Contribution to Special Issue: 'Towards a Broader Perspective on Ocean Acidification Research'
Original Article

Effects of elevated CO_2 levels on eggs and larvae of a North Pacific flatfish

Thomas P. Hurst[1]*, Benjamin J. Laurel[1], Jeremy T. Mathis[2], and Lauren R. Tobosa[3]

[1]Fisheries Behavioral Ecology Program, Resource Assessment and Conservation Engineering Division, Alaska Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, Hatfield Marine Science Center, Newport, OR 97365, USA
[2]Pacific Marine Environmental Laboratory, National Oceanic and Atmospheric Administration, Seattle, WA 98115, USA
[3]Hatfield Marine Science Center, Oregon State University, Newport, OR 97365, USA

*Corresponding author: tel: +1 541 867 0222; fax: +1 541 867 0136; e-mail: thomas.hurst@noaa.gov

The Bering Sea and Gulf of Alaska support a number of commercially important flatfish fisheries. These high latitude ecosystems are predicted to be most immediately impacted by ongoing ocean acidification, but the range of responses by commercial fishery species has yet to be fully explored. In this study, we examined the growth responses of northern rock sole (*Lepidopsetta polyxystra*) eggs and larvae across a range of CO_2 levels (ambient to 1500 μatm) to evaluate the potential sensitivity to ocean acidification. Laboratory-spawned eggs and larvae were reared at 8°C in a flow-through culture system in which CO_2 levels were maintained via computer-controlled injection of CO_2 into a seawater conditioning tank. Overall, we observed only minor effects of elevated CO_2 level on sizes of northern rock sole larvae. Size at hatch differed among offspring from four different females, but there was no significant effect of CO_2 level on egg survival or size at hatch. In three separate larval growth trials, there was little effect of CO_2 level on growth rates through the first 28 d post-hatch (DPH). However, in the one trial extended to 60 DPH, fish reared at the highest CO_2 level had lower condition factors after 28 DPH, suggesting that larvae undergoing metamorphosis may be more sensitive to environmental hypercapnia than earlier pre-flexion stages. These results suggest that while early life stages of northern rock sole are less sensitive to ocean acidification than previously examined flatfish, they may be more sensitive to elevated CO_2 levels than a previously studied gadid with a similar geographic range.

Keywords: climate change, early life history, flatfish, growth rate, hypercapnia, ocean acidification.

Introduction

Ocean acidification occurs as anthropogenically released CO_2 dissolves from the atmosphere into surface waters of the world's oceans (e.g. Feely *et al.*, 2004; Sabine *et al.*, 2004; Gattuso and Hansson, 2011), reducing the pH of seawater and the availability of carbonate ions. Significant concern has arisen that ocean acidification will disrupt the functioning of marine ecosystems and reduce the productivity of important fishery resources (Cooley and Doney, 2009; Denman *et al.*, 2011). The high-latitude seas of the North Pacific Ocean are of particular concern because they are predicted to be acutely affected by both acidification and continued warming (Fabry *et al.*, 2009; Mathis *et al.*, 2011a, 2014). Ocean acidification in the Arctic Ocean and Bering Sea is expected to be exacerbated by natural processes such as sea ice melt, river run-off, and the

respiration of organic matter over the shallow continental shelves (Mathis, 2011b; Mathis and Questel, 2013). The most recent estimates from the coupled climate-ocean model NCAR CESM1-BGC (Lindsay *et al.*, 2014) with atmospheric CO_2 emissions from the IPCC RCP 8.5 scenario (van Vuuren *et al.*, 2011) are that pH of Gulf of Alaska and Bering Sea surface waters have already decreased by 0.10 units and will decrease an additional 0.35 units by the end of the century (see Mathis *et al.*, 2014 for additional details).

Experimental studies on marine organisms have demonstrated a range of effects from elevated CO_2 ("environmental hypercapnia") and reduced pH (Fabry *et al.*, 2008; Kroeker *et al.*, 2010). In general, fish are expected to be more resilient to the direct physiological effects of ocean acidification than invertebrates with carbonate exoskeletons (Pörtner *et al.*, 2004; Melzner *et al.*, 2009a). Although recent

studies have found that elevated CO_2 may disrupt the behavioral and sensory biology of juvenile fish (Leduc *et al.*, 2013), growth and condition of juvenile and adult stage fish appear generally resilient to the effects of ocean acidification (Ishimatsu *et al.*, 2008; Melzner *et al.*, 2009b; Hurst *et al.*, 2012). Egg and larval stages, however, are expected to be more sensitive to decreased pH because of their high surface to volume ratio and lack of specialized mechanisms for acid–base regulation (Kikkawa *et al.*, 2003; Ishimatsu *et al.*, 2008). Whereas several studies have found negative effects of elevated CO_2 on development or survival of larval fish (Baumann *et al.*, 2012; Frommel *et al.*, 2012; Pimentel *et al.*, 2014), a number of others have found negligible effects (Munday *et al.*, 2011; Bignami *et al.*, 2013; Hurst *et al.*, 2013). Additional experiments on a range of species are still required as there is currently little understanding of which taxonomic groups or life history types might be most sensitive.

A recent analysis evaluated the vulnerability of Alaskan communities to potential ocean acidification-induced declines in fishery productivity based on predicted regional degree of acidification, reliance on different fishery resources for commercial and subsistence harvests, and degree of community resilience (Mathis *et al.*, 2014). Sensitivity of major resource groups (shellfish, salmon, other marine fish) was ranked based on the likelihood of direct and indirect (via the foodweb) impacts of ocean acidification on population productivity. The greater sensitivity of Alaskan crabs is supported by experimental observations of negative effects on several species (Long *et al.*, 2013a,b). However, the assumption in that study that marine fish would be generally robust to the effects of ocean acidification was based primarily on theoretical responses of fish species as direct empirical observations on ocean acidification responses exist for only one of Alaska's harvested fish species (walleye pollock *Gadus chalcogrammus*; Hurst *et al.*, 2012, 2013). Experimental studies on a broad range of species with differing life histories are clearly required to refine such analyses of the ecosystem and socio-economic impacts of ocean acidification.

In this study, we examined the potential sensitivity of northern rock sole (*Lepidopsetta polyxystra*) to the direct effects of ocean acidification in a series of laboratory experiments rearing eggs and larvae under ambient and elevated CO_2 levels. Northern rock sole are commercially harvested in the Bering Sea and Gulf of Alaska groundfish fisheries. Through the 1990s, annual harvests (combined with southern rock sole *Lepidopsetta bilineata*) averaged 27 000 mt, but have increased to an average of 58 000 mt since 2008 in response to a strong market for the roe. In addition to their commercial importance, northern rock sole were selected for study because they differ from the previously studied walleye pollock in several reproductive and early life history traits. Northern rock sole mature around 7 years of age and spawn over the continental shelf in winter and spring (Stark and Somerton, 2002). Females produce a single batch of demersal eggs which are semi-adhesive during the early stages of development (Laurel and Blood, 2011). Larvae are found in the upper 30 m of the water column (Lanksbury *et al.*, 2007), undergo transformation, and settle into inner coastal nursery areas at a size of ~15 mm (Cooper *et al.*, 2014; Laurel *et al.*, 2014). Previous work has indicated that regional environmental and climatic conditions during the larval period influence recruitment rates and juvenile growth trajectories (Doyle *et al.*, 2009; Hurst *et al.*, 2010).

