

Use of a Free Ocean CO₂ Enrichment (FOCE) System to Evaluate the Effects of Ocean Acidification on the Foraging Behavior of a Deep-Sea Urchin

James P. Barry,* Chris Lovera, Kurt R. Buck, Edward T. Peltzer, Josi R. Taylor, Peter Walz, Patrick J. Whaling, and Peter G. Brewer

Monterey Bay Aquarium Research Institute, 7700 Sandholdt Road, Moss Landing, California 95039, United States

 *Supporting Information*

ABSTRACT: The influence of ocean acidification in deep-sea ecosystems is poorly understood but is expected to be large because of the presumed low tolerance of deep-sea taxa to environmental change. We used a newly developed deep-sea free ocean CO₂ enrichment (dp-FOCE) system¹ to evaluate the potential consequences of future ocean acidification on the feeding behavior of a deep-sea echinoid, the sea urchin, *Strongylocentrotus fragilis*. The dp-FOCE system simulated future ocean acidification inside an experimental enclosure where observations of feeding behavior were performed. We measured the average movement (speed) of urchins as well as the time required (foraging time) for *S. fragilis* to approach its preferred food (giant kelp) in the dp-FOCE chamber (-0.46 pH units) and a control chamber (ambient pH). Measurements were performed during each of 4 trials (days $-2, 2, 24, 27$ after CO₂ injection) during the month-long period when groups of urchins were continuously exposed to low pH or control conditions. Although urchin speed did not vary significantly in relation to pH or time exposed, foraging time was significantly longer for urchins in the low-pH treatment. This first deep-sea FOCE experiment demonstrated the utility of the FOCE system approach and suggests that the chemosensory behavior of a deep-sea urchin may be impaired by ocean acidification.



INTRODUCTION

Deep-sea organisms inhabit the largest, most diverse, ecosystems on Earth,² with dark, cold conditions that are stable compared to surface waters, but are increasingly altered by ocean acidification and other climate-related processes. Megafauna, including urchins, sea stars, gastropod molluscs, and others with calcium carbonate shells, often dominate deep-sea sediment communities and play important roles in the global carbon cycle.^{3,4} Considering the existing low carbonate saturation of most deep-sea environments and projected expansion of corrosive deep-sea waters as global ocean acidification intensifies,⁵ the energetic costs of shell formation by deep-sea taxa will increase, requiring additional food, energy reallocation, or reduced calcification.⁶ Ocean acidification can also affect other aspects of physiological performance, leading to changes in growth, survival, and reproduction.^{7,8} Altered chemosensory behavior and feeding efficiency upon low-pH exposure have been observed in molluscs,⁹ fishes,^{10–12,8,17–19,12,13} crustaceans,^{14,15} and asteroid echinoderms¹⁶ and is likely to reduce foraging success and decrease energy intake, thereby amplifying the stress associated with ocean acidification for deep-sea calcifiers.⁶

Our understanding of the consequences of ocean acidification is based principally on short-term laboratory studies of

single species, nearly all from ocean surface waters. Though clearly important, such studies have inherent biases that may misrepresent the potential acclimation and adaptation of organisms to gradual changes in ocean conditions.¹⁷ More advanced, *in situ* approaches, such as mesocosms,^{18,19} natural carbon dioxide venting sites,^{20,21} and FOCE systems^{1,22,23} may provide a broader perspective concerning the effects of changing ocean conditions on marine organisms and assemblages in a variety of naturally varying ocean settings.

FOCE systems provide precise control of seawater pH within experimental enclosures to support manipulative experiments to evaluate the effects of ocean acidification on species or assemblages of organisms on the seabed.²³ Enclosures in FOCE systems are partially open to ambient waters so that experimental organisms will experience natural changes in most physical parameters, such as planktonic abundance, light, food, and flow, while pH is regulated. pH within enclosures may be regulated as a constant or altered as an offset of ambient pH variation. This latter approach may be particularly

