



Behavioural responses to hydrostatic pressure in selected echinoderms suggest hyperbaric constraint of bathymetric range

Justine Ammendolia¹ · Jean-François Hamel² · Annie Mercier¹

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Abstract

Although hydrostatic pressure is one of the most prominent abiotic drivers of faunal bathymetric ranges, it is one of the least understood. To better understand hyperbaric constraints on depth distributions, we explored responses to high pressure of adult echinoderms from eastern Canada. The sea urchin *Strongylocentrotus droebachiensis*, the sea star *Leptasterias polaris* and the sea cucumber *Cucumaria frondosa* were exposed to various pressures within and beyond their known bathymetric distribution (i.e. surface pressure; pressure at the midpoint in bathymetric distribution; pressure at twice the maximum depth in the species bathymetric distribution) for different durations (24 h, 72 h, 9 days). Survival was compromised by exposure to the highest pressure levels, with the sea urchin exhibiting the highest mortality. After 72-h exposure, mortality was observed for sea urchin and sea cucumber individuals while all sea star individuals survived. Following 9-day exposure of sea star individuals to high pressure, 100% mortality occurred. Exposure to pressures typical of natural distributions had little effect on the selected health and motor metrics. Pressures atypical of natural ranges negatively affected the motor functions of all species, irrespective of exposure duration. During and after 24-h exposure, feeding was reduced in sea urchin and sea cucumber individuals, but was unchanged in sea star individuals. Overall, there were no clear signs of acclimation to high pressure following sustained periods of exposure in any species. These results highlight constraints applied by hydrostatic pressure beyond current bathymetric ranges on life-sustaining behaviours in echinoderms. The potential of long-lived echinoderms to survive downward migration to greater depths is species-specific, suggesting there may be winners and losers in the face of near-future climate-driven migration patterns.

Introduction

Hydrostatic pressure is a fundamental mediator of biological processes (Macdonald 1997). It plays a critical role in defining oceanic environments as it forms a continuous linear gradient from the surface to the abyss (Pradillon 2011).

Specifically, pressure in the ocean increases by 0.1 MPa (1 MPa = 10 bar \approx 10 atm) for every 10 m of depth (Somero 1992). The oceanic depth of 200 m generally marks the continental shelf break, establishing an arbitrary subdivision between shallow-water and deep-sea environments (Hessler 1974; Thistle 2003). Since 95% of the oceans' volume lies below 200 m, pressure is a prominent abiotic factor that marine life must cope with (Miyake et al. 2012; Pradillon and Gail 2007).

The range of bathymetric distributions that different species occupy may vary from dozens to hundreds of metres (Tyler and Young 1998), broadly separated into stenobathic (restricted) and eurybathic (widespread) distributions, which may be driven by adaptations to a complex array of biotic and abiotic variables. In addition to greater pressure, factors that characterize deeper water masses include: minimal nutrients from primary production, low temperature, darkness and relatively stable water chemistry (Carney 2005; Childress 1995; Pradillon et al. 2004). The suggested primary variables responsible for controlling limits to depth

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✉ Justine Ammendolia
jammendolia@mun.ca

¹ Department of Ocean Sciences, Memorial University, St. John's, NL, Canada

² Society for Exploration and Valuing of the Environment, Portugal Cove-St. Philips, NL, Canada

distributions are pressure and temperature (Brown and Thatje 2014; Carney 2005). Although a considerable number of studies have examined the effects of combined thermal-pressure levels, our overall understanding of the biological effects of pressure remains comparatively rudimentary (e.g. Tyler et al. 2000; New et al. 2014; Aquino-Souza et al. 2008; Tyler and Young 1998; Ravaux et al. 2003, 2009).

There is increasing incentive to study both the ecological and physiological pressure thresholds of marine species in light of impending climate changes. While the combination of anthropogenic and climatic factors is expected to impact both shallow-water and deep-sea environments, coastal ecosystems are anticipated to suffer the greatest changes, especially close to the poles (Brierley and Kingsford 2009; Harley et al. 2006; Hoegh-Guldberg and Bruno 2010). Among other ecological impacts, relatively rapid shifts in physico-chemical conditions (e.g. temperature, pH, UV radiation) over decadal scales are expected to drive the migration of marine species poleward and downward towards colder and/or more stable refuges (Doney et al. 2012; Graham et al. 2007; Harley et al. 2006; Perry et al. 2005).

Fundamentally, high pressure can have complex effects on the biochemical and physiological activities of biological systems by directly altering their volume (Abe et al. 1999; Somero 1992). Generally, high pressure can alter the speed of the reaction responsible for converting reactants to products (e.g. generation of enzymes and lipid membranes) (Somero 1992). Exposure to high pressures can also have complex effects on the volume of biological systems that facilitate reactions; for instance, high pressures can enhance the rate of reactions by decreasing the system volume (Abe et al. 1999). By contrast, exposure to low pressure has been found to accelerate the rate of reactions (Hochachka and Somero 1984; Somero 1992). Both shallow-water and deep-sea species are capable of modifying the structure and composition of cell membranes (Hazel and Williams 1990; Pond et al. 2014). For instance, under high pressure, species can cope by increasing the proportions of phospholipid fatty acids to enhance the integrity and fluidity of membrane structure (Pond et al. 2014). Generally shallow-water species can reduce their membrane fluidity, whereas deep-species maintain fluidity by modifying the homeoviscous structure (Somero 1992). Thus, the inability to maintain reaction rate equilibrium can manifest as impairment in neural and muscular functionality in metazoans (Brown and Thatje 2018; Macdonald 1984). Exposure to high pressure can depress neurotransmission in crustaceans through the inhibition of *N*-methyl-D-aspartate (NMDA) receptors activity, with implications in synaptic transmission and the functionality of the neuromuscular complex (Brown and Thatje 2018).

Complex interactions between pressure and other abiotic factors are already modulating bathymetric distributions (Macdonald 1997), highlighting the need to tease out the

broad pressure thresholds of marine species through laboratory studies. In general, the study of mechanisms underlying piezophily (barophily) in marine species is logistically challenged by the rarity of high-pressure vessels capable of maintaining large volumes of water under flow-through conditions and the difficulty in testing deep-sea taxa under laboratory conditions (e.g. Miyake et al. 2007; Shillito et al. 2001, 2015). A number of laboratory studies have explored how shallow-water species cope with pressure that exceeds their natural bathymetric distributions (see review by Brown and Thatje 2014). They have shown that the response of species to pressure exposure occurs across hierarchical levels of physiological organisation. High-pressure exposure experiments on crustaceans have induced mechanistic challenges such as: dramatic decreases in heart rate from cardiac stress (Brown et al. 2017; Robinson et al. 2009), depression in metabolic rate as a result of poor membrane functionality (Thatje et al. 2010), and higher oxygen consumption relative to surface rates, indicative of internal stress (Morris et al. 2015a, b; Thatje and Robinson 2011). Such problems can trigger the high-pressure neurological syndrome (HPNS), which was originally described in vertebrates (Bowser-Riley 1984) and later observed in invertebrates exposed to pressure conditions beyond their natural bathymetric range (Somero 1992). HPNS causes chronic stress symptoms such as paralysis, spasms and uncoordinated movements (e.g. Morris et al. 2015a; Oliphant et al. 2011; Wilcock et al. 1978). Overall, pressure negatively impacts the neurological and muscular pathways of many organisms (Morris et al. 2015a). Exposure to pressures exceeding the natural range of shrimp has been found to elicit complications that hinder inhibitory or excitatory motor activity (Wilcock et al. 1978). In the early stages of exposure to such pressures, motor activity undergoes a stage of hyper-excitability (spasms, convulsions and twitches) but is immediately followed by a reduction in coordination of movements and mobility (e.g. Macdonald and Gilchrist 1978; Macdonald 1997; Wilcock et al. 1978). The term “pressure-paralysis” has been used to describe the point at which convulsive thresholds are met and there is a subsequent absence of motor activity (Macdonald and Gilchrist 1978). Therefore, examining the behavioural responses of species is an effective means of determining their ability to maintain internal homeostasis (Thatje et al. 2010). Other investigations have reported that stressful pressure conditions induce a “loss of equilibrium” in which individuals are reduced to a moribund state, notably in species of shrimp and crabs (e.g. Morris et al. 2015a; Oliphant et al. 2011; Shillito et al. 2004, 2006). The reduced motor activity (escape response of “tail-flicking”) of the shallow-water shrimp *Palaemonetes varians* from acute durations of high-pressure exposure has been attributed to unsustainable metabolic demands (Morris et al. 2015c). Although survival to various pressures was shown to be possible, whether

individuals are capable of surviving in an ecological context, e.g. by maintaining the ability to escape predators or forage to exploit food sources, has not been fully explored.