Because vital rates of early life history stages can dramatically influence population productivity, the experiments presented here focus on determining the effects of elevated CO_2 levels on size-at-hatch and larval growth rates. To facilitate direct comparison of species responses, we applied the same methodologies used in a recent study of walleye pollock (Hurst *et al.*, 2013). In multiple independent experiments, northern rock sole eggs and larvae were reared across a range of CO_2 levels to determine stage-specific sensitivity to environmental hypercapnia. Treatments were designed to reflect changes from ambient conditions predicted to occur in the North Pacific over the next century. The medium CO_2 addition treatments targeted an increase of ~400 µatm CO_2 (~0.35 pH decrease; IPCC, 2014; Mathis *et al.*, 2014), with low CO_2 addition treatments reflecting a nearer-term increase of ~150 µatm CO_2 (~0.10 pH decrease). High CO_2 treatments (to 1500 µatm) were included to better understand the overall physiological sensitivity of northern rock sole to CO_2 level (Riebesell *et al.*, 2010).

Methods

Rearing system

Eggs and larvae were incubated in a flow-through rearing system 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 ~7.5). The second tank was maintained at the ambient CO_2 level (pH~8.10). Water from these conditioning tanks was pumped to two header tanks for the "high" and "ambient" CO_2 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 ("low" and "medium" CO_2 treatments). Each header tank gravity-fed 1000 ml egg incubation beakers or 100 l rearing tanks at 50 and 500 ml min^{-1}, respectively. 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 by chemical analysis of water 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). During part of the first egg batch incubation and part of the third larval trial, water samples were not available; during these periods, 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 during adjacent periods (all model $r > 0.75$).

Parental broodstock

Eggs for these experiments were produced by a captive broodstock of northern rock sole at the Alaska Fisheries Science Center laboratory in Newport, Oregon. Fish for the broodstock were collected as adults (32–40 cm total length) from coastal waters of Kodiak Island, Alaska (57°46′N 152°21′W), and reared in the laboratory (see Laurel and Blood, 2011, for additional details). Unique-identifier passive-integrated transponder tags were injected into the dorsal

Table 1. Conditions during experimental exposures of northern rock sole (*L. polyxystra*) 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)
Eggs[a,b]					
Ambient	7.7 ± 0.5	2036.3 ± 25.2	2140.7 ± 38.9	8.15 ± 0.07	296 ± 56
Low CO$_2$	7.8 ± 0.5	2068.1 ± 34.7	2163.5 ± 45.3	8.02 ± 0.11	423 ± 126
Medium CO$_2$	7.8 ± 0.5	2105.0 ± 28.2	2170.3 ± 55.8	7.83 ± 0.13	698 ± 243
High CO$_2$	7.7 ± 0.6	2165.4 ± 20.8	2189.1 ± 46.3	7.57 ± 0.18	1281 ± 320
Larvae—trial 1					
Ambient	8.3 ± 0.4	1979.0 ± 36.2	2155.0 ± 61.8	8.14 ± 0.09	300 ± 76
Low CO$_2$	8.3 ± 0.5	2007.4 ± 45.3	2144.3 ± 51.3	8.03 ± 0.14	403 ± 138
Medium CO$_2$	8.1 ± 0.04	2060.3 ± 40.1	2140.3 ± 71.9	7.86 ± 0.19	656 ± 337
High CO$_2$	8.3 ± 0.4	2147.8 ± 28.8	2151.8 ± 58.3	7.59 ± 0.17	1219 ± 477
Larvae—trial 2					
Ambient	8.0 ± 0.5	1975.1 ± 46.4	2178.0 ± 46.3	8.20 ± 0.07	255 ± 42
Low CO$_2$	8.0 ± 0.03	2045.0 ± 36.3	2199.1 ± 59.3	8.07 ± 0.06	351 ± 44
Medium CO$_2$	7.9 ± 0.4	2086.7 ± 34.5	2177.1 ± 53.8	7.92 ± 0.09	518 ± 105
High CO$_2$	8.1 ± 0.3	2144.7 ± 20.9	2158.5 ± 27.0	7.63 ± 0.03	1054 ± 76
Larvae—trial 3[b]					
Ambient	8.2 ± 0.8	2086.7 ± 84.6	2218.8 ± 67.1	8.01 ± 0.17	549 ± 211
Low CO$_2$	8.1 ± 0.8	2125.8 ± 87.8	2217.1 ± 84.8	7.90 ± 0.019	697 ± 284
Medium CO$_2$	8.2 ± 0.8	2175.5 ± 75.9	2212.5 ± 84.0	7.75 ± 0.19	978 ± 361
High CO$_2$	8.1 ± 0.8	2239.8 ± 87.7	2218.1 ± 80.0	7.54 ± 0.16	1541 ± 493

[a]Conditions are the averages of the mean and standard deviation in conditions observed during each of the four egg incubation trials. Response metrics are plotted against batch-specific CO$_2$ levels in Figure 2.
[b]Water samples during parts of egg incubation 1 and larval trial 3 were not available. During these periods, 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 periods. Carbonate system parameters (DIC, dissolved inorganic carbon; TA, total alkalinity; temperature; and salinity) were measured 2–3 times per week and used to calculate pH and pCO$_2$.

musculature of each fish. The fish were held in a 6 m tank under a seasonally varying temperature and photoperiod. Temperatures in the spawner tank were maintained at 7–10°C during summer and reduced to 4–5°C during winter; light was provided on a seasonal cycle varying from 14.5 h light during summer to a minimum of 9.5 h before the spawning season. During the 2013 spawning season, water samples were collected from the broodstock tank once per week and analysed to determine CO$_2$ (305 ± 69 μatm s.d.) and pH (8.13 ± 0.09) conditions as described above. Although we did not monitor pH or CO$_2$ levels in the adult broodstock tanks regularly outside the spawning season, measurements of ambient seawater during experiments in Hurst *et al.* (2012) demonstrate strong seasonal variation. During summer, when coastal upwelling brings CO$_2$-rich water to the surface (Gruber *et al.*, 2012), ambient CO$_2$ levels can reach more than 700 μatm during prolonged upwelling events.

