in press.

Maneja, R., Frommel, A. Y., Browman, H. I., Clemmensen, C., Geffen, A. J., Folkvord, A., Piatkowski, U., *et al.* 2012. The swimming kinematics of larval Atlantic cod, *Gadus morhua* L. are resilient to elevated pCO_2. Marine Biology, in press.

Mathis, J. T., Cross, J. N., and Bates, N. R. 2011. Coupling primary production and terrestrial runoff to ocean acidification and carbonate mineral suppression in the Eastern Bering Sea. Journal of Geophysical Research, 116: C02030.

Mathis, J. T., Evans, W., Sabine, C. L., Juranek, L. W., Stockwell, D. A., Shake, K. L., Weingartner, T. J., *et al.* The physical and biological controls on CO_2 fluxes and carbonate mineral saturation states in the northern Gulf of Alaska. Journal of Geophysical Research, in press.

McElhany, P., and Busch, D. S. 2012. Appropriate pCO_2 treatments in ocean acidification experiments. Marine Biology, in press.

Melzner, F., Gutowska, M. A., Langenbuch, M., Dupont, S., Lucassen, M., Thorndyke, M. C., Bleich, M., *et al.* 2009. Physiological basis for high CO_2 tolerance in marine ectothermic animals: preadaptation through lifestyle and ontogeny? Biogeosciences, 6: 2313–2331.

Miller, G. M., Watson, S-A., Donelson, J. M., Mccormick, M. I., and Munday, P. L. 2012. Parental environment mediates impacts of increased carbon dioxide on a coral reef fish. Nature Climate Change, 2: 856–861.

Monk, J., Puvanendran, V., and Brown, J. A. 2006. Do different light regimes affect the foraging behaviour, growth and survival of larval cod (*Gadus morhua* L.)? Aquaculture, 257: 287–293.

Mueter, F. J., Bond, N. A., Ianelli, J. N., and Hollowed, A. B. 2011. Expected declines in recruitment of walleye pollock (*Theragra chalcogramma*) in the eastern Bering Sea under future climate change. ICES Journal of Marine Science, 68: 1284–1296.

Munday, P. L., Crawley, N. E., and Nilsson, G. E. 2009a. Interacting effects of elevated temperature and ocean acidification on the aerobic performance of coral reef fishes. Marine Ecology Progress Series, 388: 235–242.

Munday, P. L., Dixson, D. L., Donelson, J. M., Jones, G. P., Pratchett, M. S., Devitsina, G. V., and Doving, K. B. 2009b. Ocean acidification impairs olfactory discrimination and homing ability of a marine fish. Proceedings of the National Academy of Sciences of the United States of America, 106: 1848–1852.

Munday, P. L., Donelson, J. M., Dixson, D. L., and Endo, G. G. K. 2009c. Effects of ocean acidification on the early life history of a tropical marine fish. Proceedings of the Royal Society of London. Series B, Biological Sciences, 276: 3275–3283.

Munday, P. L., Gagliano, M., Donelson, J. M., Dixson, D. L., and Thorrold, S. R. 2011a. Ocean acidification does not affect the early life history development of a tropical marine fish. Marine Ecology Progress Series, 423: 211–221.

Munday, P. L., Hernaman, V., Dixson, D. L., and Thorrold, S. R. 2011b. Effect of ocean acidification on otolith development in larvae of a tropical marine fish. Biogeosciences, 8: 1631–1641.

Nilsson, G. E., Dixson, D. L., Domenici, P., Mccormick, M. I., Sorensen, C., Watson, S. A., and Munday, P. L. 2012. Near-future carbon dioxide levels alter fish behaviour by interfering with neurotransmitter function. Nature Climate Change, 2: 201–204.

Orr, J. C., Fabry, V. J., Aumont, O., Bopp, L., Doney, S. C., Feely, R. A., Gnanadesikan, A., *et al.* 2005. Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. Nature, 437: 681–686.

Pane, E. F., and Barry, J. P. 2007. Extracellular acid-base regulation during short-term hypercapnia is effective in a shallow-water crab, but ineffective in a deep-sea crab. Marine Ecology Progress Series, 334: 1–9.

Parker, L. M., Ross, P. M., and O'Connor, W. A. 2011. Populations of the Sydney rock oyster, *Saccostrea glomerata*, vary in response to ocean acidification. Marine Biology, 158: 689–697.

Parker, L. M., Ross, P. M., O'Connor, W. A., Borysko, L., Raftos, D. A ., and Portner, H. O. 2012. Adult exposure influences offspring response to ocean acidification in oysters. Global Change Biology, 18: 82–92.

Porter, S. M., and Bailey, K. M. 2007a. The effect of early and late hatching on the escape response of walleye pollock (*Theragra chalcogramma*) larvae. Journal of Plankton Research, 29: 291–300.

Porter, S. M., and Bailey, K. M. 2007b. Optimization of feeding and growth conditions for walleye pollock *Theragra chalcogramma* (Pallas) larvae reared in the laboratory. AFSC Processed Report 2007–06. Alaska Fisheries Science Center, NOAA-NMFS, Seattle. 20 pp.

Pörtner, H. O. 2008. Ecosystem effects of ocean acidification in times of ocean warming: a physiologist's view. Marine Ecology Progress Series, 373: 203–217.

Pörtner, H. O. 2010. Oxygen- and capacity-limitation of thermal tolerance: a matrix for integrating climate-related stressor effects in marine ecosystems. Journal of Experimental Biology, 213: 881–893.

Pörtner, H. O., Langenbuch, M., and Reipschlager, A. 2004. Biological impact of elevated ocean CO_2 concentrations: lessons from animal physiology and earth history. Journal of Oceanography, 60: 705–718.

Riebesell, U., Fabry, V. J., Hansson, L., and Gattuso, J-P. (Eds) 2010. Guide to Best Practices for Ocean Acidification Research and Data Reporting. Publications Office of the European Union. Luxembourg. 258 pp.

Ries, J. B., Cohen, A. L., and McCorkle, D. C. 2009. Marine calcifiers exhibit mixed responses to CO_2-induced ocean acidification. Geology, 37: 1131–1134.

Sabine, C. L., Feely, R. A., Gruber, N., Key, R. M., Lee, K., Bullister, J. L., Wanninkhof, R., *et al.* 2004. The oceanic sink for anthropogenic CO_2. Science, 305: 367–371.

Simpson, S. D., Munday, P. L., Wittenrich, M. L., Manassa, R., Dixson, D. L., Gagliano, M., and Yan, H. Y. 2011. Ocean acidification erodes crucial auditory behaviour in a marine fish. Biology Letters, 7: 917–920.

Smart, T. I., Duffy-Anderson, J. T., Horne, J. K., Farley, E. V., Wilson, C. D., and Napp, J. M. 2012. Influence of environment on walleye pollock eggs, larvae, and juveniles in the southeastern Bering Sea. Deep-Sea Research Part II: Topical Studies in Oceanography, 65–70: 196–207.

Steinacher, M., Joos, F., Frolicher, T. L., Plattner, G. K., and Doney, S. C. 2009. Imminent ocean acidification in the Arctic projected with the NCAR global coupled carbon cycle–climate model. Biogeosciences, 6: 515–533.

Yamamoto-Kawai, M., McLaughlin, F. A., Carmack, E. C., Nishino, S., and Shimada, K. 2009. Aragonite undersaturation in the Arctic Ocean: effects of ocean acidification and sea ice melt. Science, 326: 1098–1100.

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