The phylum Echinodermata has commonly been used in pressure studies because its members are ubiquitous in marine benthic ecosystems across depths, they exhibit a diversity of complex life histories, and many of them act as keystone species (e.g. Gage and Tyler 1991; Aquino-Souza et al. 2008; Villalobos et al. 2006; Young and Tyler 1993; Young et al. 1996, 1997). Many echinoderms are also suspected to be extremely long-lived, in the order of decades and even centuries (Ebert and Southon 2003), and therefore, may be susceptible to experience decadal-scale changes in ocean conditions resulting from anthropogenic and climate-related stressors. Young, Tyler and their colleagues have done an impressive amount of work on early stages of shallow-water echinoderms across a wide latitudinal gradient, generally supporting the ability of echinoderm larvae to survive pressures that are beyond the natural bathymetric range of adults (e.g. Young and Tyler 1993; Young et al. 1996, 1997; Aquino-Souza et al. 2008; Villalobos et al. 2006; Tyler and Young 1998). It has been suggested that larvae could successfully invade deeper waters and establish populations within one generation (Tyler and Young 1998; Tyler and Dixon 2000). However, most of the current knowledge about the interactions of marine organisms with pressure is limited to pre-metamorphic life stages (before settlement). Why adults of these species have not been found at such depths in nature remains unclear (Morris et al. 2015a). Until recently, studies were limited to testing adults under static water volumes for short periods of pressure exposure (<24 h) (e.g. Company and Sardà 1998; Mickel and Childress 1982a, b). The study of larvae was facilitated by their small sizes, whereas the larger body sizes and more developed metabolisms of adults required larger vessels capable of maintain flow-through conditions (Brooke and Young 2009; Shillito et al. 2015).

The objective of the present study was to address knowledge gaps in our understanding of how different marine species cope with pressure by examining the acute and sustained responses of adult echinoderms to hydrostatic pressure using a suite of indicators. Given that climate-driven changes (rising seawater temperatures and ocean acidification) may drive vertical migrations, it is important to assess the ability of different species to colonize and thrive in increasingly deeper waters (Brown and Thatje 2015; Morris et al. 2015b). Focal species were selected based on their broad distribution in temperate, cold and polar subtidal environments: the sea urchin *Strongylocentrotus droebachiensis*, the sea star *Leptasterias polaris*, and the sea cucumber *Cucumaria frondosa*. These species also exemplify three of the main echinoderm classes (Echinoidea, Asteroidea, Holothuroidea, respectively), three feeding modes (omnivory,

carnivory, herbivory, respectively) and very different body forms that contribute to different modes of locomotion (test/exoskeleton, rigid calcareous body, weakly calcareous soft body, respectively).

The study specifically tested the hypotheses that if species are limited in their spatial distribution by pressure, behaviours will be more severely (and permanently) impaired upon exposure to pressures (depths) beyond their natural distribution ranges. Second, if species can acclimate relatively quickly (at the individual level, within a generation) to increased pressure, negative responses measured after sustained exposure (72–216 h) will be weaker than those measured after short exposure (24 h).

Materials and methods

Collection and maintenance

Adult specimens of the sea urchin *S. droebachiensis* (40–102 g; 33.1–66.6 mm test diameter) the sea star, *L. polaris* (23–114 g; 6.0–11.2 mm major axis), the sea cucumber *C. frondosa* (1–3 g, immersed weight; 81–171 mm contracted length) and the sea star *Solaster endeca* were collected via scuba diving at depths of 10–15 m between May 2013 and November 2014 off the coast of Newfoundland, eastern Canada (47.08°N, 52.95°W). All species were transferred to laboratory holding facilities and maintained in tanks (20–2000 L) under a flow-through system of unfiltered seawater (7–150 L h⁻¹). Individuals were kept close to large windows with naturally fluctuating light (maximum of 25–40 lx). A chilling unit (Universal Marine Industries, 5 hp) kept water suitably cold from July to October (<10 °C). Individuals were transferred to holding tanks held between 4 and 6 °C for 1–3 weeks prior to experiments. Starvation was implemented during this period to decrease metabolic variability among individuals of a given species and among the three species tested (New et al. 2014). Only healthy individuals without obvious damage to tissues, spines or arms were selected for the experiments.

Equipment and experimental conditions

Two flow-through stainless steel incubators of 19 L (IPOCAMP™, Autoclave, France) that can be pressurized to a maximum of 30 MPa (or 300 bar), equivalent to ~3000 m depth, were used (Shillito et al. 2001). They were maintained under flow through at 20 L h⁻¹ for the duration of each trial. Temperature was controlled and measured by probes (Huber CC 240, Offenburg, Germany) situated at the inlet and outlet of the IPOCAMP (±1 °C). Sophisticated hyperbaric vessels such as the IPOAMP are rare commodities (only seven such units exist in the world, two of which were used here).

Prior to each trial, the IPOCAMP was run for at least 2 h to allow for conditions to stabilize and reach the desired temperature before inserting the individuals in the vessel (New et al. 2014; Oliphant et al. 2011). Fibre optic light guides (KL 1500 LCD, Schott, USA) were placed in two of the view-ports to uniformly illuminate the interior (~100 lx), as confirmed from verification with an endoscope (Fort Dourdan, France) placed in the third view-port. When required, visual observations were recorded with a microscope camera (AxioCam ERc 5S, Zeiss, Germany) fitted to the endoscope.

Rather than mimicking the deep-sea environment, the experiments were designed to test the effect of pressure alone while keeping the other parameters as close to natural conditions as possible. Temperature was adjusted to match holding conditions during acclimation (~6 °C), which are representative of natural subtidal (and deep-sea) oceanic temperatures at the initial time of experimentation (DFO 2009). A 12:12 photoperiod was selected to allow for equal levels of diurnal/nocturnal activity. A multi-probe system (556 MPS, YSI Environmental, USA) was used to measure temperature (5.4–6.8 °C), salinity (33–33.5), dissolved oxygen (93.4–132.4 mg L⁻¹) and pH (7.8–8.0) in the vessels (see Electronic Supplementary Material Table S1 for detailed data).

Exposure to pressure

Based on known bathymetric ranges for each species, pressures levels were separated into: surface pressure (sea surface), mid-bathymetric range pressure (middle of known bathymetric distribution) and high pressure (twice the known maximum depth of occurrence; Table 1). Bathymetric ranges of occurrence within North Atlantic Canadian waters were determined from records accessed on the World Register of Marine Species (WoRMS Editorial

Board 2014). Searches for maximum and minimum depth distributions only considered records of more than three samples collected and identified post-1980, or posterior to any major taxonomic revision (Brodie et al. 2013; Smithsonian Institution 1974). A transition period for pressurization was allowed by manually setting the experimental pressure following increments of 2 MPa every 6 min; the same method was used to depressurize the vessels. This rate is comparable to that used in previous studies (New et al. 2014; Oliphant et al. 2011). It was selected in an effort to reduce stress to individuals as drastic changes in pressure over shorter intervals (used in some previously published studies of a similar nature: Ding et al. 2007; Yoshiki et al. 2008) have been found to cause DNA damage in hydrothermal vent worms, *Paralvinella grasslei* (Dixon et al. 2002).

Responses of the three focal species were first tested in the context of acute 24-h exposure to the three pressure levels (described above). Based on biomass constraints of the vessels, each 24-h trial consisted of 6 individuals for the sea urchin and sea star (run twice with a total of 12 per treatment, all different individuals), whereas each 24-h trial testing the sea cucumber consisted of 3 individuals (run twice with a total of 6 per treatment). Duplicate runs were carried out either simultaneously or within 48 h, once in each of the two IPOCAMP vessels, to minimize any tank effects. To confirm the absence of any temporal variation, control treatments were repeated whenever a full set of experiments (for a species and a level) could not be completed within 8 days (for technical reasons). Inside the vessel, sea urchin and sea star individuals were separately housed in perforated meshed clear containers (760 mL; 100- μ m nylon mesh), which were stacked vertically at random. Individuals of the sea cucumber were uncontained and placed on three separate levels of the holder designed for the IPOCAMP (Ravaux et al. 2003).

Sustained exposure experiments were conducted to test responses to surface and high-pressure levels over a continuous period of 72 h (3 days). All three species were tested together as they do not pose predatory threats to each other and can be found in abundance in the same locations in eastern Newfoundland. Each of the two IPOCAMP vessels contained two individuals of each species isolated on different levels of the holder (for vessel design, refer to Ravaux et al. 2003). Control and experimental conditions were tested simultaneously using the two vessels: one set at surface pressure (0.1 MPa) and the other at high pressure (24 MPa, i.e. the average of the high pressures tested in the 24-h experiments for all focal species). The trials were replicated three times, for a total of six individuals of each species per treatment. Placement of the species on the levels was mirrored in the control and experimental vessels, and was flipped across the runs to account for potential vertical variability in conditions (water flow inside the vessels).

