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GLOBAL CHANGES IN A MATERIAL WORLD

Repeated Hyposalinity Pulses Immediately and Persistently Impair the Sea Urchin Adhesive System

Austin M. Garner [*,†,1,2], Andrew J. Moura [*,†,2], Carla A. Narvaez [‡,§], Alyssa Y. Stark [*,3] and Michael P. Russell[*,3]

[*]Department of Biology, Villanova University, Villanova, PA 19085, USA; [†]Department of Biology & BioInspired Institute, Syracuse University, Syracuse, NY 13244, USA; [‡]Friday Harbor Laboratories, University of Washington, Friday Harbor, WA 98250, USA; [§]Department of Biology, Rhode Island College, Providence, RI 02908, USA

[2]These authors contributed equally to this work.
[3]These authors contributed equally to this work as senior authors.

[1]E-mail: agarner@syr.edu

Synopsis Climate change will increase the frequency and intensity of extreme climatic events (e.g., storms) that result in repeated pulses of hyposalinity in nearshore ecosystems. Sea urchins inhabit these ecosystems and are stenohaline (restricted to salinity levels ~32‰), thus are particularly susceptible to hyposalinity events. As key benthic omnivores, sea urchins use hydrostatic adhesive tube feet for numerous functions, including attachment to and locomotion on the substratum as they graze for food. Hyposalinity severely impacts sea urchin locomotor and adhesive performance but several ecologically relevant and climate change-related questions remain. First, do sea urchin locomotion and adhesion acclimate to repeated pulses of hyposalinity? Second, how do tube feet respond to tensile forces during single and repeated hyposalinity events? Third, do the negative effects of hyposalinity exposure persist following a return to normal salinity levels? To answer these questions, we repeatedly exposed green sea urchins (*Strongylocentrotus droebachiensis*) to pulses of three different salinities (control: 32‰, moderate hyposalinity: 22‰, severe hyposalinity: 16‰) over the course of two months and measured locomotor performance, adhesive performance, and tube foot tensile behavior. We also measured these parameters 20 h after sea urchins returned to normal salinity levels. We found no evidence that tube feet performance and properties acclimate to repeated pulses of hyposalinity, at least over the timescale examined in this study. In contrast, hyposalinity has severe consequences on locomotion, adhesion, and tube foot tensile behavior, and these impacts are not limited to the hyposalinity exposure. Our results suggest both moderate and severe hyposalinity events have the potential to increase sea urchin dislodgment and reduce movement, which may impact sea urchin distribution and their role in marine communities.

Introduction

Sea urchins mediate community structure in shallow coastal communities (Steneck 2020). On coral reefs, sea urchin herbivory reduces the abundance of algal competitors, facilitating coral growth (Levitan 1988; Idjadi et al. 2010; Levitan et al. 2023). In kelp forests, temperate shallow rocky reefs, and seagrass meadows, however, sea urchins exert strong grazing pressure on foundational species, decreasing primary productivity and eliminating habitat-forming macroalgae and plants (Steneck 2020). Nearshore sea urchins endure intense variation in the hydrodynamic and abiotic conditions (i.e., waves, currents, temperature, salinity, and air exposure) of their native habitat, which likely pose challenges to locomotion, attachment, and grazing activities. Thus, examining how sea urchins respond biomechanically to fluctuating environmental conditions is essential for understanding their natural history.

Sea urchin success as consumers is largely mediated by their adhesive tube feet—extensible hydrostatic skeletons comprised of a flexible, contractable stem terminating in an adhesive disc. Tube feet secure sea urchins to the substratum and facilitate locomotion via

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a duo-gland adhesive/de-adhesive system (Flammang 1996). The coordinated use of hundreds of tube feet enables sustained attachment under forces exerted by waves and predators. Under large enough forces, sea urchins are dislodged from the substratum through a combination of adhesive failure of the tube foot disc and catastrophic failure of the tube foot stem (Santos and Flammang 2008; Stark et al. 2020). Sea urchins move using a combination of spines and tube feet, where repeat attachment-detachment cycles of tube feet allow sea urchins to pull themselves along surfaces of various orientations (Lawrence 1987; Domenici et al. 2003). Previous work focused on understanding the influence of hydrodynamics (e.g., Kawamata 1998; Konar 2000; Gagnon et al. 2003; Cohen-Rengifo et al. 2017; Cohen-Rengifo et al. 2019; Narvaez et al. 2022), substrate characteristics (Santos 2005; Kawamata 2012; Cho et al. 2014; Stark et al. 2020), and the presence of predators (e.g., Hagen et al. 2002; Vadas and Elner 2003; McKay and Heck 2008; Morishita and Barreto 2011; Urriago et al. 2011; Pessarrodona et al. 2019) on sea urchin movement and attachment. However, the impact of fluctuating environmental conditions like temperature and salinity on tube foot performance is still relatively unexplored (but see Cohen-Rengifo et al. 2019; Moura et al. 2023).

Pulses of hyposalinity (hyposalinity events) in nearshore marine ecosystems are becoming more frequent as climate change accelerates (IPCC 2022; Röthig et al. 2023). Sea urchins are likely more susceptible to these events (Irlandi et al. 1997; Russell 2013) because of their limited to no osmoregulatory capability (Russell 2013; Castellano et al. 2017). Recently, we quantified the effects of a single hyposalinity exposure on tube feet function in the green sea urchin, *Strongylocentrotus droebachiensis* (Moura et al. 2023). We found that tube foot performance is dramatically reduced during-exposure to a single hyposalinity event, but the salinity at which a substantial decline in performance occurs is different between tube foot functions (e.g., adhesion, locomotion). However, many wild *S. droebachiensis* are repeatedly exposed to temporary hyposalinity events, so an examination of how sea urchin tube feet respond during and after repeated exposure is needed. Previous studies found that populations exposed to different hyposalinity regimes (e.g., estuaries vs. open ocean) exhibit differences in righting response (time to right following overturning) and mortality rate under hyposalinity, suggesting local acclimation to this environmental stressor (Himmelman et al. 1984; Drouin et al. 1985).

