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Evaluating the acclimation capacity of two keystone Antarctic echinoderms to coastal freshening

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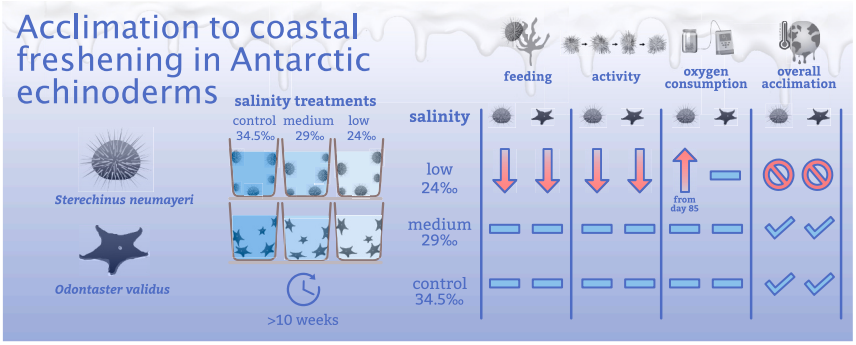
HIGHLIGHTS

- Antarctic echinoderms can successfully acclimate to a reduced salinity of 29 ‰.
- Long-term survival at 24 ‰ is unlikely, risking Antarctic food-webs with freshening.
- Significant mass loss observed in animals exposed to low salinity (24 ‰).
- Low metabolic rates may enhance Antarctic echinoderms tolerance to freshening.

GRAPHICAL ABSTRACT



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ABSTRACT

Coastal freshening in the Southern Ocean is expected to increase under projected climate scenarios. As a major environmental stressor, prolonged reduced salinity could pose a significant challenge to Antarctica's endemic echinoderms. Acclimatising to low salinity may be crucial for their continued survival as climate change accelerates, yet little is currently known about their capacity to do so. The sea star *Odontaster validus* and sea urchin *Sterechinus neumayeri,* two of the most ecologically important and abundant echinoderms of the shallow Antarctic seas, were exposed to reduced salinities (29 ‰ and 24 ‰) for at least 71 days after a stepwise dilution from 34.5 ‰. Feeding, faecal production (*S. neumayeri* only) and activity coefficient were significantly impacted at 24 ‰ and did not recover to control levels in either species. Oxygen consumption remained similar to control levels (34.5 ‰) across both treatments and species until day 85, when a significant increase was observed in *S. neumayeri* at 24 ‰. Coelomic fluid osmolality was near isosmotic with external salinities in both species, while coelomocyte composition and concentration were unaffected by reduced salinities (*S. neumayeri* only). Both species demonstrated the capacity to tolerate lower salinities that may be expected with climate change, with successful acclimation demonstrated at 29 ‰. Although survival rates were high at 24 ‰, significant reductions in mass and the failure of metrics to return to control levels suggest that long-term survival at 24 ‰ is unlikely, potentially impacting Antarctic food-web dynamics and ecological interactions.

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1. Introduction

Accelerated warming in Antarctica is driving an increase in the rate of coastal freshening (the lowering of seawater salinity) due to melting of sea ice, glaciers and ice-shelves, reduced sea ice production and increased precipitation (Jacobs et al., 2002; Hellmer et al., 2011; Haumann et al., 2016; Swart et al., 2018). In particular, the Western Antarctic Peninsula (WAP) has already experienced the highest rate of warming of any region globally (Vaughan et al., 2003; González-Herrero et al., 2022), which has contributed to reductions in coastal salinity (Hellmer et al., 2011; Haumann et al., 2016). Varying salinity gradients can be a major environmental stressor for marine invertebrates (Kinne, 1964; Freire et al., 2011b), due to the metabolic costs associated with adjusting cellular level compositions in order to balance osmotic gradients (Sokolova et al., 2012b; Rivera-Ingraham and Lignot, 2017) among other factors. For this reason salinity plays a crucial role in determining the physiology, distribution and survival of marine invertebrates (Kinne, 1964). The fjordic coastline of the WAP is currently rich in biodiversity and benthic abundance (Grange and Smith, 2013). However, predictions from Arctic fjords (which are considered to be at a later stage of climate warming than Antarctic fjords), suggest that coastal freshening could lead to distinct salinity stratification in the water column (Bianchi et al., 2020), potentially threatening these biodiversity hotspots (Grange and Smith, 2013). For example, in Greenland, inner fjord salinities often remain below 25 ‰ down to at least 10 m (Sejr et al., 2017, 2022) indicating that Antarctic fjords may experience similar conditions as climate warming advances.

Reductions in seawater salinity due to coastal freshening is a concern for Antarctica's endemic marine organisms which have evolved in a thermally stable and predictable environment over millions of years (Peck, 2018). Their capacity to acclimatise to environmental change, particularly warming, is generally considered poor and the time required for acclimation is considerably longer compared to similar temperate species (Peck et al., 2014). Evidence of low salinity acclimatisation abilities in Antarctic marine organisms, is sparse. Studies that have investigated long-term tolerance to low salinity demonstrate poor acclimation capacity. For example the Antarctic limpet *Nacella concinna* reached 50 % mortality at 20 ‰ after 60 days (Navarro et al., 2020), while the intertidal Antarctic notothenid teleost, *Harpagifer antarcticus* demonstrated reduced capacity to maintain homeostasis when exposed to low salinities for one week (Vargas-Chacoff et al., 2021).

Of particular concern are Antarctica's endemic echinoderm species. As a strictly marine phylum, echinoderms are often considered to have poor euryhaline abilities, lacking distinct excretory organs and possessing highly permeable body walls (Hyman, 1955; Binyon, 1972; Santos-Gouvea and Freire, 2007). However, numerous species live in low salinity environments, such as brackish waters (Russell, 2013; Turner and Meyer, 1980) and the intertidal (Freire et al., 2011a; Castellano et al., 2018). Aquarium studies have demonstrated wide variation in short-term low salinity tolerance levels across different taxa (Russell, 2013; Stickle and Diehl, 1987), while several studies have demonstrated that echinoderms have the capacity to acclimate to lower salinity over longer periods (Barrett et al., 2024a; Russell, 2013; Shirley and Stickle, 1982a). However, the physiological and behavioural response of echinoderms to low salinity, can differ dependant on the duration and rate of salinity reduction (e.g., Barrett et al., 2024a). This correlates with mechanistic differences in the acute response to low salinity, compared to that of the gradual process of cellular reorganisation necessary for acclimation in osmoconformers (Khlebovich, 2017; Rivera-Ingraham and Lignot, 2017). In echinoderms under acute hypoosmotic stress, the establishment of transient ionic and osmotic gradients between the coelomic fluid and external seawater, appear to provide a 'buffer' to internal tissue, allowing time for cellular volume control to take place (Barrett et al., 2024a; Freire et al., 2011a; Santos et al., 2013). Over longer periods of exposure to low salinity in osmoconformers, cellular solute composition and concentration can be modified through changes to membrane bound transporters favouring the release of specific 'osmolytes' from the cytoplasm (Gilles, 1987; Meng et al., 2013; Barrett et al., 2022; Podbielski et al., 2022). In addition, structural changes to the cell cytoskeleton may support changes in osmotic pressure (Pedersen et al., 2001; Barrett et al., 2022). However, the genetic mechanisms underlying acclimatisation to lower salinity in echinoderms is yet to be established.

There have been no long-term studies on their capacity of Antarctic echinoderms to acclimatise to low salinity. However, short-term tolerance to hypoosmotic exposure has been evaluated in a number of species with varying results (Barrett et al., 2024b; Cowart et al., 2009; Pearse, 1967). For example, multiple holothurian species demonstrate high tolerance to low salinity (24-h LD_{50} = 11.5 ‰) in contrast to the Antarctic brittle star *Ophionotus victoriae*, where mortality was >50 % at 19 ‰ after 24 h (Barrett et al., 2024b).

The sea star, *Odontaster validus* and sea urchin *Sterechinus neumayeri* are both common circumpolar inhabitants of the nearshore Antarctic benthic ecosystem (Dell, 1972; Pearse, 2013). *Odontaster validus* is a subtidal asteroid normally found at depths of between 15 and 200 m, however it can be found from the shoreline down to depths of over 2000 m (Dearborn, 1977; Pearse, 2013). It is considered an opportunistic omnivore with diverse feeding strategies from predatory to detrital feeding (see McClintock, 1994), and has been considered a keystone species (Kidawa et al., 2010; McClintock et al., 1988; McClintock et al., 2008; although see Pearse, 2013). *Sterechinus neumayeri* is a shallow-water regular echinoid which inhabits both the intertidal and subtidal zones down to 450 m (Brey, 1991). It is most abundant in depths of <35 m (Dell, 1972; Barnes and Brockington, 2003; Waller et al., 2006) where it is the dominant echinoid in the near-shore benthic ecosystem (Brockington, 2001). Considered an omnivorous grazer and predator, its diet ranges from diatoms and macroalgae to scallops, bryozoans and even seal faecal material (Pearse and Giese, 1966; McClintock, 1994). Due to its high abundance and diverse feeding strategies, it significantly impacts community structure (Brey, 1991) and plays a key role in regulating the food web in Antarctic benthic ecosystems (Sporta Caputi et al., 2020). Consequently, it is considered a keystone species (Ortiz et al., 2017; Sporta Caputi et al., 2020; Détrée et al., 2023). Tolerance to acute low salinity has been demonstrated in both species, with *O. validus* the more tolerant of the two (Barrett et al., 2024b; Pearse, 1967). The calculated 24-h LD_{50} (salinity which is lethal to 50 % of exposed animals) was 13.4 ‰ for *O. validus* and 16.5 ‰ for *S. neumayeri* (Barrett et al., 2024b). However, early life stages of *S. neumayeri* appear to be highly sensitive to subtle decreases in salinity, with 99 % mortality observed in embryos exposed to a drop in salinity from 34 to 30 ‰ (Cowart et al., 2009).

This study aims to assess the acclimation capacities of *O. validus* and *S. neumayeri*, to lower salinity levels (29 ‰ and 24 ‰) over a period of at least ten weeks, as might be predicted under future freshening scenarios in Antarctica. Assessments of whole animal physiological metrics [mortality, oxygen consumption, feeding, faecal production (*S. neumayeri* only) and changes in mass] and behavioural assessments (ability to right), will be combined with cellular and body fluid measurements [coelomic fluid osmolality and coelomocytes composition and concentration (*S. neumayeri* only)] to assess evidence of low salinity acclimation. This is the first study to assess the low salinity acclimation capacity of any Antarctic echinoderm.

