Biogeosciences Discuss., 10, 8313–8341, 2013
www.biogeosciences-discuss.net/10/8313/2013/
doi:10.5194/bgd-10-8313-2013

Biogeosciences Discussions Open Access

Physiological compensation for environmental acidification is limited in the deep-sea urchin *Strongylocentrotus fragilis*

J. R. Taylor, C. Lovera, P. J. Whaling, K. R. Buck, E. F. Pane, and J. P. Barry

Monterey Bay Aquarium Research Institute, Moss Landing, California, USA

Received: 15 April 2013 – Accepted: 26 April 2013 – Published: 17 May 2013

Correspondence to: J. R. Taylor (josi@mbari.org)

Published by Copernicus Publications on behalf of the European Geosciences Union.

Abstract

Anthropogenic CO_2 is now reaching depths over 1000 m in the Eastern Pacific, overlapping the Oxygen Minimum Zone (OMZ). Deep-sea animals – particularly, calcifiers – are suspected to be especially sensitive to environmental acidification associated with global climate change. We have investigated the effects of hypercapnia and hypoxia on the deep-sea urchin *Strongylocentrotus fragilis*, during two long-term exposure experiments (1 month and 4 month) at three levels of reduced pH at in situ O_2 levels of approx. 10 % saturation, and also to control pH at 100 % O_2 saturation. During the first experiment, internal acid-base balance was investigated during a one-month exposure; results show *S. fragilis* has limited ability to compensate for the respiratory acidosis brought on by reduced pH, due in part to low non-bicarbonate extracellular fluid buffering capacity. During the second experiment, longer-term effects of hypercapnia and variable O_2 on locomotion, feeding, growth, and gonadosomatic index (GSI) were investigated; results show significant mortality and correlation of all measured parameters with environmental acidification at pH 6.6. Transient adverse effects on locomotion and feeding were seen at pH 7.2, without compromise of growth or GSI. Based on the expected changes in ocean pH and oxygen, results suggest extinction of *S. fragilis* in the eastern North Pacific is unlikely. Rather, we expect a shoaling and contraction of its bathymetric range.

1 Introduction

Anthropogenic CO_2 is penetrating to the deep waters of the oceans through thermohaline circulation and other vertical mixing processes, and has now reached depths over 1000 m in the Eastern Pacific (Gruber and Sarmiento, 2002; Sabine et al., 2004), overlapping the Oxygen Minimum Zone (OMZ) and driving ocean acidification (OA). Increased CO_2 concentrations reduce even further the typically low pH of OMZs, which are known to be stressful environments for many taxa. As OA and climate-related

BGD
10, 8313–8341, 2013

deoxygenation (Doney et al., 2009b; Keeling and Garcia, 2002; Stramma et al., 2011) intensifies in the future, animals in OMZs are expected to experience increased physiological stress, particularly for calcifying taxa (Dupont et al., 2010; Melzner et al., 2012; Portner et al., 2011). Deep-sea animals living in this dark, energy-poor environment have evolved with moderate to mild temporal variation in biochemical conditions, but very large spatial variation in biochemical conditions. In particular, taxa spanning a range of depths within and beyond an OMZ experience very large ranges of temperature, pH, oxygen, and food; not as individuals, but as populations. The high amount of genetic diversity presumably required for a population to persist throughout a bathymetric range including an OMZ suggests we should expect fairly high tolerance to at least the extent of conditions seen within its bathymetric range. We expect the apparent limitation in tolerance of OMZ taxa to global climate change is not a product of evolutionary constancy, but rather is a product of the limited energy (food and oxygen) available to cope with the stress of environmental change (Barry et al., 2011; Pane and Barry, 2007; Seibel and Walsh, 2003).

Environmental acidification can affect an individual marine animal's energy budget by disrupting homeostatic processes; parameters including growth rates, reproduction, and nutritional status may consequently be reduced (Dupont et al., 2012; Portner, 2012; Uthicke and Fabricius, 2012). The extent to which a marine animal can regulate extracellular fluid acid-base balance thus offers a reasonable predictor of the extent to which behavior, growth, and reproduction may be affected by increases in environmental acidity (Seibel and Walsh, 2003; Spicer et al., 2007). Proper function of enzymes, including those necessary for homeostatic processes, demands precise internal pH conditions; precise regulation of acid-base balance is typically far better honed in more phylogenetically derived animals (e.g., bony fishes as compared to aquatic invertebrates). Studies have shown major species-dependence in the acid-base regulatory capacity of sea urchins (Calosi et al., 2013). Shallow-living sea urchins *Psammechinus miliaris* and *Echinus esculentus* were shown to perfectly compensate the respiratory acidosis induced by emersion (Spicer et al., 1988). Similarly, *Strongylocentrotus droebachiensis*,

BGD
10, 8313–8341, 2013

a taxa experiencing considerable environmental variability, is able to fully or partially compensate extracellular pH changes by accumulation of bicarbonate (Stumpp et al., 2012). However, taxa distributed around a CO_2 vent revealed very different capacities for accumulation of extracellular bicarbonate between sea urchins *Arbacia lixula* and *Paracentrotus lividus* (Calosi et al., 2013). To our knowledge, the ability of deep-sea urchin taxa to regulate extracellular acid-base balance is yet unknown.

Ocean acidification typically does not happen as a solitary environmental change; it is often accompanied by warming temperatures and less frequently by hypoxia. Each of these parameters can act simultaneously on individual physiology; occasionally in a way that offsets negative effects of one stressor, but additive (or synergistic) negative effects are also observed. For example, concomitant low O_2 and high CO_2 interfere with aquatic respiration as O_2 becomes more difficult to obtain from the hypoxic water and CO_2 becomes more difficult to excrete to the water along a weaker concentration gradient. The scientific and popular literature is rich with examples of the effects of multiple climate stressors on aquatic animals (Crain et al., 2009; Howell, 2012; Najjar et al., 2010; Wahl, 2009); however, the OA literature has yet to be enriched by the addition of practical multiple stressor studies.

Finally, calcification is one of the more clearly visible processes adversely affected by rising ocean acidity. Larval exposure to elevated CO_2 conditions has been shown to be particularly detrimental to sea urchins (Byrne et al., 2013; Dupont et al., 2010; Hofmann et al., 2010; Kurihara et al., 2012); however, even adults of some taxa (e.g., echinoderms and crustaceans) use carbonate from their test to buffer extracellular fluid acidification (Kroeker et al., 2010, 2013; Melzner et al., 2009; Shirayama, 2005).

A high priority motivating research concerning the effects of OA on marine animal physiology is to understand more fully the potential impacts of OA on ecosystem services important to society. For most, the deep-sea is unseen and unheard, but society depends upon the continuing function and health of deep-sea ecosystems for many fisheries and more fundamentally for their role in key biogeochemical cycles (Armstrong et al., 2010; Danovaro et al., 2008; Dell'Anno and Danovaro, 2005;

BGD
10, 8313–8341, 2013

Jørgensen and Boetius, 2007; Suttle, 2007). Unfortunately, measuring and valuing deep-sea ecosystem functions and services is difficult, as our quantitative knowledge of these goods and services, and their interactions, is very limited (Armstrong et al., 2010).

The deep-sea fragile urchin, *Strongylocentrotus fragilis*, is a member of the Strongylocentrotidae, a worldwide, and largely shallow water family, that inhabits the upper continental slope along the eastern North Pacific, ranging in depth from 200–1200 m (in seawater chemistry described in Fig. 1) off central California. It is a key member of the benthic megafaunal community on the upper slope, where it is an important detritivore. The bathymetric range of *S. fragilis* spans a fairly large gradient in temperature, oxygen, pH, and presumably food supply, each of which may affect its individual performance and population distribution. We hypothesize that the physiological performance of *S. fragilis* will vary considerably over the large range of environmental variability across its bathymetric range, and it will be most vulnerable to ocean acidification and hypoxia in the core of the OMZ. Two experiments have been used to test this hypothesis. The first addresses the ability of *S. fragilis* to regulate internal acid-base balance at near- and far-future levels of environmental CO_2. The second set of experiments addresses the behavioral, nutritional, and growth consequences of similar levels of CO_2 on *S. fragilis*, and also examined whether this taxon is able to improve upon baseline rates of locomotion, feeding, and growth, on exposure to surface levels of O_2 rather than typical OMZ levels.