Here, we extend our previous work by exploring: (1) the possibility of adhesive and locomotor performance acclimation to repeated pulses of hyposalinity; and (2) the potential for the negative effects of hyposalinity to persist after the hyposalinity exposure ends. To do this, we exposed sea urchins to pulses of three different salinity treatments (control: 32‰; moderate hyposalinity: 22‰; severe hyposalinity: 16‰) four times over two months and measured maximum locomotor speed and disc tenacity (adhesive force per unit disc surface area) during the hyposalinity exposure and after sea urchins were returned to normal salinity (32‰). In addition to adhesive failure of the tube foot disc, mechanical failure of the tube foot stem often contributes to dislodgment in sea urchins. Thus, we also examined how tube feet respond to tensile forces during and after repeated hyposalinity events. Specifically, we measured tube foot stem breaking force, tube foot total extension, tube foot spring constant (a measure of stiffness), and the work to break tube feet (a measure of energy absorption capacity); we collectively refer to these tensile parameters as tube foot tensile behavior. We hypothesized hyposalinity would negatively impact sea urchin locomotor and adhesive performance and tube foot tensile behavior. Additionally, we predicted that the negative impacts of hyposalinity on sea urchin performance and tube foot tensile behavior would be less severe at later exposures compared to earlier exposures (suggesting acclimation) and that any negative effects on performance and tube foot tensile behavior would persist after exposure.

Methods

Sea urchin collection and maintenance

We collected sea urchins (49.98 ± 1.64 mm diameter, $n = 27$) from 9 m depth near the University of Washington Friday Harbor Laboratories on San Juan Island, Washington (48°32′ 26.2392″ N, 123°0′ 40.3128″ W) on October 31, 2021, and held them in flow-through sea tables for 12 days before shipping them to Villanova University. Immediately upon arrival, we transferred them to a 1000-L recirculating seawater system containing artificial seawater (Crystal Sea® Marinemix; Marine Enterprises International, Baltimore, MD, USA). Sea urchins were housed separately in PVC enclosures with false bottoms to allow seawater circulation (Fig. 1A). Each sea urchin received aerated seawater from dedicated taps above each enclosure that flowed through the false bottom and was fed rehydrated kelp (Wel-Pac) *ad libitum* when not undergoing a salinity exposure. We cleaned the sea table and monitored water temperature and salinity daily, rotated enclosures among taps, and monitored water chemistry (Ca, Mg, pH, dKH, P, and NH_3) weekly. Sea urchins were held in the laboratory for 119 days before starting experimental treatments.