Male and female fish were allowed to ripen naturally without the use of hormonal injection. When females showed external signs of egg ripening (distended abdomen), they were captured from the tank and checked for spawning condition by gently squeezing the abdomen. If eggs did not flow freely, the fish was returned to the tank and re-checked at 3–4 d intervals. When eggs flowed freely, they were collected in a clean, dry container and fertilized with milt from three male fish randomly selected from the broodstock (total of nine males used across all maternal egg batches). Gametes were mixed for 1 min and repeatedly rinsed with ambient pH seawater to remove excess milt and disperse egg clumps. In total, egg batches were collected from five different females with offspring used in egg incubation trials (*n* = 4) and/or larval growth trials (*n* = 3; Table 2).

Egg incubation experiments

From each egg batch used for incubation experiments, three replicate samples of 0.5 ml of eggs (~450 eggs) were incubated in each of the four CO$_2$ treatments. Temperatures were maintained at 8°C

Table 2. Northern rock sole (*L. polyxystra*) egg batches used for experimental rearing of eggs and larvae at ambient and elevated CO$_2$ levels.

Egg batch	Spawn date	DPF sampling hatchers	DPH larval growth
1	26 March 2012	18	–
2	7 February 2013	–	3–33
3	25 February 2013	21	–
4	4 March 2013	21	3–17
5	22 March 2013	21	3–60

Eggs from each female were fertilized with the milt from three males. Eggs from batches 1, 3, 4, and 5 were incubated at multiple CO$_2$ levels and sampled at 18 or 21 d post-fertilization (DPF) for determination of hatch success and size at hatch. Independently, eggs from batches 2, 4, and 5 were incubated at ambient CO$_2$ levels until 3 DPH then reared at ambient and elevated CO$_2$ levels for 14–57 d.

during the incubation period. Each egg sample was incubated in a 1000 ml beaker with 220 μm mesh bottom suspended in a temperature- and pH-regulated tank which served as a water bath. A constant flow of 50 ml min^{-1} of pH-regulated water from the header tank was passed through a 10 μm particle filter into each incubation beaker to maintain pH at the targeted treatment level. The mesh bottoms of the beakers allowed the water to flow over the eggs and out of the incubation beakers into the water bath. The eggs were introduced into the incubation beakers at ambient pH within 10 min of fertilization, after which the flow of pH-controlled water from the header tanks was initiated and pH was adjusted to the treatment pH over a period of ~30 min.

The eggs were left undisturbed in the incubation beakers and allowed to hatch. Hatching occurred between 13 and 18 d after fertilization, consistent with previous observations at similar temperatures (Laurel and Blood, 2011). On day 21 (day 18 for egg batch 1),

all beakers were sampled to determine the number and size of hatched larvae in each replicate. All surviving larvae were counted and scanned for malformation (curved or twisted shape; Laurel and Blood, 2011). Because dead eggs were difficult to distinguish from the shed chorion of hatched larvae, estimates of hatching success were based on the number of hatched larvae counted during sampling and the estimated number of eggs introduced into the incubation beakers. A subsample of 15 larvae was randomly selected from each replicate for size measurements. These fish were digitally photographed under a dissecting microscope. An image analysis system was used to measure standard length (SL), myotome height at the anus (MH), and yolk area (YA) from the photographs. After photographing, subsampled larvae were pooled into groups of five larvae ($n = 3$/replicate) and dried for >24 h at 50°C for determination of dry weight (DW). In trial 1, dry weights were measured on groups of ten larvae independently sampled from all hatched larvae (i.e. the same fish were not used for length and weight measures). Because of the variation in amount of residual yolk at hatch, condition factors of newly hatched larvae were based on MH rather than DW (Hurst et al., 2013). Larval condition index was calculated as the fractional deviation of each fish from a linear relationship between MH and SL (MH_{dev}). The mean value of each metric in each replicate incubation beaker was used as the level of observation in these analyses. Because the CO_2 levels in specific treatments varied across egg batches, ANCOVA with CO_2 level as the covariate and egg batch as a random main effect was used to examine variation in hatch success, fraction of curved larvae, and size-at-hatch metrics (SL, DW, MH_{dev}, and YA).

Larval growth experiments

Three independent larval rearing experiments were conducted with offspring from different egg batches (Table 2). Rearing techniques generally followed those used by Laurel et al. (2014) describing thermal effects on growth of northern rock sole larvae and by Hurst et al. (2013) in ocean acidification experiments on larval walleye pollock. After fertilization, all the eggs to be used in a larval growth trial were incubated together at ambient CO_2 levels ($\sim$300 µatm) in a single layer in a 175 l tank maintained at 6°C. Three days after the estimated midpoint of hatching, yolk sac larvae were gently scooped from the surface water of the egg incubation tank in batches of 200–300 larvae, warmed to 8°C over a 1 h period and introduced to larval rearing tanks. All tanks in each trial were stocked with the same estimated number of larvae, but the stocking density varied across trials due to differences in the cumulative number of larvae available for the experiment (range 1200–2500 fish per tank, Table 2). These stocking densities are similar to those used by Laurel et al. (2014) and below levels expected to induce density-dependent effects on growth rates (Baskerville-Bridges and Kling, 2000). Larvae were reared in black, 100 l tanks with weak up-welling circulation maintained by light aeration and positioning the in-flow (500 ml min^{-1}) at the bottom centre of the tank. Light was provided by overhead fluorescent bulbs on a 12:12 h light:dark photoperiod at a level of 6.7 µE m^{-2} s at the water surface. Prey was introduced 1 d after stocking. Larvae were reared on rotifers (Brachionus plicatilis) enriched with Algamac 3050 (Aquafauna, Hawthorne, CA, USA) which were supplied at densities of 5 prey ml^{-1} twice daily. After 4 weeks of rearing in trial 3, rotifers were supplemented with microparticulate dry food (Otohime A, Marubeni Nisshin Feed Co., Tokyo) 2–3 times per day.

Trial 1 was ended after 4 weeks of rearing (33 d post-hatch, DPH) and all remaining larvae were counted to estimate survival rates.

Owing to lower initial numbers and lower overall survival rates in trial 2, this trial was ended and all surviving fish counted after only 2 weeks of experimental rearing (at 17 DPH). However, the larger numbers of larvae used in trial 3 (and rearing system availability) allowed us to extend two of the three replicates in each treatment through later stages of larval development (to 60 DPH). After 4 weeks of rearing (at 32 DPH), all tanks ($n = 12$) were sampled to determine fish length and weight. The number of larvae surviving to 32 DPH in each tank was estimated by averaging independent visual counts made by two observers (see Laurel et al., 2014, for additional details). An estimated instantaneous daily mortality rate for each tank in all trials was determined from the initial stocking density, the number of fish removed during sampling, and number of survivors after 4 weeks of rearing (2 weeks in trial 2). Because estimated mortality rates were highly variable across tanks and trials and not normally distributed, analysis was performed on ranked values. The estimated mortality rates were ranked within each experimental trial (1 lowest, 12 highest) and compared with treatment CO_2 level across experiments using Spearman rank correlation ($n = 36$ tanks).