2 Methods

2.1 Urchin collection and maintenance

S. fragilis were captured by suction sampler from the Remotely Operated Vehicle (ROV) *Ventana*, operated from the R/V *Point Lobos*, from depths of approx. 500 to 1000 m in Monterey Bay, CA (Fig. 2), during late 2010 and early 2011. Once aboard the R/V *Point*

BGD
10, 8313–8341, 2013

Lobos, animals were kept in 5 °C seawater for 3–5 h until arrival at the Monterey Bay Aquarium Research Institute (MBARI). Urchins were then held in climate-controlled environmental chambers in the laboratory, in an approx. 50 L acrylic tank receiving flow-through chilled seawater pumped in from Monterey Bay (5.5 ± 0.5 °C, 220 ± 20 µM O_2, 34 ± 1 ppt salinity).

2.2 Experimental design

During both experiments, individual urchins were held in 1 L glass jars overflowing with seawater with the desired chemistry at a rate of approx. 30 mL min^{-1}, for the duration of the experiment. Target conditions for seawater oxygen and pH were maintained throughout the study period by a PC-manipulated, Gas-Controlled Aquarium (GCA) system supplying seawater to the laboratory's climate-controlled environmental chamber. In brief, oxygen and pH for each seawater treatment holding tank were controlled using a combination of sensors (Aanderra oxygen optodes and Honeywell pH probes) and membrane contactors connected to recirculation pumps and gas sources. A LabVIEW software system integrated with mass flow controllers for oxygen, carbon dioxide, and nitrogen sources allows real-time, automated regulation of gas concentrations in each tank (Barry et al., 2008).

Experimental seawater conditions were monitored throughout using a logging system for pH, oxygen, and temperature sensors in the seawater head tanks, and from samples collected directly from jars containing urchins (pH and Total CO_2 (C_{CO_2}) measurements). During regular spot-sampling, treatment and control conditions within jars were checked using a Thermo Scientific Orion 5-Star handheld meter with an optode and temperature compensated pH probe. The Orion optode was calibrated using a two-point method at temperature (approx. 6 °C). The probe comes with a sleeve to provide a 100 % saturation calibration point; a sodium dithionate solution was used to generate a 0 % saturation point. An Aanderraa optode was also used to crosscheck the oxygen (% saturation, µM) measurements determined by the GCA system. During jar sampling, the water flow delivery line was removed and the probes and cables (bound

BGD
10, 8313–8341, 2013

together) were lowered mid-way into the 1 L volume of seawater in the jars, easily avoiding the animal contained therein. Output was given 5 min to stabilize, after which values from each probe were recorded.

During animal sampling events, jar seawater and tap samples were also collected in glass, gas-tight Scintillation vials with negligible headspace and analyzed within one hour, using the same protocols by which concurrently sampled animal fluids were analyzed. For these samples, C_{CO_2} was measured by non-dispersive infrared analysis (LI-COR model 6262), as detailed by Friederich et al. (2002) In brief, samples were acidified (5 % phosphoric acid) and the stripped gas introduced into an infrared analyzer. A standard curve was created using sodium carbonate (dried for 4 h at 250 °C before making carbonate standards), and resultant values standardized to certified reference material (A. Dickson CRM, Scripps Institute of Oceanography, La Jolla, CA). Seawater pH was measured by spectrophotometry using the indicator dye m-cresol purple (Clayton and Byrne, 1993; SOP 6b of Dickson et al., 2007; Part 1 of Riebesell et al., 2010).

2.3 Experiment one: internal acid-base balance

Experiment one was used to measure the ability of *S. fragilis* to regulate internal acid-base balance under a range of environmental conditions, and evaluate the hypothesis that its acid-base regulatory capacity is weak. The impact of hypercapnia on urchin acid-base balance was examined over a 31 day period. Four treatments (30 individuals per treatment) were defined as $pH_{(total\ scale)}$ 8.0, 7.5, 7.1, and 6.7 at in situ O_2 (25 µM) and 5 °C. No animals were fed during the experimental period.

2.3.1 Blood buffering capacity

The non-bicarbonate buffering capacity (β) of extracellular fluids was determined for urchins collected at the same time, but not undergoing other experimentation. Samples of extracellular fluids were drawn anaerobically through the oral membrane into ice-cold

BGD
10, 8313–8341, 2013

gas-tight Hamilton syringes rinsed with a modified Crab Ringer comprised of NaCl (460 mM), KCl (10 mM), $CaCl_2$ (20 mM), $MgCl_2$ (9.5 mM), adjusted to pH 7.8 (Lang and Gainer, 1969; Pane and Barry, 2007). Fluid samples were then centrifuged aerobically at 4000 × g at 5 °C for 1 min to remove debris, added (200 µL) to round-bottom flasks for equilibration with humidified gas mixtures of CO_2 and nitrogen from pre-analyzed cylinders (Airgas), and allowed to equilibrate for 90 min in a shaking cooler at 5 °C. Samples were then drawn into gas-tight syringes and $pH_{(total\ scale)}$ was measured using a microelectrode and in-line reference electrode (Microelectrodes) thermostatted to 5 °C and coupled to an Accumet (Fisher Scientific) pH meter. pH was standardized with Radiometer Analytical precision buffers adjusted to the ionic strength of *S. fragilis* extracellular fluid (~ 1050 mOsm). C_{CO_2} was measured as for seawater samples by non-dispersive infrared analysis (LI-COR).

Extracellular fluid CO_2 tension (P_{aCO_2}) and [HCO_3^-], based on measured pH and CCO_2, were calculated by rearrangement of the Henderson–Hasselbach equation with values for CO_2 solubility (αCO_2) and apparent pK (pK′) at the appropriate temperature taken from Boutilier et al. (1984) and Truchot (1976). βs were derived from linear regression of pH-bicarbonate plots.

2.3.2 Blood chemistry

Five animals were terminally sampled from each pH treatment 5 days before treatment conditions were achieved; and on days 0 (when treatment conditions were achieved after a 5 day ramp to conditions), 1, 3, 7, and 31 of treatment. Extracellular fluids were attained while animals were gently held by hand at the top of their respective jars in the environmental chamber, aboral surface just below the water's surface. Approx. 2 mL fluid was drawn anaerobically through the oral membrane into ice-cold gas-tight Hamilton syringes rinsed with the modified Crab Ringer described above. Extracellular fluids were held on ice in their sampling syringes until measurement of pH by the spectrophotometric method (Clayton and Byrne, 1993; Dickson et al., 2007; Riebesell et al., 2010). Samples were then prepared in triplicate for DIC measurements by

BGD
10, 8313–8341, 2013

non-dispersive infrared analysis (LI-COR, as above) by diluting 300 µL extracellular fluids in 3300 µL Nanopure water in glass scintillation vials (by Hamilton syringe through a septum with negligible head-space). A 16-gauge needle was used to vent the septa, and another fed sample into the LI-COR. A standard curve was created using three sets of four standards prepared as above, in parallel, and measured before, during, and after extracellular fluid samples. Resultant values were standardized to certified reference material (A. Dickson CRM, Scripps Institute of Oceanography, La Jolla, CA).