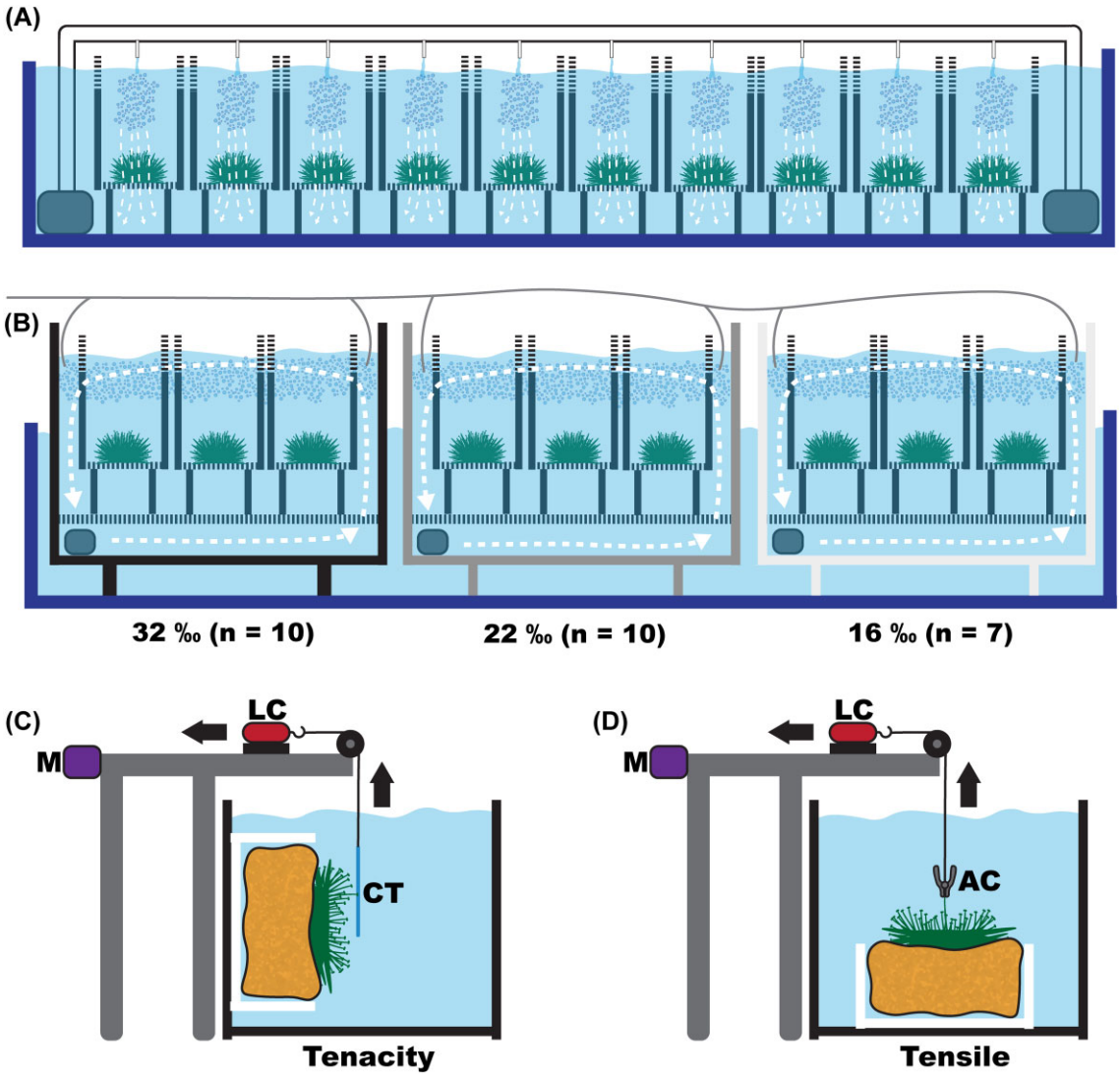

Fig. 1. Schematic of the recirculating seawater table configuration for *S. droebachiensis* housing and exposure to hyposalinity pulses. (A) Sea urchins were individually housed in enclosures fitted with mesh tops and bottoms (dashed black lines). Submersible pumps (gray rounded rectangles) supplied seawater to dedicated taps above each enclosure, aerating and circulating the seawater through the sea urchin enclosures (dotted white arrows). (B) During the hyposalinity exposures, sea urchins were placed in plastic bins based on their treatment group (indicated by the black, dark gray, and light gray bins) and exposed to hyposalinity pulses as described in the methods. Plastic bins were placed in the seawater table to maintain temperature but did not share water with the seawater table or one another. A submersible pump (gray rounded rectangles) and air diffusers (gray lines) provided water circulation and aeration in the plastic bins, respectively. (C) Tenacity tests were conducted using a custom-built apparatus composed of a load cell (LC) on a mobile trackway actuated by a DC motor (M). A glass capillary tube (CT) was connected to the load cell via monofilament thread fed through a pulley system. Sea urchins were placed into a small, seawater-filled container and restrained with their oral surfaces facing outward in a sponge-packed PVC collar attached to the side of the container. When a single tube foot attached to the capillary tube, the load cell was displaced backward on the apparatus, and the adhesive force of the tube foot recorded. (D) Tensile tests were conducted similarly to tenacity tests, but the PVC collar was oriented on the bottom of the container, and the glass capillary tube was replaced with an alligator clamp (AC). The clamp was placed on the distal end of a single tube foot, the load cell displaced until the tube foot broke, and force-time data recorded.

Experimental design

We divided sea urchins into three groups of equal mean test diameter (ANOVA: SS = 8.28, MS = 4.14, $F_{2,26} = 0.057$, $P = 0.94$) and variance (Levene's Test: df = 2, F = 0.17, $P = 0.85$) and randomly assigned them to one of three salinity exposure treatments: 32‰ (mean ± SE: 49.63 ± 3.02 mm diameter, $n = 10$), 22‰ (mean ± SE: 50.90 ± 2.56 mm diameter, $n = 10$), and 16 ‰ (mean ± SE: 49.15 ± 3.87 mm diameter, $n = 7$). We collected 30 individuals, intending to split them evenly

among treatment groups ($n = 10$) but one individual assigned to the 16‰ treatment died soon after the experiment began, and two other individuals assigned to the 16‰ treatment were (unintentionally) used for another experiment. We removed these individuals from the analyses.

Salinity exposures

Salinity exposures occurred biweekly for eight weeks (four exposures). Sea urchins (inside their enclosures) were placed into one of three 100-L plastic bins (81.6 cm length × 48.6 cm width × 34.9 cm height) based on treatment (32, 22, or 16‰). The bins were filled with filtered 32‰ seawater and suspended in the sea table to maintain ambient temperature. A small submersible pump and air diffusers were placed in each bin for circulation and aeration (Fig. 1B). For the two hyposalinity treatments, the salinity in the bins was lowered at a constant rate of 1.99 ± 0.03‰ every 10 min by adding chilled deionized water. After the exposure, sea urchins were returned to 32‰ at a rate of 1.99 ± 0.04‰ every 10 min by adding hypersaline seawater to the hyposalinity bins. Equivalent amounts of chilled 32‰ seawater were added to the 32‰ treatment bin while raising and lowering the salinity of the hyposalinity bins. Given the constant rate of salinity reduction, hyposalinity exposure duration differed between treatment groups; sea urchins were exposed to 22‰ for 24 h and 16‰ for 23 h, accounting for the 30 additional minutes required to lower salinity from 22 to 16‰ and raise seawater from 16 to 22‰. This ensured individuals across treatments had an identical rate of salinity change and recovery time. After sea urchins were returned to 32‰, they were removed from the plastic bins (inside their enclosures) and placed back into the recirculating seawater table. Sea urchins were not fed for the duration of the exposure.

Performance measurements

We measured maximum locomotor speed (mm s^{-1}) and tube foot disc tenacity (adhesive force per unit area [MPa]) 20 h after exposure to treatment salinity (during-exposure) and 20 h after sea urchins were returned to 32‰ seawater (post-exposure). Performance measures were performed on every sea urchin within each salinity treatment group (32 and 22‰: $n = 10$; 16‰: $n = 7$). Performance measurements during-exposure were conducted in seawater at each group's treatment salinity, while those post-exposure were conducted at 32‰ seawater. Tube foot disc area was measured 24 h prior to each salinity exposure in 32‰ seawater.

Locomotor performance

Maximum speed

Locomotor performance was assessed by placing sea urchins at one end of a 10-gallon glass aquarium and dispensing 10 mL of 65°C deionized water at the ambulacral column opposite the desired direction of movement. Preliminary trials indicated sea urchin movement was reliably cued by 65°C deionized water without resulting in observable harm to epithelial tissue. As described in Moura et al. (2023), sea urchin movement was recorded at one frame per second by a DSLR camera (Nikon D5600; Nikon USA, Melville, NY, USA) positioned beneath the aquarium with a 1 cm^2 scale in view. Frames for the first 30 s of movement were isolated at 1-s intervals (resulting in 30 frames) and analyzed in ImageJ using the MTrackJ plugin (Meijering et al. 2012). We tracked the center of the sea urchin's exposed jaw across the 30 frames and calculated speed between successive frames. Maximum locomotor speed (cm s^{-1}) was calculated as the maximum frame-to-frame speed recorded in each trial (Moura et al. 2023).

Tube foot adhesive performance measurements

Disc surface area

To estimate the mean tube foot disc area (mm^2), sea urchins were restrained in a sponge-packed PVC collar topped with a glass Petri dish held in a 3-L container of 32‰ seawater (Narvaez et al. 2020) 24 h prior to each salinity exposure. Once at least 10 tube foot discs attached to the glass, the discs were photographed with a 1 mm scale (Nikon D5600; Nikon USA, Melville, NY, USA). We randomly selected 10 attached discs, measured their area in ImageJ, and calculated the mean disc area (mm^2) per individual.

Disc tenacity

Disc tenacity (maximum adhesive force per unit area [MPa]) of oral tube feet was assessed using the method described by Narvaez et al. (2020). Disc adhesive force was measured by restraining sea urchins in a sponge-packed PVC collar mounted to the side of a 3-L container of seawater. A digital 1 N capacity load cell (FUTEK LSB 200; Irvine, CA, USA) was mounted to a custom-built motorized track and connected to a monofilament thread ending in a glass capillary tube (Fig. 1C). The glass capillary tube was presented to the extended tube feet. Once a single tube foot disc attached, adhesive force was measured by the motorized load cell pulling the capillary tube at a constant rate (2.59 cm s^{-1}) until detachment. The peak force value (N) was recorded as the maximum adhesive force of the

tube foot. The adhesive force of three tube feet was measured both during- and post-exposure. The maximum value of adhesive force at each time point was used to calculate maximum disc tenacity by dividing the highest adhesive force by the mean disc area of that sea urchin measured prior to salinity exposure.