Table 1 Bathymetric ranges (m) of focal species with corresponding pressure tested

Species	Known depth range (m)	Pressure tested (MPa)
Sea urchin <i>Strongylocentrotus droebachiensis</i>	0–1200	Surface (0.1) Mid-bathymetric range (6) High (24)
Sea star <i>Leptasterias polaris</i>	0–1100	Surface (0.1) Mid-bathymetric range (5) High (22)
Sea cucumber <i>Cucumaria frondosa</i>	0–1300	Surface (0.1) Mid-bathymetric range (6.5) High (26)

Note that for all three focal species, the maximum depth of occurrence is based on only one record that met the search criteria described in the methods

A long-term behavioural feeding experiment was conducted on the sea star, during which individuals were maintained under sustained pressure for a period of 216 h (9 days). The IPOCAMP was also run in flow-through mode so that there was a constant supply of fresh seawater in the header tank, and the protocols for experimental pressurization followed those outlined for 24-h trials. The 216-h experimental period tested the control (surface pressure) and the species-specific high pressure to determine if/how individuals are able to acclimate to pressures atypical of their distributions (Table 2). Simultaneously in each vessel, feeding was quantified from four individuals who were randomly selected and placed within separate containers stacked in the IPOCAMP. Another group of three sea star individuals were placed on a flat platform situated directly on top of the stacked containers to monitor behaviour throughout the experiment using the endoscope camera described earlier.

Response metrics (dependent variables) were measured, including various morphometrics, indices and behaviours (see below for their descriptions), at different times for

the various pressure levels and durations tested (Table 2). Pre-trial responses were tested at least 2 h before the start of the trials, post-trial responses were measured just after removal from the experimental vessel, while post-recovery responses were measured 7 days post-trial. A summary of the responses of each of the three species to the various experimental conditions is presented in Table S2 (Electronic Supplementary Material).

Response metrics

Weight variations

As change in weight is considered a stress indicator that measures variations in fluid volume in echinoderms (Ferguson 1992), the initial and final wet weights of sea urchin and sea star individuals were used to determine the net weight loss/gain associated with 24 h experimental trials: animals that died post-trial were excluded from these calculations (Table 2). Weight variations of the sea cucumber were not

Table 2 Summary of experiments, including trial conditions and response variables measured

Species	Trial duration (h)	Pressure	Response variables	Time of response measurement ^a
Sea urchin <i>Strongylocentrotus droe-</i> <i>bachiensis</i>	24	Surface (0.1) Mid-bathymetric range (6) High (24)	Ingestion indices Righting movement Survival (%) Weight	During/post-trial Pre-trial, post-trial Post-trial Pre-trial, post-trial
	72	Surface (0.1) High (24)	Righting movement Survival (%)	Pre-trial, post-trial, post-recovery Post-trial, post-recovery
Sea star <i>Leptasterias polaris</i>	24	Surface (0.1) Mid-bathymetric range (5) High (22)	Ingestion indices Righting movement Survival (%) Behaviour Weight	During/post-trial Pre-trial, post-trial, post-recovery Post-trial, post-recovery During/post-trial Pre-trial, post-trial
	72	Surface (0.1) High (22)	Righting movement Survival (%)	Pre-trial, post-trial, post-recovery Post-trial, post-recovery
	216	Surface (0.1) High (22)	Ingestion indices Righting movement Survival (%) Behavioural observations Weight	During/post-recovery Pre-trial, post-trial, post-recovery Post-trial, post-recovery During/post-trial Pre-trial
Sea cucumber <i>Cucumaria frondosa</i>	24	Surface (0.1) Mid-bathymetric range (6.5) High (26)	Anchorage time Cloacal openings Predator-escape response Time to feed Survival (%) Weight	Pre-trial, post-trial, post-recovery Pre-trial, post-trial, post-recovery Post-trial Post-trial Post-trial, post-recovery Pre-trial
	72	Surface (0.1) High (26)	Anchorage time Cloacal openings Predator-escape response Time to feed Survival (%)	Pre-trial, post-trial, post-recovery Pre-trial, post-trial, post-recovery Post-trial Post-trial Post-trial, post-recovery

Note that all response variables listed for a given trial duration were measured at the two or three pressures outlined

^aTime of measurement: pre-trial (activity prior to pressure exposure), during (while being exposed to pressure), post-trial (immediately after pressure exposure), post-recovery (7 days after pressure exposure)

assessed because obtaining the post-trial weights would have caused stress that would have affected the four behavioural experiments that followed exposure.

Righting times

Following exposure to pressures (24 and 72 h), righting times were tested at surface pressure in sea urchin and sea star individuals (not applicable in the sea cucumber because of its cylindrical body morphology) to assess the condition of the individuals post-trial (Table 2), since this response is indicative of health (Taylor et al. 2014). Immediately after removal from the vessels, individuals were placed in separate 20-L tanks with seawater maintained at ~ 6 °C. After a 10-min acclimation period, individuals were tested for their righting response by turning them 180° onto their aboral surface. Time to right was recorded as the time required to turn back onto oral surface. Monitoring was ended after 2 h; if the response was incomplete after this delay, the time to right was scored as > 120 min.

The protocols for the 216-h exposure experiments were slightly modified from the shorter duration experiments because the possible post-trial effects of containment on individuals from the experimental design were taken into account. No significant difference was found between the righting responses of the two groups (contained and free-moving within the vessel) over any exposure duration (Text S1, Electronic Supplementary Material) so the data were pooled for analysis ($n = 7$).

Ingestion index and feeding posture

For studies involving the carnivorous sea star and grazing sea urchin, ingestion indices were measured during the 24-h trials. In addition, feeding postures were determined for both 24 and 72-h trials in sea star individuals, and their behaviour and feeding was monitored using video during the 216-h trial, along with their ingestion index. Since sea cucumbers are suspension feeders and it was not possible to implement a method for quantifying the ingestion of plankton in the setup used, the impact of pressure on their feeding required a different method (detailed below).

When sea urchin individuals were introduced into the experimental chambers, each individual was placed directly on piece of kelp (*Laminaria digitalis*), a typical food source for this species (Meidel and Scheibling 1999). The kelp fragment had been thoroughly blotted and cut to obtain an initial wet weight of 10 g. At the end of the trial, residual food was removed, blotted and weighed again. The ingestion index was calculated as the weight of food consumed (mg) on the average weight of the individual (g) per day. Pressure had no effect on the weight of the offered food (Text S2, Electronic Supplementary Material). Wet weights of sea urchin

(*S. droebachiensis*) individuals before and after each trial were averaged to obtain the mean wet weight used to calculate the ingestion indices.

For sea star individuals, a single mussel *Mytilus edulis* (4–5 cm shell length) was offered to each individual in the experimental chamber, in line with previous diet studies (Rochette et al. 1994). To examine consumption without foraging effort, the mussels were opened and the tissue attached to the abductor muscles offered on one shell. All loose fragments were removed so only one intact piece of flesh was present (mean initial tissue weight: 6.0 ± 2.6 g). Following the completion of the experiments, the residual food was removed blotted and weighed to determine the amount of food ingested, as previously described. Preliminary experiments had been conducted to determine the percent weight variation generated by pressure on mussel flesh and a correction was applied to account for it when calculating the ingestion index (Text S2, Electronic Supplementary Material).

Upon depressurization and removal of sea star individuals from the vessel after the 24 and 72-h trials, each container was immediately observed to determine the position of the individuals relative to the food item and whether active feeding could be scored. These observations were only possible for the sea star because of their conspicuous feeding mechanism of everting their stomach. In total, four different positions were noted to describe feeding: (1) arms wrapped around the mussel shell and stomach everted; (2) wrapped around the mussel shell and no everted stomach; (3) arms not wrapped around the mussel shell and stomach everted; and (4) arms not wrapped around the mussel shell and no everted stomach.

For the 216-h trials (only performed with the sea star), ingestion indices of contained individuals were measured according to the techniques outlined above, except two mussels were provided (mean initial tissue weight: 14.6 ± 3.1 g). Further, the three uncontained individuals were offered a total of four live mussels; this time mussels were intact (with shell) so that qualitative observations could be made about foraging effort/behaviour. As these individuals were free inside the vessels, their feeding could be observed in vivo using an endoscope (described previously) twice every 24 h (10:00, 16:00) for a continuous 10-min period, starting from the initial time of pressurization (for a total of 20 min per day in each treatment).

Post-trial and post-recovery monitoring

All experimental individuals were examined post-trial, and following data collection (see metrics above), they were placed in long-term recovery under conditions matching their pre-trial conditions. The sea star was more formally tested after all experiments were completed (24, 72 and 216 h): individuals from the different treatments were

maintained in 20-L tanks under the pre-trial holding conditions. Exactly 168 h (7 days) post-trial, their righting response was measured following the methods described previously. Post-trial righting recovery periods have been found to be a useful means of assessing sea star physiological health (Appelhans et al. 2014). At the beginning of post-trial and post-recovery periods survival (%) was also recorded along with qualitative observations of abnormal body condition. The other two species were kept in recovery for the same duration, but only qualitatively monitored.

Health indices in sea cucumber

The following health indices for the sea cucumber were visually assessed as per previous studies (Gianasi et al. 2015; Verkaik et al. 2016): feeding, cloacal opening, escape response and anchorage. These were used as the anatomy and general behaviour of the sea cucumber differ markedly from the two other echinoderms studied here. Pre-trial anchorage time was determined for a maximum of 30 min after which individuals not anchored were not used for the trials (after exposure to 0.1 MPa healthy sea cucumber anchorage took ≤ 2 min). In post-trial measurements, individuals that had not anchored after this delay were scored as > 30 min. Once all of the individuals were successfully anchored, or following the maximum delay mentioned above, cloacal opening was measured for a period of 5 min. This measure is a proxy of respiration rate as it indicates the rate of water exchange in the respiratory tree (Doyle and McNeill 1964; Gianasi et al. 2015).