2.4 Experiment two: behavior and nutritional status

The second of our two experimental groups of *S. fragilis* was used to investigate the impacts of hypercapnia and O_2 availability on locomotion, feeding, growth, and reproductive capacity during approx. 4 months (140 d) exposure. Urchins collected from a depth of 660 m at Sponge Ridge were allowed two days to acclimate *en masse* in aquaria as described above. The health of 150 urchins (< 48 mm diameter) was evaluated prior to their inclusion in the experiment using a flip test by turning them aboral side down and monitoring the time required to 'right' themselves (oral side down). These 150 urchins were segregated haphazardly into 5 groups (30 individuals per group) and each individual placed in a 1 L jar. The jars (A–E; 1–30) were then connected to water supply lines of the Gas Controlled Aquaria (GCA) as described above. The five treatments were defined as $pH_{(total\ scale)}$ 7.9, 7.6, 7.2, and 6.7 at in situ O_2 (25 µM), and pH 7.9 at surface O_2 (220 µM). All treatments were applied at the in situ temperature of 5 °C. In each treatment, two feeding regimes were applied; odd numbered urchins were fed kelp to satiation, while even numbered urchins were fasted during the experimental period.

BGD
10, 8313–8341, 2013

2.4.1 Locomotion

Video analysis flip test methods

The day following urchin segregation into jars, video recordings were made of a flip test for each animal. Down-looking video through the open-top jar was collected using simple web cameras in waterproof housings connected to a PC. With this setup, 8–10 individuals were monitored simultaneously. The time taken for each individual to right itself was noted, with recordings stopped if the individuals failed to turn over within two hours. Routinely over the course of the experiment, individuals from all treatments were rotated through the video station and evaluated using a flip test.

2.4.2 Feeding

Urchins in the feeding treatment groups (odd numbered jars in treatments A–E) were fed weighed stipes of the kelp *Macrocystis pyrifera* to satiation; kelp in jars was replaced every 4–12 days and weighed for calculations of feeding rates. Visual evidence of kelp consumption (Fig. 3b) was also recorded to document feeding frequency; this was used as a Y/N measure of feeding during the feeding period. The weight change of kelp upon cutting and exposure to seawater was quantified in parallel for each feeding period, and found to be negligible. A validated blotting technique was used to ensure consistency in attaining kelp weights on removal from treatment jars.

2.4.3 Growth

Photo sizing methods

All urchins included in this experiment were digitally photographed (Fig. 3a) in anticipation of sizing animals from images. Once urchins had been initially evaluated for their ability to right themselves and placed in their assigned jars, a digital 35 mm camera mounted on a tripod and operated via infrared remote was used to take photographs of

BGD
10, 8313–8341, 2013

each animal against a solid black background. Two internal metric scales, one on the background and one on a wand allowing its placement along the major axis mid-line of the animal, were included in each image. Midway through the experiment this process was repeated. At the termination of the experiment (half of each treatment group at 30 and the other half at 140 days), digital images were again collected. As before overhead shots were taken of the aboral surface of the animal; additionally, images of the oral side were taken at this time. Animals were then removed from jars and measured to the nearest 0.1 mm using calipers; these measurements were used to validate image measurements.

2.4.4 Reproductive capacity

Gonadosomatic index (GSI) was measured as a proxy for reproductive capacity. Urchin whole body wet weight was measured in the urchins sacrificed after 140 days of treatment exposure; gonads were also extracted and weighed separately in order to calculate the terminal GSI (percentage of gonad to whole body wet weight) for each animal at this timepoint.

2.5 Statistical analyses

All data were analyzed using StatPlus (AnalystSoft) software. Each mean value is reported with its standard error of mean (mean ± SEM). One-way analysis of variance (ANOVA) was used to test the impact of the treatment conditions on parameters measured in experiment one, as these data met assumptions for normality and equal variance as determined by the Shapiro–Wilk test (Shapiro and Wilk, 1965) and the F Test for equal variance. In experiment two, most data did not meet assumptions of normality; the Mann–Whitney *U* test was thus used to determine statistical significance as detailed below.

BGD
10, 8313–8341, 2013

3 Results

3.1 Water chemistry

GCA logging and spot-sampling revealed that target treatment conditions for experiments one and two were maintained ±5 µM O_2, 0.5 °C, and 0.05 pH units.

3.1.1 Experiment one: internal acid-base balance

In all treatments except pH 8.0, extracellular fluids mirrored closely the pH of treatment seawater. As shown in pH-bicarbonate (Davenport) diagrams (Fig. 4), a pattern of significant (ANOVA, $F = 9.68$, 30.46, and 30.55 for pH 7.5, 7.1, and 6.7, respectively; $p < 0.0001$ in all cases) hypercapnic-induced acidosis persists in *S. fragilis*. Within the first 24 h of all three hypercapnic exposures, extracellular fluid chemistry was titrated in an acidic direction, roughly along the non-bicarbonate buffering (β) line and consistent with CO_2-derived respiratory acidosis (Fig. 4). Metabolic compensation of respiratory acidosis is typical of aquatic regulators and is characterized by large gains in bicarbonate concentration and movement in an alkalotic direction across P_{CO_2} isopleths; there was evidence of this type of compensation only in animals from the pH 7.5 treatment, and even in this case compensation was incomplete after 31 days (Fig. 4b, two-tailed t test, $t_{crit} = 2.45$, $p < 0.05$). No mortality occurred in experiment one.

3.2 Experiment two: behavior and nutritional status

3.2.1 Mortality

In the pH 6.6 group, there was one mortality (3.3 %) before the first terminal samples were taken (day 30) and four before the final terminal samples were taken on day 140. In the pH 7.2 group, three mortalities occurred before the final sampling day. In the pH 7.6 group, there was one mortality before day 140. In the pH 7.9 group, no

BGD
10, 8313–8341, 2013

mortality occurred. In the pH 7.9/high O_2 group, there were two mortalities before the final sampling day.

3.2.2 Locomotion

Time required for an overturned urchin to right itself (flip time) increased for the pH 6.6 treatment after 6 days (two-tailed t test, $t_{crit} = 1.99$, $p < 0.05$), and remained increased for all timepoints after day 16 (two-tailed t test, $t_{crit} = 1.99$, $p < 0.0001$), compared to pre-treatment flip times. Increased flip response time was also seen in *S. fragilis* exposed to pH 7.2 for 16 days (two-tailed t test, $t_{crit} = 1.99$, $p < 0.001$).

3.2.3 Feeding

During the entire experimental period, urchins fed significantly less frequently (Fig. 6a) than the pH 7.9/high O_2 group when exposed to pH 6.6 (Mann–Whitney U test, $U = 289$, $p < 0.001$) and pH 7.2 (Mann–Whitney U test, $U = 234$, $p = 0.002$). Urchins at pH 6.6 consumed significantly less kelp than pre-treatment at most timepoints after 10 days of exposure to treatment conditions (Fig. 6b, denoted by "+"). Feeding rates of urchins at pH 6.6 were also significantly less than those of urchins at pH 7.9/high O_2 at timepoints after 10 days of exposure (Fig. 6b, denoted by "*"). Urchins exposed to pH 7.2 also fed less often than urchins at pH 7.9/high O_2 at timepoints 16 (Mann–Whitney U test, $U = 48$, $p = 0.037$) and 37 (Mann–Whitney U test, $U = 42$, $p = 0.024$) days into the experiment (Fig. 6b). The F Test for equal variances revealed significantly greater variation in feeding rates of animals at pH 7.6 during the first week of exposure ($F = 40$, $p < 0.001$), as compared with pre-treatment variance (Fig. 6b).