Tube foot tensile behavior

Tube foot tensile behavior was assessed by restraining sea urchins in a sponge-packed PVC collar mounted to the bottom of a 3-L container of seawater and allowing tube feet to extend naturally. A single tube foot was clamped by a metal clip inlaid with sandpaper (Blast-Case Steel Toothless Alligator Clips, John Miller, Inc.) at the distal tip. Clamped tube feet were pulled in tension at a constant rate (2.59 cm s^{-1}) until breaking using the custom-built motorized track described above (Fig. 1D). The metal clip was connected to a 1 N digital load cell (FUTEK LSB 200, Irvine, CA, USA) by a fishing line (12 lb Shakespeare Omniflex; Columbia, SC, USA). Instances where tube feet slipped out of the clip were omitted from our dataset and we did not observe tube feet breaking where the clamp was placed. During each pull, data were continuously recorded as force-time curves. Force-time curves were then converted to force-extension curves by multiplying time elapsed by the rate of displacement (2.59 cm s^{-1}). Force data were smoothed in R using a cubic spline (package *stats*) prior to the extraction of tensile parameters.

Tensile properties are typically extracted from stress-strain curves. These curves explain mechanical behavior under tension, independent of sample geometry. Stress and strain are calculated from force and extension, respectively, but require measures of both cross-sectional area and length. In echinoderm tube feet, these parameters are often estimated using histological preparations and imaging (e.g., Santos and Flammang 2005), which were not possible given our experimental setup and logistics. Therefore, we opted to use force-extension curves to describe variation in these tensile parameters we collectively call tube foot tensile behavior: tube foot breaking force (N), tube foot total extension (cm), tube foot spring constant (N m^{-1}), and work to break tube feet (mJ). Tube foot breaking force was defined as the maximum force required to cause tube foot stem failure (Santos and Flammang 2005; Cohen-Rengifo et al. 2017, 2019; Narvaez et al. 2020, 2022). Tube foot total extension was estimated by calculating the difference in extension between breaking and when force reached 0.0015 N (*sensu* Santos and Flammang 2005). This force threshold was chosen because this is when previous work would measure the initial length of tube feet, though we were unable to make length measurements here (Santos and Flammang 2005). Tube foot spring constant is a measure of tube foot stiffness and was estimated as the slope of the linear portion of the J-shaped force-displacement curve just prior to tube foot stem failure (similar to calculating Young's modulus from a stress-strain curve). The work (or energy) to break tube feet was estimated by measuring the area under the force-displacement curve via the *numpy.trapz* function in the *NumPy* package in Python (similar to calculating toughness from a stress-strain curve). Tensile parameters of three tube feet per urchin were measured during and after salinity exposures on the same randomly selected subset of sea urchins (32 and 22‰: $n = 5$; 16‰: $n = 4$). The lower sample size of the 16‰ salinity treatment was a result of one of the sea urchin deaths indicated above. We calculated the maximum tensile parameters of three tube feet per sea urchin during- and post-exposure. We were unable to collect data on tube foot tensile behavior during the first exposure because of malfunctions of the digital load cell.

Statistics

All statistical analyses were performed using R (R Core Team 2023). We used linear mixed effects models via the *lmer* function in the *lme4* R package (Bates et al. 2015) to examine the impacts of salinity, exposure number, and their interaction on locomotor performance (maximum speed), tube foot disc tenacity, and tensile parameters (breaking force, total extension, spring constant, and work). Analyses included during-exposure and post-exposure data separately. Individual sea urchins were modeled as a random effect in all linear mixed effects analyses to account for repeated measurements on individuals across exposures. In the case of significant fixed effects, Tukey's Honest Significant Difference (HSD) tests were used to determine differences among groups via the *emmeans* package. In the case of significant interaction terms, pairwise comparisons were limited to tests of simple effects. Assumptions of normally distributed residuals and homogeneity of variances were tested with Shapiro-Wilk and Levene's tests, respectively (Supplementary Tables S1 and S2). Several response variables were log transformed to meet assumptions (see variables indicated with $*$ in Supplementary Table S1).Disc tenacity failed to meet the normality assumptions even using transformed data. Linear mixed effects analyses, however, are robust to violations of this assumption (Schielzeth et al. 2020), so we conducted these analyses on the transformed data nonetheless. Disc area failed to meet the homogeneity of variance and normality assumptions even using transformed data. Therefore, we used non-parametric Kruskal-Wallis tests to examine the impact of repeated exposures and salinity on

Table 1 Linear mixed model tables for maximum speed, disc tenacity, breaking force, total extension, spring constant, and work for both during-exposure and post-exposure measurements.

