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The distribution and behaviour of *Patiriella mortenseni* and *P. regularis* in the extreme hyposaline conditions of the Southern New Zealand Fiords

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Abstract

Echinoderms are a stenohaline phylum, however some species can tolerate varying degrees of exposure to hyposaline conditions. These exposures typically are limited in duration to a few hours to no more than a day or two, and hyposalinity levels range from ~8‰ to <30‰. The unique climatological and hydrographic conditions in Fiordland of southwest New Zealand produce a near-freshwater layer (Low Salinity Layer = LSL) on top of marine (34‰) water. The relative depth of the LSL varies both tidally and with rainfall. This halo-boundary presents a particularly stressful osmotic challenge to organisms found in shallow water. A series of transects in Doubtful Sound and the more northern Milford Sound quantified differences in density and bathymetric distribution of two species of seastars. The surveys revealed that *Patiriella mortenseni* occurs primarily below the LSL whereas *Patiriella regularis* is found well within the range of the LSL. Video observations of *P. regularis* over a rising tide were correlated with salinity measurements and showed a strong correlation between increased salinity and general vertical displacement upwards of the seastars observed moving. Activity coefficients (AC) were used to estimate the degree of neuro-muscular coordination and stress levels of these seastars in two hyposalinity experiments. Reciprocal transplants showed that *P. regularis* performed equally well in deeper, marine-salinity waters, as in shallow hyposalinity conditions, whereas *P. mortenseni* showed significantly impaired performance in the hyposaline conditions. These performance estimates were refined in a second experiment by quantifying AC of both species in a seawater dilution series. To rule out the possibility of heretofore unknown osmoregulatory capacity, we exposed both species to 0‰, 5‰, 15‰, 25‰, and 34‰ and recorded the osmolarity of the body fluid after 24 h. *P. mortenseni* died at salinities below 25‰. In contrast, *P. regularis* survived and the body fluid was nearly isotonic with the surrounding water. Two final experiments established the hyposalinity-tolerance limits of *P. regularis*. In a righting-time (RT) experiment we exposed *P. regularis* to hyposaline water of 5‰ for: 12, 24, 36, 48, 60, 84, and 133 h and recorded righting times immediately after re-immersing them in sea water. All individuals survived and the mean RT ranged from 18 min to 27 h (control group = 1.6 min). Finally, a lethal-exposure experiment to 0‰ (deionized water) confirmed that *P. regularis* can withstand far greater levels of extreme hyposalinity than has been reported for any other echinoderm.

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1. Introduction

The fiords of south western New Zealand are notable for their topography of steep cliffs dropping to deep water with a sharp halocline separating the near-freshwater stratum on the surface from the marine waters below. This low salinity layer

(LSL) is of variable thickness from <1 m to greater than 12 m depth (Gibbs, 2001). The depth of this layer varies both spatially and temporally, depending on distance from the entrance of the fiord, rainfall in the catchment, or with tidal rise and fall. The layer is often particularly deep in Doubtful Sound because of additional input of fresh water from a hydroelectric power dam discharging into Deep Cove at the head of the fiord.

The submarine cliffs throughout the fiords are characterized by a low diversity of species within depths subjected to lower salinity (Boyle et al., 2001) but a rich and highly diverse assemblage of organisms occurs below this layer (Grange et al.,

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1981; Smith and Witman, 1999). Echinoderms, particularly the echinoid *Evechinus chloroticus* (Val.) and the large seastar *Coscinasterias muricata* (Verrill, 1867) are common at shallow depths immediately beneath the LSL and their feeding activities have an important role in structuring the shallow water communities to depths of 5–20 m (Witman and Grange, 1998; Villouta et al., 2001). Two other seastars are common in the shallow subtidal of fiordland. The recently described *Patiriella mortenseni* (O'Loughlin et al., 2002), is sympatric with *E. chloroticus* and *C. muricata* below the LSL. In shallower water, well within the LSL however, the small cushion star *Patiriella regularis* (Verrill, 1867) is very common throughout Doubtful and Milford Sounds.