2. Methods

2.1. Experimental animals

Both species were collected in the austral summer of 2021/2022 by SCUBA divers at depths between 10 and 20 m near the British Antarctic Survey's (BAS) Rothera research station, Adelaide Island (67°34'07"S, 68°07°30"W). After collection, they were transferred to the Rothera aquarium, and subsequently transported to the UK in a temperature-

controlled, containerised aquarium. In the UK, they were held in an aquarium system maintained at −0.3 ± 0.2 °C and 34.5 ‰, in a 12:12 h light: dark regime, for approximately 6—12 months prior to the experiment. Both species were fed once a week on a frozen krill diet.

2.2. Experimental set-up

Three salinity treatments were selected to evaluate acclimation capacity of the two species over a 71-day (*O. validus*) and 85-day (*S. neumayeri*) period: 34.5 ‰ (control), 29 ‰ (medium), 24 ‰ (low). The two reduced salinity treatments were selected based on the results of their 24-h short-term tolerance (Barrett et al., 2024b), data from the Rothera Time Series [surface salinity ranged from 28.4 ± to 35.2 ± 0.03 ‰ from December 2012 to January 2021 (Clarke et al., 2022)], approaches from previous salinity studies on Antarctic marine animals (e.g., Navarro et al., 2019; Navarro et al., 2020; Park et al., 2020) and future freshening predictions from Arctic fjords (e.g., Grange and Smith, 2013; Sejr et al., 2017, 2022). For *O. validus*, 108 animals with an arm radius (from the tip of arm to the centre of mouth) of 25–63 mm were randomly divided between the three salinity treatments and distributed between three replicate 60-L tanks per treatment ($n = 12$ per tank; $n = 36$ per treatment, 9 tanks in total). For *S. neumayeri*, 120 animals with a test diameter of 15–37 mm were split between four replicate 50-L tanks per treatment ($n = 10$ per tank; $n = 40$ per treatment; 12 tanks in total). A protein skimmer (REEF- Skim Nano 100 AC, TMC Ltd) and bio-filter (ZB-150, Ziss) with an attached airline were fitted to each tank.

Animals were habituated for 8 (*O. validus*) or 12 days (*S. neumayeri*) before tanks were diluted using distilled water in a stepwise approach, reducing salinity by 2 ‰ per day until experimental salinities were reached (as per Barrett et al., 2024a; Shirley and Stickle, 1982b). A conductivity probe (CDC40101, Hach) was used for measuring salinity. Within each experiment floating trays were added to tanks to isolate individual animals for repeated measurements. For *O. validus*, 3 animals per tank were isolated in trays, equalling 9 per treatment; for *S. neumayeri* 2 animals per tank were isolated in trays, equalling 8 per treatment. In the *S. neumayeri* experiment, data could not be collected during the week of day 43. Therefore, the data from day 50 represents two weeks' worth of observations, which have been averaged.

Water chemistry was monitored twice weekly using JBL aquarium test kits (JBL, Germany). Ammonium (NH_4^+), nitrates (NO_3^-) and nitrites (NO_2^-) were kept below recommended levels ($NH_4^+ < 0.05$ mg/L^{-1}, $NO_3^- \sim 1$ mg/L^{-1}, $NO_2^- < 0.025$ mg/L^{-1}), for each treatment by refreshing seawater (pre-diluted and chilled) twice weekly between 20 and 30 L per tank. Water pH was monitored weekly using a pH probe prior to water changes (Hach, Germany) (*S. neumayeri* control: pH 7.97 ± 0.05, medium: 7.93 ± 0.05, low: 7.92 ± 0.05, mean ± s.e.m., $n = 12$; *O. validus* control: pH 7.98 ± 0.03, medium: 7.92 ± 0.03, low: 7.93 ± 0.03, mean ± s.e.m., $n = 10$). Tank temperatures were maintained at ~ − 0.4 °C (*O. validus*: -0.45 ± 0.03 °C mean ± s.e.m. $n = 72$; *S. neumayeri* − 0.42 ± 0.02 °C mean ± s.e.m., $n = 158$) and checked daily.

2.3. Mortality

Mortality was assessed daily. Animals unable to right within 24 h and lack of movement of tube feet (*S. neumayeri*) or arms (*O. validus*) after stimulus were declared dead.

2.4. Buoyant weight change and percentage ash free dry mass (AFDM)

To assess for changes in mass in *O. validus*, buoyant weight was measured in each isolated sea star before and after the experimental period following seven days without feeding. Buoyant weight is considered insensitive to changes in water content of the animal (Jokiel et al., 1978), which can occur in sea stars due to the lack of an exoskeleton. For *S. neumayeri*, percentage AFDM was used as an end point assessment of the difference in organic mass to inorganic mass per isolated urchin over the experimental period (following Barrett et al., 2024a). This was expressed as:

$$\%AFDM = \frac{AFDM}{(AFDM + ash\ mass)}\ x\,100$$

2.5. Oxygen consumption

Oxygen consumption was measured in the isolated urchins seven days before salinity dilution began, and then on day 1, 8, 15, 29, 57, 71 (*O. validus* only) and 85 (*S. neumayeri* only) after the experimental salinity dilution was reached (hereafter referred to as sample points). Closed bottle respirometry techniques were used to measure oxygen consumption following Barrett et al. (2024a). Each animal was placed in a 300 mL glass respirometry chamber with a 4 mm mesh covering, allowing oxygenated water to circulate but preventing animals from escaping. Animals were left for one hour to habituate within the chamber and ensure the metabolic effects of handling had subsided, before the lids were sealed and a first measurement of the water oxygen saturation levels were measured. A Fibox-4 fibre optic oxygen sensor system (PreSens, Germany) was used to record the chamber water oxygen saturation. After approximately four hours for *O. validus* or six hours for *S. neumayeri*, a second oxygen saturation measurement was taken. Three additional empty chambers were sealed and assessed over the same time period to measure any background microbial metabolic activity. These values were then used to correct the calculated rates for each animal. Animal volume was measured by way of water displacement following Morley et al. (2016) and each chamber volumes was adjusted for animal volume to produce a respired water volume for each calculated value of oxygen consumption. At the end of the experimental period animals were selected for destructive sampling to obtain AFDM. Animals were sacrificed by being placed in a − 80 °C freezer, then dried in a convection oven at 60 °C for at least 48 h until a consistent mass was obtained. Dried animals were weighed, then placed in a muffle furnace for six hours at 475 °C to obtain an ash mass value. AFDM was calculated by subtracting the ash mass from dry mass. For *O. validus*, AFDM was measured in all isolated animals ($n = 9$ per treatment). For *S. neumayeri*, AFDM was determined from a wet mass vs. AFDM regression using a combination of isolated and tank animals ($n = 10$ per treatment). Due to the change in ratio of organic mass to inorganic mass observed at the end of the experiment, linear regressions between pre-experiment wet mass and post-experiment mass were used to determine modelled AFDM at each timepoint.

For additional confirmation of the impact of salinity on oxygen consumption in *S. neumayeri*, a repeat experiment was implemented. A single end point sample of oxygen consumption was measured after 78 days of exposure to experimental salinities which was consistent with the previous trial (see Fig. S1).

2.6. Righting ability (activity coefficient)

Sea urchins and sea stars will attempt to right themselves if inverted with the time taken to right being a widely used metric to assess echinoderm activity rates (Russell, 2013; Stickle and Diehl, 1987). Time taken to right in seconds was converted to an activity coefficient (AC = 1000/ righting time in seconds) (after Lawrence and Cowell, 1996) and assessed weekly in all treatments for both species including seven days before salinity dilution began. For *O. validus* repeat measurements were made on the same isolated sea stars ($n = 9$ per treatment), while for *S. neumayeri* between 8 and 12 urchins were randomly sampled per treatment. The maximum time allowed for righting was 30 min for *O. validus* and 60 min for *S. neumayeri*, which equals a minimum AC value of 0.55 for *O. validus* (1000/1800 s) and 0.28 for *S. neumayeri* (1000/3600 s).

2.7. Feeding

Feeding trials began within the habituation period while all treatments were at ambient salinity (34.5 ‰) to provide a baseline feeding rate (two weeks for *S. neumayeri* and one week for *O. validus*), and then commenced weekly. Feeding was not measured in the final week in either species. The artificial diet fed to the animals (Vitalis Marine Grazer) constituted 28 % protein obtained from fish, algae, molluscs and crustacean derivatives and had been previously used in *S. neumayeri* feeding experiments (De Leij, 2021). The feed was also tested on *O. validus* in a pre-experiment feeding trial to ensure suitability. The isolated animals were fed pre-dried and weighed pellets once a week throughout the experimental period and allowed to feed for 48 h before any remaining food was removed. The remaining food was rinsed twice in distilled water to remove saltwater, dried for 24 h in a convection oven and weighed. The feeding rate was calculated by subtracting the remaining dry mass of food from the initial dry mass and dividing by the ADFM of the animal (mg food ingested mg animal AFDM^{-1} week^{-1}). The free roaming tank animals were fed weekly and allowed to feed ad libitum. The amount of food provided was at least $\geq$ 50 mg animal^{-1} week^{-1} for *S. neumayeri* and $\geq$ 100 mg animal^{-1} week^{-1} in *O. validus* to ensure consumption was not limiting. In *O. validus*, an additional metric of feeding behaviour was recorded after 24 h by turning over each sea star and visually assessing whether food was being ingested.

2.8. Faecal production

Faecal collection for *S. neumayeri* began on day 1 after acclimation salinities were reached and represented faecal production from the prior week of feeding. Faeces were collected weekly from all isolated urchins (n = 8 per treatment) by pipette and transferred to a microcentrifuge vial. Faeces were then rinsed twice with distilled water to remove saltwater and dried in a convection oven for 24 h and weighed. Dried faeces were then ashed in a muffle furnace for 6 h at 475 °C and weighed. Ash free dried mass (AFDM) was calculated by subtracting the ash mass from the dried mass. Faecal production was not assessed in *O. validus* due to the lack of coherence in the faeces produced by this species.