Response variable	Measurement time	Predictor	SS	MS	NumDF	DenDF	F	*P*
Maximum speed	During-exposure	Exposure number	5.71	1.90	3	72	16.88	< **0.0001**
		Salinity	6.47	3.23	2	24	28.67	< **0.0001**
		Exposure number∗salinity	1.75	0.29	6	72	2.59	**0.025**
	Post-exposure	Exposure number	2.27	0.76	3	72	5.39	**0.002**
		Salinity	2.02	1.01	2	24	7.19	**0.004**
		Exposure number∗salinity	0.24	0.04	6	72	0.29	0.94
Disc tenacity	During-exposure	Exposure number	0.004	0.001	3	70.80	1.68	0.18
		Salinity	0.08	0.04	2	23.96	44.38	< **0.0001**
		Exposure number∗salinity	0.01	0.002	6	70.75	2.15	0.06
	Post-exposure	Exposure number	0.003	0.001	3	69.62	1.46	0.23
		Salinity	0.01	0.01	2	23.03	9.27	**0.001**
		Exposure number∗salinity	0.003	0.0005	6	69.58	0.74	0.62
Breaking force	During-exposure	Exposure number	0.05	0.02	2	33	0.51	0.61
		Salinity	1.05	0.53	2	33	10.71	**0.0003**
		Exposure number∗salinity	0.40	0.10	4	33	2.02	0.11
	Post-exposure	Exposure number	0.004	0.001	3	44	0.34	0.80
		Salinity	0.062	0.031	2	44	8.35	**0.0008**
		Exposure number∗salinity	0.009	0.002	6	44	0.41	0.87
Total extension	During-exposure	Exposure number	0.15	0.07	2	33	2.00	0.15
		Salinity	0.28	0.14	2	33	3.72	**0.03**
		Exposure number∗salinity	0.09	0.02	4	33	0.62	0.65
	Post-exposure	Exposure number	1.55	0.52	3	33.00	2.52	0.07
		Salinity	0.05	0.02	2	11.00	0.12	0.89
		Exposure number∗salinity	1.01	0.17	6	33.00	0.82	0.56
Spring constant	During-exposure	Exposure number	0.22	0.11	2	22	1.44	0.26
		Salinity	1.10	0.55	2	11	7.37	**0.009**
		Exposure number∗salinity	0.35	0.09	4	22	1.16	0.36
	Post-exposure	Exposure number	207.56	69.19	3	33	1.11	0.36
		Salinity	319.42	159.71	2	11	2.56	0.12
		Exposure number∗salinity	417.80	69.63	6	33	1.11	0.38
Work	During-exposure	Exposure number	0.03	0.02	2	22	0.18	0.83
		Salinity	0.40	0.20	2	11	2.27	0.15
		Exposure number∗salinity	0.70	0.18	4	22	1.97	0.13
	Post-exposure	Exposure number	0.54	0.18	3	44	1.46	0.24
		Salinity	1.73	0.86	2	44	7.00	**0.002**
		Exposure number∗salinity	0.03	0.00	6	44	0.03	0.9998

Bold values indicate significant *P*-values ($P < 0.05$).

disc area (Supplementary Table S3). For all statistical analyses, $\alpha = 0.05$.

Results

Locomotor performance

Maximum speed

During-exposure maximum speed varied significantly as a function of exposure number, salinity, and the interaction between exposure number and salinity (Table 1; Fig. 2A). We describe the salient results of the interaction between exposure number and salinity below—complete statistical results (e.g., pairwise comparisons) can be found in Fig. 2A and the Supplementary Information (Supplementary Table S4). Across all four exposures, sea urchins exposed to 16‰ seawater had significantly lower during-exposure maximum speed than those exposed to 32‰ seawater (all comparisons

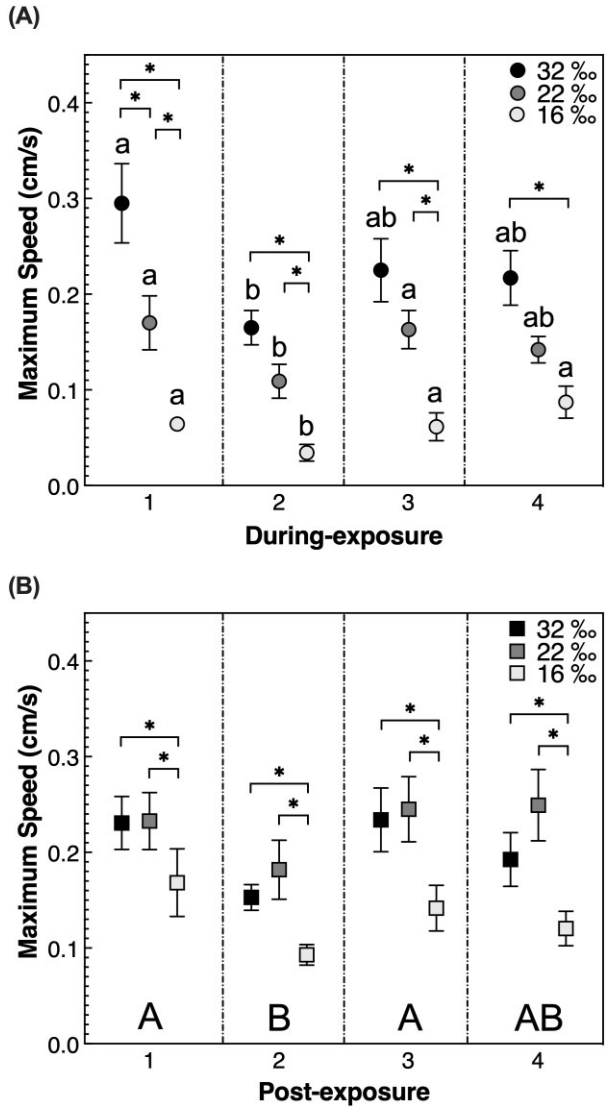

Fig. 2. The impacts of repeated hyposalinity exposures on green sea urchin (*S. droebachiensis*) locomotor performance. (A) During-exposure maximum speed was significantly impacted by the interaction between exposure number and salinity treatment. Groups connected by a bracket and ∗ indicate significant differences between salinity treatment groups within a particular exposure (i.e., within exposures 1–4; $P < 0.05$). Lowercase letters indicate statistical differences in maximum speed across different exposures within a particular salinity treatment group (i.e., within 32, 22, and 16‰ treatments; $P < 0.05$). (B) Post-exposure maximum speed was significantly impacted by salinity and exposure number. There was no significant interaction between exposure number and salinity, thus all pairwise comparisons indicated in this panel are of main effects only (i.e., exposure number or salinity individually). Groups connected by a bracket and ∗ indicate significant differences between salinity treatment groups ($P < 0.05$); significant differences were consistent across exposures (i.e., no interaction). Uppercase letters indicate statistical differences in maximum speed across different exposures (exposures 1–4; $P < 0.05$).

$P < 0.05$). During-exposure maximum speed of sea urchins exposed to 16‰ seawater was significantly lower than those exposed to 22‰ during the first through third exposures (all comparisons $P < 0.05$). During-exposure maximum speed of sea urchins exposed to 22‰ was generally between that of the other salinity treatments, although statistical differences varied depending on exposure number. Maximum speed of all sea urchins decreased significantly during the second exposure compared to the first exposure (all comparisons $P < 0.05$) but returned to first exposure levels during the third and fourth exposures (all comparisons $P > 0.05$).