Echinodermata is the only major animal phylum restricted to marine habitats. Of the approximately 7000 species of echinoderms (Brusca and Brusca, 2003), <1% are found in habitats where salinities fluctuate below the levels found in normal seawater (see Table 1 in Stickle and Diehl, 1987). Echinoderms lack specialized excretory organs and the capacity for osmoregulation (Hyman, 1955; Binyon, 1966; Binyon, 1972a, Shumway, 1977). A few species are infrequently found in brackish waters but show physiological and neuro-muscular indications of stress under hyposaline conditions (Binyon, 1972b; Diehl, 1986; Forcucci and Lawrence, 1986; Shirley and Stickle, 1982a,b; Stickle and Diehl, 1987; Watts and Lawrence 1990). The reported exposures to hyposaline conditions for 38 species of echinoderms range between 8 and 29‰ (Stickle and Diehl, 1987). These exposures tend to be seasonally episodic with durations spanning a few hours to days, but rarely exceed one week. The species with the greatest reported tolerance for hyposalinity is the amphiuroid brittlestar, *Ophiophragmus filigraneus* (Turner and

Meyer, 1980). Although this species is the only echinoderm restricted to estuarine habitats and tolerates salinities as low as 8‰ (Thomas, 1961), its respiratory, excretory, and regenerative performances are significantly better under marine salinity conditions (Talbot and Lawrence, 2002).

The sharp salinity discontinuity in the fiords of southwest New Zealand presents an exceptional osmotic challenge to shallow-water organisms, but especially to osmoconforming echinoderms. The discovery of *P. regularis* living well within this layer raises intriguing questions in regard to its ability to survive in this habitat. *P. regularis* may be tolerating conditions of reduced salinity or migrating out of the LSL when the tide ebbs.

In this study we describe the distribution patterns of both *P. regularis* and its congener *P. mortenseni* in Doubtful and Milford Sounds, and examine the tolerance of both species to conditions of lowered salinity.

2. Materials and methods

2.1. Field sites and depth distribution

Bathymetric distribution data were collected from a series of depth-transects in two fiords (Fig. 1): Doubtful Sound (45 15'S 166 51'E) and Milford Sound (44 34'S 167 48'E). One site (14) was in Thompson Sound which has a separate northern opening to the West coast but a south-eastern confluence with Doubtful Sound. In each transect, photographs were taken of a 0.25 m² quadrant placed down the near-vertical rock walls every 2 m starting at 0 m. Individual seastars from 7 replicate transects at each of 10 sites in Doubtful Sound, and 4 sites in Milford Sound, were identified and counted from the photographs (Fig. 1).

2.2. Experimental animals

Both *P. regularis* and *P. mortenseni* were collected from 4–15 m in Doubtful Sound. Individuals were held in their ambient seawater in sealed buckets when transported through the LSL to the surface. Seastars used in the osmolarity, mortality, and extended-hyposalinity exposure experiments, were transported in full salinity seawater to the Portobello Marine Laboratory. These seastars were held in tanks provided with running, filtered seawater from Otago Harbor (34‰) until used in experimental trials.

2.3. Time-lapse video

To monitor movements of *P. regularis* within the LSL during salinity-tidal fluxes a video camera was set up on July 26, 2005 at Espinosa Point in Doubtful Sound (Fig. 1, 45 19' 36 S 167 00' 52 E), a site where seastars were particularly abundant (42 per 0.25 m² sd 11.18, $n=5$). The camera was positioned toward a steeply sloping rock face at a depth of 5.5 m in a position where movements of seastars were mainly vertical i.e. any movement was likely to subject them to higher or lower salinity. The camera was set to record on time lapse, 2 s for every