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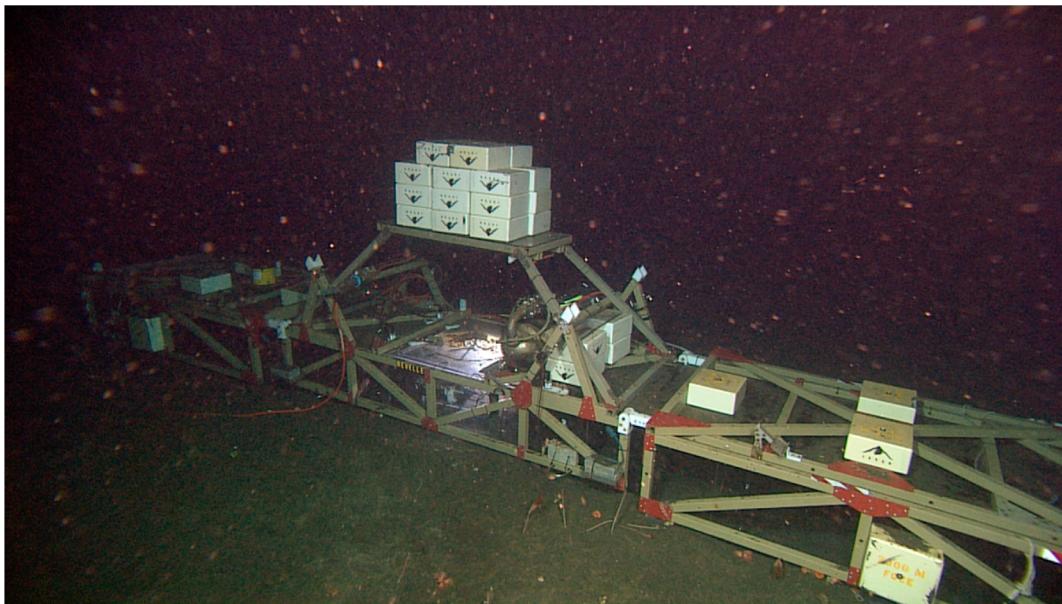


Figure 1. Image of the dp-FOCE system on the seafloor at 885 m depth off Monterey Bay, California. The fiberglass frame is approximately 5 m long \times 1 m wide. The experimental chamber within which pH is controlled is the center section beneath the white floatation blocks. A light inside the chamber is on in this image. Speckles on image are suspended sediment and detrital aggregates sinking toward the seabed.

important for sites with high natural pH variability (e.g., diel pH variation in some coastal temperate sites²⁴). FOCE technology has only recently become available and applied in field experiments,^{1,22} and is expected to become an important research tool complementing other methods for research on the consequences of ocean acidification.

Deep-sea animals and ecosystems are generally considered to be vulnerable to environmental change,^{25–27} yet few studies have examined the effects of ocean acidification on deep-sea taxa,²⁸ in part because of the cost and technical complexities of deep-sea operations. In this study, we report the results of the first biological experiment performed using a deep-sea FOCE system¹ to evaluate the influence of simulated ocean acidification on the foraging time and movement of a common bathyal echinoid, the deep-sea fragile urchin, *Strongylocentrotus fragilis*.

MATERIALS AND METHODS

Study Organism: Deep-Sea Fragile Urchin. The deep-sea fragile urchin, *Strongylocentrotus fragilis* (formerly *Allocentrotus fragilis*^{29–31}), is a common echinoid on the upper continental slope (ca. 200–1200 m depth) in the northeastern Pacific. *S. fragilis* forages across surface sediments on detrital material such as diatoms, foraminifera, algae, and animal remains.^{32,33} Observations using remotely operated vehicles (ROVs) by Harrold et al.,³⁴ and this study indicate that *S. fragilis* prefers algal detritus, particularly giant kelp, *Macrocystis pyrifera*, based on aggregations of *S. fragilis* feeding on *M. pyrifera*. Many echinoids, including strongylocentrotids, are known to respond to chemical cues and exhibit aversion or preference for various food types,^{35,36} but it is not known how ocean acidification may affect the foraging behavior of *S. fragilis* or other deep-sea megafauna.