Post-exposure maximum speed varied significantly as a function of exposure number and salinity treatment. There was no significant exposure number-salinity interaction on post-exposure maximum speed (Table 1; Fig. 2B). Sea urchins exposed to 32 and 22‰ seawater had significantly greater post-exposure maximum speeds than those exposed to 16‰ (32 vs. 16‰: $P = 0.02$; 22 vs. 16‰: $P = 0.004$). Post-exposure maximum speeds of sea urchins exposed to 22 and 16‰ seawater were not statistically different from one another ($P = 0.77$). Maximum speeds of all sea urchins were significantly lower following the second exposure compared to those following the first ($P = 0.003$) and third exposures ($P = 0.007$). Maximum speeds following the first, third, and fourth were not statistically different from one another (all comparisons $P > 0.05$). Maximum speeds following the fourth exposure were not statistically different from those following the second exposure ($P = 0.10$).

Tube foot adhesive performance

Disc tenacity

Tube foot disc area did not vary significantly as a function of exposure or salinity (Supplementary Table S3 and Fig. S1). During-exposure disc tenacity varied significantly as a function of salinity but not as a function of exposure number or the exposure number-salinity interaction (Table 1; Fig. 3A). During-exposure disc tenacity was significantly lower in sea urchins exposed to 16‰ compared to those exposed to 32‰ ($P < 0.0001$) and 22‰ seawater ($P < 0.0001$). Sea urchins exposed to 32 and 22‰ seawater did not vary significantly in disc tenacity during-exposure ($P = 0.33$).

Post-exposure disc tenacity varied significantly as a function of salinity but not as a function of exposure number or the exposure number-salinity interaction (Table 1; Fig. 3B). Post-exposure disc tenacity was significantly lower in sea urchins exposed to 16‰ compared to those exposed to 32‰ ($P = 0.001$) and 22‰

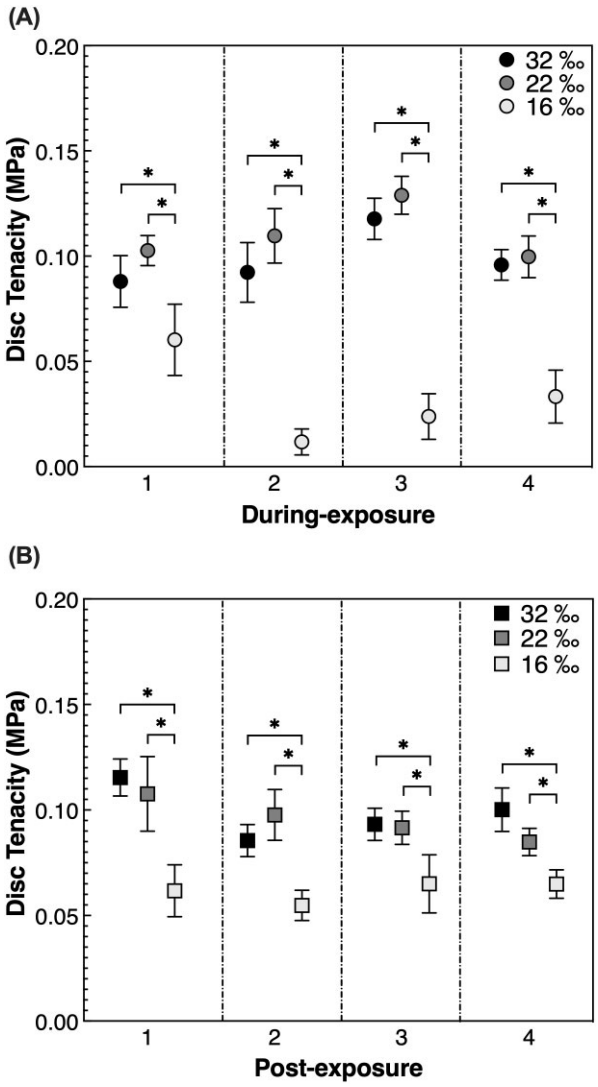

Fig. 3. The impact of repeated hyposalinity exposures on disc tenacity of *S. droebachiensis* tube feet. During-exposure (A) and post-exposure (B) disc tenacity were significantly affected by salinity. Groups connected with a bracket and ∗ indicate significant differences between salinity treatment groups (i.e., within 32, 22, and 16‰ treatments; $P < 0.05$). There was no significant interaction between exposure number and salinity, thus all pairwise comparisons indicated in this figure are of the main effect of salinity regardless of exposure number.

seawater ($P = 0.004$). Sea urchins exposed to 32 and 22‰ seawater did not vary significantly in disc tenacity post-exposure ($P = 0.90$).

Tube foot tensile behavior

Parameters describing tube foot tensile behavior (tube foot breaking force, extension, spring constant, and work) did not vary significantly as a function of exposure number or the interaction between exposure number and salinity (Table 1) for both during- and post-exposure values. During-exposure, tube foot breaking force and spring constants varied significantly as a function of salinity (Tables 1 and 2). Tube feet of sea urchins exposed to 16‰ ($P = 0.008$) and 22‰ ($P = 0.004$) seawater broke at significantly lower forces than those exposed to 32‰ seawater. Tube feet of sea urchins exposed to 16‰ seawater had significantly lower spring constants than those exposed to 32‰ seawater ($P = 0.008$); tube feet of sea urchins exposed to 22‰ seawater had intermediate spring constants closer in magnitude to the sea urchins exposed to 16‰ seawater but were not statistically distinct from either treatment group (32 vs. 22‰: $P = 0.07$; 22 vs. 16‰: $P = 0.36$). During-exposure tube foot extension did not differ significantly across salinity treatments (Tables 1 and 2), but the tube feet of sea urchins exposed to 22 and 16‰ seawater tended to have greater extension than those exposed to 32‰ seawater. During-exposure work to break tube feet did not differ significantly between salinity treatments (Tables 1 and 2).

Tube foot breaking force of sea urchins exposed to 22 and 16‰ seawater continued to be significantly lower than those exposed to 32‰ seawater post-exposure ($P = 0.01$ for both comparisons). Tube foot extension and spring constants did not differ significantly amongst treatment groups post-exposure (Tables 1 and 2), while the work to break tube feet was significantly lower in sea urchins exposed to 22 and 16‰ seawater than those exposed to 32‰ seawater ($P = 0.02$ for both comparisons).