Table 1
Density measures. Site numbers refer to the map in Fig. 1

Site	<i>P. regularis</i>		<i>P. mortenseni</i>	
	Density (# m ⁻²)	<i>N</i>	Density (m ⁻²)	<i>N</i>
<i>Milford Sound</i>				
1	2.67	28	2.38	25
2	0.00	0	0.00	0
3	0.00	0	0.00	0
4	1.52	17	1.05	11
<i>Doubtful Sound</i>				
5	2.38	25	0.38	6
6	0.38	4	0.89	14
7	0.67	7	1.51	23
8	1.52	16	1.14	18
9	8.19	86	4.44	70
10	0.29	3	0.25	4
11	0.00	0	0.25	4
12	8.59	88	0.20	3
13	0.10	1	0.00	0
14	2.86	30	1.84	29

Density estimates based on counts of seastars from the 0.25 m² photo-quadrants and total area for each species was based only on the depths where they were found. *Patiriella regularis* was found between 2–14 m and 4–14 m in Milford and Doubtful Sounds respectively. *Patiriella mortenseni* was found between 4 and 20 m in both sites. Densities were adjusted for missing photo-quadrants: Site 7 (one each at 18 and 20 m) and Site 12 (one each at 12 and 20 m).

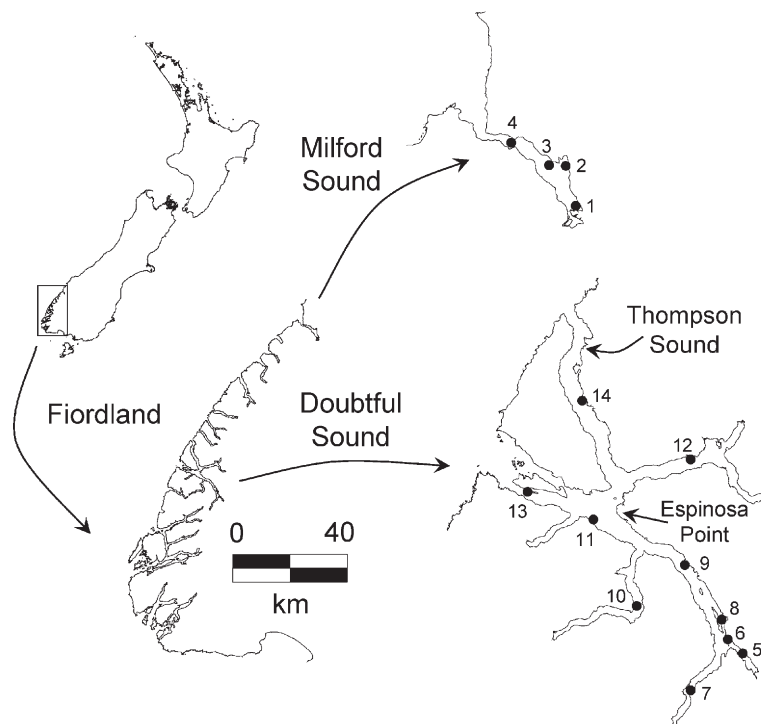


Fig. 1. Study areas. The Fiordland National Park along the southwest coast of New Zealand consists of 14 glacially carved fiords and includes our two sampling areas: Doubtful Sound and Milford Sound. Bathymetric sampling sites are numbered and plotted in the fiords. The reciprocal transplant experiment was conducted at Espinosa Point.

minute. Battery life in the cold waters allowed continuous recording for up to 4 h before the camera had to be retrieved and the battery replaced. A continuously recording (1 reading per second) conductivity sensor (SeaBird SBE 25) was placed to the side and within the field of view of the video and the stainless steel frame held in position with large stones. A meter stick was placed vertically along the wall in the field of view for several minutes of the recording to allow calibration of vertical distances moved. The positioning of the equipment allowed the simultaneous correlation of seastar movement with changes in salinity. The camera was deployed at 09:26 and retrieved at 15:04 (predicted low tide 0.3 m at 09:25; high water 3.1 m at 15:46).

The video tape was transferred to a computer file and viewed on a 21 inch-flatscreen monitor. A centimeter scale grid was created on the monitor using the captured image of the meter stick from the video. Using this grid, individual seastars were tracked by plotting their positions on the grid every 30 min. Vertical displacements of individuals that moved for each time interval were calculated by subtracting the vertical position at the start of the interval from vertical position at the end of the interval.