Time to tentacle deployment was monitored post-trial and was considered an indirect proxy of time to feed. Measurements were made while the water flow to the tanks was interrupted briefly to maintain food concentrations (Gianasi et al. 2015). The food consisted of 3 mL (100% concentration) of a commercial algal feed (Phytofeast-Feast® Live) comprising six phytoplankton species (*Pavlova* sp., *Isochrysis* sp., *Thalassiosira weissflogii*, *Tetraselmis* sp., *Nannochloropsis* sp., and *Synechococcus* sp.) with cell sizes ranging from 1 to 15 μm in diameter, which is well accepted by this sea cucumber according to Gianasi et al. (2015). Time required for each individual to open its tentacles and begin feeding was recorded. Individuals that had not started feeding after 60 min were scored as > 60 min (after exposure to 0.1 MPa, feeding response took ≤ 6 min).

Following the feeding experiment, individuals were transferred to a tank without suspended food with flow-through seawater before testing the predator response. One of its known predator, the sea star *S. endeca* (So et al. 2010), was placed directly on the sea cucumber. Time needed to initiate the escape response and its various stages, e.g. swelling, elongation and contraction, was monitored for a total of 15 min (Gianasi et al. 2015; So et al. 2010).

Statistical analysis

The comparison of means was carried out using Student's *t* tests, one-way or two-way analyses of variance (ANOVAs) after confirming homogeneity of variance. When statistically significant differences occurred, post hoc multiple comparisons tests were conducted using the Holm-Sidak method. Where equal variance was violated, a Mann-Whitney *U* test or a Kruskal-Wallis ANOVA on ranks, followed by Dunn's post hoc tests as appropriate, was used. Significance levels were considered at $P < 0.05$ and all analyses were carried out using Sigma Plot version 11.0 (Systat Software, USA).

Results

Sea urchin *Strongylocentrotus droebachiensis*

Following the 24-h trials, all individuals survived exposure to surface and mid-bathymetric range pressures ($100 \pm 0\%$, $n=4$ runs; and $100 \pm 0\%$, $n=2$ runs, respectively), whereas only $50 \pm 0\%$ ($n=2$ runs) of them survived exposure to high pressure beyond the known bathymetric distribution (Table 3). In survivors, spines were brittle and easily broken upon light handling. Deleterious effects on sea urchin behaviour occurred both during and after acute exposure. Feeding significantly decreased as pressure increased (Kruskal-Wallis test, $H_2=26.56$, $P < 0.001$) whereby feeding indices were lower at high than at either surface ($P=0.001$) or mid-bathymetric range pressure ($P < 0.001$), but did not vary significantly between the latter ($P=0.312$), which are within the known depth range of the species (Fig. 1a). Post-trial righting times were significantly affected by pressure (Fig. 1b; Kruskal-Wallis test, $H_2=7.80$, $P=0.020$) with shorter righting times at mid-bathymetric range pressure than at high pressure ($P=0.009$). There was no significant difference in times between exposure to surface and mid-bathymetric range pressures ($P=0.131$) or between surface and high pressure ($P=0.065$). Overall, pressure did not significantly affect body weight of surviving individuals; the difference in mean weight after exposure to high, mid-bathymetric range and surface pressures from the initial weights were reduced on average by: -0.02 ± 0.05 g ($n=2$ runs), -0.22 ± 0.35 g ($n=2$ runs) and -0.17 ± 0.38 g ($n=4$ runs), respectively (Kruskal-Wallis test, $H_2=1.80$, $P=0.406$).

No mortality occurred post-trial following the sustained 72-h pressure exposure. However, post-recovery survival for individuals exposed to high pressure was marginally lower ($83 \pm 29\%$, $n=3$ runs) than following exposure to surface pressure ($100 \pm 0\%$, $n=3$ runs) but in a non-significant manner. There was a significant interaction between pressure levels and time (pre-trial, post-trial, post-recovery) on time to right (Fig. 1c; ANOVA, $F_{2,35}=4.71$, $P=0.017$). Based

Table 3 Survival rates (%; post-trial and post-recovery) associated with the 24, 72 and 216-h pressure exposure trials

Species	Trial duration (h)	Pressure	Post-trial survival (%) ^a	Post-recovery survival (%) ^b
Sea urchin <i>Strongylocentrotus droebachiensis</i>	24	Surface (0.1)	100	NT
		Mid-bathymetric range (6)	100	NT
		High (24)	50	NT
	72	Surface (0.1)	100	100
		High (24)	100	83.3
Sea star <i>Leptasterias polaris</i>	24	Surface (0.1)	100	100
		Mid-bathymetric range (5)	100	100
		High (22)	92	83.3
	72	Surface (0.1)	100	100
		High (22)	100	100
216		100	86	
		51.7	0	
Sea cucumber <i>Cucumaria frondosa</i>	24	Surface (0.1)	100	100
		Mid-bathymetric range (6.5)	100	100
		High (26)	100	100
	72	Surface (0.1)	100	100
		High (26)	100	83.3

Variables that were not tested are indicated with NT

^aSurvival was measured post-trial (immediately after removal from experimental vessel)

^bSurvival was measured post-recovery (7 days after removal from experimental vessel)

on independent analyses, righting post-trial took significantly longer after exposure to high than to surface pressure (Paired *t* test, $t = -3.86$, $df = 10$, $P = 0.003$); after high-pressure exposure, righting was longer post-trial than pre-trial (Mann–Whitney *U* test, $U = 2.00$, $df = 10$, $P = 0.009$) whereas there was no significant difference with post-recovery values (Paired *t* test, $t = 2.21$, $df = 2$, $P = 0.052$), indicating weak if not absent recovery after a week.

The results of a two-way ANOVA on the effect of pressure levels (surface, high) and trial duration (24, 72 h) on the post-trial time to right showed significantly slower responses after exposure to high than surface pressure (ANOVA, $F_{1,47} = 8.40$, $P = 0.006$); but exposure duration had no effect (ANOVA, $F_{1,47} = 3.36$, $P = 0.074$). See Electronic Supplementary Material Table S2 for a summary of all results.

Sea star *Leptasterias polaris*

All sea star individuals survived the acute 24-h exposure to surface or mid-bathymetric range pressures. Post-trial survival to high pressure for 24 h was $92 \pm 12\%$ ($n = 2$ runs), and among those, $50 \pm 23\%$ were unable to right and displayed uncoordinated tube feet movements. After a week, there was mortality among the individuals exposed to the high-pressure treatment (Table 3). Pressure level of acute exposure had a significant effect on weight of sea star (ANOVA, $H_2 = 11.94$, $P = 0.003$), whereby weight loss was greater at high pressure than either mid-bathymetric range ($P = 0.018$) or surface pressure ($P < 0.001$); but not between the latter ($P = 0.880$). Mean difference in weights

of surviving individuals following pressure exposures were reduced from initial weights by: -4.32 ± 0.05 g ($n = 2$ runs), -1.09 ± 1.12 g ($n = 2$ runs) and -0.24 ± 1.0 g ($n = 4$ runs), respectively. By contrast, feeding was not significantly affected by pressure over 24 h (Fig. 2a; ANOVA, $F_{1,45} = 0.40$, $P = 0.669$). Immediately after the removal the majority of individuals exposed to surface pressure (58%, $n = 2$ runs) exhibited active feeding behaviour, their arms were wrapped around the mussel and they had everted stomachs. This was greater than the proportion of individuals exposed to mid-bathymetric range and high pressures (Table 4; 42 and 25%, respectively; $n = 2$ runs). Inversely, individuals that did not demonstrate active feeding behaviour (arms not around the mussel or no everted stomachs) were mostly observed after exposure to mid-bathymetric range and high pressures (50 and 33%, respectively; $n = 2$ runs) as opposed to individuals exposed to surface pressure (25%, $n = 2$ runs). The 24-h exposure to pressure significantly affected the righting times (ANOVA, $H_2 = 22.98$, $P < 0.001$), which were slower after high than mid-bathymetric range ($P = 0.001$) or surface pressure exposures ($P < 0.001$), but responses did not vary between the latter (Fig. 2b; $P = 0.502$). Post-recovery righting time was not significantly different across pressure treatments (Fig. 2c; Kruskal–Wallis test, $H_2 = 1.09$, $P = 0.579$), and individuals consistently took less time to right post-recovery than post-trial ($H_1 = 31.91$, $P < 0.001$), indicative of improved condition after a week.