2.9. Coelomic fluid osmolality and coelomocyte analysis in S. neumayeri

Coelomic fluid was extracted from randomly selected free-roaming tank animals (n = 4 for *O. validus*; n = 5 for *S. neumayeri*) in each treatment on days 1, 8, 15, 29, 57 and 85 for *S. neumayeri* and days 1, 8, 22, 36, and 64 for *O. validus* after the experimental salinity dilution was reached. Sub-lethal sampling was conducted by inserting a syringe with a 21-gauge needle into the coelomic cavity (after Reinardy and Bodnar, 2015). Between 0.5 and 1 mL of coelomic fluid was extracted per animal. Triplicate tank water samples were taken at the same sampling point to provide a mean tank water osmolality value. Coelomic fluid and seawater osmolality was measured on a Vapro 5600 vapour pressure osmometer (ELITech Group).

Coelomocytes were collected at the last sample point (day 85) from *S. neumayeri* (n = 5 per treatment). Coelomic fluid was collected as previously described, with samples kept on ice until measurements were taken to prevent cell clumping. Cell concentration, differential cell counts (red spherulocytes and clear cells) and cell viability were assessed on a Neubauer haemocytometer using a light microscope. The percentage viable cells was assessed by mixing coelomic fluid with 0.5 % Trypan Blue (ratio 1:1) and the number of dead blue cells and clear live cells counted (as per Barrett et al., 2024a; Liu et al., 2022). Due to logistical constraints, cell counts could not be included at each coelomic fluid sampling timepoint. Therefore, a single end-point sampling was chosen to assess the impact of acclimation across treatments in *S. neumayeri*. Cell counting was not conducted for *O. validus* because it was incorporated into the *S. neumayeri* experiment only after the *O. validus* study had been completed.

2.10. Hypoosmotic shock

To assess the resilience of the acclimated animals to hypoosmotic shock, a subset of animals (n = 6 per treatment for *O. validus; n* = 5 per treatment for *S. neumayeri*) were directly transferred to a single 19 ‰ salinity tank per treatment for a duration of seven hours. Oxygen consumption was assessed over the first 3 h in *O. validus* and the first 6 h in *S. neumayeri* as previously described. Activity coefficient was assessed after 6 h in 19 ‰ as previously described. For *S. neumayeri*, the righting challenge was additionally extended to 90 min which equals a minimum AC value of 0.19 (1000/5400 s).

2.11. Statistics

One-way analysis of variance (ANOVA) was implemented when comparing the response means between salinity treatments at specific timepoints, followed by Holm's adjusted correction to account for multiple comparisons. Assumptions of normality and homogeneity of variance were formally tested using Shapiro–Wilk test and Levene's test, respectively. When the residuals of the data were not normally distributed, data were either log, square or cube root transformed and reassessed. With continued violations of normality in the residuals, non-parametric Kruskal-Wallis tests were implemented. For data where the variance of residuals across groups was unequal, Welch's ANOVA tests were used. For significant ANOVA, Welch's ANOVA, or Kruskal-Wallis tests, post-hoc tests were performed to determine significant differences between group pairings, using Tukey's test, Games-Howell test, and Dunn's test, respectively. Two-way ANOVA tests were performed when comparing the response variable over different time points including their interaction. When repeat measurements were performed on the same animal, the model was adapted to include the individual animal as a random factor. When the assumptions of normality were violated in two-way models and data transformation was unsuccessful, an aligned rank transformation ANOVA (ART ANOVA) was implemented as a non-parametric alternative. One-sample *t*-tests were performed when comparing coelomic fluid osmolality values with mean tank water osmolality. Two sample *t*-tests were performed when comparing oxygen consumption values before and after hypoosmotic shock. Chi-squared tests were performed on mortality data. Percentage data (e.g., percentage AFDM and coelomocyte metrics) were arcsine transformed prior to statistical analysis. Statistical tests were considered significant if $P < 0.05$. All analyses were carried out in R (version 4.2.2).

3. Results

3.1. Tank effects

There were no significant variations between tanks regarding oxygen consumption, activity coefficient and feeding rate in either the *O. validus* or *S. neumayeri* experiment, therefore each individual animal was treated as a biological replicate (Table S1).

3.2. Mortality

O. validus – Over the experimental period, there were three recorded mortalities in the low salinity treatment. There were no mortalities in the medium and control salinities. Overall, salinity had a marginal but significant impact on mortality (Chi-squared: $\chi^2_2 = 6.2$, $P = 0.046$) (Fig. 1A; Table S1).

S. neumayeri – Mortalities were recorded in all three treatments over the experimental period: four in both the control and medium salinities, and nine in the low salinities. However, salinity had no significant impact on mortality (Chi-squared: $\chi^2_2 = 3.4$, $P = 0.18$) (Fig. 1B; Table S1).

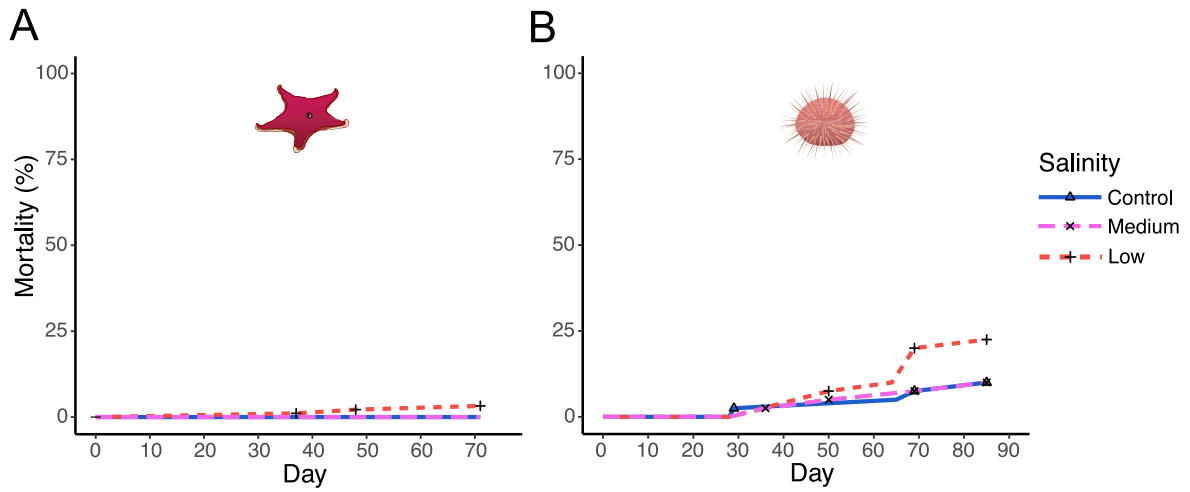

Fig. 1. Cumulative percentage mortality of A) *Odontaster validus* and B) *Sterechinus neumayeri* over the course of the experimental period. Symbols (triangle, cross and plus sign) correspond to recorded mortalities.

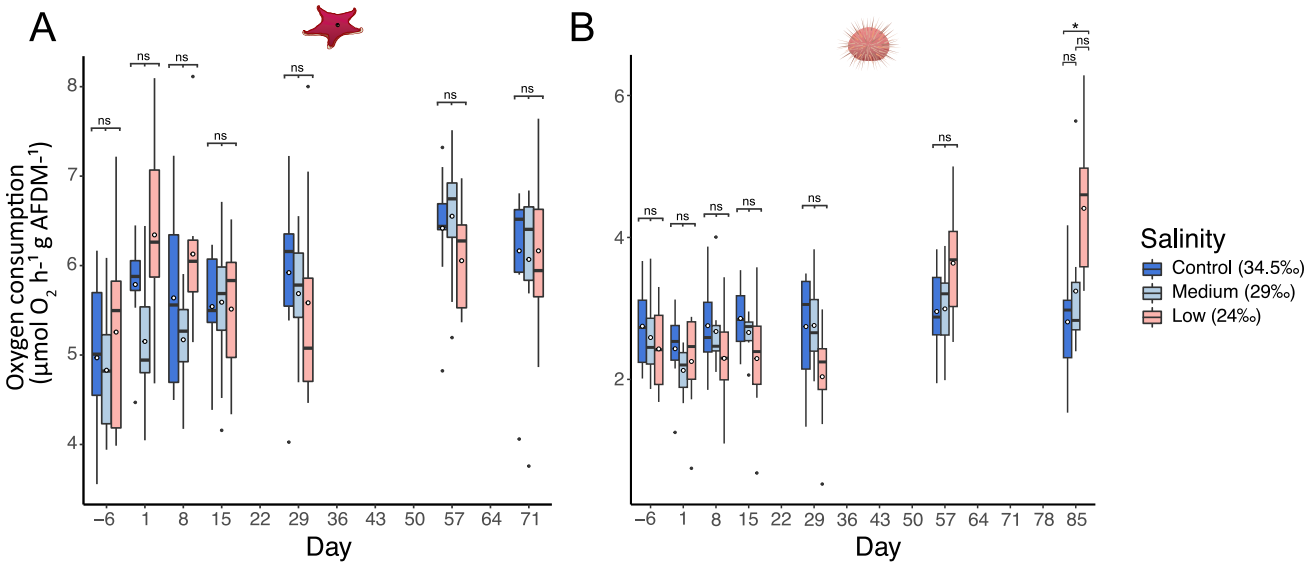

Fig. 2. Rate of oxygen consumption of A) *Odontaster validus* and B) *Sterechinus neumayeri* at time points during exposure to different salinity conditions. Results are shown for one-way ANOVA/Welch's ANOVA and post hoc Tukey test (*S. neumayeri:* day 85) between salinity treatments at each time point (n.s., not significant; $^*P < 0.05$; $n = 9$ for *O. validus* and $n = 8$ for *S. neumayeri* biological replicates at each time point and treatment).

3.3. Buoyant weight change

O. validus – Salinity had a significant impact on the change in mean (±s.e.m.; n = 8–9) buoyant weight (low: −1.09 ± 0.03 g; medium: −0.004 ± 0.01 g; control: −0.006 − 0.02 g) (One-way ANOVA: $F_{(2,23)} = 6.9$, $P = 0.004$). There were significant differences between the low and control (Tukey: $P < 0.01$) and low and medium salinities (Tukey: $P = 0.009$) (Table S1).