Specimen Collection. Because *S. fragilis* is not abundant near the Monterey Accelerated Research System (MARS) site at 890 m depth, urchins for this experiment were collected from a nearby location 1 day prior to the initiation of the experiment. Urchins were collected by ROV from 650 m depth on 9/19/

2011 and were held overnight in chilled seawater aquaria aboard the ship. A “flip test”,⁴⁹ commonly used to assess the general health of urchins, was performed the next morning for each urchin used in the experiments. Sixty healthy, similar size (~55 mm test diameter) individuals of *S. fragilis* were divided between two mesh containers with hinged lids to serve as “holding pens” for urchins to be placed in either the dp-FOCE or control enclosures (Figure 2). These pens were placed in seawater in the ROV sample drawer immediately prior to the launch of the ROV dive used to initiate the behavior experiment. Urchins were not fed during the month-long experimental period.

FOCE System. The experiment was performed using a FOCE system (dp-FOCE) connected to the MARS node at a depth of 890 m off Monterey Bay, CA. This cabled observatory system provides power and Ethernet communications from the MARS node to the shore. Further details concerning the study site are provided in Supporting Information.

The dp-FOCE system¹ was used to regulate the CO₂/pH conditions within an experimental enclosure where measurements of urchin behavior were performed. Briefly, the dp-FOCE system injects a sufficient amount of CO₂-enriched seawater into the inlet stream of a flume-like enclosure (2 \times 1 \times 0.5 m) on the seafloor to maintain a specified offset in the pH of waters in the enclosure. Thrusters (chain-driven propellers) at each end of the enclosure were used to maintain water flow through the flume at speeds similar to near-bottom currents at this site (~4–5 cm·s⁻¹). pH was measured inside and outside the FOCE enclosure at 10 s intervals throughout the experiment. Thus, conditions within the dp-FOCE enclosure were enriched in CO₂, but otherwise very similar to ambient conditions, including flow (~5 cm⁻¹) of ambient seawater through the chamber. The dp-FOCE chamber has an access panel on the top and is open to the sediment on the bottom. A camera system mounted in the chamber was programmed to record ~15 s of video at 5 min intervals. Owing to logistic constraints, measurements of urchin movement for the High-CO₂ treatment were limited to a single dp-FOCE enclosure. A

similar FOCE assembly without CO₂-enriched conditions was placed ~10 m away and was used as a “control” treatment for measurements of urchins under ambient pH conditions. Although the experimental section of the control enclosure was identical to the FOCE enclosure (i.e., a rectangular box without ends or a bottom section), it lacked the long wings and propeller-driven thrusters of the FOCE chamber. Thus, flow through the experimental section of the control was less unidirectional and controlled by typically oscillatory bottom currents.

pH was not recorded within the control chamber and was assumed to be nearly identical to the ambient pH outside the dp-FOCE chamber because of the continuous flushing of the largely open enclosure by bottom currents. As is typical of most deep-sea environments, variation in ambient pH at this site is known to be very low. Daily and seasonal variation are both ca. 0.03 units, based on a 15 month-long time series of high frequency (~0.1 Hz) pH measurements at this site during the development phase of the FOCE system.³⁷ Thus, we were confident that the pH perturbations produced in the FOCE chamber represented offsets from the ambient, but unmeasured conditions in the Control chamber. For most shallow water environments, high natural pH variability (e.g.,²⁴) would necessitate pH measurement within all experimental chambers.

Experimental Design. The FOCE system was used to maintain a pH offset of ca. -0.46 units in the FOCE enclosure (Figure 1) for a month-long period. During this period experimental trials (~60 h long) were used to evaluate the foraging and movement of *S. fragilis* in both the FOCE (pH ~7.14; pCO₂ ~3255 ppm) and control (ambient pH ~7.63; pCO₂ ~1028 ppm) enclosures. Four trials (trials 1–4, initiated after -2, 2, 24, and 27 days of exposure to high-CO₂ or control pH treatments, respectively) were performed to measure foraging behavior during the month-long experiment. No observations were available for the control enclosure during trial 3.