3.2.4 Growth

After 30 days of exposure to treatment conditions, changes in body width were significantly correlated (negatively) only with pH 6.6 fed animals (Fig. 7a; Mann–Whitney U test, $U = 52$, $p = 0.005$); change in body height was correlated (negatively) with pH 6.6

BGD
10, 8313–8341, 2013

in both fed (Fig. 7b, Mann–Whitney U test, $U = 49$, $p = 0.015$) and fasted (Fig. 7b, Mann–Whitney U test, $U = 3$, $p = 0.006$) animals during the 30 day treatment period. After 30 days, no significant effects of feeding regime alone on width or height were observed (i.e., within each treatment, no difference among fed and fasted animals).

Significantly greater width was seen after 140 days treatment exposure in fed urchins as compared with fasted urchins (Fig. 7c) at pH 7.2 (Mann–Whitney U test, $U = 7$, $p = 0.046$), 7.6 (Mann–Whitney U test, $U = 54$, $p = 0.003$), pH 7.9 (Mann–Whitney U test, $U = 62$, $p = 0.002$) and pH 7.9/high O_2 (Mann–Whitney U test, $U = 2$, $p = 0.007$). Conversely, urchins at pH 6.6 (Fig. 7c, Mann–Whitney U test, $U = 21$, $p = 0.570$) did not show correlation of width and feeding after 140 days of treatment exposure. Changes in height (Fig. 7d) were significantly correlated (positively) with feeding only in urchins exposed to pH 7.9 at high O_2 levels (Fig. 7d, Mann–Whitney U test, $U = 4$, $p = 0.015$). After 140 days, fed (Fig. 7d, Mann–Whitney U test, $U = 35$, $p = 0.005$) and fasted (Fig. 7d, Mann–Whitney U test, $U = 42$, $p = 0.003$) urchins at pH 6.6 showed correlation (negative) of pH and change in height.

3.2.5 Reproduction

S. fragilis exposed to pH 6.6 and fed for 140 days showed significantly lower GSI (Mann–Whitney U test, $U = 29$, $p < 0.05$) when compared with animals from all other treatments (Fig. 8). Fasted animals from all treatments showed GSI values statistically equivalent to zero (Fig. 8, two-tailed, one-sample t tests, $p > 0.05$).

4 Discussion

S. fragilis has negligible ability to regulate acid-base balance during environmental acidification, substantiated by the low non-bicarbonate buffer capacity of its extracellular fluids (Fig. 4). At each reduced pH treatment condition *S. fragilis* showed an uncompensated respiratory acidosis, which increased in intensity as treatment pH

BGD
10, 8313–8341, 2013

decreased. This result is in accordance with findings for shallower living Strongylocentrotidae (Spicer et al., 2007, 2011; Stumpp et al., 2012), and is not surprising considering the lack of available internal body compartments for ion exchange and thereby movement of H^+ for acid-base balance regulation (Portner, 1993). *S. fragilis* is largely devoid of internal structures, visibly only possessing a thin, relatively straight-thru gastrointestinal tract and variable gonadal mass (typically with GSI of approx. 5 %; see Fig. 8) within otherwise non-compartmentalized extracellular fluids. The pH along the bathymetric range of *S. fragilis* drops to near pH 7.6 in the OMZ (Fig. 1). On exposure to environmental acidification of only 0.1 pH units below this in situ value, *S. fragilis* was still unable to fully compensate a respiratory acidosis after 30 days (Fig. 4). The consequences of prolonged systemic acidosis are addressed by experiment two, in which results show that among the parameters measured the effects of a presumably slight extracellular acidosis are negligible at pH 7.6. However, during further reduction of pH to a treatment 0.4 units less than the current OMZ low of pH 7.6, prolonged systemic acidosis (seen in Fig. 4) is correlated with significant adverse impacts- at least transiently- on locomotion and feeding (see treatment pH 7.2 of Figs. 5 and 6, respectively).

Adding to the complexity of the story, Dupont et al. (2012) show that although *Strongylocentrotus drobacheiensis* is impaired by elevated pCO_2 up to 4 months, by 16 months it has fully compensated and has acclimated in many ways. This result is in accordance with our measurements of transient adverse effects at treatment pH 7.2, but suggests that acclimation may be possible on much longer time scales (i.e. 4–16 months) than we considered. We expect that *S. fragilis* exposed to pH 7.2 for upwards of 4 months may see continued improvements in metrics of physiological compensation of acidosis.

The results of experiment two suggest *S. fragilis* is vulnerable to adverse effects of OA via impaired locomotion, reduced feeding, decreased somatic growth and reduced reproductive capacity. Effects on locomotion (Fig. 5) and feeding (Fig. 6) are transient at pH 7.2, a level of OA feasible in the OMZ by the end of the century

BGD
10, 8313–8341, 2013

(Caldeira and Wickett, 2003; Doney et al., 2009a, b). However, at pH 6.6 the effects on locomotion and feeding are more catastrophic and persistent, and are accompanied by decreased somatic growth and a significant decline in reproductive capacity.

Not surprising but perhaps most worrisome for the persistence of *S. fragilis* at its current bathymetric range is the adverse impact of fasting on reproductive capacity (Fig. 8). Presumably, GSI is prioritized over any other process in the energy budget, such that the population persists, although very few studies have examined energy budget priorities in sea urchins. Those available show that reproductive growth in fact tends *not* to be prioritized (Stumpp et al., 2012) unless food quality (i.e., protein content) is high (Poorbagher et al., 2010). The ability to search for, consume, and assimilate prey items is thus of utmost concern for the persistence of *S. fragilis*; effective locomotion is an essential aspect of the detritivore lifestyle and may be compromised by OA.

Based on the expected impact of global climate change on ocean pH and oxygen, our results suggest that we should not expect a mass die-off and ultimate extinction of *S. fragilis* in the eastern North Pacific; rather, we expect to see a contraction of its bathymetric range. *S fragilis* will doubtfully have a problem at the shallow end of its bathymetric range (i.e., 200–300 m, see Fig. 1) in the future, but perhaps will suffer a decline in the heart of the OMZ (i.e., 400–1000 m). While a shoaling of the *S. fragilis* bathymetric range seems likely, we have only examined one taxon of a complex ecosystem (Barry et al., 2011; Loreau et al., 2001; Russell et al., 2012). It is possible that while *S. fragilis* may better tolerate the chemistry of the shallower range, competition in fact may be a more considerable limitation in the new depth range. The OMZ, while generally considered an energetically limited, arduous area of the ocean to inhabit, has seemingly few competing taxa.

The effect of global climate change on deep-sea ecosystems and the services they provide society is undoubtedly a complex issue in need of further investigation. While the impacts of OA on individual animal physiology and population dynamics are becoming better understood, consequent changes in taxa interactions and resultant community structure are important next considerations.

BGD
10, 8313–8341, 2013

Acknowledgements. The authors thank the David and Lucile Packard Foundation for support of MBARI researchers.

References

Armstrong, C. W., Foley, N., Tinch, R., and VanDenHove, S.: Ecosystem Goods and Services of the Deep Sea, Deliverable 6.2 of the Project HERMIONE – Hotspot Ecosystem Research and Man's Impact on European Seas, p. 68, 2010.

Barry, J. P., Lovera, C., Okuda, C., Nelson, E., and Pane, E. F.: A Gas-Controlled Aquarium System for Ocean Acidification Studies, IEEE Xplore, 978–1–4244–2126–8/08, 2008.

Barry, J. P., Widdicombe, S., and Hall-Spencer, J. M.: Effects of Ocean Acidification on Marine Biodiversity and Ecosystem Function, edited by: Gattuso, J.-P. and Hansson, L., Oxford University Press, 192–209, 2011.

Boutilier, R. G., Heming, T. A., and Iwama, G. K.: Appendix: Physicochemical Parameters for use in Fish Respiratory Physiology, in: Fish Physiology, Academic Press, edited by: Hoar, W. S. and Randall, D. J., 10, Part A, 403–430, doi:10.1016/S1546-5098(08)60323-4, 1984.