During the first 4 weeks of each trial, samples of 15–20 larvae were drawn from each replicate tank at weekly intervals. Additional sampling was conducted in the two extended duration replicates in each treatment of trial 3 at 47 and 60 DPH. Sampled larvae were digitally photographed under a dissecting microscope and measurements were made of SL and MH. After photographing, subsampled larvae were pooled into groups of five larvae ($n = 3$ per replicate) and dried for 24 h at 50°C for determination of dry weight (DW). Because DW was not measured separately for each fish, DW condition factors (DW_{dev}) were estimated for each tank on each sampling date as the per cent deviation from the observed relationship between mean SL and mean DW. The underlying relationship was described by fitting a negative exponential smoothing function to the SL-DW data as ontogenetic patterns in growth and development of the larvae resulted systematic departures from simple exponential, power, or polynomial functions (based on patterns in residuals). The mean size of fish in each replicate tank was used as the level of observation in statistical analyses. Within each experiment, fish size (SL and DW) and condition (DW_{dev}) were analysed with two-way ANOVA with CO_2 level and sampling date as main effects. Post hoc least significant difference (LSD) tests were used to indicate differences among treatments on specific sampling dates.

Results

Egg incubation experiments

Hatching success was high among incubation beakers, with estimated hatch rates averaging 91%. Estimated hatch rates did not differ significantly among egg batches (ANCOVA, $F_{3,42} = 1.45$, $p = 0.241$) and were not affected by incubation CO_2 level ($F_{1,42} = 0.11$, $p = 0.747$). The incidence of deformed ("curled") larvae in the first egg batch was estimated to be <2% but was not scored for individual hatching containers. Among the three later batches, incidence of deformed larvae was not affected by incubation CO_2 level ($F_{1,32} = 0.03$, $p = 0.859$), but did differ among egg batches ($F_{2,32} = 12.55$, $p < 0.01$; highest in batch 4 at 2.8%). There were no significant interactions between batch and CO_2 level on hatch rate or incidence of deformity ($p > 0.50$).

There was no consistent effect of incubation CO_2 level on any of the size-at-hatch metrics across the four egg batches (Figure 1). There was no significant main effect of CO_2 level on any of the
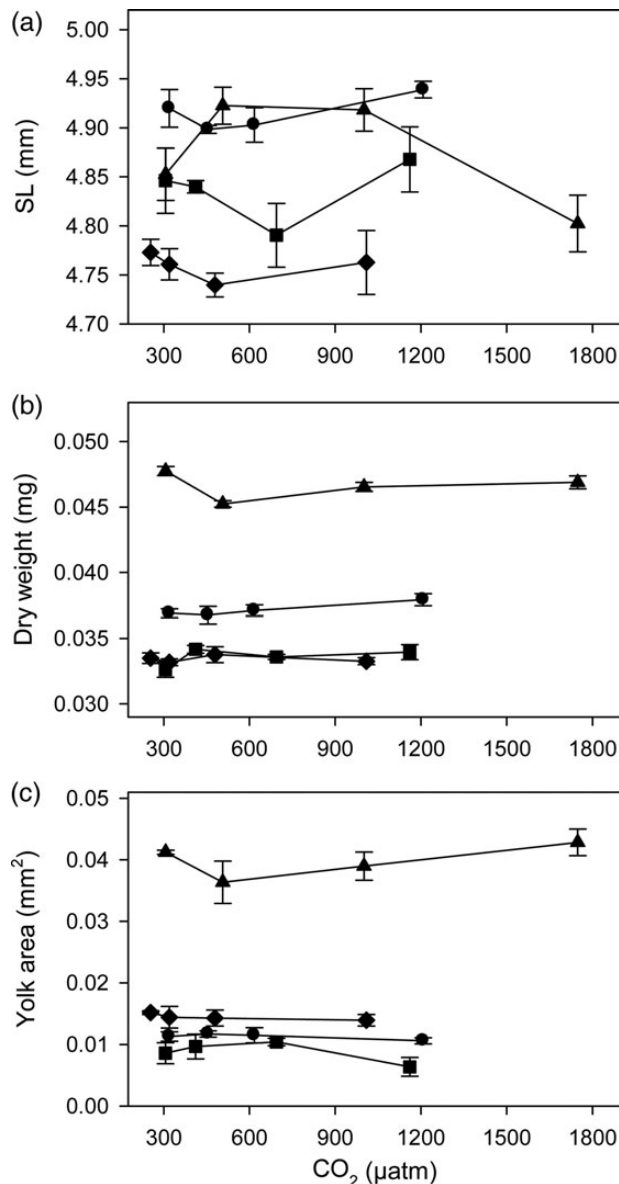
Figure 1. Size at hatch metrics of northern rock sole (*L. polyxystra*) larvae as a function of incubation CO$_2$ level. Points are the mean values ($\pm$ s.e.) of three replicate incubation beakers in each CO$_2$ treatment. Symbols represent batches collected from different females from a laboratory broodstock: batch 1, filled triangle; batch 3, filled circle; batch 4, filled square; batch 5, filled diamond. The higher dry weights and yolk areas in egg batch 1 were due to earlier sampling (18 DPH) than in subsequent batches (21 DPH).

metrics (all $p > 0.1$, Table 3) but all differed significantly among egg batches ($p < 0.01$). Most clearly, larvae from egg batch 1 were significantly heavier and had much larger yolk areas than fish from the other egg batches because they were sampled at 18 rather than 21 d after fertilization.

Larval growth experiments

Larval mortality rates were based on estimated initial densities and direct counts of larvae surviving after 4 weeks (2 weeks in trial 2). Estimated mortality rates were highly variable across the experiment.

Table 3. Analysis of covariance of hatching metrics of four batches of northern rock sole (*L. polyxystra*) eggs incubated across a range of CO$_2$ levels.

Measure	Factor	d.f.	F	p-value
Standard length				
	CO$_2$ level	1	0.87	0.357
	Egg batch	3	25.09	<0.01
	Error	43	–	–
Dry weight				
	CO$_2$ level	1	2.64	0.112
	Egg batch	3	492.36	<0.01
	Error	42	–	–
Condition (MH$_{dev}$)				
	CO$_2$ level	1	0.39	0.149
	Egg batch	3	23.88	<0.01
	Error	43	–	–
Yolk area				
	CO$_2$ level	1	6.81	0.768
	Egg batch	3	285.79	<0.01
	Error	43	–	–

Egg batch is treated as a random main effect. Response variables are the mean value measured in three replicate incubation beakers for each batch $\times$ CO$_2$ treatment.

Because estimated mortality rates were significantly higher in trial 2 than in trials 1 and 3 (Kruskal–Wallis ANOVA $H = 24.35$, $p < 0.01$), there was no overall effect of CO$_2$ level on estimated mortality rates (Figure 2a; Spearman rank correlation, $p < 0.50$). However, when tank mortality rates were ranked within each trial, there was a trend towards greater mortality rates in treatments with higher CO$_2$ levels, although this trend was not statistically significant (Figure 2b; Spearman $R = 0.322$, $p = 0.056$).