3.4. Percentage AFDM

S. neumayeri – Salinity had a significant impact on the ratio of organic mass to inorganic mass (AFDM to dry mass) following the acclimation period (expressed as the mean percentage AFDM: low 17.51 ± 0.86 %; medium 27.1 ± 1.28 %; control 26.84 ± 1.55 %) (One-way ANOVA: $F_{(2,54)} = 17.4$, $P < 0.0001$). Animals in low salinity had a significantly lower percentage of AFDM compared to animals in control salinity and medium salinity (Tukey: both $P < 0.0001$), while there were no significant differences between animals in medium and control salinity (Table S1).

3.5. Oxygen consumption

O. validus – The impact of salinity on rates of oxygen consumption had no overall significant effects (Two-way ANOVA with repeated measures: $F_{(2,24)} = 1.1$, $P = 0.34$); however, the interaction between time points and salinity was significant (Two-way ANOVA with repeated measures: $F_{(12,144)} = 1.96$, $P = 0.03$), in addition to the effects of time (Two-way ANOVA with repeated measures: $F_{(6,144)} = 10.9$, $P < 0.0001$). Analysis of oxygen consumption at each timepoint demonstrated no significant impact between salinity treatments (Fig. 2A; Table S1).

S. neumayeri– The impact of salinity on rates of oxygen consumption had no overall significant effects (Two-way ART ANOVA with repeated measures: $F_{(2,23)} = 0.2$, $P = 0.8$); however, the interaction between time points and salinity was significant (Two-way ANOVA with repeated measures: $F_{(12,126)} = 4.6$, $P < 0.0001$), in addition to the effects of time (Two-way ANOVA with repeated measures: $F_{(6,126)} = 14.5$, $P < 0.0001$). Analysis of rates of oxygen consumption at each timepoint demonstrated

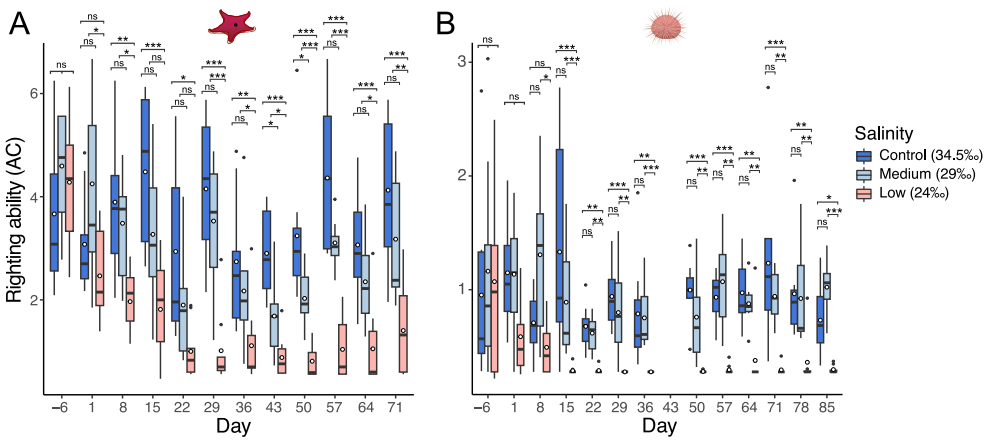

Fig. 3. Righting ability (activity coefficient) of A) *Odontaster validus* and B) *Sterechinus neumayeri* at time points during exposure to different salinity conditions. Results are shown for one-way ANOVA/Welch's ANOVA/Kruskal-Wallis and relevant post hoc test between salinity treatments at each time point (n.s., not significant; $*P < 0.05$, $**P < 0.01$, $***P < 0.001$; $n = 9$ for *O. validus* and $n = 8$–12 for *S. neumayeri* biological replicates at each time point and treatment). Note: a smaller AC value indicates longer righting times.

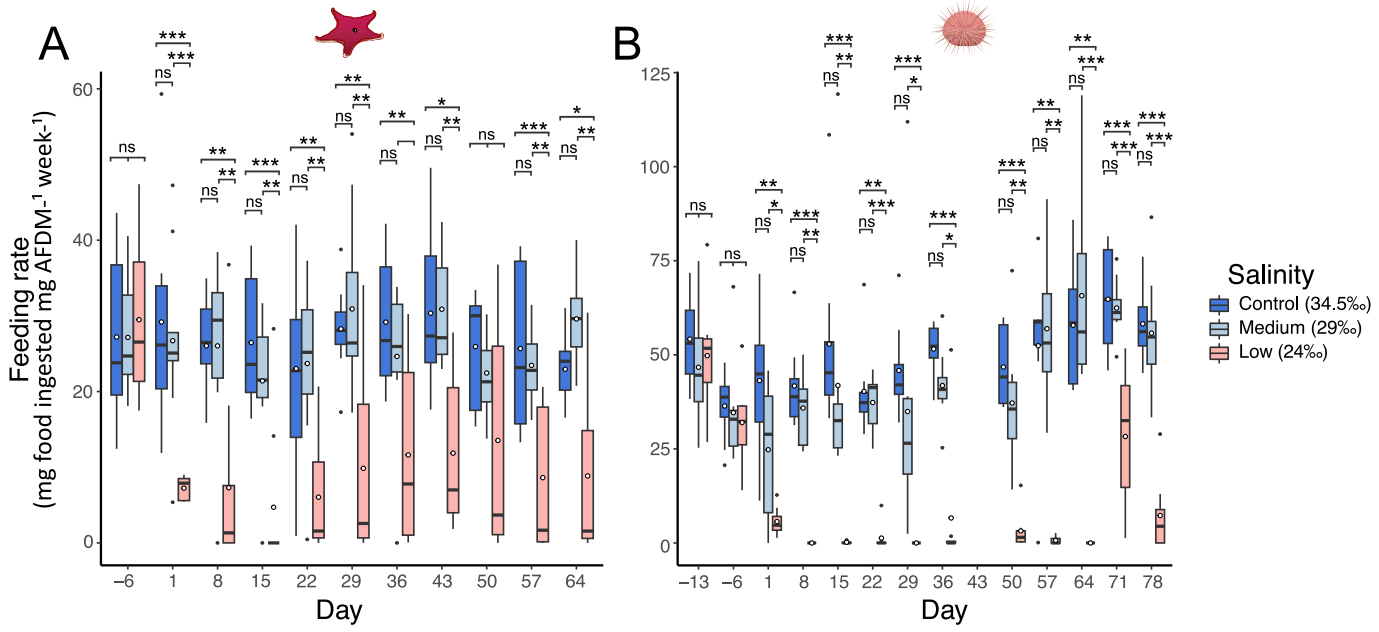

Fig. 4. Feeding rate of A) *Odontaster validus* and B) *Sterechinus neumayeri* at time points during exposure to different salinity conditions. Results are shown for one-way ANOVA/Welch's ANOVA/Kruskal-Wallis and relevant post hoc test between salinity treatments at each time point (n.s., not significant; $*P < 0.05$, $**P < 0.01$, $***P < 0.001$; $n = 9$ for *O. validus* and $n = 8$ for *S. neumayeri* biological replicates at each time point and treatment). Feeding was calculated as mg food ingested/mg animal AFDM/week.

salinity had a significant impact on day 85 only (One-way ANOVA: $F_{(2,21)} = 6.4$, $P = 0.046$), where there were significant differences between the control and low salinity treatments (Tukey: $P = 0.01$) (Fig. 2B; Table S1). In the repeat experiment (day 78), salinity had a significant impact on the rates of oxygen consumption (One-way ANOVA: $F_{(2,20)} = 9.6$, $P = 0.001$), with significant differences between the control and low salinity treatments (Tukey: $P = 0.007$) and between the medium and low salinity treatments (Tukey: $P = 0.001$) (Fig. S1; Table S1).

3.6. Righting ability (activity coefficient)

O. validus – Mean AC rates varied significantly between salinity treatments and time points, including their interaction (Two-way ANOVA with repeated measures: Salinity $F_{(2,24)} = 30.8$, $P < 0.0001$; Time points $F_{(11,264)} = 14.6$, $P < 0.0001$; Salinity:Time points $F_{(22,264)} = 3.5$, $P < 0.0001$). At each time point, except on day −6, salinity had a significant impact on mean AC (One way ANOVA and Welch's ANOVA: all $P < 0.05$). Righting times were significantly longer in low salinity compared to the control at all time points from day 8 (Tukey and Games-Howell: all $P < 0.05$) and compared to medium salinity from day 1 (Tukey and Games-Howell: all $P < 0.05$), with the exception of day 15 and 22 where they were not significant. Righting times were significantly longer in medium salinity compared to the control on day 43 (Games-Howell: $P = 0.02$) and day 50 (Tukey: $P = 0.02$) only (Fig. 3A; Table S1).

S. neumayeri – Mean AC rates varied significantly between salinity treatments and time points, including their interaction (Two-way ART ANOVA: Salinity $F_{(2,296)} = 107.4$, $P < 0.0001$; Time points $F_{(12,296)} = 2.8$, $P < 0.01$; Salinity:Time points $F_{(24,296)} = 1.1$, $P = 0.04$). At each time point, except on day −6 and 1, salinity had a significant impact on

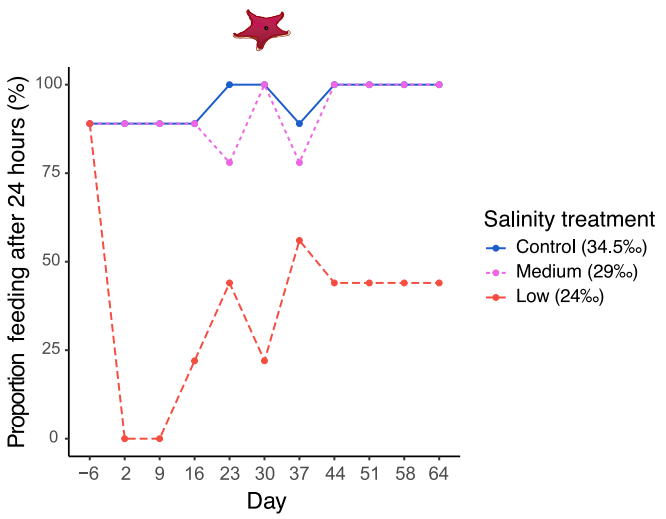

Fig. 5. Percentage of *Odontaster validus* feeding 24 h after food given over the experimental period.

mean AC (One way ANOVA and Welch's ANOVA and Kruskal-Wallis: all $P < 0.05$). Righting times were significantly longer in low salinity compared to the control at all time points from day 15 (Dunn and Games-Howell: all $P < 0.02$) and compared to medium salinity from day 8 (Dunn and Games-Howell: all $P < 0.02$). There were no significant differences in AC between medium salinity and control at any point in the experiment (Fig. 3B; Table S1).