Foraging Behavior Assays. Urchin foraging time and movement speed were measured from video recordings of urchins held within urchin raceways (Figure 2) positioned in both the dp-FOCE and Control enclosures. Each raceway (52 cm wide, 67 cm long, with 14 cm high walls) had 5 lanes (10 cm wide) allowing simultaneous measurement of up to 5 urchins. Raceways had mesh-covered openings at both ends and a mesh lid to allow water movement, but impede urchin escape. For each trial, 5 urchins were positioned in separate lanes at one end of a raceway in both the FOCE and Control enclosures. The effects of elevated CO₂ exposure were measured as (1) “foraging time”, the time required for individual urchins to move across the raceway to a small bundle of *M. pyrifera*, and (2) “speed”, the average movement speed for each urchin for hours 0–2 and 48–50 of each trial.

Trial 1 was initiated on 9/20/2011 using the ROV *Doc Ricketts*. Upon arrival of the ROV at the seafloor, 5 urchins were removed from one holding pen and positioned at one end of 5 lanes of a raceway, which was placed in view of the camera within the dp-FOCE chamber. A small bundle of giant kelp (*M. pyrifera*; collected from the shore; similar size and condition for each trial) was positioned inside the dp-FOCE enclosure at the upstream end of the flow path, adjacent to the raceway at the end opposite the starting position of the urchins (urchins could not access the kelp). The holding pen was then placed into the dp-FOCE enclosure so that the urchins used in subsequent trials were exposed to low-pH conditions through the entire

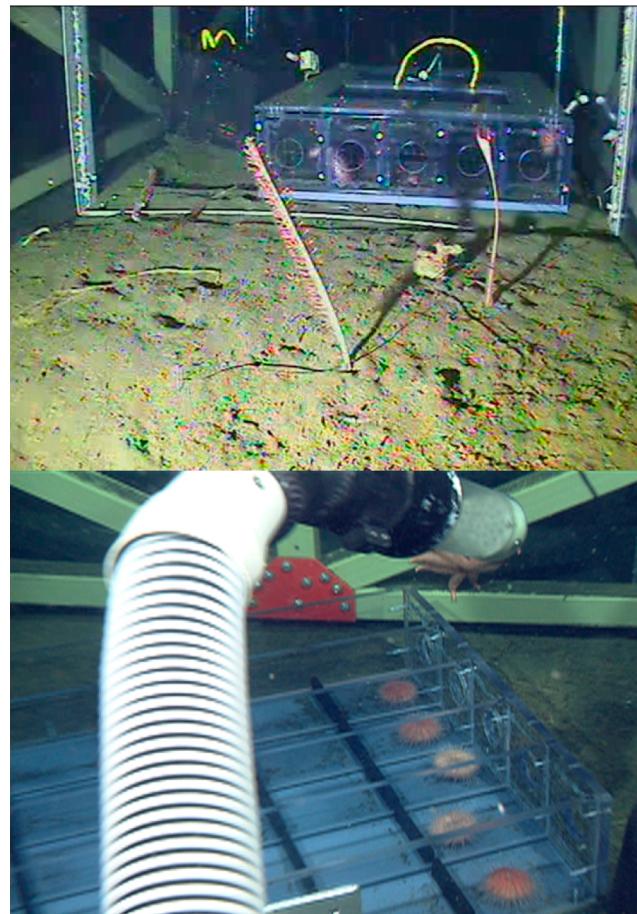


Figure 2. Images of urchin raceway. Top photograph shows the raceway inside the Control enclosure during the setup of a Trial. Note the urchins at the facing end of the raceway and the holding pen (black mesh container) in the upper left. Sea pens and animal burrows are visible on the sediment covered seabed. Bottom image shows the raceway while loading urchins using the robotic manipulator arm of the remotely operated vehicle. The white tubing is part of the suction sampler used to collect and place urchins. The dp-FOCE frame is visible in the background.

experimental period. This process was repeated at the control enclosure, using urchins from the control holding pen.