All individuals exposed to sustained high and surface pressure for 72 h survived, both immediately post-trial and also post-recovery (Table 3). However, the motor functions

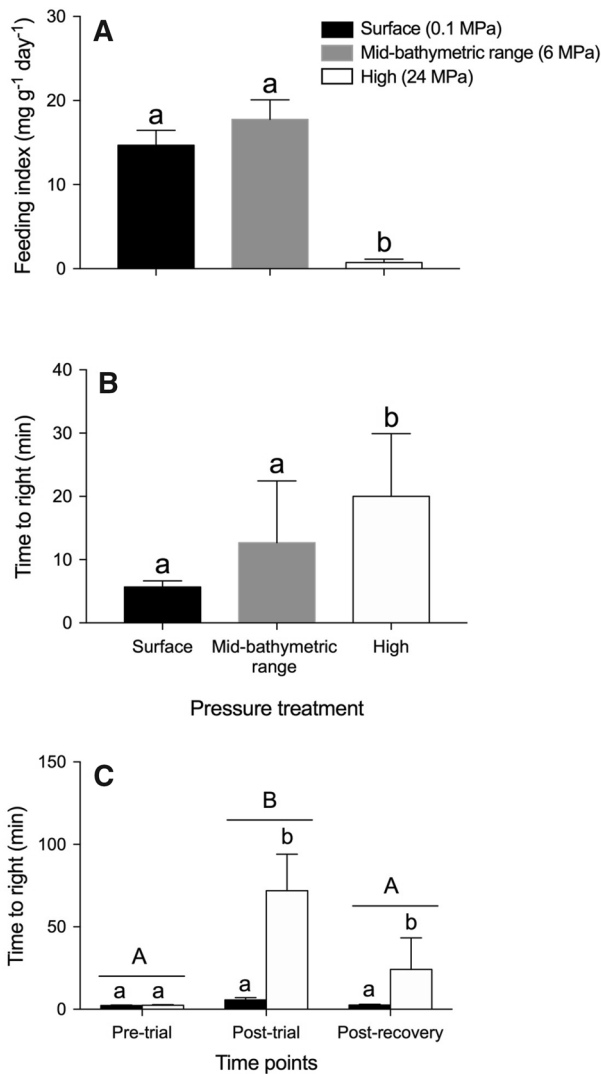


Fig. 1 Responses of the sea urchin *Strongylocentrotus droebachiensis* to pressures within and beyond their natural bathymetric distribution. **a** Feeding index (mean ± SE, n = 12–24) during 24-h exposure. **b** Time to right (mean ± SE, n = 12–24) immediately after the 24-h trial. **c** Comparison of time to right (mean ± SE, n = 6) pre-trial, post-trial or post-recovery for 72-h exposure. Means with different letters are significantly different (Kruskal-Wallis test, P < 0.05). Lower case letters (a, b) correspond to results within treatments and capital letters (A, B) to results between treatments

were negatively impacted (Fig. 3a, b). There was a significant interaction between the effects of time and pressure on righting times (Fig. 2c; ANOVA, $F_{2,35} = 5.73$, $P = 0.008$), which led to independent analyses within each factor. Based on the latter, it took longer to right after exposure to high than surface pressure post-trial (paired *t* test, $t = 6.20$, $df = 10$, $P < 0.001$) but no difference between pressure levels occurred for either pre-trial ($t = -0.39$, $df = 10$, $P = 0.700$) or post-recovery (Fig. 2b, c; $t = 0.71$, $df = 10$, $P = 0.493$). There was no effect of pressure level on the righting time in post-recovery individuals (Mann–Whitney *U* test, $U = 6.00$,

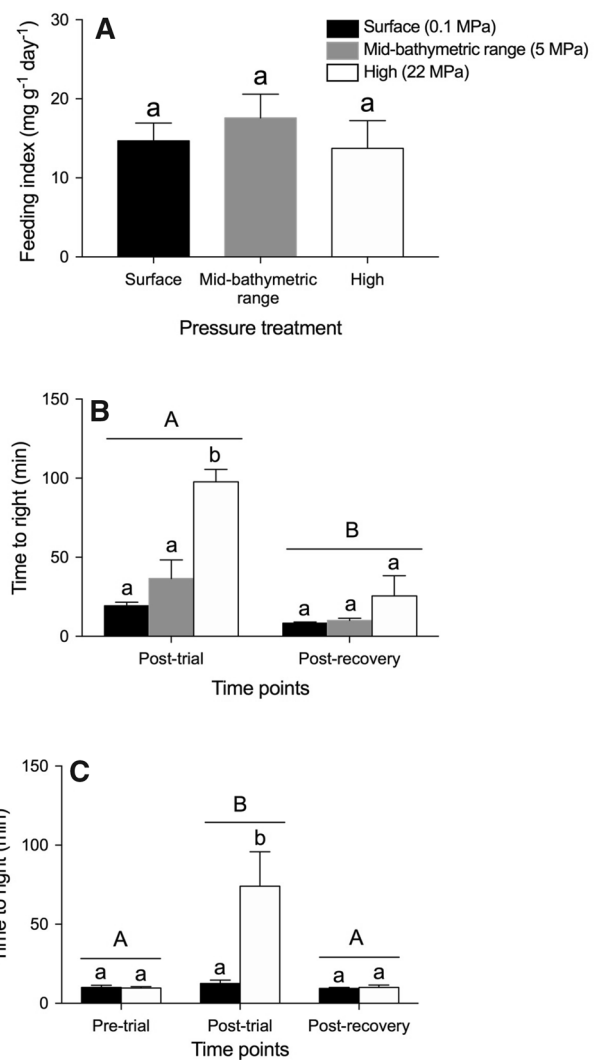


Fig. 2 Responses of the sea star *Leptasterias polaris* to pressures within and beyond their natural bathymetric distribution. **a** Feeding indices (mean ± SE, n = 12–24) during 24-h exposure. **b** Time (mean ± SE, n = 12–24) required to right immediately after the 24-h trial. **c** Time (mean ± SE, n = 6) required to right pre-trial, post-trial or post-recovery for 72-h exposure to pressure. Means with different letters are significantly different (Kruskal-Wallis test, P < 0.05). Lower case letters (a, b) correspond to results within treatments and capital letters (A, B) to results between treatments

$df = 10$, $P = 0.065$). In two-way ANOVAs examining pressure levels (surface, high) and exposure duration (24, 72 h), post-trial righting times were affected by pressure ($F_{1,47} = 6.26$, $P = 0.016$) but not time ($F_{1,47} = 1.69$, $P = 0.201$). Individuals took longer to right after exposure to high pressure than to surface pressure for both 24-h ($P < 0.001$) and 72-h experiments ($P < 0.018$).

Following sustained 216-h pressure exposure, all individuals exposed to surface pressure survived, compared to only 57% of those exposed to high pressure. Following 7-day recovery, all individuals exposed to surface

Table 4 Proportion of sea star *Leptasterias polaris* individuals found in the various feeding positions after exposure to three pressure levels

Pressure	Proportion of individuals (%)			
	Arms around mussel; stomach everted	Arms around mussel; no stomach everted	Arms not around mussel; stomach everted	Arms not around mussel; no stomach everted
Surface (0.1)	58.3	0	16.7	25.0
Mid-bathymetric range (5)	41.7	8.3	0	50.0
High (22)	8.3	25.0	33.3	33.3

Pressure did not affect the final feeding positions of sea stars after 24-h ($H_2 = 2.47$, $P = 0.291$)

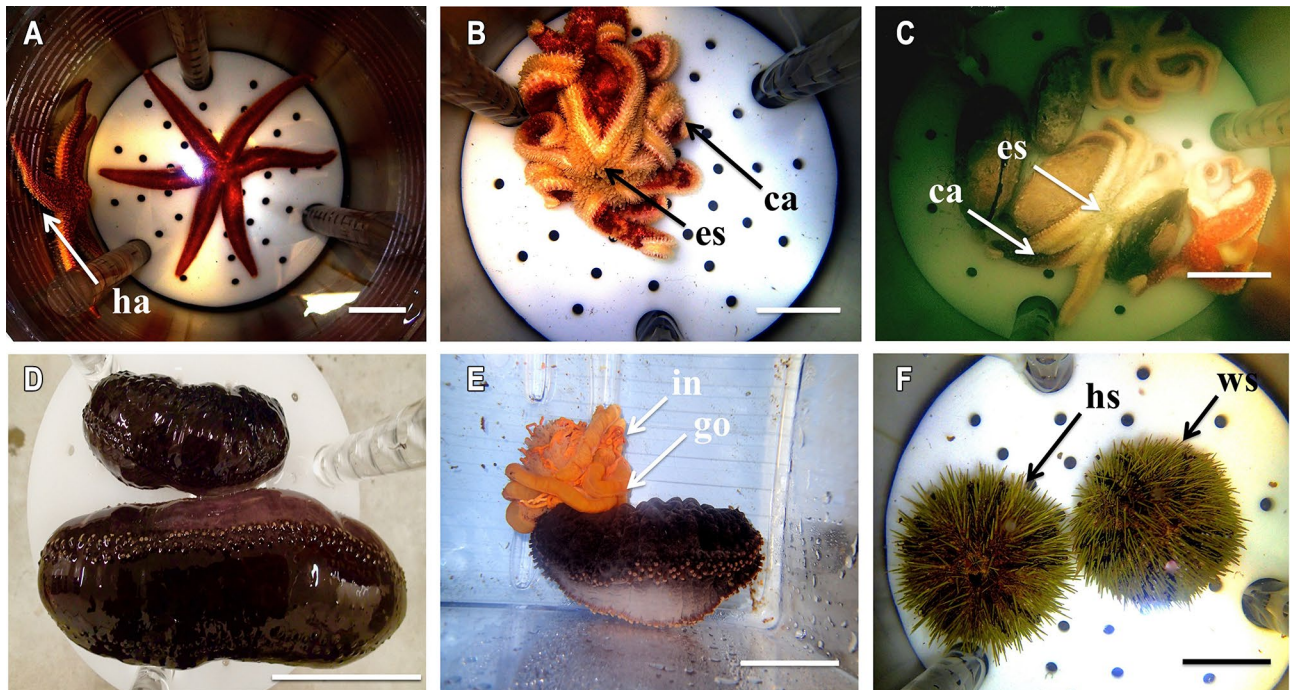


Fig. 3 Post-trial condition of individuals immediately after pressure exposure for 72 and 216 h. **a** Sea stars *Leptasterias polaris*; after exposure to surface pressure (0.1 MPa) for 72 h, individuals were climbing and had healthy arms (ha) indicative of good health. **b** Sea stars; after exposure to high pressure (25 MPa) for 72 h individuals all had convoluted arms (ca) and everted stomachs (es). **c** Sea stars after exposure to high pressure (22 MPa) for 216 h; individuals all had convoluted arms (ca) and everted stomachs (es). **d** Healthy sea cucumbers *Cucumaria frondosa* after exposure to surface pressure