3.7. Feeding

O. validus – Mean feeding rates varied significantly between salinity treatments, time points and their interactions (Two-way ART ANOVA with repeated measures: Salinity $F_{2(2256)} = 81.1$, $P < 0.0001$; Time points $F_{(10,256)} = 4.2$, $P < 0.01$; Salinity:Time points $F_{(20,256)} = 1.76$, $P = 0.025$). At each time point, except on day −6 and 50, salinity had a significant impact on feeding (One way ANOVA and Welch's ANOVA: all $P < 0.05$). Feeding rates were significantly lower in low salinity compared to the control at all time points from day 1 (Tukey and Games-Howell: all $P < 0.04$), and the medium salinity at all time points from day 1 (Tukey and Games-Howell: all $P < 0.04$), except on day 50 where they were not significant. There were no significant differences in feeding between medium salinity and control at any point in the experiment (Fig. 4A; Table S1). The number of individual sea stars feeding after 24 h dropped from 100 % before dilution to 0 % after dilution in the low salinity treatment, before stabilising at 44 % from day 44 to 64. Feeding in the medium group followed a similar pattern to the control group, stabilising at 100 % in both groups from day 44 to 64 (Fig. 5).

S. neumayeri – Mean feeding rates varied significantly between salinity treatments, time points and their interactions (Two-way ART

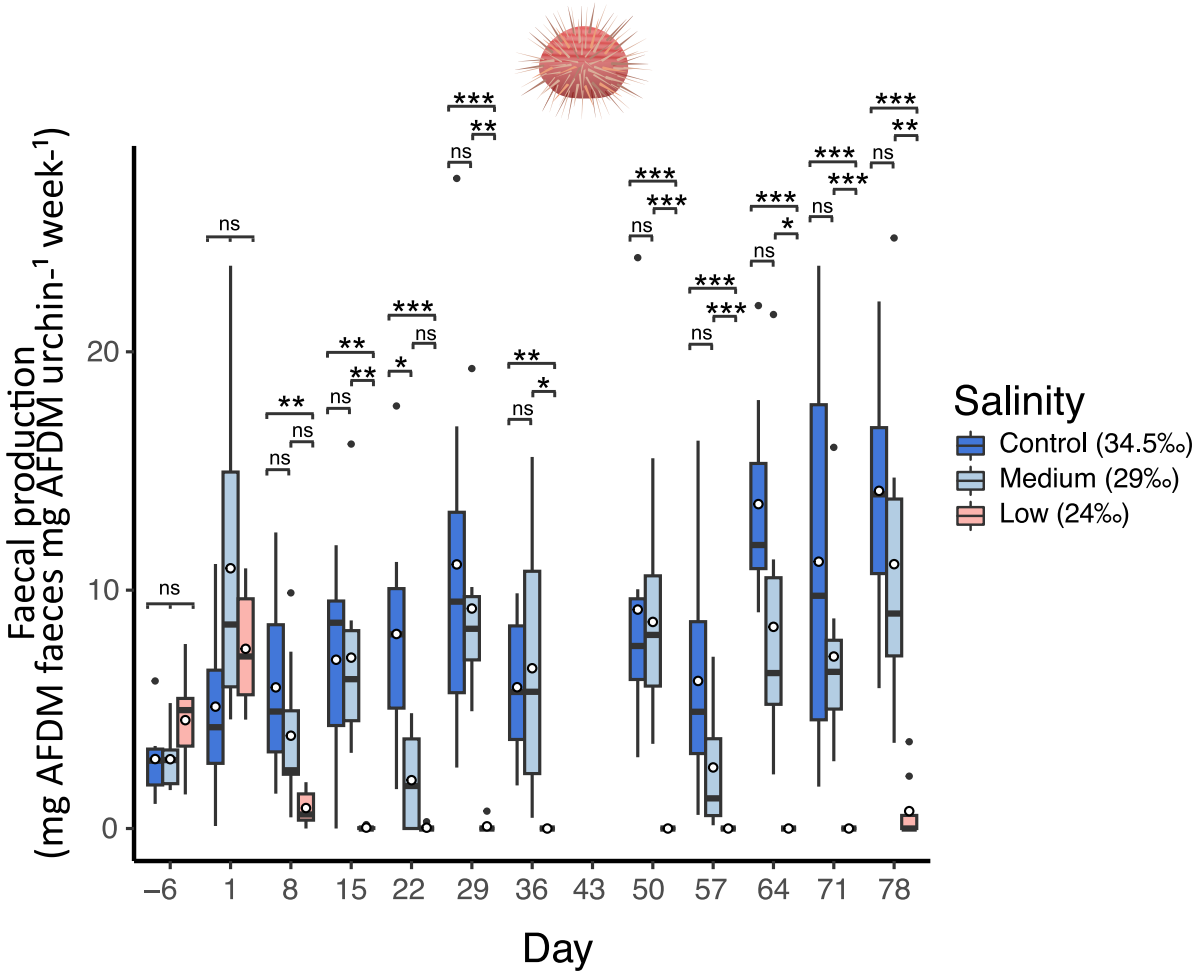

Fig. 6. Rate of faecal production of *Sterechinus neumayeri* at time points during exposure to different salinities. Results are shown for one-way ANOVA/Welch's ANOVA/Kruskal-Wallis and relevant post hoc test between salinity treatments at each time point (n.s., not significant; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; $n = 8$ biological replicates at each time point and treatment).

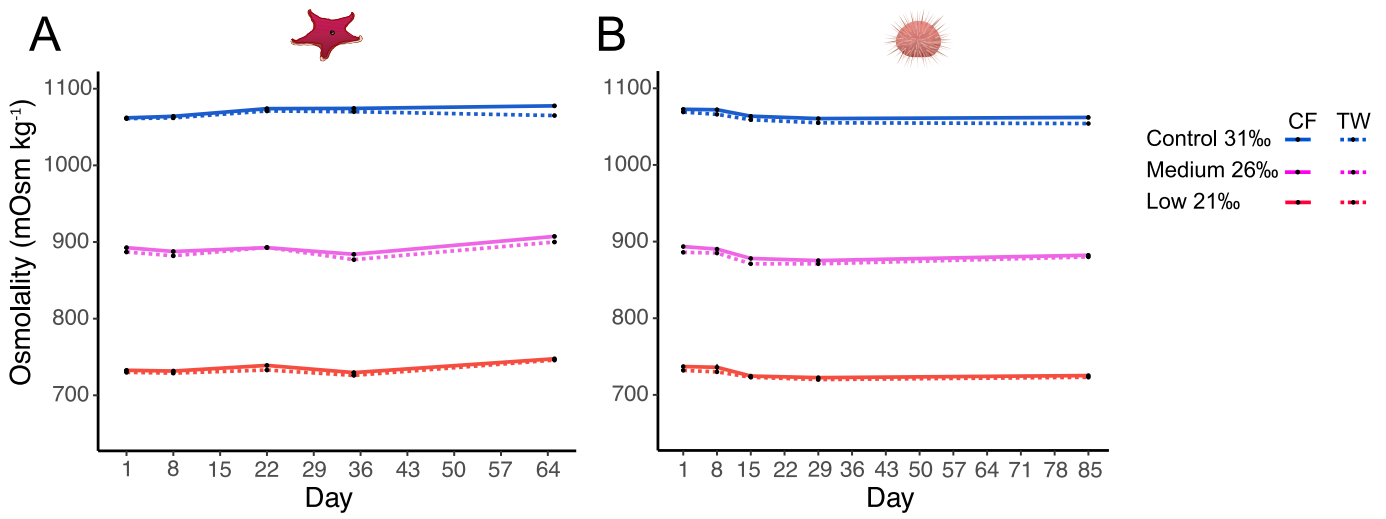

Fig. 7. Coelomic fluid osmolality of A) *Odontaster validus* and B) *Sterechinus neumayeri* versus tank water osmolality. Data are mean ± s.e.m. coelomic fluid (CF; $n = 4$ for *O. validus* and $n = 5$ for *S. neumayeri* biological replicates for each salinity and time point) and tank water (TW; $n = 3$ technical replicates for each salinity and time point) osmolality at time points during exposure to different salinity conditions.

Table 1
Coelomocyte analysis after 85 days of exposure of in *Sterechinus neumayeri* to different salinities.

Treatment	*n*	Mean test diameter (mm)	Mean wet weight (g)	Cell viability (%)	Red spherule cells (% of total coelomocyte concentration)	Total coelomocyte concentration (cells mL^{-1})	s.e.m.
Control (34.5 ‰)	5	23 ± 1	5.7 ± 0.7	96.58 ± 1.2	10.42 ± 4.3	1.45E+07	1.45E+07
Medium (29 ‰)	5	24 ± 1.5	6.0 ± 0.6	95.22 ± 0.7	13.05 ± 3.2	3.69E+06	8.25E+05
Low (24 ‰)	5	21 ± 1.2	4.3 ± 0.7	95.98 ± 0.8	12.88 ± 3.4	1.50E+06	5.31E+05

Data are means±s.e.m. There were no significant differences (one-way ANOVA) between salinity treatments in terms of cell viability ($F_{(2,12)} = 0.31$, $P = 0.74$), proportion of red spherulocytes ($F_{(2,12)} = 0.31$, $P = 0.74$) and total coelomocyte concentration ($F_{(2,12)} = 3.88$, $P = 0.05$).

ANOVA with repeated measures: Salinity $F_{(2,23)} = 63.6$, $P < 0.0001$; Time points $F_{(12,252)} = 18.2$, $P < 0.0001$; Salinity:Time points $F_{(24,252)} = 7.9$, $P < 0.0001$). At each time point, except on day −13 and 6, salinity had a significant impact on feeding (One way ANOVA, Welch's ANOVA and Kruskal-Wallis: all $P < 0.01$). Feeding rates were significantly lower in low salinity compared to the control (Tukey, Dunn and Games-Howell: all $P < 0.002$) and medium salinity (Tukey, Dunn and Games-Howell: all $P < 0.05$) at all time points from day 1. There were no significant differences in feeding between medium salinity and control at any point in the experiment (Fig. 4B; Table S1).