Despite the differences in stocking density and observed mortality rates, growth rates were generally similar across the three larval trials. There were no significant differences between trials in SL or DW after 2 weeks of growth (both $F_{2,30} < 1.0$, $p > 0.40$); after 4 weeks of growth, fish in trial 3 were slightly heavier than the fish in trial 1 ($F_{1,19} = 5.12$, $p = 0.036$), but there was no significant difference in length ($F_{1,19} = 1.62$, $p = 0.389$).

Throughout the three experimental trials, there were only minor differences in growth rates of northern rock sole larvae across CO$_2$ treatments (Figure 3). In trials 1 and 2, there was no significant main effect of CO$_2$ level or interaction with age on SL or DW (Table 4). In trial 3, which was extended to 60 DPH, there was a significant effect of CO$_2$ level with fish in the low CO$_2$ treatment being significantly longer than fish in the elevated CO$_2$ treatments, especially after 25 DPH (significant *post hoc* LSD tests among treatments at 25, 32, and 60 DPH). There was also a significant effect of CO$_2$ and significant interaction with age on DW in trial 3. Again, these responses were driven by the heaviest fish being in the low CO$_2$ treatment, especially after 25 DPH (significant differences among treatments at 47 and 60 DPH). The interaction was caused by fish in the ambient treatment which tended to be large through the first month of the experiment, were small on days 32 and 47, and large on the final sample at 60 DPH.

Dry weight condition factors were based on deviations of the relationship between tank mean SL and DW from samples pooled across the three experiments. Statistical patterns varied across the three trials (Table 4), but lower condition factors were generally observed in treatments with elevated CO$_2$ levels. In trial 1, there was a significant interaction between age and CO$_2$ level on condition factor, with the lowest condition factors observed in either the
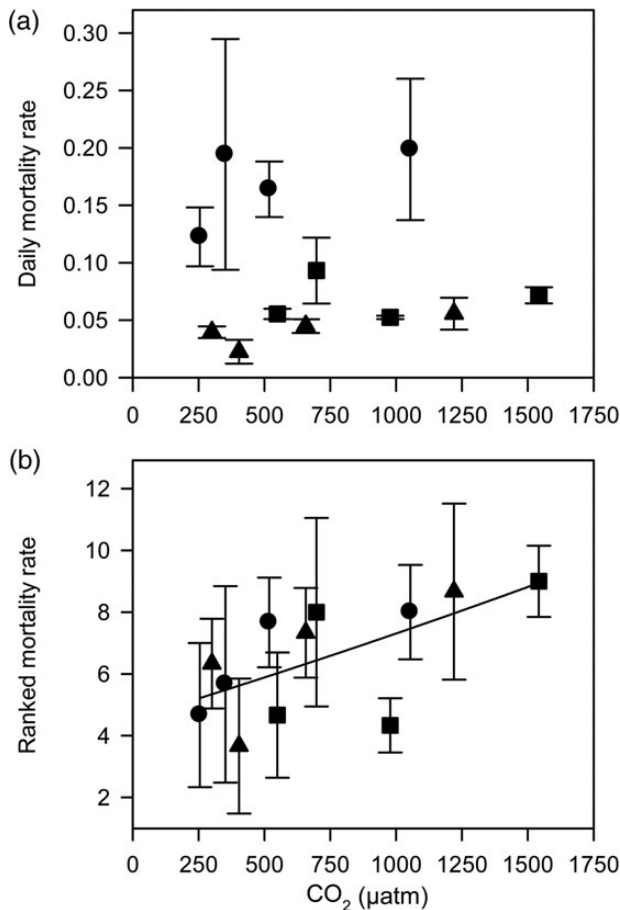
Figure 2. Mortality rates of northern rock sole (*L. polyxystra*) larvae as a function of CO_2 level. Values are mean ($\pm$ s.d.) of estimated mortality rates (a) and ranked mortality rates (1–12) within each experimental trial (b). Symbols represent trial 1, filled triangle; trial 2, filled circle; trial 3, filled square, reared from egg batches 2, 4, and 5, respectively.

medium or high CO_2 treatment at each sampling date (significant differences among treatments at 12, 19, and 26 DPH, but not 33 DPH; Figure 3). In trial 2, there were no significant effects of age or CO_2 level on condition factor. The differences among treatments were most dramatic during the extended trial 3 when there was a significant main effect of CO_2 treatment on condition factor; lowest values were observed in the high CO_2 treatment (Table 4) diverging markedly after 30 DPH (Figure 3). *Post hoc* LSD tests indicated that condition factor in the high CO_2 treatment was lower than the ambient and low CO_2 treatments at 47 DPH and lower than all other treatments at 60 DPH.

Discussion

Experimental work has demonstrated a wide range of biological effects stemming from exposure to elevated environmental CO_2 levels associated with ongoing ocean acidification. The nature and magnitude of these effects have been shown to vary widely among species groups (Fabry *et al.*, 2008; Wittmann and Pörtner, 2013). While fish as a group appear less sensitive to some of the direct effects of high CO_2 than calcifying invertebrates, negative effects on the early life stages and disruptions of behavioral and sensory biology have been demonstrated in a diverse group of species (Leduc *et al.*, 2013). Results of the experiments presented here

suggest that during the first few weeks of life, northern rock sole are not negatively impacted by CO_2 levels that would be encountered in Alaskan surface waters over the next few decades (Mathis *et al.*, 2014). These results are generally similar to those observed for early life stages of walleye pollock, the only other commercially important North Pacific fish species examined to date (Hurst *et al.*, 2012, 2013). However, two observations suggested that northern rock sole larvae may be less resilient to high CO_2 levels than larval walleye pollock, despite having been tested across a narrower range of CO_2 levels. When reared for longer periods (trial 3), northern rock sole larvae in the highest CO_2 treatments were significantly smaller and in poorer condition than fish in the lower CO_2 treatments. In addition, although it was not statistically significant, we observed a trend of larvae in higher CO_2 treatment tanks generally having higher estimated mortality rates than fish at lower CO_2 levels, a pattern not observed in similarly conducted walleye pollock experiments (Hurst *et al.*, 2013). As these experiments addressed only the direct effects of high CO_2 on growth and survival northern rock sole larvae, a more comprehensive evaluation of the impacts of ocean acidification on the productivity of this resource species requires consideration of interactions with other environmental stressors and the behavioral and sensory deficits observed in other species (Leduc *et al.*, 2013; Gräns *et al.*, 2014). Below, we discuss these results in the context of other temperate and boreal marine fish species and the considerations of applying laboratory exposure studies to ocean acidification effects on natural populations.