(0 MPa). **e** Sea cucumber; after exposure to high pressure (25 MPa) only one individual was found eviscerated upon removal from the IPOCAMP; gonads (go) and intestines (in) are visible. **f** Sea urchins *Strongylocentrotus droebachiensis*; individual on the left was exposed to 0.1 MPa and had healthy spines (hs); individual on the right was exposed to high pressure (25 MPa) and had weak spines (ws); note that these individuals were tested in different pressure vessels. Scale bars on all photos represent 4 cm

pressure were still alive, and only one individual did not right (of seven individuals), whereas all individuals that had been exposed to high pressure were dead. None of the individuals exposed to high pressure for 216 h were able to successfully right immediately post-trial. Feeding was not significantly affected by pressure during the 216-h exposure (see Electronic Supplementary Material, Table S3; ANOVA, $F_{1,43} = 2.63$, $P = 0.113$). All individuals displayed some level of activity over the first 3 days

but, by day 6, all of them were on the bottom, scattered among the mussels, and no further movement was detected for the remainder of the experiment (Fig. 3c). Post-trial, the arms and central disks of both the free-moving and contained individuals from the high-pressure treatments were softened to the point of fragmentation upon handling. In several instances, arms were completely detached. See Electronic Supplementary Material Table S2 for a summary of all results.

Sea cucumber *Cucumaria frondosa*

All sea cucumber individuals survived the acute 24-h trials and recovery period, irrespective of pressure level, but post-trial feeding response was significantly affected by pressure level (Fig. 4a; Tables 3, 4; Kruskal–Wallis test, $H_2=9.96$, $P=0.007$). Specifically, tentacle extension was delayed after exposure to mid-bathymetric range compared to surface pressure ($P=0.002$) as it was when comparing high and surface pressure ($P=0.015$); there was no difference between mid-bathymetric range and high pressure ($P=0.937$).

Following the sustained 72-h trials, there also was $100 \pm 0\%$ ($n=3$ runs) immediate survival (Fig. 3d), but post-recovery survival was down to $83 \pm 29\%$ ($n=3$ runs) for individuals exposed to high pressure (Table 4). Only one individual died from high-pressure exposure (through the evisceration of the gonads; Fig. 3e); this did not occur with any other individuals in the study. Pressure level did not have any significant effect on the feeding response after 72-h exposure (Fig. 5a; Mann–Whitney U test, $U=12.00$, $df=2$, $P=0.394$). When examining the combined effect of trial duration (24, 72 h) and pressure level (surface, high) on feeding response, there was no effect of the former (ANOVA, $F_{1,23}=1.91$, $P=0.182$) but a significant influence of the latter ($F_{1,23}=9.97$, $P=0.005$). After 24-h exposure, the feeding response was faster for individuals exposed to surface than high pressure ($P=0.002$), but there was no difference after 72-h exposure ($P=0.385$).

Since there was no difference in feeding between either of the tested times, there is no direct evidence for acclimation to pressure.

The frequency of cloacal movement (cloacal respiration) following 24-h trials was significantly affected by pressure level (Fig. 4b; ANOVA, $F_{2,53}=7.15$, $P=0.002$) and time (pre-trial, post-trial, post-recovery; $F_{2,53}=15.03$, $P<0.001$), with a significant interaction between these factors ($F_{2,53}=7.05$, $P\leq 0.001$). In independent analyses, cloacal movement post-trial was higher after exposure to surface than high pressure (Paired t test, $t=8.17$, $df=10$, $P\leq 0.001$) and for individuals exposed to mid-bathymetric range than high pressure (Mann–Whitney U test, $U=5.50$, $df=10$, $P=0.041$) but not between surface and mid-bathymetric range pressure ($U=13.00$, $df=10$, $P=0.466$). Time relative to high-pressure exposure had a significant effect on cloacal movement; there was significantly less movement post-trial than both pre-trial (paired t test, $t=8.97$, $df=10$, $P=0.017$) and post-recovery ($t=6.82$, $df=10$, $P=0.025$); but no difference between pre-trial and post-trial ($t=2.15$, $df=10$, $P=0.05$). Findings from the 72-h trials were similar to those from 24-h trials. Cloacal movement was influenced by pressure level (ANOVA, $F_{1,35}=4.60$, $P=0.040$) and time ($F_{2,53}=6.08$, $P=0.006$), but with an interaction between the two (Fig. 5b; $F_{2,53}=4.21$, $P=0.025$). Independent analyses showed that cloacal movement was more frequent after exposure to surface than to high pressure but only post-trial (paired t test, $t=5.41$, $df=10$, $P<0.001$); values did not differ between pre-trial pressure levels (paired t test, $t=-1.57$,

Fig. 4 Behaviours of the sea cucumber *Cucumaria frondosa* measured either at pre-trial, post-trial or post-recovery following 24-h exposure to pressures within and beyond their natural distribution (mean \pm SE, $n=6$). **a** Time to initiate feeding. **b** Cloacal movements in a 5-min interval. **c** Time to initiate escape from predator. **d** Time to anchor firmly to substrate. Means with different letters are significantly different (Kruskal–Wallis test, $P<0.05$). Lower case letters (a, b) correspond to results within treatments and capital letters (A, B) to results between time treatments

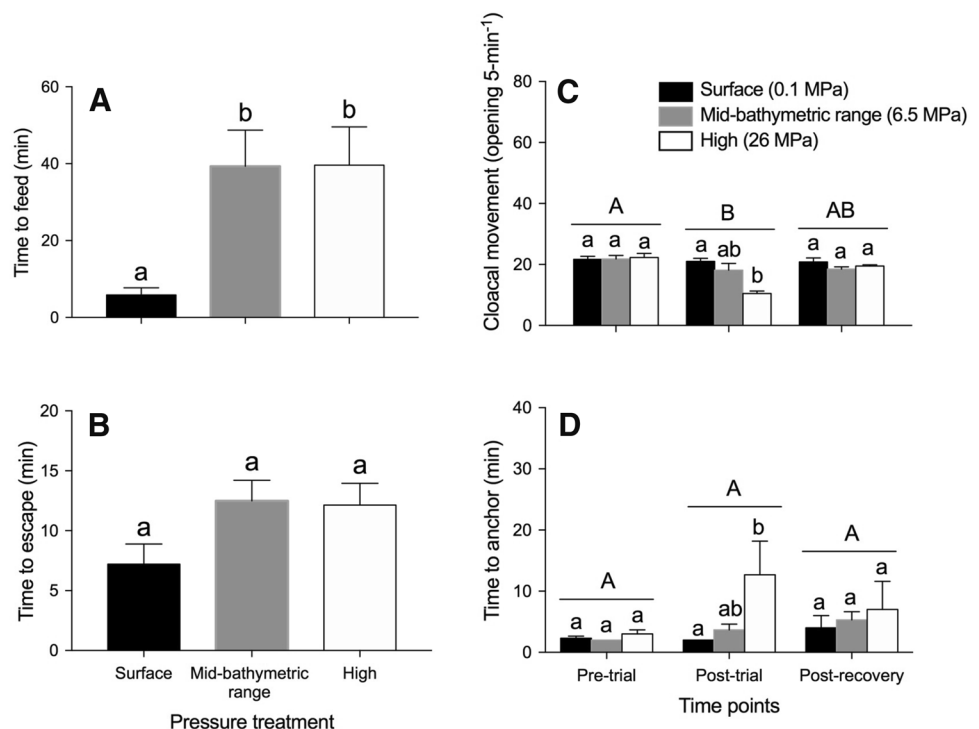
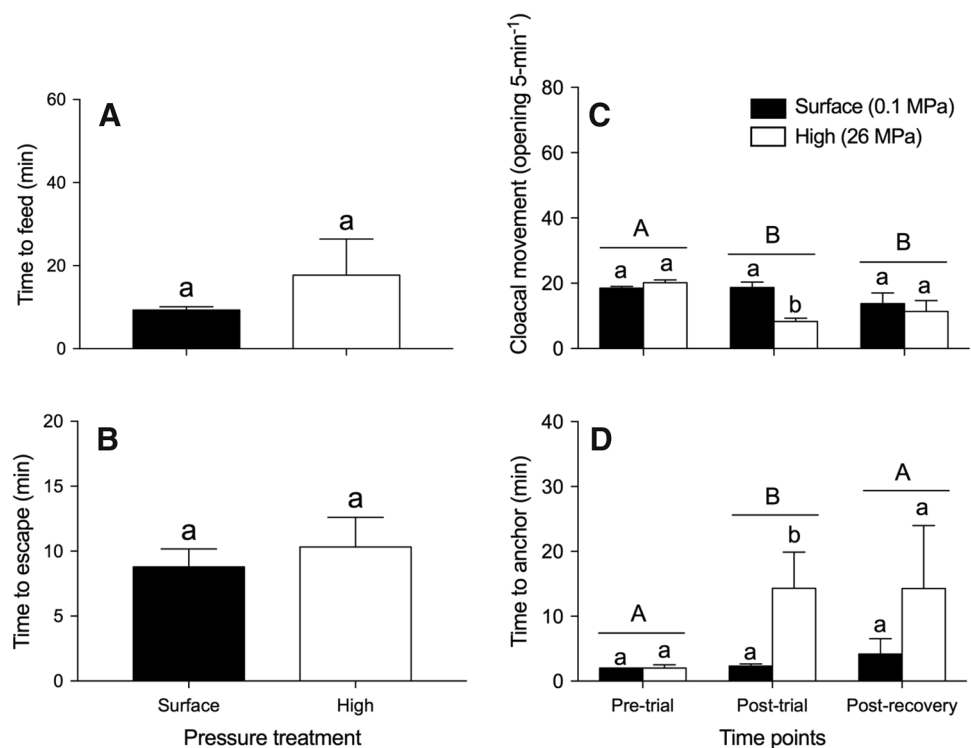