3.8. Faecal production

S. neumayeri – Mean faecal production varied significantly between salinity treatments, time points and their interactions (Two-way ART ANOVA with repeated measures: Salinity $F_{(2,21)} = 111.5$, $P < 0.0001$; Time points $F_{(11,231)} = 8.8$, $P < 0.0001$; Salinity:Time points $F_{(22,231)} = 6.2$, $P < 0.0001$). At each time point, except on day −6 and 1, salinity had a significant impact on faecal production (One way ANOVA, Welch's ANOVA and Kruskal-Wallis: all $P < 0.05$). Faecal production was significantly lower in low salinity compared to the control at all time points from day 8 (Tukey, Dunn and Games-Howell: all $P < 0.006$), and the medium salinity at all time points from day 15 (Tukey and Games-Howell: all $P \leq 0.05$). Faecal production was significantly lower on day 22 in the medium group compared to the control (Games-Howell: $P = 0.011$), but there were no significant differences at any other timepoint. (Fig. 6; Table S1).

3.9. Coelomic fluid osmolality

O. validus – Mean coelomic fluid osmolality differed significantly between salinity treatments at each sample time point (Two-way ANOVA: Salinity $F_{(2,30)} = 68{,}903.22$, $P < 0.0001$; Tukey: all $P < 0.0001$). At each sample time point within each salinity treatment, coelomic fluid was always isosmotic or slightly hyperosmotic compared to the tank water. Hyperosmotic coelomic fluid was only significantly different (all one-sample *t*-tests) from tank water on day 65 in the control ($t_2 = 8.7$, $P = 0.01$) and days 8 ($t_2 = 5.3$, $P = 0.03$), 35 ($t_2 = 12.1$, P 0.007), and 65 ($t_2 = 22$, $P = 0.002$) in medium salinity (Fig. 7A; Table S1).

S. neumayeri – Mean coelomic fluid osmolality differed significantly between salinity treatments at each sample time point (Two-way ANOVA: Salinity $F_{(2,50)} = 44{,}450.6$, $P < 0.0001$; Tukey: all $P < 0.0001$). At each sample time point within each salinity treatment, coelomic fluid was always isosmotic or slightly hyperosmotic compared to the tank water. Hyperosmotic coelomic fluid was only significantly different (all one-sample *t*-tests) from the tank water on day 29 ($t_2 = 4.6$, $P = 0.02$) and 85 ($t_2 = 3.2$, $P = 0.03$) in the control, days 1 ($t_2 = 3.4$, $P = 0.043$), 8 ($t_2 = 5.4$, $P = 0.006$) and 15 ($t_2 = 4.8$, $P = 0.009$) in medium salinity, and days 1 ($t_2 = 2.8$, $P = 0.047$) and 85 ($t_2 = 3.8$, $P = 0.02$) in the low salinity (Fig. 7B; Table S1).

3.10. Coelomocytes

S. neumayeri – Salinity had no significant impact on total coelomocyte number, percentage viable cells or percentage red spherulocytes (Table 1, Table S1).

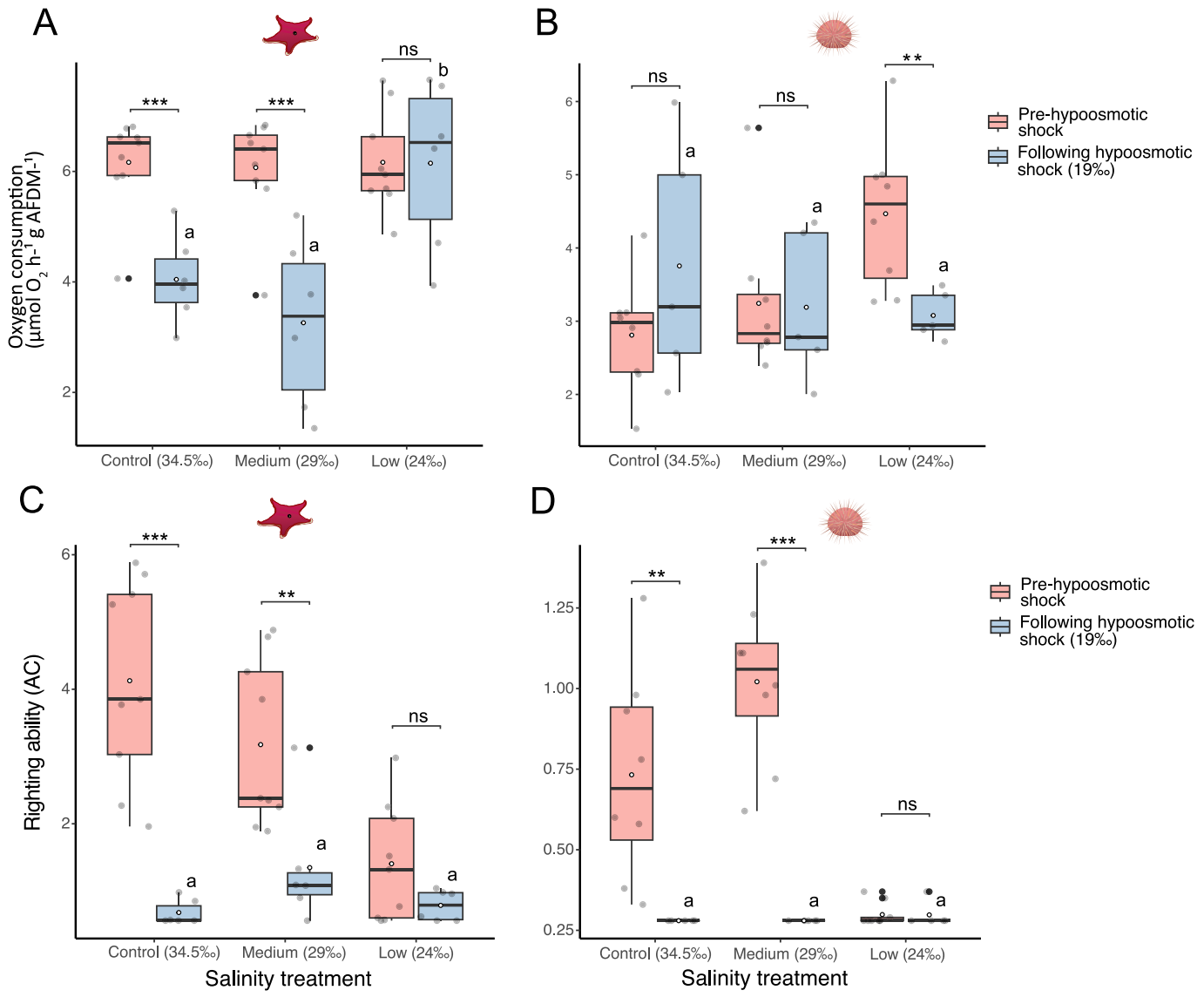

Fig. 8. Hypoosmotic shock–animals were exposed to different salinity treatments for 71 days (*O. validus*) and 85 days (*S. neumayeri*) followed by an acute immersion in 19 ‰ salinity. Oxygen consumption of A) *Odontaster validus* and B) *Sterechinus neumayeri* before (red boxes: $n = 9$ for *O. validus* and $n = 8$ for *S. neumayeri* biological replicates) and after hypoosmotic shock (blue boxes: $n = 6$ for *O. validus* and $n = 5$ for *S. neumayeri* biological replicates) following acclimation to reduced salinity. Righting ability (activity coefficient (AC)) of C) *O. validus* and D) *S. neumayeri* before (red boxes: $n = 9$ for *O. validus* and $n = 8$ for *S. neumayeri* biological replicates) and after hypoosmotic shock (blue boxes: $n = 6$ for *O. validus* and $n = 5$ for *S. neumayeri* biological replicates) following acclimation to reduced salinity. Results are shown for two-sample *t*-test between pre-hypoosmotic shock and following hypoosmotic shock for each salinity treatments (n.s., not significant; $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; and results for one-way ANOVA/Kruskal-Wallis and post hoc Tukey test between salinity treatments following hypoosmotic shock (different letters indicating significant differences $P < 0.05$). Biological replicates are represented by grey circles, means by open circles and outliers by filled black circles.

3.11. Hypoosmotic shock

O. validus – Following the acclimation period, mean oxygen consumption after direct immersion in 19 ‰ varied significantly between treatments (One-way ANOVA: $F_{(2,15)} = 7.6$, $P = 0.005$), with a significantly higher rate in the low salinity compared to both control and medium (Tukey: both $P < 0.04$); however, there was no significant difference in oxygen consumption between control and medium salinity. Both the control and medium salinities had significantly lower rates of oxygen consumption after immersion in 19 ‰ compared to their prior mean value on day 71 (Two sample t-test: Control $t_5 = 4.8$, $P < 0.001$; Medium $t_{13} = 4.4$, $P < 0.001$), while there was no significant difference in the low salinity treatment (Fig. 8A; Table S1).

There was no significant difference in mean AC between treatments following direct immersion in 19 ‰ (Fig. 8C; Table S1). Righting was completed in 3 of 6 control animals, 5 of 6 animals in medium salinity and 4 of 6 animals in low salinity. Both the control and medium salinities had significantly lower mean AC values after immersion in 19 ‰ compared to their prior mean value on day 71 (Two sample *t*-test: Control $t_8 = 6.8$, $P < 0.001$; Medium $t_{13} = 3.8$, $P < 0.01$) while there was no significant difference in the low salinity treatment (Fig. 8C; Table S1).

S. neumayeri – There were no significant differences in mean oxygen consumption between salinity treatments after direct immersion in 19 ‰ following the acclimation period. Oxygen consumption was significantly lower in the low treatment after immersion in 19 ‰ compared to the prior mean value on day 85 (Two sample t-test: $t_9 = 3.5$, $P < 0.01$), however there were no significant differences in the medium and control treatments (Fig. 8B; Table S1).