Byrne, M., Ho, M. A., Koleits, L., Price, C., King, C. K., Virtue, P., Tilbrook, B., and Lamare, M.: Vulnerability of the calcifying larval stage of the Antarctic sea urchin *Sterechinus neumayeri* to near-future ocean acidification and warming, Glob. Change. Biol., doi:10.1111/gcb.12190, 2013.

Caldeira, K. and Wickett, M. E.: Oceanography: anthropogenic carbon and ocean pH, Nature, 425, 365, doi:10.1038/425365a, 2003.

Calosi, P., Rastrick, S. P. S., Graziano, M., Thomas, S. C., Baggini, C., Carter, H. A., Hall-Spencer, J. M., Milazzo, M., and Spicer, J. I.: Distribution of sea urchins living near shallow water CO_2 vents is dependent upon species acid–base and ion-regulatory abilities, Mar. Pollut. Bull., in press, doi:10.1016/j.marpolbul.2012.11.040, 2013.

Clayton, T. D. and Byrne, R. H.: Spectrophotometric seawater pH measurements: total hydrogen ion concentration scale calibration of m-cresol purple and at-sea results, Deep-Sea Res. Pt. I, 40, 2115–2129, 1993.

Crain, C. M., Halpern, B. S., Beck, M. W., and Kappel, C. V.: Understanding and managing human threats to the coastal marine environment, Ann. NY Acad. Sci., 1162, 39–62, doi:10.1111/j.1749-6632.2009.04496.x, 2009.

BGD
10, 8313–8341, 2013

Danovaro, R., Gambi, C., Dell'Anno, A., Corinaldesi, C., Fraschetti, S., Vanreusel, A., Vincx, M., and Gooday, A. J.: Exponential decline of deep-sea ecosystem functioning linked to benthic biodiversity loss, Curr. Biol., 18, 1–8, doi:10.1016/j.cub.2007.11.056, 2008.

Dell'Anno, A. and Danovaro, R.: Extracellular DNA plays a key role in deep-sea ecosystem functioning, Science, 309, 2179, doi:10.1126/science.1117475, 2005.

Dickson, A. G., Sabine, C. L., and Christian, J. R.: Guide to Best Practices for Ocean CO_2 Measurements, PICES Special Publication 3, 191 pp., 2007.

Doney, S. C., Balch, W., Fabry, V. J., and Feely, R. A.: Ocean acidification: a critical emerging problem for the ocean sciences, Oceanography, 22, 16–25, doi:10.5670/oceanog.2009.93, 2009a.

Doney, S. C., Fabry, V. J., Feely, R. A., and Kleypas, J. A.: Ocean acidification: the other CO_2 problem, Annu. Rev. Mar. Sci., 1, 169–192, doi:10.1146/annurev.marine.010908.163834, 2009b.

Dupont, S., Ortega-Martínez, O., and Thorndyke, M.: Impact of near-future ocean acidification on echinoderms, Ecotoxicology, 19, 449–62, doi:10.1007/s10646-010-0463-6, 2010.

Dupont, S., Dorey, N., Stumpp, M., Melzner, F., and Thorndyke, M.: Long-term and trans-life-cycle effects of exposure to ocean acidification in the green sea urchin *Strongylocentrotus droebachiensis*, Mar. Biol., in press, doi:10.1007/s00227-012-1921-x, 2012.

Friederich, G., Walz, P., Burczynski, M., and Chavez, F.: Inorganic carbon in the central California upwelling system during the 1997–1999 El Niño–La Niña event, Prog. Oceanogr., 54, 185–203, doi:10.1016/S0079-6611(02)00049-6, 2002.

Gruber, N. and Sarmiento, J. L.: Biogeochemical/Physical Interactions in Elemental Cycles, edited by: Robinson, A. R., McCarthy, J. J., and Rothschild, B. J., John Wiley and Sons, New York, 337–399, 2002.

Hofmann, G. E., Barry, J. P., Edmunds, P. J., Gates, R. D., Hutchins, D. A., Klinger, T., and Sewell, M. A.: The effect of ocean acidification on calcifying organisms in marine ecosystems: an organism-to-ecosystem perspective, Annu. Rev. Ecol. Syst., 41, 127–147, doi:10.1146/annurev.ecolsys.110308.120227, 2010.

Howell, P.: The status of the southern New England lobster stock, J. Shellfish Res., 31, 573–579, doi:10.2983/035.031.0217, 2012.

Jørgensen, B. B. and Boetius, A.: Feast and famine-microbial life in the deep-sea bed, Nature Rev. Microbiol., 5, 770–781, doi:10.1038/nrmicro1745, 2007.

BGD
10, 8313–8341, 2013

Keeling, R. F. and Garcia, H. E.: The change in oceanic O_2 inventory associated with recent global warming, P. Natl. Acad. Sci. USA, 99, 7848–53, doi:10.1073/pnas.122154899, 2002.
Kroeker, K. J., Kordas, R. L., Crim, R. N., and Singh, G. G.: Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms, Ecol. Lett., 13, 1419–1434, doi:10.1111/j.1461-0248.2010.01518.x, 2010.
Kroeker, K. J., Kordas, R. L., Crim, R., Hendriks, I. E., Ramajo, L., Singh, G. S., Duarte, C. M., and Gattuso, J.-P.: Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming, Glob. Change. Biol., 19, 1884–1996, doi:10.1111/gcb.12179, 2013.
Kurihara, H., Takano, Y., Kurokawa, D., and Akasaka, K.: Ocean acidification reduces biomineralization-related gene expression in the sea urchin, *Hemicentrotus pulcherrimus*, Mar. Biol., 159, 2819–2826, doi:10.1007/s00227-012-2043-1, 2012.
Lang, M. A. and Gainer, H.: Isosmotic intracellular regulation as a mechanism of volume control in crab muscle fibers, Comp. Biochem. Physiol., 30, 445–456, 1969.
Loreau, M., Naeem, S., Inchausti, P., Bengtsson, J., Grime, J. P., Hector, A., Hooper, D. U., Huston, M. A., Raffaelli, D., Schmid, B., Tilman, D., and Wardle, D. A.: Biodiversity and ecosystem functioning: current knowledge and future challenges, Science, 294, 804–808, doi:10.1126/science.1064088, 2001.
Melzner, F., Gutowska, M. A., Langenbuch, M., Dupont, S., Lucassen, M., Thorndyke, M. C., Bleich, M., and Pörtner, H.-O.: Physiological basis for high CO_2 tolerance in marine ectothermic animals: pre-adaptation through lifestyle and ontogeny?, Biogeosciences, 6, 2313–2331, doi:10.5194/bg-6-2313-2009, 2009.
Melzner, F., Thomsen, J., Koeve, W., Oschlies, A., Gutowska, M. A., Bange, H. W., Hansen, H. P., and Körtzinger, A.: Future ocean acidification will be amplified by hypoxia in coastal habitats, Mar. Biol., in press, doi:10.1007/s00227-012-1954-1, 2012.
Najjar, R. G., Pyke, C. R., Adams, M. B., Breitburg, D., Hershner, C., Kemp, M., Howarth, R., Mulholland, M. R., Paolisso, M., Secor, D., Sellner, K., Wardrop, D., and Wood, R.: Potential climate-change impacts on the Chesapeake Bay, Estuar. Coast. Shelf S., 86, 1–20, doi:10.1016/j.ecss.2009.09.026, 2010.
Pane, E. and Barry, J.: Extracellular acid-base regulation during short-term hypercapnia is effective in a shallow-water crab, but ineffective in a deep-sea crab, Mar. Ecol.-Prog. Ser., 334, 1–9, doi:10.3354/meps334001, 2007.