Individual urchins were only used for a single trial. To initiate trials 2–4, urchins from the previous trial were removed from the raceway and placed in the ROV sample drawer for shipboard size measurements. Each raceway was then repopulated with individuals from the high-CO₂ or control holding pens. Thus, urchins in trials 2–4 had been exposed to high-CO₂ or control pH levels for 2, 24, and 27 days, respectively.

Each experimental trial lasted for ~60 h, during which urchins were free to move within their lane in each raceway. Upon initiation of an experimental trial, time-lapse images were recorded at 5 min intervals from both the FOCE and Control enclosures using cameras having an oblique view of the raceway. Imagery from each trial was analyzed to determine the time required for each animal visible (typically 3+ ind.) to arrive at the end of the raceway adjacent to the kelp, presumably in response to its odor plume. In addition, for each trial we determined the mean speed of movement for each urchin, regardless of direction, based on the average of

movement speeds measured during two 2-h periods (0–2, 48–50 h).

Analysis. Univariate ANOVA performed using SPSS V.22 software was used to analyze variation in the time (h) required for urchins to move from their start position to the opposite end of the raceway (foraging time) and the average speed of movement ($\text{cm}\cdot\text{h}^{-1}$) during each trial. Two-factor, fixed effects ANOVA using CO_2 -treatment (high- CO_2 or control) and days exposed (trial) as factors was applied to data from trials 1 (before CO_2 addition), and trials 2–4 (2, 24, and 27 days exposure to high- CO_2 , respectively). All raw data sets were transformed by \log_{10} prior to analysis to correct for non-normality.

RESULTS AND DISCUSSION

The dp-FOCE system was very effective in maintaining high- CO_2 conditions within the high- CO_2 enclosure. The pH offset averaged -0.46 pH units (S.D. = 0.057) with an average pH of 7.14 in the high- CO_2 treatment (Figure 3). pH measured

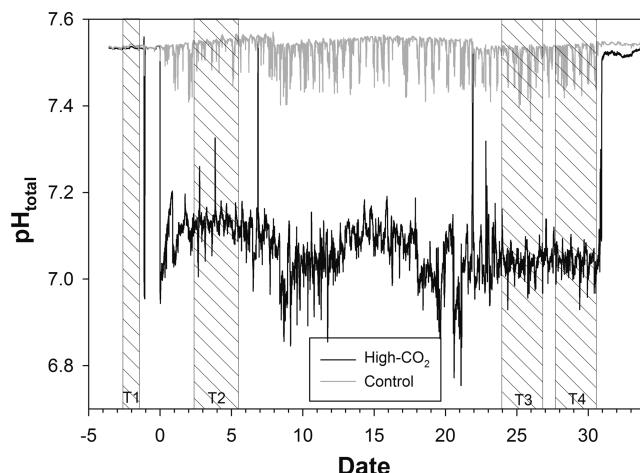


Figure 3. pH data recorded during the dp-FOCE urchin feeding behavior experiment. Black line indicates pH measured within the dp-FOCE chamber (high- CO_2 treatment) at 10 s intervals during the experiment. Gray line indicates pH measurement from outside the dp-FOCE chamber. T1 indicates the period for trial 1, prior to pH reduction in the dp-FOCE chamber. T2–T4 indicate trials 2–4, respectively.

immediately outside (i.e., within 10 cm) the dp-FOCE chamber (ambient) averaged 7.549 (S.D. = 0.061) and varied by ca. 0.15 pH units. Average, naturally varying pH at the MARS site, based on year-long, high frequency measurements is 7.625, with an SD of 0.011,¹ and was assumed to represent conditions within the control chamber. The slightly lower pH measured outside the FOCE chamber is almost certainly due to the periodic overlap of the exhaust plume from the dp-FOCE enclosure with the external “ambient” pH sensor very nearby.