Acidification effects in flatfish

Understanding the mechanisms responsible for species-specific differences in sensitivity to ocean acidification of fish early life stages will be critical to predicting ecosystem impacts. Unfortunately, there remain too few studies across the range of phylogenetic groups and life history guilds to draw firm conclusions about the factors that may influence species-specific sensitivity to the elevated CO_2 levels associated with ocean acidification. Flatfish (Pleuronectiformes) are a monophyletic group with a worldwide distribution in boreal, temperate, and tropical seas (Munroe, 2005). Flatfish's unique life history includes a metamorphosis more dramatic than that of most other fish. This complex development may make this species group vulnerable to environmental stressors and a potentially useful model group to explore the effects of ocean acidification in marine fish. In addition, due to interest in aquaculture of these species for commercial and conservation purposes, rearing and culture techniques are being established for an increasing number of flatfish species, facilitating laboratory experimentation and cross-species comparisons.

To date, there have been only two published reports of the effects of high CO_2 on the eggs and larvae of marine flatfish (Chambers *et al.*, 2014; Pimentel *et al.*, 2014). Among the three studied species, northern rock sole may be the least sensitive to elevated CO_2 levels as the effects were limited to a non-significant trend towards increased larval mortality rate and reduced growth and condition in postflexion larvae. Conversely, Senegalese sole (*Solea senagalensis*) appear to be most sensitive to elevated CO_2 levels, exhibiting significantly reduced growth and survival of larvae at elevated CO_2 levels ($\sim$1600 μatm; Pimentel *et al.*, 2014). Responses in summer flounder (*Paralichthys dentatus*) were less severe than observed in Senegal sole but potentially more severe than those observed in northern rock sole as exposure to elevated CO_2 levels ($\sim$1800 and 4714 μatm) resulted in reduced hatching success and increased abnormalities in the livers and trunk musculature, but these were not associated with reductions in growth rate (Chambers *et al.*, 2014).
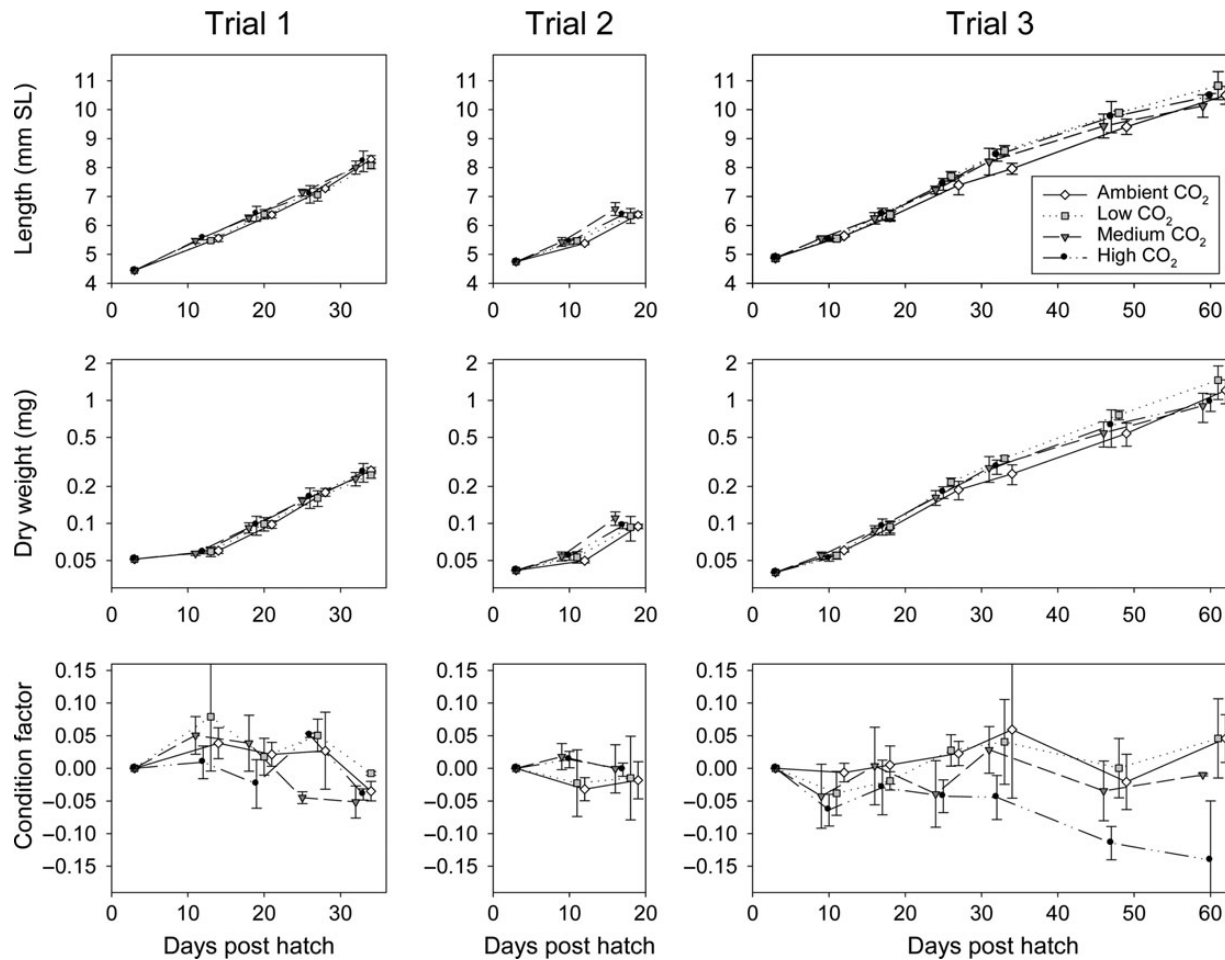
Figure 3. Growth and condition of northern rock sole (*L. polyxystra*) larvae reared at ambient and elevated CO_2 levels in three independent trials. Points represent mean ($\pm$ s.d.) of replicate tanks within each treatment. Note: all tanks sampled on the same days in each trial, points offset horizontally for clarity.

Table 4. Analysis of variance of size and dry weight-condition factor of northern rock sole (*L. polyxystra*) larvae reared across a range of CO_2 levels.

Factor	d.f.	Standard length F	p-value	Dry weight F	p-value	Condition (DW_{dev}) F	p-value
Trial 1							
CO_2 level	3	1.83	0.161	2.03	0.129	3.08	0.041
Day	3	475.18	<0.001	246.04	<0.001	11.14	<0.001
$CO_2 \times$ day	9	0.45	0.899	0.52	0.849	2.39	0.034
Error	32	–	–	–	–	–	–
Trial 2							
CO_2 level	3	0.79	0.157	1.305	0.307	1.42	0.273
Day	1	264.45	<0.001	133.35	<0.001	0.05	0.830
$CO_2 \times$ day	9	0.94	0.445	0.85	0.485	0.32	0.808
Error	32	–	–	–	–	–	–
Trial 3							
CO_2 level	3	6.32	0.001	5.68	0.002	12.07	<0.001
Day	5	592.99	<0.001	136.49	<0.001	2.68	0.035
$CO_2 \times$ day	15	1.01	0.461	2.01	0.040	1.06	0.416
Error	40	–	–	–	–	–	–

Response variables are the tank mean value measured on each sampling date in each of three replicate rearing tanks in each experiment.