BGD
10, 8313–8341, 2013

Poorbagher, H., Lamare, M. D., and Barker, M. F.: Effects of nutrition on somatic growth and reproductive strategy of the sea urchin *Pseudechinus huttoni*, Mar. Biol. Res., 6, 292–301, doi:10.1080/17451000903233797, 2010.

Portner, H.-O.: Multicompartmental Analyses of Acid-Base and Metabolic Homeostasis During Anaerobiosis: Invertebrate and Lower Vertebrate Examples, edited by: Hochachka, P. W., CRC Press, 139–157, 1993.

Portner, H.-O.: Integrating climate-related stressor effects on marine organisms: unifying principles linking molecule to ecosystem-level changes, Mar. Ecol.-Prog. Ser., 470, 273–290, 2012.

Portner, H.-O., Gutowska, M. A., Ishimats, A., Lucassen, M., Melzner, F., and Seibel, B. A.: Effects of ocean acidification on nektonic organisms, edited by: Gattuso, J.-P. and Hansson, L., 154–175, Oxford University Press, L., 2011.

Riebesell, U., Fabry, V. J., Hansson, L., and Gattuso, J.-P.: Guide to best practices for ocean acidification, EUR (Publications Office of the European Union), Luxembourg, 2010.

Russell, B. D., Harley, C. D. G., Wernberg, T., Mieszkowska, N., Widdicombe, S., Hall-Spencer, J. M., and Connell, S. D.: Predicting ecosystem shifts requires new approaches that integrate the effects of climate change across entire systems, Biol. Lett., 8, 164–166, doi:10.1098/rsbl.2011.0779, 2012.

Sabine, C. L., Feely, R. A., Gruber, N., Key, R. M., Lee, K., Bullister, J. L., Wanninkhof, R., Wong, C. S., Wallace, D. W. R., Tilbrook, B., Millero, F. J., Peng, T.-H., Kozyr, A., Ono, T., and Rios, A. F.: The oceanic sink for anthropogenic CO_2, Science, 305, 367–371, doi:10.1126/science.1097403, 2004.

Seibel, B. A. and Walsh, P. J.: Biological impacts of deep-sea carbon dioxide injection inferred from indices of physiological performance, J. Exp. Biol., 206, 641–650, doi:10.1242/jeb.00141, 2003.

Shapiro, S. S. and Wilk, M. B.: An analysis of variance test for normality (complete samples), Biometrika, 52, 591–611, 1965.

Shirayama, Y.: Effect of increased atmospheric CO_2 on shallow water marine benthos, J. Geophys. Res., 110, C09S08, doi:10.1029/2004JC002618, 2005.

Spicer, J. I., Taylor, A. C., and Hill, A. D.: Acid-base status in the sea urchins *Psammechinus miliaris* and *Echinus esculentus* (Echinodermata: Echinoidea) during emersion, Mar. Biol., 99, 527–534, doi:10.1007/BF00392560, 1988.

Spicer, J. I., Raffo, A., and Widdicombe, S.: Influence of CO_2-related seawater acidification on extracellular acid–base balance in the velvet swimming crab *Necora puber*, Mar. Biol., 151, 1117–1125, doi:10.1007/s00227-006-0551-6, 2007.

Spicer, J. I., Widdicombe, S., Needham, H. R., and Berge, J. A.: Impact of CO_2-acidified seawater on the extracellular acid–base balance of the northern sea urchin *Strongylocentrotus dröebachiensis*, J. Exp. Mar. Biol. Ecol., 407, 19–25, doi:10.1016/j.jembe.2011.07.003, 2011.

Stramma, L., Prince, E. D., Schmidtko, S., Luo, J., Hoolihan, J. P., Visbeck, M., Wallace, D. W. R., Brandt, P., and Körtzinger, A.: Expansion of oxygen minimum zones may reduce available habitat for tropical pelagic fishes, Nat. Clim. Change, 2, 33–37, doi:10.1038/nclimate1304, 2011.

Stumpp, M., Trübenbach, K., Brennecke, D., Hu, M. Y., and Melzner, F.: Resource allocation and extracellular acid-base status in the sea urchin *Strongylocentrotus droebachiensis* in response to CO_2 induced seawater acidification, Aquat. Toxicol., 110–111, 194–207, doi:10.1016/j.aquatox.2011.12.020, 2012.

Suttle, C. A.: Marine viruses-major players in the global ecosystem, Nature Rev. Microbiol., 5, 801–812, doi:10.1038/nrmicro1750, 2007.

Truchot, J.-P.: Carbon dioxide combining properties of the blood of the shore crab, *Carcinus maenas* (L.): CO_2-dissociation curves and Haldane effect, J. Comp. Physiol. B, 112, 283–293, doi:10.1007/BF00692299, 1976.

Uthicke, S. and Fabricius, K. E.: Productivity gains do not compensate for reduced calcification under near-future ocean acidification in the photosynthetic benthic foraminifer species *Marginopora vertebralis*, Glob. Change. Biol., 18, 2781–2791, doi:10.1111/j.1365-2486.2012.02715.x, 2012.

Wahl, M. (Ed.): Marine Hard Bottom Communities, Springer, Berlin, Heidelberg, 2009.

BGD
10, 8313–8341, 2013

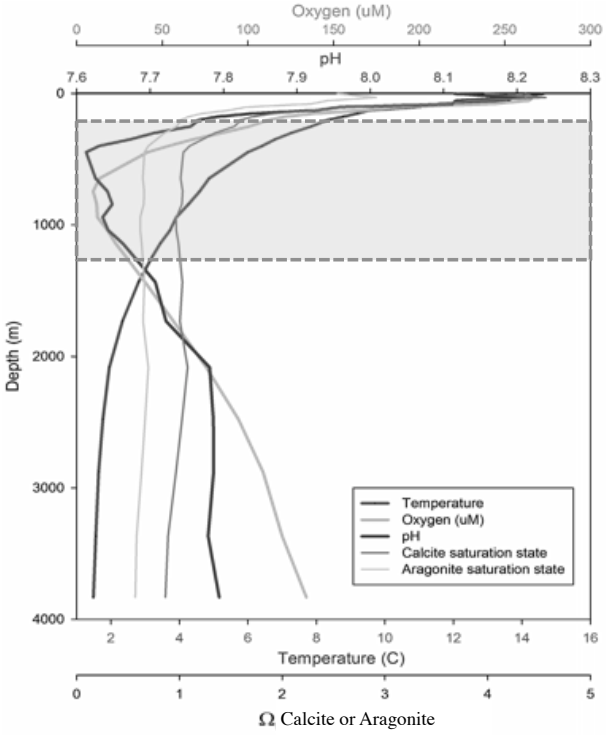

Fig. 1. *S. fragilis* lives at depths of 200–1200 m in Monterey Bay, CA, in seawater with chemistry described in the shaded area of the plot.