Discussion

Hyposalinity events in nearshore marine ecosystems will become more frequent and intense as climate change intensifies (IPCC 2022; Röthig et al. 2023). Stenohaline organisms, such as sea urchins, are particularly susceptible to these extreme climatic events (Irlandi et al. 1997; Russell 2013), as they generally lack the, or have a limited, ability to physiologically adapt to salinity (Russell 2013; Castellano et al. 2017). Recently, we quantified the consequences of hyposalinity exposure on the performance of green sea urchin (*S. droebachiensis*) tube feet (Moura et al. 2023), anatomical structures that are essential for survival because they function in adhesion, locomotion, feeding, respiration, and sensing (Lawrence 1987; Flammang 1996; Leddy and Johnson 2000). We found hyposalinity dramatically reduced tube foot performance, but the degree of performance degradation depended on the coordination requirements of the activity. Here we examined the response of green sea urchin (*S. droebachiensis*) locomotor performance, adhesive performance, and tube foot tensile behavior to repeated pulses of hyposalinity to answer three key questions raised by our previous work

Table 2 Green sea urchin (*S. droebachiensis*) tube feet tensile behavior parameters as a function of measurement time (during- or post-exposure) and salinity treatment.

Response variable	Measurement time	Salinity		
		32‰	22‰	16‰
Breaking force (N)	During-exposure	0.28 ± 0.02^{a}	0.20 ± 0.01^{b}	0.20 ± 0.02^{b}
	Post-exposure	0.31 ± 0.01^{a}	0.24 ± 0.01^{b}	0.24 ± 0.01^{b}
Extension (cm)	During-exposure	1.88 ± 0.07	2.26 ± 0.11	2.25 ± 0.16
	Post-exposure	2.06 ± 0.11	1.98 ± 0.13	1.97 ± 0.12
Spring constant (N/m)	During-exposure	40.10 ± 3.38^{a}	29.30 ± 1.44^{ab}	25.64 ± 2.90^{b}
	Post-exposure	45.90 ± 1.84	38.81 ± 2.02	38.05 ± 2.42
Work (mJ)	During-exposure	1.28 ± 0.1	0.95 ± 0.08	1.28 ± 0.22
	Post-exposure	1.44 ± 0.09^{a}	1.08 ± 0.06^{b}	1.06 ± 0.08^{b}

Values are means ± s.e.m. Means with different letters indicate statistical differences.

(Moura et al. 2023). First, does the performance of sea urchin tube feet acclimate to repeated pulses of hyposalinity? Second, how does tube foot tensile behavior respond to both single and repeated hyposalinity events? Third, are the negative effects of hyposalinity limited to the exposure, or are they persistent following a return to normal salinity levels?

Potential for acclimation to hyposalinity events

We expected tube foot performance and properties in sea urchins repeatedly exposed to hyposalinity to improve over time and become similar to those of the normal salinity treatment (i.e., 32‰) if acclimation of these parameters occurred. We did not observe these particular trends in our data, suggesting that we have no support for the acclimation of sea urchin locomotion, adhesion, and tube feet tensile behavior to repeated hyposalinity events. This result is surprising because previous work found populational differences in *S. droebachiensis* righting response, an activity that requires substantial tube foot coordination, to variable salinity conditions (Himmelman et al. 1984). Furthermore, the growth rate of *S. droebachiensis* acclimates to repeated exposure to hyposalinity in the laboratory (Russell 2013). The physiological mechanisms driving acclimation to hyposalinity in these parameters are unknown. Multiple hypotheses exist predicting how echinoderms may acclimate to and tolerate osmotic stress, including the involvement of heat shock proteins (Russell 2013) and physiological or behavioral modifications (e.g., reduction of surface area by muscle contraction; Castellano et al. 2018; Arribas et al. 2022). These responses have been documented in echinoderms (Vidolin et al. 2002; Meng et al. 2011; Castellano et al. 2018), but they have not yet been connected to whole organism outcomes such as acclimation in righting responses and growth (Russell 2013; Arribas et al. 2022). Furthermore, it is unclear why these processes would not extend to the aspects of tube foot performance studied here (i.e., locomotion, disc tenacity, and tensile behavior). Future work is clearly needed to identify the mechanistic bases of acclimation in sea urchin functional responses to hyposalinity and whether they explain the lack of acclimation observed here.

Although we did not detect acclimation to hyposalinity exposures, we did observe that all sea urchins (including those exposed to 32‰ seawater) had significantly reduced locomotor performance during the second exposure compared to the first exposure (Fig. 2A). Although we cannot explain this unexpected result, one hypothesis is that the stress induced by handling and/or other experimental protocols (Bose et al. 2019) resulted in this decrease in performance, with sea urchins adjusting to this stress after the second exposure. In any case, locomotor performance returned to first exposure levels during the third and fourth exposures, suggesting that this finding has little impact on our results and interpretations.

Negative effects of hyposalinity—during-exposure

The impacts of hyposalinity on locomotor and adhesive performance are consistent with our previous work on this topic. Moura et al. (2023) found that locomotor performance decreased linearly with decreases in salinity, while adhesive performance was maintained until relatively severe hyposalinity levels (i.e., 16‰ seawater). In this study, we observed that sea urchins attained significantly lower maximum speeds during-exposure to 16‰ seawater relative to those exposed to 32 and 22‰ seawater (Fig. 2A). Although maximum speed did not differ significantly between the sea urchins exposed to 32 and 22‰ seawater consistently throughout our experiment, we observed a general trend of decreasing maxi-

mum speed with decreasing salinity during each exposure. Disc tenacity did not differ significantly between the sea urchins exposed to 32 and 22‰ seawater but was significantly reduced in the sea urchins exposed to 16‰ seawater (Fig. 3A), which is consistent with our previous work (Moura et al. 2023). The physiological and/or behavioral mechanisms explaining the impacts of hyposalinity on locomotor and adhesive performance are not clear (Moura et al. 2023) but may be the result of the dilution of essential ions for effective neuromuscular functioning (Carafoli 2005; Hill et al. 2012), generation of adhesive bonds (Hennebert et al. 2015; Lebesgue et al. 2016), or adhesive glue secretion (Lebesgue et al. 2016). Hyposalinity also results in sea urchins taking up more water in their tissues, which may also impact tube foot performance (Santos et al. 2013).