The differences observed across flatfish species suggest that caution needs to be applied when making broad generalizations about the physiological sensitivity of fish guilds, but suggest a pattern in species sensitivity which has not been previously examined. The differences observed among flatfish species in magnitude of effects from high CO_2 may be related to thermal regimes and intrinsic development rates: Senegal sole is a subtropical species with rapid development rates. Perhaps the more slowly developing temperate and boreal species such as summer flounder and northern rock sole are less sensitive to the detrimental effects of hypercapnia. As indicated in extended rearing with northern rock sole, it is also possible that the negative effects of elevated CO_2 may take longer to manifest or that it is the later metamorphic-stages that are more sensitive to high CO_2 stress (Pope *et al.*, 2014). We suggest that comparisons across widely distributed taxonomic groups could be especially informative in identifying general patterns of sensitivity to ocean acidification and that comparisons consider the role of thermal guild as well as taxonomic classification.

Experimental considerations

A number of factors should be considered when inferring or modelling population and community level responses to ocean acidification based on experimental exposures to environmental

hypercapnia. These studies represent acute exposures to rapidly altered conditions and do not allow the opportunity for acclimation or adaptation to more gradual, long-term environmental change. In addition, no individual study is sufficiently comprehensive to evaluate the effects of elevated CO_2 across the full range of life history stages and physiological and behavioural responses. This can lead to over- or underestimation of overall species sensitivity as observed sensitivity (or resiliency) in one life stage or trait may not predict a similar degree of sensitivity in other stages or traits (Maneja *et al.,* 2012; Cripps *et al.,* 2014).

In particular, we suggest that experimental evaluations of sensitivity of marine fish larvae should be extended to encompass later developmental stages which may be more sensitive (Pope *et al.,* 2014). In early studies, Kikkawa *et al.* (2003) found that the median lethal CO_2 levels were higher in early stage "pre-flexion" larvae than later stage "post-flexion" larvae in both red sea bream (*Pagrus major*) and Japanese whiting (*Sillago japonica*). More recent studies with ocean acidification-relevant CO_2 levels have identified negative effects in later stages that were not evident in the first few weeks of life (Frommel *et al.,* 2012; but see Maneja *et al.,* 2012). This extended rearing will be especially important in studies of cold-water species with slow development rates.

The evolutionary and environmental history of the source population should also be considered in evaluating species-specific sensitivity to projected ocean acidification. Several studies have found differential responses among tested populations (e.g. Atlantic herring *Clupea harengus*; Franke and Clemmesen, 2011; Frommel *et al.,* 2014) and parental conditioning has been shown to mitigate some of the detrimental effects of high CO_2 in early life stages (Miller, *et al.,* 2012; Murray, *et al.,* 2014; but see Schade, *et al.,* 2014). In a previous study, Hurst *et al.* (2013) cautioned that the resilience of larval walleye pollock to elevated CO_2 levels may be greater in the examined Puget Sound population than would be observed in the more northerly harvested populations. The parental broodstock of northern rock sole used in this study were collected from the central Gulf of Alaska, in the middle of the commercial range of the species. Therefore, we do not believe that population differences would limit the applicability of these results to the harvested populations in Alaskan waters.

With regard to the potential effects of parental conditioning on the responses of northern rock sole offspring to elevated CO_2, the conditions experienced by fish in our laboratory culture are not unlike those that wild fish would experience in Alaskan waters. Recent observations from the Bering Sea shelf (Mathis *et al.,* 2013) found that while surface waters in spring (experienced by larvae) had CO_2 concentrations <300 μatm, CO_2 concentrations in the bottom waters (experienced by adults) over 1000 μatm were observed for extended periods (>3 months). Therefore, while exposure to seasonally elevated CO_2 levels experienced by the parental broodstock (outside the spawning season) may have conferred some acclimation to the offspring examined in this study, this would appear to be a natural aspect of the species life history that would influence sensitivity to future increases in environmental CO_2 levels (Murray *et al.,* 2014).

Conclusions

Prediction of the ecosystem and socio-economic effects of ocean acidification depends on identifying the nature and magnitude of high CO_2 effects across a range of ecosystem components and valuable resource species (Cooley and Doney, 2009; Mathis *et al.,* 2014). Species-specific sensitivity to ocean acidification is likely to be a

function of both taxonomic and life history considerations. Experiments on the eggs and larvae of northern rock sole indicated a trend towards higher larval mortality rates and lower condition factors at elevated CO_2 levels, but only at CO_2 levels above those predicted for Alaskan waters in the next 100 years. While less severe than the effects observed in some other species (e.g. Frommel *et al.,* 2014; Pimentel *et al.,* 2014), these effects were observed in northern rock sole at CO_2 levels that did not induce any negative effects in walleye pollock, another commercially important fishery species in the Gulf of Alaska and Bering Sea (Hurst *et al.,* 2012, 2013). Comparing the results observed here for northern rock sole with those of other flatfish species, we suggest that thermal guild and developmental rate may be critically relevant traits determining the degree of sensitivity among species. Finally, we caution that these experiments have addressed only one aspect of the potential effects of ocean acidification on this species. Future work should focus on high-CO_2 interactions with other environmental factors and the potential for sensory and behavioural deficits (Leduc *et al.,* 2013; Gräns *et al.,* 2014) which may be the primary pathways of action of ocean acidification in some fish species.

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.

Bignami, S., Sponaugle, S., and Cowen, R. K. 2013. Response to ocean acidification in larvae of a large tropical marine fish, *Rachycentron canadum*. Global Change Biology, 19: 996–1006.

Chambers, R. C., Candelmo, A. C., Habeck, E. A., Poach, M. E., Wieczorek, D., Cooper, K. R., Greenfield, C. E., *et al.* 2014. Effects of elevated CO_2 in the early life stages of summer flounder, *Paralichthys dentatus*, and potential consequences of ocean acidification. Biogeosciences, 11: 1613–1626.

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

Cooper, D. W., Duffy-Anderson, J. T., Norcross, B. L., Holladay, B. A., and Stabeno, P. J. 2014. Northern rock sole (*Lepidopsetta polyxystra*) juvenile nursery areas in the eastern Bering Sea in relation to hydrography and thermal regimes. ICES Journal of Marine Science, 71: 1683–1695.

Cripps, G., Lindeque, P., and Flynn, K. J. 2014. Have we been underestimating the effects of ocean acidification in zooplankton? Global Change Biology, 20: 3377–3385.

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.

Doyle, M. J., Picquelle, S. J., Mier, K. L., Spillane, M. C., and Bond, N. A. 2009. Larval fish abundance and physical forcing in the Gulf of Alaska, 1981–2003. Progress in Oceanography, 80: 163–187.