BGD
10, 8313–8341, 2013

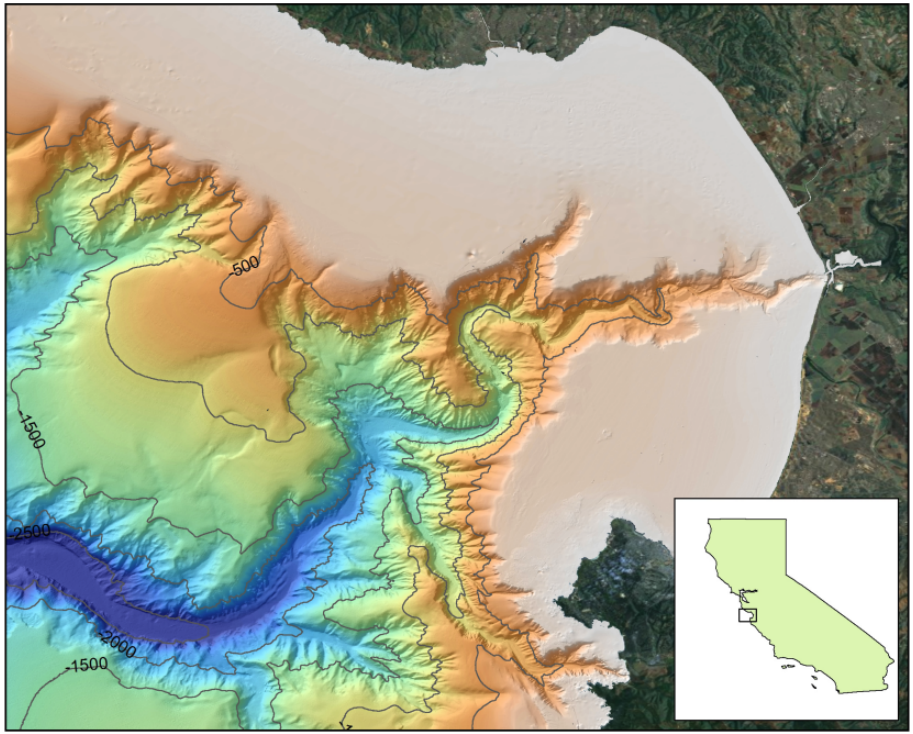

Fig. 2. *S. fragilis* were collected from Monterey Bay, CA, at depths of 500–1000 m, during ROV dives in late 2010 and early 2011.

BGD
10, 8313–8341, 2013

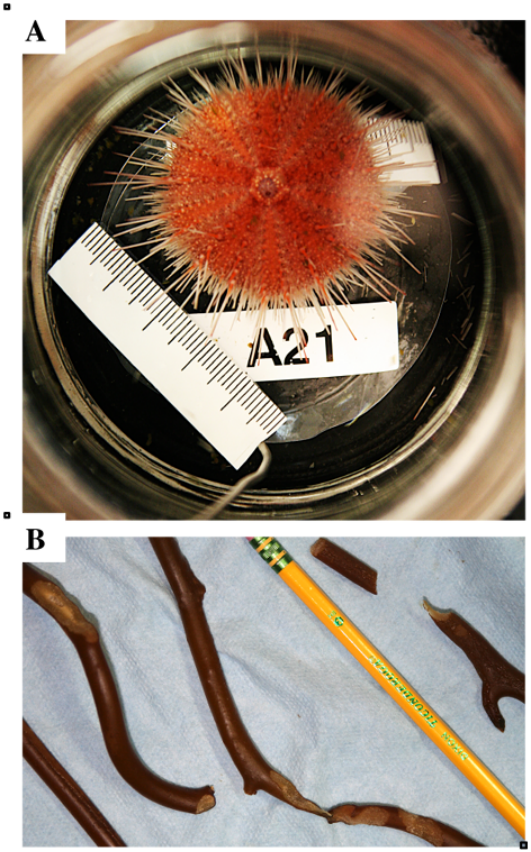

Fig. 3. *S. fragilis* were measured by validated photo imaging **(A)** techniques before, during, and after exposure to Experiment 2 conditions. Urchins in the feeding treatment groups were fed weighed kelp to satiation; kelp in jars was replaced every 4–8 days and weighed for consumed mass. Evidence of kelp consumption **(B)** was also recorded.

BGD
10, 8313–8341, 2013

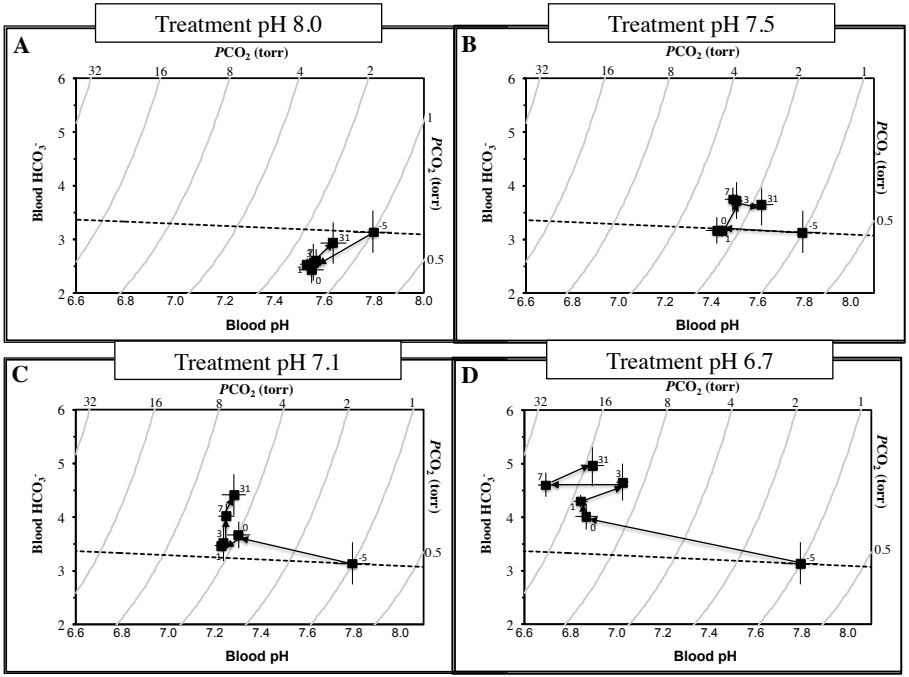

Fig. 4. *S. fragilis* acid-base balance, depicted by Davenport diagrams showing blood pH and HCO_3^- before (−5 d) and during (0, 1, 3, 7, and 31 d) exposure to pH 8.0 **(A)**, 7.5 **(B)**, 7.1 **(C)**, and 6.7 **(D)**. The straight dashed line represents the non-bicarbonate buffering (β) line of *S. fragilis* extracellular fluid; PCO_2 isopleths are shown for reference. Each point is a mean ± SEM of 5 determinations, with the sampling time in days indicated.

BGD
10, 8313–8341, 2013

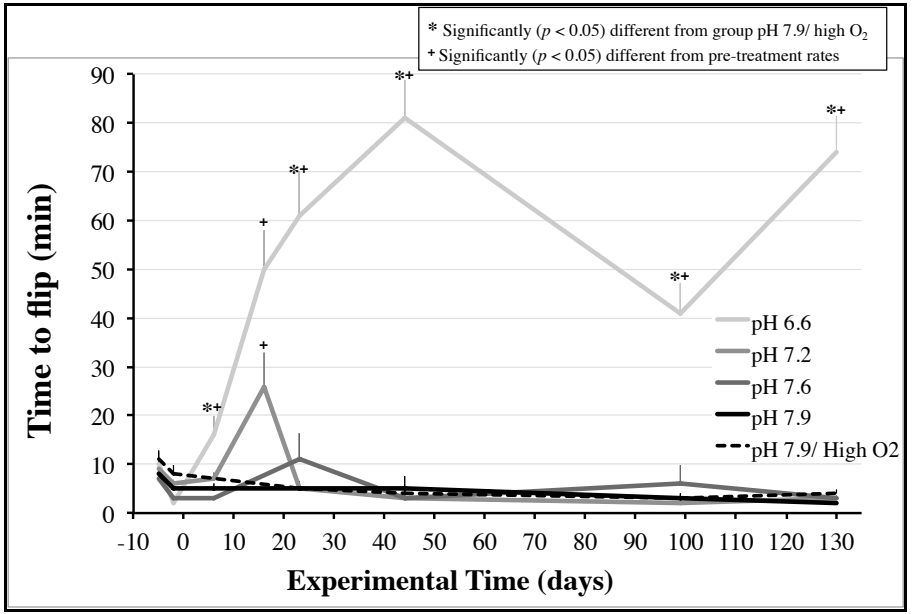

Fig. 5. Time for *S. fragilis* to right is shown before and during exposure to pH 7.9, 7.6, 7.1, and 6.6 at in situ O_2 levels, and pH 7.9 with 100 % O_2 saturation. Times are shown as mean ± SEM.