Following acclimation, mean AC values measured over 60 min were not significantly different between treatments after immersion in 19 ‰ (Fig. 8D; Table S1). One animal from the low salinity group completed righting after 60 min, while none in the control or medium treatments

did so. However, mean AC values measured over 90 min were significantly different between treatments (Kruskal-Wallis: $\chi^2_2 = 6.9, P = 0.03$), with significantly faster righting times (higher AC values) in low salinity compared to the control and medium (Dunn: both $P < 0.02$) (Fig. S2; Table S1). Three urchins in the low treatment righted themselves within 90 min, while none in the control or medium treatments did so. Both the control and medium salinities had significantly lower mean AC values after immersion in 19 ‰ compared to their prior mean value on day 85 (Two sample t-test: Control $t_7 = 4$, $P < 0.01$; Medium $t_7 = 8.3$, $P < 0.0001$) while there was no significant difference in the low salinity treatment (Fig. 8D; Table S1).

4. Discussion

The capacity to acclimate in response to rapid environmental change is considered of paramount importance for slow-growing organisms with long generation times, such as Antarctic marine species, to counter the threat posed by climate change. (Somero, 2010; Peck et al., 2014). An ability to acclimate to low salinity, has clearly been demonstrated in both species of Antarctic echinoderm in this study, with animals in the medium salinity treatment (29 ‰) showing no significant behavioural or physiological differences compared to control animals by the end of the experimental period. However, at the lower salinity (24 ‰), although survival was high, both species displayed responses which suggested that the stabilisation of physiological functions had not occurred within the experiment.

4.1. Mortality, buoyant weight change and percentage AFDM

Overall mortality rates were relatively low indicating a high capacity to tolerate the lowest salinity for an extended period in both species. Barrett et al. (2024b) demonstrated acute 24-h LD_{50} limits to be 13.4 ‰ for *O. validus* and 16.5 ‰ for *S. neumayeri*, substantially below the low salinity level tested in this study. However, in the current study mortality rates were higher in both low salinity groups by the end of the experimental period compared to control animals (significantly higher in *O. validus*), suggesting extended exposure at 24 ‰ may be unsustainable and below acclimation limits for both species. This is exemplified by significant reductions in mass observed in both species at low salinity, indicating impaired growth and likely tissue catabolism. These findings mirror observations from previous studies on chronic exposure to low salinity in both echinoids [*Echinus esculentus* (Barrett et al., 2024a); *Psammechinus miliaris*, and *Strongylocentrotus droebachiensis*, (Podbielski et al., 2022)] and asteroids [*Asterias rubens* (Podbielski et al. (2022); *Leptasterias hexactis* (Shirley and Stickle, 1982a)].

4.2. Oxygen consumption, activity, feeding and faeces production

In both species, oxygen consumption remained at similar levels to control animals for the first 71 days under reduced salinities. Under acute hypoosmotic shock, Barrett et al. (2024b) also demonstrated no change in oxygen consumption at 29 ‰ in both species and at 24 ‰ in *S. neumayeri*. However, in *O. validus* they observed a significant reduction in oxygen consumption at salinities ≤24 ‰, which was also a common pattern in other Antarctic echinoderm species (Barrett et al., 2024b). A reduction in metabolic rate in response to stress is considered a mechanism to conserve energy and allocate resources to core homeostatic functions (Sokolova et al., 2012a). The return to metabolic rates similar to controls at 24 ‰ in *O. validus* over a longer period in the current study suggests either a stabilising adjustment due to acclimation or additive costs related to osmoregulation that balance out the overall metabolic rate. Exposure to chronic low salinity in temperate echinoderms have caused varying response patterns in regard to oxygen consumption. Reductions were observed in the echinoid *L. hexactis* when exposed to 28 days at 15 ‰ and 20 ‰ (Shirley and Stickle, 1982b) and *S. droebachiensis* after 14 days at 15 ‰ (Sabourin and Stickle, 1981). However, Barrett et al. (2024a) demonstrated increased oxygen consumption in the echinoid *E. esculentus* after 5 days at low salinity (21 ‰), which persisted over the 25-day experiment. For *S. neumayeri*, there was a significant rise in oxygen consumption for animals held at low salinity by day 85 (similarly observed in the repeat experiment on day 78), matching the response pattern seen in *E. esculentus* by Barrett et al. (2024a), but only after a substantially extended period of stasis. An increase in oxygen consumption implies an increase in energy requirements for metabolic functions. As echinoderms are osmoconformers with extracellular fluids that are generally isosmotic with the external medium, acclimation involves active cellular volume regulation to adjust the osmotic and ionic balance within cells (Rivera-Ingraham and Lignot, 2017). These changes may include modifications to membrane-bound transporters, structural changes and amino acid metabolism, all incurring metabolic costs (Gilles and Delpire, 1997; Rivera-Ingraham and Lignot, 2017; Podbielski et al., 2022). Resting metabolic rates in Antarctic marine invertebrates are generally substantially lower than for temperate and tropical species (Peck, 2005), which consequently means a slower accumulation of detrimental metabolic waste, while stored reserves can stretch further (Peck et al., 2010). The substantial delay in the rise in oxygen consumption for *S. neumayeri* at low salinity, compared to the relatively rapid increase seen in the temperate urchin *E. esculentus*, suggests that Antarctic urchins were able to offset the increased metabolic demands of osmoregulation. Instead, their low metabolic rate as a consequence of the cold environment, enabled reliance on stored reserves for a protracted period before reserves ran critically low (demonstrated by the loss in organic matter), and homeostatic metabolic demands increased. Barrett et al. (2024b) demonstrated that metabolic rates in multiple Antarctic echinoderm species increase at critical lower salinity limits, suggesting that *S. neumayeri* in the current study were near failure. Indeed, mortality reached ~25 % at the time of increased oxygen consumption.

Although metabolic rates across the first 71 days appears to be unaffected by reduced salinity, activity and feeding rates were severely impacted under the lowest salinity in both species. In both species a similar pattern was observed; significant reductions in AC after the first 8–15 days in the low salinity treatment compared to control, with levels remaining low over the first 50 days. This was followed by slight increases in AC towards the later weeks, especially in *O. validus*, however, rates did not return to control levels. Reductions in AC have been demonstrated in both species under acute hypoosmotic stress (Barrett et al., 2024b). For *O. validus* this was at ≤24 ‰ (Barrett et al., 2024b) differing from the current study on day 1 (where AC rates were undistinguishable across treatments), but consistent from day 8, suggesting that the stepwise dilution process provides time for cellular volume regulation to occur. AC rates for *S. neumayeri* under acute low salinity (Barrett et al., 2024b) were consistent with the current study over the first two weeks of treatment. Reduced righting rates are a frequent observation in echinoderm salinity studies upon exposure to reduced salinity [e.g., in echinoids: *S. droebachiensis* (Sabourin and Stickle, 1981; Moura et al., 2023), *E. esculentus* (Barrett et al., 2024a) and asteroids *Lytechinus variegatus* (Lawrence, 1975) and *Luidia clathrata* (Forcucci and Lawrence, 1986)]. Barrett et al. (2024a) and Sabourin and Stickle (1981) demonstrated that echinoderms could not return to control AC levels after chronic low salinity exposure, a finding similar to both Antarctic echinoderms in the current study. The inability to recover righting performance under chronic low salinity implies a reduction in functional capacity and suggests that energy allocation to activity is constrained (Stickle and Diehl, 1987). This is likely due to an increase in homeostatic maintenance as a result of elevated osmoregulatory costs. Even a significant uptake in oxygen consumption at the end of the experimental period in *S. neumayeri* did not correlate with increased AC rates, similar to the findings for *E. esculentus* (Barrett et al., 2024a). This implies a tipping point has been reached where there is a physiological limitation to resisting the continued impact of salinity stress and suggests that continued exposure, at least in *S. neumayeri,* will result in

failure.

Feeding was impacted directly after step-wise dilution to low salinity and failed to recover to comparable control rates in both species. Similar results were observed in the echinod *E. esculentus* following exposure to low salinity over 25 days (Barrett et al., 2024a), while a reduction in feeding in response to low salinity has also been demonstrated in the asteriods *L. hexactis* (Shirley and Stickle, 1982a), *Pisaster ochraceus* (Held and Harley, 2009) and *Luidia clathrata* (Forcucci and Lawrence, 1986). Since feeding in urchins and sea stars involves neuromuscular coordination (Hyman, 1955), the parallel reduction in activity suggests an increasing inability to feed due to decreased movement, as previously observed in echinoderms under hypoosmotic stress. (Barrett et al., 2024a; Forcucci and Lawrence, 1986; Held and Harley, 2009).

The cessation of feeding in *S. neumayeri* under low salinity (24 ‰) was reflected in the cessation of faeces produced. Under medium salinity (29 ‰) there was a reduction in faecal production after the first three weeks, with subsequent recovery to control levels. However, mean faecal production remained consistently lower than the control in the medium animals from day 57, although this was not significant. Changes in faecal production as result of environmental change can reflect the speed of movement of food through the gut as reported in *S. neumayeri* (De Leij et al., 2022). As feeding in the medium salinity treatment was very similar to rates in the control, the lower mass of faeces produced, at least on day 22, suggests either a slower movement of food through the gut or a greater absorbance of organic material as a response to reduced salinity. The subsequent return to levels similar to controls, indicates successful acclimation after the initial adjustment.

In both species at low salinity, there was an increase in feeding after an initial drastic reduction. In *O. validus*, this was observed from day 22; however, it appears that just under half of the animals exhibited this response (Fig. 5), suggesting a degree of acclimation in some individuals. In *S. neumayeri* there was an increase in the last two weeks of the experiment, coinciding with an increase in oxygen consumption compared to control levels. The specific dynamic action of feeding (SDA) which represents the energy expended on ingestion, digestion and absorption of food is often characterised by a rapid rise in metabolic rate following feeding (Secor, 2009). Although measures to minimise the effect of SDA on oxygen consumption were taken by leaving a five day gap between the removal of food and respirometry measurements being made, Antarctic marine ectotherms are known to have post-prandial rises in metabolism that persist for considerably longer than those in warmer species (Peck, 1998). This, in addition to the likely slower movement of food through the gut (as seen in the medium treatment) may go some way to accounting for the observed higher rates of oxygen consumption in *S. neumayeri*. However, the drastic reduction in organic body composition by over one-third in the low salinity urchins suggests that high catabolism – needed to counter the lack of calorie intake while maintaining metabolic rates similar to controls – may ultimately prove fatal over longer periods if the high metabolic rates persist.