We also tested the impacts of hyposalinity on the tensile behavior of tube feet by measuring the force and extension of tube feet under tension until breaking. Overall, tube feet of sea urchins exposed to 22 and 16‰ seawater break at significantly lower forces (Table 2), have generally lower spring constants (Table 2), but absorb similar amounts of energy (i.e., work; Table 2) compared to sea urchins exposed to 32‰ seawater. A linear mixed effects analysis revealed total tube foot extension varied significantly as a function of salinity but post-hoc Tukey pairwise comparisons showed no significant differences between groups. Nevertheless, tube feet exposed to 22 and 16‰ seawater have generally greater extension than those of sea urchins exposed to 32‰ seawater (Table 2). Collectively, these findings suggest that tube feet are easier to break in hyposaline conditions yet have greater extension and a lower spring constant, which results in relatively similar energy absorption capacities. It is possible that changes in material properties are not driving the effects on tensile behavior, as we were unable to quantify geometrically independent values of stress and strain. Tube foot morphology typically scales with test diameter (Parvez et al. 2018; Narvaez et al. 2020), and our treatment groups had nearly equivalent mean test diameters and variances. Despite this, hyposalinity may differentially result in morphological changes that we were not able to capture in our experiment (e.g., stem thickness, differential impact on tissues associated with tensile properties) and thus may be driving or contributing to the changes in tensile behavior we observed. Although we cannot ascribe the mechanisms of the changes in tensile behavior, our measurements are both ecologically and functionally relevant as they directly describe how tube feet as an integrated unit will respond to mechanical loads in these conditions. Future work on this topic should implement experimental methods to confirm the relative contributions of morphology and material properties in tube foot tensile behavior under varying hyposalinity conditions.

Negative effects of hyposalinity—post-exposure

After 20 h in 32‰ seawater, the locomotor and adhesive performance of sea urchins exposed to 16‰ seawater continued to be negatively impacted by severe hyposalinity, indicating that impairment caused by hyposalinity extends well beyond the initial exposure (Figs. 2B and 3B). Although this difference is significant, sea urchins exposed to 16‰ seawater appear to regain a limited amount of locomotor and adhesive performance 20 h post-exposure. These hyposalinity "aftereffects" are interesting considering the ions essential for effective neuromuscular functioning (Carafoli 2005; Hill et al. 2012), adhesive bond generation (Hennebert et al. 2015; Lebesgue et al. 2016), or glue secretion (Lebesgue et al. 2016) have returned in 32‰ seawater. These findings suggest that there are additional mechanisms at work that have longer term implications, resulting in reduced locomotor and adhesive performance in hyposaline conditions.

Tube foot breaking force also remained significantly reduced in sea urchins exposed to 22 and 16‰ seawater 20 h post-exposure (Table 2), while total tube foot extension (Table 2) and spring constant (Table 2) were no longer impacted. This shift in tensile behavior post-exposure resulted in the tube feet of sea urchins exposed to 22 and 16‰ seawater having significantly reduced work to break (Table 2). As such, sea urchins exposed to these salinity treatments not only continued to have reduced breaking force 20 h post-exposure, but also had significantly reduced energy absorption capacities. The mechanical properties of sea urchin tube feet are largely the result of connective tissue, which can be modified by the ionic environment. Previous work demonstrated the absence of calcium increases the compliance of sea urchin tube feet (Santos et al. 2005). Thus, the increased extensibility and decreased stiffness during-exposure and subsequent return to normal values post-exposure may be explained by the reduced concentration of calcium in hyposaline conditions and the return of these ions post-exposure. However, the persistence of a reduction in breaking force post-exposure suggests other temporary or potentially irreversible cellular or subcellular changes impact the structural integrity of the tube foot stem. Again, we cannot discount the differential effect of hyposalinity on tube foot morphology that may contribute to or drive differences in tensile behavior post-exposure. Future research is needed to understand the morphological, physiological, and cellular mechanisms responsible for changes in locomotor and

adhesive performance and tensile behavior during and after hyposalinity events.

Our findings suggest that exposure to hyposalinity may increase the risk of dislodgment for sea urchins by predators and hydrodynamic forces, consistent with our previous work (Moura et al. 2023). We found no ability of sea urchins to acclimate their tube feet biomechanics to hyposalinity exposure, at least under the short time frames examined in this study. Longer term studies have demonstrated acclimation in green sea urchin growth. Russell (2013) subjected juvenile green sea urchins to 8 hyposalinity events over 16 weeks. These sea urchins showed negative effects on growth for the first 4 exposures but acclimated in exposures 5 through 8. Therefore, longer term studies may demonstrate the acclimation of tube feet performance and properties. Nevertheless, hyposalinity exposures were performed on a total of four occasions over the course of 2 months, simulating four events at which dislodgement or other fitness impacts could have occurred should sea urchins have experienced these salinities in their natural habitat. As such, we posit that the time frame studied here is an ecologically relevant time span given that significant decrements in performance even after a single exposure could have substantial negative effects on an individual (i.e., being dislodged from a substrate or unable to move to forage or relocate).

The inclusion of tube foot tensile behavior in this study resulted in the discovery that tube feet can remain attached and absorb similar amounts of energy at moderate salinity levels (e.g., 22‰) but that they are more susceptible to breaking under tensile loads. These findings suggest that moderate hyposalinity may have similar impacts on sea urchin dislodgment risk as severe hyposalinity events. Reduced breaking force and maintained adhesive performance in moderate hyposalinity conditions may even present themselves as a greater challenge for sea urchins post-exposure than severe hyposalinity. The catastrophic failure of tube feet, as opposed to adhesive failure, may impose greater energetic demands as sea urchins may need to reallocate energy to the regrowth of damaged structures (Narvaez et al. 2020, 2022). Our work also indicated that the negative effects of hyposalinity on tube foot biomechanics are not limited to the hyposalinity event. Some of these effects are maintained (reduced speed, disc tenacity, breaking force) or novel (reduced work to breaking) post-exposure, potentially increasing the window of dislodgement and impacts on fitness. As climate change continues to increase the intensity and frequency of atmospheric river events and nearshore freshwater input (IPCC 2022; Röthig et al. 2023), sea urchins are likely to experience more numerous and severe hyposalinity events. Our work indicates that the anatomical structures sea urchins rely on for many critical organismal functions are severely impacted by hyposalinity. Thus, we may expect to observe shifts in the distribution of sea urchins in the future, which may directly affect their ecological communities.

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Supplementary data

Supplementary Data available at ICB online.

Conflict of interest

The authors have no conflicts of interest to declare.

Data availability

Data and code for this study are available in the figshare repository: https://doi.org/10.6084/m9.figshare.24556705.v2.

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