Control of pH within high- CO_2 treatments in previous deep-sea studies has been problematic, making it difficult to identify threshold pH levels responsible for changes in high- CO_2 treatment groups.³⁸ The dp-FOCE system effectively mimicked the performance of free air CO_2 enrichment (FACE) systems, which have generated much of our understanding concerning the effects of rising atmospheric CO_2 levels on terrestrial plant communities³⁹; FACE systems inspired the development of FOCE methods.¹

Although the dp-FOCE system functioned as planned, weaknesses in the experimental design, including the use of a single chamber for the high- CO_2 and control treatments, limits the inferential power of the experiment. Ideally, the design would include multiple FOCE enclosures configured for analysis using an ANOVA design comparing replicated pH treatments and ambient control levels, or a regression design in which multiple FOCE chambers would be operated over a range of pH offsets.⁴⁰ However, the high cost of developing and operating FOCE systems in the deep-sea and the general complexities of deep-sea research restricted the experiment to comparisons between one treatment chamber and one control chamber.

Replication was restricted to multiple lanes of each raceway within dp-FOCE and control chambers, and among experimental trials. Absence of replication for treatment and control chambers and the restriction that pH could only be regulated in a single chamber results in confounding of the effects of treatments (i.e., pH) with the effects of different chambers (i.e., the single dp-FOCE and Control chambers), in addition to the nonindependence among lanes within the same enclosure (pseudoreplication, *sensu* Hurlbert⁴¹). To evaluate the effects of pH on urchin movement, we must assume that the effects of the chambers alone were negligible or at least small compared to the effects of pH changes, and were similar for the dp-FOCE and Control enclosures. If true, then any detectable differences in urchin behavior were related principally to their response to changes in seawater pH. Although we cannot be certain these assumptions are valid, we are confident that the handling of urchins and characteristics of the dp-FOCE and control enclosures (other than pH and flow control) were very similar.

Urchin foraging time varied considerably among individuals and among treatments, ranging from less than 1 h to over 100 h (Figure 4). Why such large variation? It is possible that differences in hunger, handling effects, or other unknown factors contributed to the large variability, though our unquantified observations of *S. fragilis* during laboratory studies also indicate large variation in activity among individuals. Bottom currents at this site vary tidally in both direction and

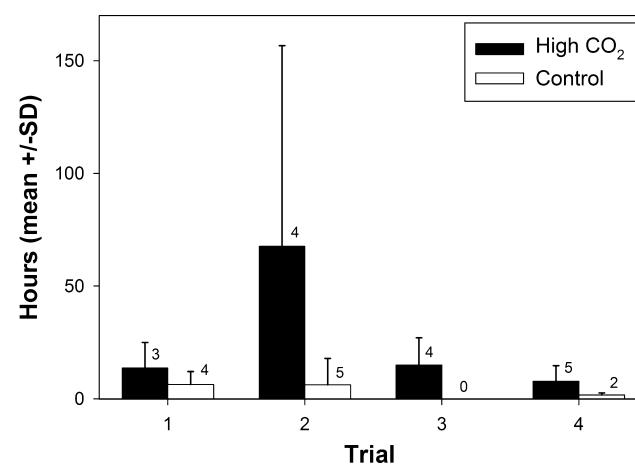


Figure 4. Urchin foraging time. Bars represent mean time (hours \pm SD) required for urchins to travel along raceway lanes and arrive near the kelp bundle. Trial 1 was performed before CO_2 -release. Trials 2–4 were initiated 2, 24, and 27 days after the pH offset was established. Black bars indicate high- CO_2 treatment. White bars indicate ambient pH (control treatment). Numbers above bars indicate the sample size of urchins.

speed, ranging from ~5 to 15 cm s⁻¹ and may have led to variation in the detection of odor plumes from the kelp bundles.

There was no significant interaction detected between the effects of CO₂ treatment and trial (time exposed) on foraging time (Table 1). The power of this test was weak, however, with

Table 1. Two-Way ANOVA Results for Analysis of Urchin Foraging Speed^a

factor	type IV SS	df	M.S.	F-ratio	p-value
CO ₂	2.263	1	2.263	5.366	0.031
trial	0.588	3	0.196	0.465	0.710
trial-CO ₂	1.112	2	0.556	1.318	0.290
error	8.435	20	0.442		

^aThe factor CO₂ included the high-CO₂ and control levels. The time for urchins to reach the kelp (i.e. foraging time) was greater in the high-CO₂ treatment. No statistically significant variation was detected among trials or for the CO₂-trial interaction.

only a 25% likelihood of detecting a significant interaction. No difference in foraging time was detected among trials ($F(3,20) = 0.46$, $p = 0.71$), suggesting that urchins did not acclimate over the time scales of the experiment. Similar results have been reported for numerous taxa (see below). Owing to the limited replication and high variability among individuals, power to detect a significant effect of time was only 6%. Nor did the urchins in the control chamber reduce foraging time over the experiment, as might be expected if starvation over the month-long experiment stimulated foraging behavior.