Fig. 5 Behaviours of the sea cucumber *Cucumaria frondosa* measured either at pre-trial, post-trial or post-recovery following 72-h exposure to surface pressure and high pressure, i.e. twice the known maximum depth of occurrence (mean \pm SE, $n=6$). **a** Time to initiate feeding. **b** Cloacal movements in a 5-min interval. **c** Time to initiate escape from predator. **d** Time to anchor firmly to substrate. Means with different letters are significantly different (Kruskal–Wallis test, $P<0.05$). Lower case letters (a, b) correspond to results within treatments and capital letters (A, B) to results between time treatment



$df=10$, $P=0.147$) or post-recovery (Mann–Whitney U test, $U=13.00$, $df=10$, $P=0.485$), indicating a dissipation of high pressure impacts on cloacal respiration after a week. For high-pressure trials, there was more frequent cloacal movement pre-trial than either post-trial (paired t test, $t=-7.94$, $df=10$, $P<0.001$) or post-recovery (Mann–Whitney U test, $U=2.00$, $df=10$, $P=0.009$); but no difference between post-trial and post-recovery (Fig. 5b; Mann–Whitney U test, $U=12.00$, $df=10$, $P=0.394$), indicating a persistence of the effects of high pressure after a week. A two-way ANOVA showed that pressure level had a significant effect on cloacal movement ($F_{1,24}=79.32$, $P<0.001$) but trial duration did not (24 vs. 72 h; $F_{1,24}=3.70$, $P=0.069$). Cloacal movement was less frequent for individuals exposed to high than to surface pressure after both 24 h (paired t test, $t=6.35$, $df=1$, $P<0.001$) and 72 h ($t=6.25$, $df=1$, $P<0.001$).

Pressure did not have any significant effect on the predator-escape response following the 24-h (Fig. 4c; ANOVA, $F_{2,17}=2.89$, $P=0.087$) or 72-h (Fig. 5c; Kruskal–Wallis test, $H_1=0.92$, $P=0.394$) trials. A two-way ANOVA confirmed that trial duration (24, 72 h) and pressure (surface, high) did not have any effect on the time to escape predator ($F_{1,23}=0.003$, $P=0.952$; $F_{1,23}=3.150$, $P=0.091$, respectively). By contrast, pressure level had a significant effect on the time needed to anchor to the substrate in the 24-h trial (ANOVA, $F_{2,23}=3.94$, $P=0.027$) but there was no effect of time (Fig. 4d; pre-trial, post-trial, post-recovery; $F_{2,23}=2.54$, $P=0.090$). Anchoring times were only

significantly slower following exposure to high vs. surface pressure (Kruskal–Wallis test, $H_1=7.44$, $P=0.004$). Following the 72-h exposure, anchorage time was also significantly influenced by pressure level (ANOVA, $F_{1,35}=4.55$, $P=0.041$) but not time ($F_{2,35}=1.96$, $P=0.159$); and there was no interaction between the two (Fig. 5d; $F_{2,35}=1.64$, $P=0.210$). Individuals took longer to anchor following exposure to high than to surface pressure, but immediately post-trial only ($P=0.002$). A two-way ANOVA showed that anchoring time was significantly affected by pressure (surface, high) ($F_{1,23}=8.40$, $P=0.009$) but not trial duration (24, 72 h) ($F_{1,23}=0.065$, $P=0.801$). Individuals were slower to attach to the substrate after exposure to high than to surface pressure for 72 h ($P=0.042$) but not 24 h ($P=0.068$). Generally, individuals took longer to anchor, both post-trial and post-recovery, following the 72 than 24-h exposure to high pressure, suggesting an inability to acclimate. See Electronic Supplementary Material Table S2 for a summary of all results.

Discussion

The results of the present study support the main hypothesis that behaviour in adults of three echinoderms species is more strongly impacted by exposure to pressures beyond than within their natural bathymetric ranges. Despite having been collected at shallow depths (10–15 m), individuals showed relative tolerance to a greater than fivefold

increase in pressure, corresponding to the middle of their known depth distributions (which ranged from 500 to 650 m in the three focal species). Generally, survival was lower post-recovery than immediately post-trial, suggesting that non-lethal physiological damage sometimes led to post-traumatic complications. The lowest survival among the tested species for trial duration ≤ 72 h was with the sea urchin, *S. droebachiensis*, suggesting that calcification or other traits related to body rigidity may drive the contrasting species-specific pressure tolerances. For trials ≤ 72 h, mortalities were only elicited by exposure to the highest pressure. There was no consistent weakening of the negative responses after longer exposures to support the hypothesis of rapid acclimation in the three species under study. Instead, all of these species exhibited variable responses to longer exposures (e.g. 24 h vs 72/216 h). The results suggest that the focal species would not likely be able to sustain long-term displacements beyond their current bathymetric range of occurrence. Overall, when they were not lethal, the highest hydrostatic pressures (representing twice their known depth of occurrence; 2200–2600 m) reduced their ability to feed, move, right and/or anchor, which is likely to impact their health and reproduction (Sokolova 2013).

The echinoderm species studied here all display a broad bathymetric profile (e.g. they are eurybathic) and can occur in environments characterized by vertical walls or steep drops, high turbulence, as well as high densities of predators. Therefore, the possibility exists for them to topple down slopes or cliff walls during storm events or while escaping predation, in a manner documented in the black turban snails (*Tegula funebris*) after encounter with a predator (Feder 1963, 1972). Based on the present study, abrupt relocation (whether active or passive) to deeper habitats beyond their current range would not allow adult echinoderms to readily resume feeding. For instance, feeding activity in the sea urchin (*S. droebachiensis*) and feeding response in the sea cucumber (*C. frondosa*) were slower and less defined during or following exposure to the highest pressure, respectively. Similarly, Thatje and Robinson (2011) found that when the shallow-water crab *Maja brachydactyla* was exposed to pressure atypical of its depth distribution, it did not feed. In contrast, limited vertical movement appears to have minimal effects on feeding. During pressure exposure that was characteristic of mid-depth distributions of a given species, sea urchin and sea star (*L. polaris*) individuals were seen to consume more food than at surface pressure. Pressure (inside the tolerance limit of a species) has been shown to induce metabolic demands and cause temporary increase in basal activity of many marine species (Schlieper 1968), thus, a benign increase in pressure may create a demand for nutrients to cope with the stress. Similarly, when exposed to slightly increased pressures within natural distributions (< 6 MPa) the shallow-water crab showed increased feeding

(Thatje and Robinson 2011). Notably, Brown et al. (2017) found similar results, whereby exposure to high pressure elicited sustained increases in metabolic rates in the crab *Lithodes maja*. Increases in respiration rates were noted when individuals were exposed to pressures slightly above those typical of their deepest distributions (7.5 vs 12.5 MPa) but the rates steeply declined when pressures representative of twice the natural distribution (20.0 MPa) were applied, presumably due to metabolic stress (Brown et al. 2017). High metabolic rates at > 1000 m have been reported for *C. frondosa*, which was assumed to reflect proximity to the species bathymetric range limit (Brown et al. 2018).