4.3. Coelomic fluid osmolality and coelomocytes

In both species coelomic fluid values were consistently isosmotic or slightly hyperosmotic to the treatment salinity, comparable to findings by Barrett et al. (2024b) for both species and the general pattern found in other echinoderms (Barrett et al., 2024a; Diehl, 1986; Vidolin et al., 2007). The maintenance of near-isosmotic osmolality with the treatment salinity throughout the experiment confirms that osmoconforming strategies are present in both species. Exposure to long periods of low salinity had no observable effects on the innate immune cells in *S. neumayeri*, similar to the temperate urchin *E. esculentus* over 25 days at low salinity (Barrett et al., 2024a). However, in the current study comparisons of total coelomocyte concentration were borderline significant between treatments, which may warrant further investigation with a higher sample size. Previous studies have demonstrated that red spherulocytes abundance can increase upon acute heat stress and under exposure to pollution (Branco et al., 2012; Pinsino and Matranga, 2015). Additionally, increases in total coelomocyte concentrations have been observed in echinoids under acute hypo-osmotic stress (*Echinometra lucunter*; Honorato et al., 2017). However, short- and medium-term exposure to low salinity were not assessed in this study and warrant further investigation.

4.4. Hypoosmotic shock

Exposure to hypoosmotic shock after the experimental acclimation period had differing responses between species. In *O. validus*, the reduction in oxygen consumption in the medium and control group mirrored the results observed by Barrett et al. (2024b) for direct immersion from the acclimated salinity to 19 ‰ over 24-h. This likely reflects the suppression of all metabolic activity to devote all available resources to homeostatic maintenance in a bid to prolong survival, while cell volume re-balancing takes place (Sokolova et al., 2012a). In the low salinity animals, the maintenance of oxygen consumption rates similar to their pre-exposure values could mean one of two things. Either, there is sufficient energy available to maintain homeostatic functioning without the need to further suppress metabolic activity; or, suppression of non-essential functions (e.g., growth, reproduction, storage etc.) is accompanied by an increase in homeostatic functions related to cellular volume re-balancing [e.g., metabolism of intracellular free amino acids (Goolish and Burton, 1989)], resulting in no net change in oxygen consumption. For *S. neumayeri,* the reduction in oxygen consumption in the low group could be indicative of the suppression of metabolic activity to prolong survival. Metabolic depression has previously not been recorded in *S. neumayeri* following acute low salinity immersion (Barrett et al., 2024b). However, since oxygen consumption of the low salinity group was elevated prior to the hypoosmotic shock, the values were only suppressed back to the control group levels, or 'normal' levels.

AC levels in the *S. neumayeri* low group were substantially higher than either medium or control animals after hypoosmotic shock within 90 min (see Fig. S2). The ability to mount a righting response in 19 ‰ indicates sufficient energy reserves available. As the osmotic change is substantially less from 24 ‰ to 19 ‰ than from either 29 ‰ or 34.5 ‰, there is likely to be less swelling and consequently less immediate stress. This was also observed in *O. validus* with 4 of 6 animals from the low group able to complete righting, providing a higher mean AC compared to the control (Low = 0.79 ± 0.09; Control = 0.68 ± 0.08, mean ± s.e.m., $n = 6$). Although this comparison was not significant, the fact that righting was still possible indicates sufficient energy was available. The significantly higher AC in the medium group compared to the control implies that, despite the apparent metabolic depression, energy could still be allocated to righting. This matches results observed in the echinoid *E. esculentus* where there was an improved ability to right in animals acclimated to reduced salinity (Barrett et al., 2024a). Perhaps the smaller osmotic change on immersion in 19 ‰ and the resulting reduced osmotic stress, allow sufficient energy to be allocated to righting in the low salinity group, which is considered a critical function (i.e., offering immediate protection from predation) with a high failure threshold (De Leij et al., 2022). The exception occurs when the osmotic gradient is large, likely causing significant swelling and temporarily impeding the ability to right.

4.5. Ecological context and conclusions

Both echinoderm species in this study were collected from Ryder Bay, Adelaide Island, on the WAP at a depth of 10-20 m. Although subtidal salinity (15 m) in the bay has remained relatively stable over the past 20 years [e.g., 33.4 ± 0.4 ‰ (mean ± s.d.) between 2001 and 2021], surface salinity (0 m) occasionally drops to ~28.5 ‰ (Clarke et al., 2022). Closer to the shoreline, and especially in enclosed regions, meltwater runoff can reduce salinity to <12 ‰ (Stockton, 1984; Waller et al., 2006; Vargas-Chacoff et al., 2021). The extensive fjordic systems

of the WAP, have been described as biodiversity hotspots for Antarctica benthic fauna due to high productivity, low impact of meltwater intrusion and low sedimentation disturbance (Grange and Smith, 2013). Increased warming and glacial melting, in addition to increased precipitation in the region (Hellmer et al., 2011; González-Herrero et al., 2024) can substantially increase the extent and thickness of low salinity layers in fjords (Bianchi et al., 2020). This may lead to long-term reductions in salinity in the fjords of the WAP, similar to the low salinities observed in the Arctic, such as those found in inner Greenland fjords where salinity levels can drop below 25 ‰ at depths of up to 10 m (Sejr et al., 2017, 2022). Based on the current study, reductions in salinity to 29 ‰ appear to be well tolerated by both echinoderms species. Successful acclimation to 29 ‰ was evident across all metrics measured by the end of the experimental period, with no differentiation between the medium and control groups. In most cases, this occurred directly after the stepwise dilution, implying that osmotic adjustments were rapid and not detrimental to the energy budget of either species. Similar results were observed in the temperate echinoid *E. esculentus* in a mid-level salinity within their tolerance range (26 ‰ previously acclimatised to 31 ‰), using a comparable experimental set up to that used here, but over a shorter timespan (Barrett et al., 2024a). In the current study, the lower salinity of 24 ‰ was clearly tolerated by both species, however, acclimation was absent in both. *Odontaster validus* appeared the more tolerant of the two at 24 ‰, based on the return to feeding in some animals, improvement in righting and stable oxygen consumption, which is consistent with it having a lower 24-h low salinity threshold compared to *S. neumayeri* (Barrett et al., 2024b). However, it was apparent that the increased rate of feeding in *O. validus* from day 22, was only exhibited by just under half the sea stars at 24 ‰ (see Fig. 5). It is unknown whether a return to previous control levels of feeding and righting ability would have been possible if the experiment had continued for longer, but it seems likely that only a subset of the population would have achieved this. From an evolutionary perspective, if a significant proportion of a population has the capacity to acclimatise, selection for these traits under increased freshening may enable longer term survival, especially for species with very large population sizes such as *O. validus* and *S. neumayeri*. However, caution should be applied to evaluating surviving individuals that have not displayed full physiological acclimation, especially in polar marine ectotherms, where innate low metabolic rates can mask other critical changes (Peck et al., 2010).

For both species, persistent coastal freshening may induce migration to deeper, higher salinity waters, relinquishing the need for acclimation capacity. Considering both are keystone species, changes in community structure as a result of migration may disrupt ecosystem interactions and food web dynamics (Sporta Caputi et al., 2024). However, righting ability and therefore activity levels were substantially reduced under low salinity in the current study, indicating that migration abilities may still depend on the capacity to acclimate.

Future research on longer-term survival, growth and reproductive capabilities of Antarctic echinoderms under low salinity would now be beneficial. However, this would be logistically challenging, requiring multi-year, multi-generation experimental systems to incorporate full lifecycles (e.g., Dupont et al., 2013; Suckling et al., 2015). A fuller picture of acclimation could be provided by examining genetic and biochemical level changes utilising different 'omics approaches such as RNA-seq and metabolomic analysis, which have yet to be examined in Antarctic echinoderms. Attention should also focus on how low salinity interacts with other climate change stressors—such as temperature, pH, and oxygen saturation—which may have additive or synergistic effects on marine organisms (Gunderson et al., 2016; Barrett et al., 2022). Multiple stressor studies on Antarctic echinoderms have yet to include low salinity, which has proven to significantly modify fitness in other Antarctic species when combined with other stressors (Navarro et al., 2020; Park et al., 2020).

What may be an advantage to Antarctic echinoderms in tolerating long durations of low salinity compared to temperate species, is their ability to maintain low metabolic rates, with *S. neumayeri* having one of the lowest on record for a regular echinoid (Brockington, 2001). Indeed, the temperate echinoid *E. esculentus*, which has a resting metabolic rate at least three times greater than *S. neumayeri* displayed elevated oxygen consumption after only five days at low salinity compared to controls (Barrett et al., 2024a). The low temperatures experienced in the Antarctic marine environment enable these echinoderms to maintain low metabolic rates under reduced salinity for a substantial period (i.e., > 2.5 months in the current experiment). Low metabolic rates require lower calorific input from feeding, and instead can be sustained through metabolising stored reserves, as demonstrated by the loss of mass. These adaptations are well known to be advantageous in the Antarctic winter environment when food is scarce. For example *S. neumayeri* can survive for up to six months without feeding, in part due to their very low metabolic rate (Brockington, 2001). Under long-term reduced salinity, this ability could allow sufficient time for a return to better salinity conditions or greater time for acclimation adjustments.

In summary, two of the most abundant macroinvertebrates in Antarctic benthic ecosystems *O. validus* and *S. neumayeri*, demonstrate long-term tolerance to the reduced salinities that may occur due to climate change-induced freshening. Both species successfully acclimated to 29 ‰, but at 24 ‰, while survival remained relatively high, neither species had fully acclimated after >2.5 months. This suggests that 24 ‰ is near the physiological limits of both species, and prolonged exposure could lead to population decline which may impact ecosystem dynamics. Nevertheless, the inherently low metabolic rates of Antarctic echinoderms may allow them to endure extended seasonal low salinity, which may be advantageous under a warming climate.

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CRediT authorship contribution statement

Nicholas J. Barrett: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Elizabeth M. Harper:** Writing – review & editing, Supervision, Conceptualization. **Lloyd S. Peck:** Writing – review & editing, Supervision, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data is publicly available here: https://github.com/njb202/Antarctic_echinoderm_acclimation

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