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

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.

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.

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.

Frommel, A. Y., Maneja, R., Lowe, D., Pascoe, C. K., Geffen, A. J., Folkvord, A., Piatkowski, U., *et al.* 2014. Organ damage in Atlantic herring larvae as a result of ocean acidification. Ecological Applications, 24: 1131–1143.

Gattuso, J-P., and Hansson, H. 2011. Ocean Acidification. Oxford University Press, Oxford.

Gräns, A., Jutfelt, F., Sandblom, E., Jonsson, E., Wiklander, K., Seth, H., Olsson, C., *et al.* 2014. Aerobic scope fails to explain the detrimental effects on growth resulting from warming and elevated CO_2 in Atlantic halibut. Journal of Experimental Biology, 217: 711–717.

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.

Hurst, T. P., Abookire, A. A., and Knoth, B. 2010. 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., Fernandez, E. R., and Mathis, J. T. 2013. Effects of ocean acidification on hatch size and larval growth of walleye pollock (*Theragra chalcogramma*). ICES Journal of Marine Science, 70: 812–822.

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. 2014. Climate Change 2014: Synthesis Report of the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. 132 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.

Lanksbury, J. A., Duffy-Anderson, J. T., Mier, K. L., Busby, M. S., and Stabeno, P. J. 2007. Distribution and transport patterns of northern rock sole, *Lepidopsetta polyxystra*, larvae in the southeastern Bering Sea. Progress in Oceanography, 72: 39–62.

Laurel, B. J., and Blood, D. M. 2011. The effects of temperature on hatching and survival of northern rock sole larvae (*Lepidopsetta polyxystra*). Fishery Bulletin, 109: 282–291.

Laurel, B. J., Haines, S. A., and Danley, C. 2014. The effects of temperature on growth, development and settlement of northern rock sole larvae (*Lepidopsetta polyxystra*). Fisheries Oceanography, 23: 495–505.

Leduc, A. O. H. C., Munday, P. L., Brown, G. E., and Ferrari, M. C. O. 2013. Effects of acidification on olfactory-mediated behaviour in freshwater and marine ecosystems: a synthesis. Philosophical Transactions of the Royal Society B—Biological Sciences, 368: 20120447.

Lewis, E., and Wallace, D. W. R. 1998. Program developed for CO_2 system calculations: ORNL/CDIAC-105.

Lindsay, K., Bonan, G. B., Doney, S. C., Hoffman, F. M., Lawrence, D. M., Long, M. C., Mahowald, N. M., *et al.* 2014. Preindustrial control and 20th century carbon cycle experiments with the earth system model CESM1(BGC). Journal of Climate, 27: 8981–9005.

Long, W. C., Swiney, K. M., and Foy, R. J. 2013a. Effects of ocean acidification on the embryos and larvae of red king crab, *Paralithodes camtschaticus*. Marine Pollution Bulletin, 69: 38–47.

Long, W. C., Swiney, K. M., Harris, C., Page, H. N., and Foy, R. J. 2013b. Effects of ocean acidification on juvenile red king crab (*Paralithodes camtschaticus*) and Tanner crab (*Chionoecetes bairdi*) growth, condition, calcification, and survival. Plos One, 8: e60959.

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, 160: 1963–1972.

Mathis, J. T., Cooley, S. R., Lucey, N., Hauri, C., Ekstrom, J., Hurst, T. P., Colt, S., *et al.* 2014. Ocean acidification risk assessment for Alaska's fishery sector. Progress in Oceanography, in press.

Mathis, J. T., Cross, J. N., and Bates, N. R. 2011a. The role of ocean acidification in systemic carbonate mineral suppression in the Bering Sea. Geophysical Research Letters, 38: GL048884.

Mathis, J. T., Cross, J. N., and Bates, N. R. 2011b. 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., Cross, J. N., Monacci, N., Feely, R. A., and Stabeno, P. J. 2013. Evidence of prolonged undersaturations in the bottom waters of the southeastern Bering Sea shelf from autonomous sensors. Deep Sea Research II, 109: 125–133.

Mathis, J. T., and Questel, J. M. 2013. Assessing seasonal changes in carbonate parameters across small spatial gradients in the Northeastern Chukchi Sea. Continental Shelf Research, 67: 42–51.

Melzner, F., Gobel, S., Langenbuch, M., Gutowska, M. A., Pörtner, H-O., and Lucassen, M. 2009a. Swimming performance in Atlantic Cod (*Gadus morhua*) following long-term (4–12 months) acclimation to elevated seawater P(CO2). Aquatic Toxicology, 92: 30–37.

Melzner, F., Gutowska, M. A., Langenbuch, M., Dupont, S., Lucassen, M., Thorndyke, M. C., Bleich, M., *et al.* 2009b. 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, 12: 858–861.

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

Munroe, T. A. 2005. Systematic diversity of the Pleuronectiformes. *In* Flatfishes: Biology and Exploitation, pp. 10–41. Ed. by R. N. Gibson. Blackwell, Oxford.

Murray, C. S., Malvezzi, A., Gobler, C. J., and Baumann, H. 2014. Offspring sensitivity to ocean acidification changes seasonally in a coastal marine fish. Marine Ecology Progress Series, 504: 1–11.

Pimentel, M. S., Faleiro, F., Dionisio, G., Repolho, T., Pousao-Ferreira, P., Machado, J., and Rosa, R. 2014. Defective skeletogenesis and oversized otoliths in fish early stages in a changing ocean. Journal of Experimental Biology, 217: 2062–2070.

Pope, E. C., Ellis, R. P., Scolamacchia, M., Scolding, J. W. S., Keay, A., Chingombe, P., Shields, R. J., *et al.* 2014. European sea bass, *Dicentrarchus labrax*, in a changing ocean. Biogeosciences, 11: 2519–2530.

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. 2010. Guide to best practices for ocean acidification research and data reporting, p. 260. Publications Office of the European Union, Luxembourg.

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.

Schade, F. M., Clemmesen, C., and Wegner, K. M. 2014. Within- and transgenerational effects of ocean acidification on life history of marine three-spined stickleback (*Gasterosteus aculeatus*). Marine Biology, 161: 1667–1676.

Stark, J. W., and Somerton, D. A. 2002. Maturation, spawning and growth of rock soles off Kodiak Island in the Gulf of Alaska. Journal of Fish Biology, 61: 417–431.

van Vuuren, D. P., Edmonds, J., Kainuma, M., Riahi, K., Thomson, A., Hibbard, K., Hurtt, G. C., *et al.* 2011. The representative concentration pathways: an overview. Climate Change, 109: 5–31.

Wittmann, A. C., and Pörtner, H-O. 2013. Sensitivities of extant animal taxa to ocean acidification. Nature Climate Change, 3: 995–1001.

Handling editor: Howard Browman