In the present study, post-trial motor functions of individuals with more rigid bodies were severely compromised with increasing pressure, regardless of exposure duration. After exposure to high pressure, individuals lacked coordination and their tube feet appeared more fragile (e.g. readily breaking off in sea urchin and sea star individuals). Since the sea urchin has a calcareous skeleton (test) it may have been limited in its ability to deform in response to the forces of compression and thus may be susceptible to physical damage, as evidenced by the easy breakage of spines after exposure to high pressure (Fig. 3f). Similarly, sea star individuals were not able to successfully attach to the substrate after high-pressure trials and their attempts to right were impeded by uncoordinated arm movements. These responses may be the result of internal damage caused by pressure, in line with the finding that chondrocyte-like cell lines exposed to elevated pressures of 10–50 MPa for short periods of time (< 24 h) displayed damage to cell cytoskeletons and dissociation of protein structures (Balny et al. 1997; Swezey and Somero 1985; Takahashi et al. 1998). These cellular and molecular changes have been observed in adult crustaceans manifesting convulsions, spasms and/or paralysis (e.g. Cottin et al. 2012; Macdonald and Gilchrist 1978; Oliphant et al. 2011; Wilcock et al. 1978). The atypical behaviours recorded in the present study are consistent with evidence that links spasms from high-pressure exposure to HPNS in other invertebrate phyla (e.g. Macdonald 1972; Oliphant et al. 2011; Thatje and Robinson 2011). Similarly in Brown et al. (2017), lithodid crabs that had been exposed to pressures atypical of their bathymetric range for 216 h were “quiescent for several days following treatment before resuming normal activity”. This observation suggests that individuals faced metabolic costs associated to high-pressure conditions and would unlikely be able to adapt over the long term.

Here, the effects of prolonged exposure to elevated pressure confirmed that, similar to results of the 24-h trials, the more calcified (hard-bodied) echinoderm species (sea urchin, sea star) were unable to acclimate to pressurized conditions over longer time period compared to the soft-bodied sea cucumber. In the case of motor responses in the sea urchin, individuals took three times longer to fully right after 72-h

trials then after 24-h trials, emphasizing the building effects of pressure exposure. Sea urchin individuals exposed to high pressure retracted their tube feet, which reportedly increases the internal pressure of the coelomic space through shifts in the hydrovascular system (Ellers and Telford 1992). The increased coelomic pressure may negatively affect the ability for sea urchin individuals to deploy tube feet or cause severe tube feet damage resulting in immobility (Ellers and Telford 1992). Although coelomic pressure was not evaluated in the present study, it is likely that sea urchin individuals under high pressure experienced increased internal coelomic pressure as the tube feet were noted to weaken and lack coordination post-trial. Given that sea urchin individuals were not able to maintain motor functions, it is unlikely that they would survive at this pressure for longer periods of time. Impaired mobility in a natural setting would disrupt foraging and predator evasion, possibly resulting in low survival. In the sea star, increased exposure time to high pressure did not necessarily intensify the negative effects of pressure on the time to right, even though righting took longer after high-pressure than surface treatments. It is possible that longer exposure to pressure could result in temporary tolerance to these conditions but not complete acclimation, as revealed by the 216-h experiments (see below). Our results, therefore, indicate that adults of the sea star exhibit complex responses to pressure. While no previous data on adults exist, pressure tolerance of larvae (measured as survival) was found to be inversely related to exposure duration in the common sea star (*Asterias rubens*) and the spiny sea star (*Marthasterias glacialis*) exposed to 20 MPa (~2000 m) (Villalobos et al. 2006). The greater survival, and weaker damages and changes in behaviour recorded in the sea cucumber, exposed to pressures within and beyond its known depth range, may be due to its poorly calcified, flexible and naturally deformable body (Lawrence 1987). The present results are in line with the fact that sea cucumbers are among the most commonly found benthic marine invertebrates across oceanic depths, down to the abyssal trenches (e.g. Amaro et al. 2009; Bett et al. 2001; MacDonald et al. 2010).

Interestingly, feeding, motor functions and other health proxies were not permanently disrupted by exposure to high pressure in most of the species and treatments. Although sea star individuals were more sluggish after exposure to high pressure relative to surface pressure for the two exposure durations, righting times were back to pre-trial values after the week-long post-trial recovery at surface pressure. By contrast, after 72-h pressure exposure, sea urchin individuals were the most severely compromised; post-recovery righting times improved from post-trial times but were still longer than the pre-trial times. Post-recovery motor tests of the sea star showed that individuals could recover from the deleterious effects of high pressure but this was not as clear for the sea urchin. In Asteroidea, muscle cells receive

sensory information about whole-body movement because of internal pressure changes in the coelomic cavity (Gardiner and Rieger 1980). Environmental hydrostatic pressure can distort such messages and inhibit activity (Gardiner and Rieger 1980). Thus, sea star individuals may have regained function of their muscle cells and their mobility under surface pressure. In the third species, the sea cucumber, not all health proxies were as severely impacted as in the other two, although anchorage and cloacal respiration were both negatively affected by high pressure, irrespective of the exposure duration. Similarly, a study of their response to low pH revealed that they were impacted in a non-lethal and reversible manner (Verkaik et al. 2016). While the 24 and 216-h treatments tested species independently, the 72-h treatments tested the three species together, but separated by the tiered levels of the vessel to prevent direct interactions. Given that the species co-occur and do not pose predatory threats to each other, behaviours are unlikely to have been altered.

While the sea star might acclimate to high pressure exposures to some degree, based on results of the 72-h exposure and on post-recovery trials, the result of the 216-h exposures to high pressure revealed that individuals were unable to right immediately post-trial, and that many individuals displayed softened tissues, peeling epidermis, as well as autotomy (arm loss), which is commonly associated with stress (Wessel et al. 2014) caused by interactions with fishing gear (Ramsay et al. 2001), predators (Ramsay et al. 2000) and parasites (Stasek 1967). Although autotomy can be a useful survival strategy, arm loss can impede motor ability such as righting and/or capture and ingestion of prey items (Ramsay et al. 2001), potentially affecting long-term survival. Furthermore, all sea star individuals were moribund/dead 1 week after this long-term trial. Internal damage was presumably extensive given that sea stars lack any defined skeleton (rather possessing hundreds of minute calcareous ossicles), so their ability to maintain internal rigidity/stability is minimal (Cavey and Märkel 1994; LeClair 1993). This suggests that despite weak plasticity was noticed when exposed to pressure over short periods of time, exposure to pressure for several days compromised their survival, suggesting their poor ability, at least at the scale tested here, to acclimate/acclimatize to deeper habitats.

Contrary to the low tolerance for high-pressure over prolonged exposure seen in the other species, the sea cucumber exhibited variable responses across behaviours, most of which did not fully support the acclimation hypothesis. Sea cucumber individuals took less time to initiate feeding and escape from predators after the 72 than 24-h exposure, indicative of acclimation to pressure. Elevated pressure severely decreased cloacal movement regardless of exposure duration, yet the potential for recovery differed based on duration of exposure. All individuals exposed for 24 h had returned to basal cloacal respiration levels after a week

of recovery, whereas those exposed for 72 h still had slower rates of cloacal opening post-recovery. Typically, cloacal respiration increases with stress in this sea cucumber species (Gianasi et al. 2015) so the reduction of water circulation may indicate internal damage to the respiratory tree, where oxygenation occurs (Doyle and McNiell 1964; Gianasi et al. 2015) or a weakening of the cloacal muscles. Internal pressure increases with body wall contractions, required for movement, and external pressure from the environment; if the pressure reaches the maximum threshold then the opening of the cloaca can result in the rupture of the cloacal wall (VandenSpiegel and Jangoux 1987). Similarly, time to anchor post-trial was significantly longer than pre-trial and post-recovery following the high-pressure exposure for 72 h but not 24 h. Post-recovery anchoring delays were still relatively longer than pre-trial values, demonstrating an intensification of the effects of pressure on tube feet activity over sustained exposures. Despite sea cucumbers are observed at all depths in the deep sea, the focal species, *C. frondosa*, is not often recorded below 300 m in the wild, which could explain why specimens exposed to higher pressure showed deviant behaviour. Interestingly, Ross et al. (2013) showed that individuals of *C. frondosa* found at depths > 850 m showed sign of impaired reproduction.

Overall our findings revealed that pressure has a complex effect on the behavioural responses of different species of echinoderms. This study provides insight into the responses of three species of echinoderms within and beyond their bathymetric distributions and hints at possible class-specific responses, which would require additional research using more representatives to be confirmed. Since echinoderms can be long-lived (Ebert and Southon 2003), it can be predicted that the cold-water species studied here will experience significant climate-driven changes within their lifetime. Furthermore, as shallow-water areas around the continental shelf are undergoing more rapid changes (increasing temperature and acidity), vertical movements of subtidal communities to more stable deeper water regions can be expected (Brown and Thatje 2014, 2015). Therefore, it is important to assess the ability of various shallow-water species to colonize and thrive in deeper waters characterized by high pressure. Given the growing threat of ocean acidification, it would be advisable (but not necessarily easily achievable) for future investigations to test exposure to combined stressors for longer durations to determine whether the trends intensify over time.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest

Ethical standards All necessary international, national and institutional protocols for the care and use of animals were followed.

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