Foraging time varied significantly among CO₂ treatments, (Table 1), averaging 4.7 times longer for animals exposed to high CO₂ waters in the FOCE enclosure (Figure 4). Power for this test was higher (69%) than for the interaction term or the effect of Trial, likely related to the relatively large effect size (0.23). If detection of the odor plume influenced foraging time, we expected that the unidirectional flow in the FOCE chamber would provide a more reliable odor cue than the control, in contrast with the observed results.

The speed of movement for urchins, regardless of direction, did not vary among treatments (average; 22.5 and 26.9 cm·h⁻¹ for the FOCE and Control chambers, respectively) even though foraging time was significantly slower for the low-pH treatment (Figure 5). Nor did movement speed vary among trials or in relation to the pH-trial interaction (Table 2).

Longer foraging time upon exposure to low-pH waters suggests that future ocean acidification may affect foraging efficiency in deep-sea urchins, with potential consequences for the populations and food webs. Food limitation is known to shape population and community structure in deep-sea seafloor ecosystems.^{42,43} Reduced foraging efficiency in these urchins could have negative consequences for growth and survival of individuals during periods when food is scarce, potentially affecting the dynamics of the entire population. Such changes in urchin populations could have cascading effects throughout the community through shifts in grazing, competition, or biological interactions, regardless of their sensitivity to ocean acidification.

On the basis of studies of related taxa, *S. fragilis* was expected to both sense and respond to *M. pyrifera* from waterborne cues. Chemosensory structures and receptors have been documented in other strongylocentrotid urchins,^{36,44,45} and several congeners of *S. fragilis* exhibit clear foraging preferences for macroalgae. *S. droebachiensis*, *S. franciscanus*, and *S. purpuratus*,

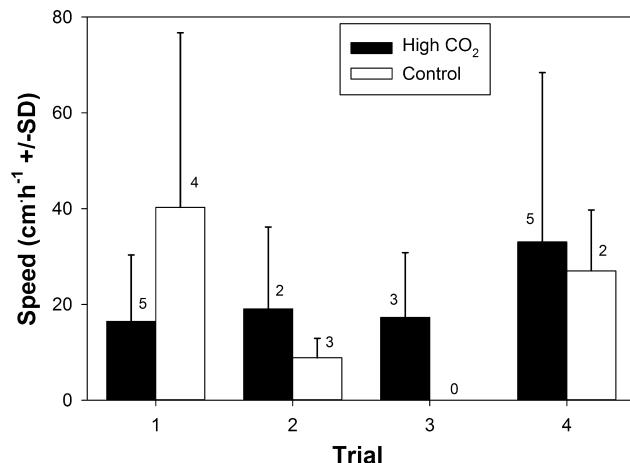


Figure 5. Urchin movement speed. Bars represent mean speed (cm·s⁻¹ ± SD) of urchins, regardless of direction, during two 2 h periods (0–2, 48–50 h) after the trial was initiated. Symbols as in Figure 4

Table 2. Two-Way ANOVA Results Regarding Average Movement Speed of Urchins^a

factor	type IV SS	df	M.S.	F-ratio	p-value
CO ₂	0.136	1	0.136	0.438	0.517
trial	0.193	3	0.064	0.207	0.890
trial-CO ₂	0.490	2	0.245	0.786	0.472
error	5.296	17	0.312		

^aIncludes trials 1, 2, and 4, for days 0, 2, 24, and 27, respectively. There was no statistically significant variation in movement speed between treatments or among trials.

have all been shown to prefer kelps, including *M. pyrifera* over other macroalgae.^{46–48} *M. pyrifera* was also the sole food source for *S. fragilis* in laboratory based studies,⁴⁹ during which it sensed, moved quickly toward, and fed on kelp (J.B., personal observation). Therefore, the increased time required for *S. fragilis* individuals to move toward giant kelp under low-pH conditions is interpreted as impairment of foraging ability, particularly with the result that average movement speed was similar among treatments.