BGD
10, 8313–8341, 2013

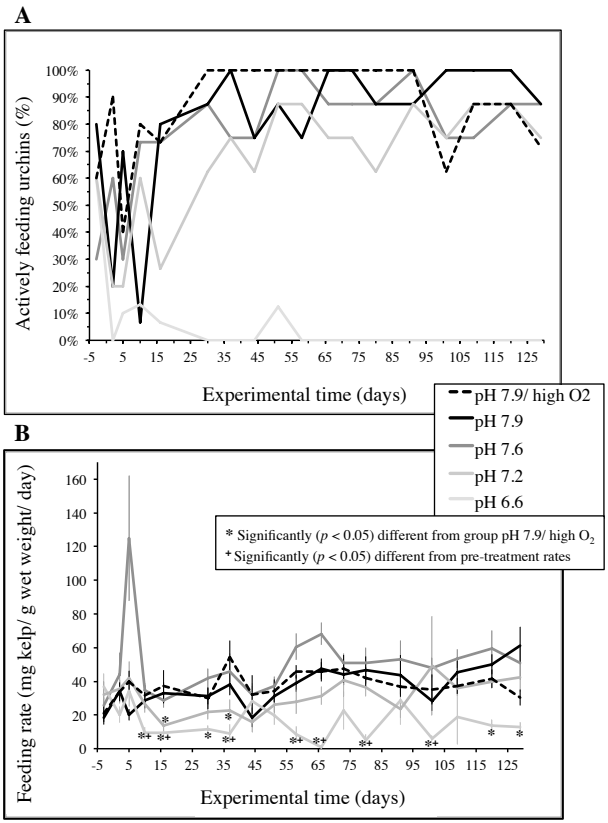

Fig. 6. *S. fragilis* feeding frequency **(A)** and rates **(B)**, before and during exposure to pH 7.9, 7.6, 7.1, and 6.7 at in situ O_2 levels, and pH 7.9 with 100 % O_2 saturation. Feeding rates are shown as mean ± SEM.

BGD
10, 8313–8341, 2013

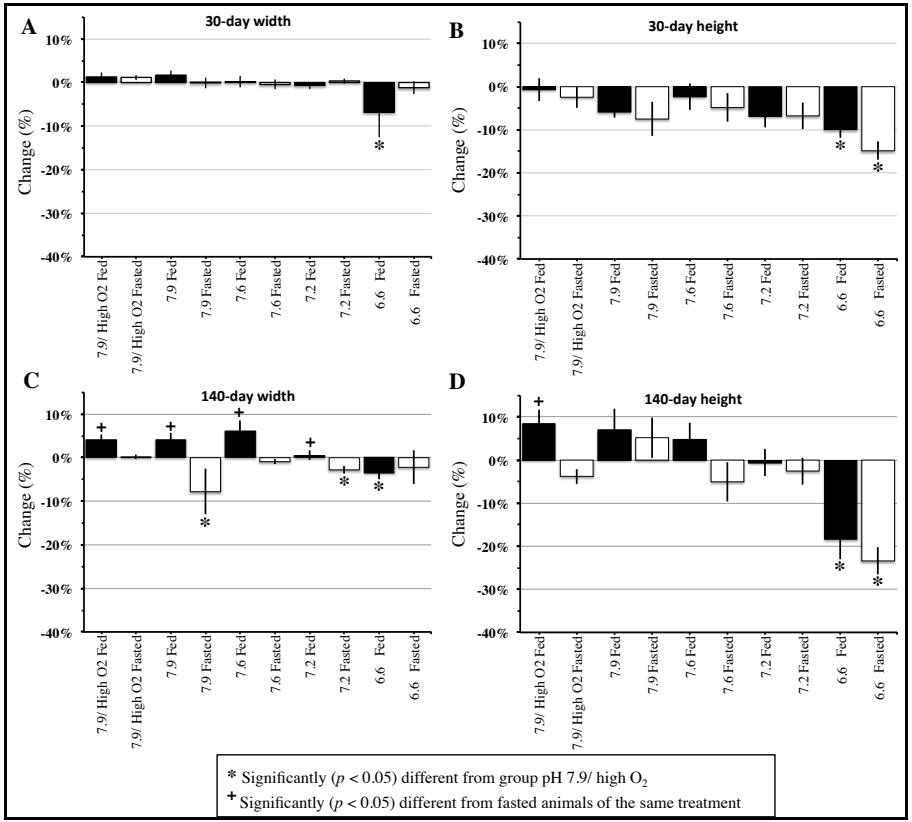

Fig. 7. *S. fragilis* changes in width (panels **A** and **C**) and height (panels **B** and **D**) are shown following 30 (**A** and **B**) and 140 (**C** and **D**) days of exposure to pH 7.9, 7.6, 7.2, and 6.6 at in situ O_2 levels, and pH 7.9 with 100 % O_2 saturation, following a feeding (filled bars) or fasting (unfilled bars) regime. Change in size is shown as mean ± SEM.

BGD
10, 8313–8341, 2013

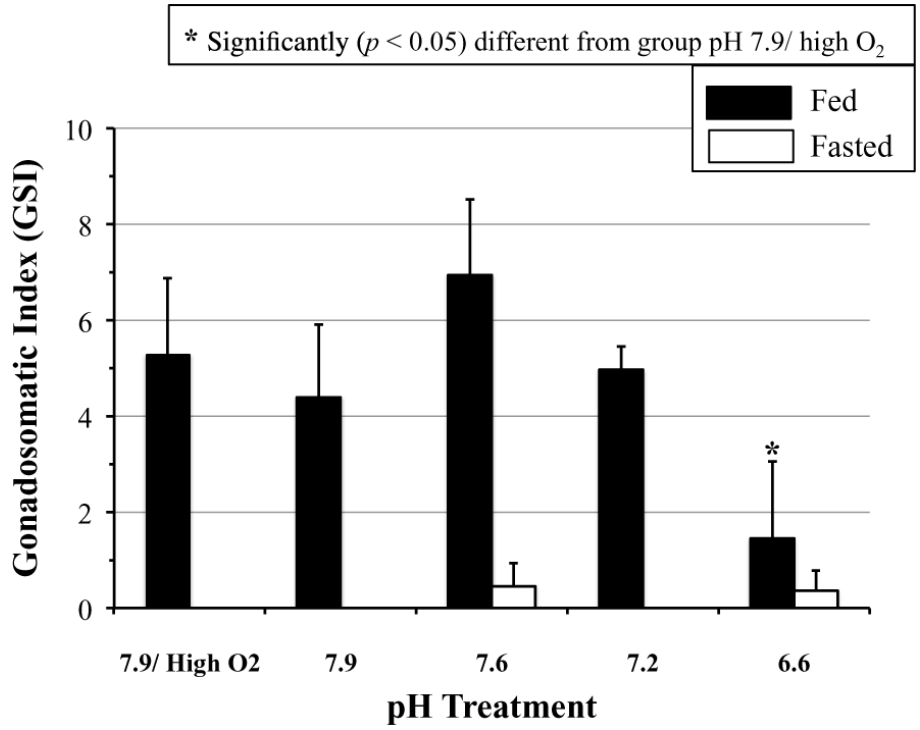

Fig. 8. *S. fragilis* Gonadosomatic Index (GSI) after 140 days of exposure to pH 7.9, 7.6, 7.2, and 6.6 at in situ O_2 levels, and pH 7.9 with 100 % O_2 saturation, following feeding and fasting regimes. GSI is shown as mean ± SEM.

BGD
10, 8313–8341, 2013