Species inhabiting environments with a wide range in pH over space or time appear to be less sensitive to low-pH exposure.^{24,50,51} *S. fragilis*, however, has been shown to be moderately tolerant of decreased pH expected with future ocean acidification, so long as ample food is available.⁴⁹ The pH reduction for the dp-FOCE-based experiment reported here was very near to that of Taylor et al.⁴⁹ (~0.4 units) who reported continued feeding and growth of *S. fragilis*. Reduced foraging speed found in this dp-FOCE study suggests that *S. fragilis* may become less effective in finding food, potentially affecting its growth, survival, and reproduction, unless acclimation or adaptation over longer time scales is possible.

The duration of the dp-FOCE experiment may have a strong influence on the response of *S. fragilis* to ocean acidification. Most experimental studies concerning the effects of ocean acidification have found little evidence of acclimation over weeks to months (e.g., fishes¹²). Form and Riebesell⁵² documented impaired growth of the deep-sea coral *Lophelia pertusa* over week-long exposure to high CO₂ waters, yet after 6 months, growth was enhanced. Similarly, Li and Gao¹⁵ found that copepods reduced feeding rates over the first 24 h exposure to a 0.3 unit reduction in pH, but rates returned to

normal or higher from 36 to 90 h. Dupont et al.⁵³ reported that fecundity in *S. droebachiensis* was reduced after 4 months exposure to low pH, but by 16 months females had acclimated fully and shown no reproductive impairment.

It remains unclear whether *S. fragilis*, a close relative of well-studied shallow-living urchins, will be able to acclimate to future ocean acidification. Deep-sea taxa are generally thought to be more sensitive to ocean acidification and other environmental changes than those inhabiting surface waters, largely because of the typically more stable conditions in deep ocean waters.^{6,54} In the upper bathyal zone (0.2–1 km) of the N.E. Pacific, however, and other regions with strong oxygen minimum zones (OMZs), there are strong gradients in temperature, oxygen, pH, and other factors from the surface to ~1000 m depth. Yet while there is strong depth-related variation, conditions at any single depth below ~200 m are very stable over time. Thus, individual organisms living at a depth in the upper bathyal are exposed to a narrow range in temperature, oxygen, and pH throughout their lives, even though the population spanning the upper bathyal zone is exposed to and must retain the genetic diversity to tolerate fairly wide ranges in these parameters (ranges: temperature, ~6.4 °C; O₂, ~100 μmols·kg⁻¹; pH, ~0.15 units). Conditions at abyssal depths (e.g., >2000 m) are far more homogeneous, and environmental variation within the abyss off California is very narrow (ranges: temperature, ~0.8 °C; O₂, ~60 μmols·kg⁻¹; pH, ~0.04 units). Thus, a true test of the sensitivity of “typical” deep ocean taxa may require study of bathyal and abyssal species.

This dp-FOCE experiment was the first use of a FOCE system for manipulative experiments evaluating the effects of ocean acidification on deep-sea organisms. FOCE systems, in general can help advance the scope of experimental studies focusing on climate-related changes in ocean conditions by enabling studies of multispecies assemblages over long time periods. Without such approaches, it will be difficult to identify indirect effects of ocean acidification and other anthropogenic stressors that arise through species interactions, which ultimately may be more important than direct effects.^{55,56}

ASSOCIATED CONTENT

Supporting Information

Details concerning the study site, the fragile deep-sea urchin, and the experimental control treatment enclosure. This material is available free of charge via the Internet at <http://pubs.acs.org>.

AUTHOR INFORMATION

Corresponding Author

*E-mail: barry@mbari.org. Phone: 831-775-1726.

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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