2.4. Activity coefficient and righting experiments

We performed field and laboratory righting experiments to assess the performance of neuro-muscular coordination and behaviour of the two species in low salinities. Seastars are normally oriented with the tube feet of the oral surface attached to the substratum. Overturning an individual so that the oral

surface faces up and the tube feet are not in contact with the substratum elicits a righting response: the seastar coordinates movement of its tube feet to contact the substratum and adjusts the position of the arms to turn over into a normal orientation. The time it takes to complete the righting response is measured as an Activity Coefficient ($AC = 1000 \times s^{-1}$) and indicates levels of stress and well-being (Lawrence and Cowell, 1996). Relatively higher AC values (longer righting times) are associated with higher levels of stress. In righting trials, individual seastars were placed oral surface up in a 500 ml container of seawater of appropriate salinity for a treatment. The maximum time allowed to right was 30 min which equals a minimum AC of 0.56 (1000/1800 s). Righting was complete when the oral surfaces of all five distal tips of the arms were in contact with the substratum.

2.5. Reciprocal transplant experiment

On July 7, 2005, we performed a 24-hour reciprocal transplant experiment at Espinosa Point in Doubtful Sound (Fig. 1) moving *P. mortensenii* into the shallow low-salinity zone (6 m) and *P. regularis* into deeper marine water (12 m). Controls of each species were handled in the same manner – collected off the substrate, held in mesh bags, and moved an appropriate distance but at the same depth where they were collected. Each of the four experimental groups had three mesh bags (replicates) and each replicate contained 5 seastars (total seastars=60). After 24 h the seastars were collected and kept in the ambient seawater from the experimental depth where they were held and immediately evaluated for a righting response.

2.6. Seawater dilution series

To estimate performance under hyposaline conditions, we evaluated AC for a salinity-dilution series (34‰, 26‰, 17‰, and 9‰ which are 100%, 75%, 50%, and 25% strength seawater). Seastars were collected from the field (6 m for *P. regularis* and 12 m depth for *P. mortenseni*) and held in full-strength seawater until righting times and AC levels were assessed within 24 h ($n=11$ individual seastars of each species for each treatment, total=88 individuals). For each salinity trial, seastars were removed from the full-strength seawater containers and immediately immersed in the treatment salinity, oral surface up.

2.7. Osmolarity measures

To assess whether hyposalinity was differentially affecting the perivisceral fluids of the two species or if there was any evidence of osmoregulation, we conducted a 2×5 factorial experiment to measure body fluid osmolarity after a 24 hour hyposalinity exposure. The first factor was species (two levels – *P. regularis* and *P. mortenseni*) and the second factor was salinity with five levels (34‰, 25‰, 15‰, 5‰, 0‰). Replicate 500 ml containers held individual seastars with seawater at ambient temperature (14 °C) and of the appropriate salinity ($n=5$ for each combination of species and salinity level, total=50 individuals). After 24 h the seastars were dissected and samples of perivisceral fluid were extracted. A Wescor 5500 Vapour Pressure osmometer was used to estimate the osmolarity of these samples.

2.8. Extended exposure experiment

During dives in Doubtful Sound we consistently observed *P. regularis* within the LSL and measured salinities of 3–5‰ at several sites in the immediate proximity of seastars. Therefore we used 5‰ in an extended-hyposalinity experiment over the course of six days (maximum exposure level). Seven sets of *P. regularis* were placed in a series of replicated ($n=5$ for each set) 500 ml containers in 5‰ seawater at ambient temperature (14 °C). Water changes in each replicate occurred every 24 h. Every 12 h for the first 2.5 days, and then again at day 4 and day 5, a set was placed in full-strength salinity seawater (34‰). Each seastar was positioned oral side up in a separate container and these containers were placed under a time-lapse video recorder. The time taken for animals to complete the righting response and commence moving within the container was determined from the videotape recordings. A single set ($n=5$) of controls was set up at 34‰ seawater and used to determine baseline AC at each time interval.

2.9. Deionized water experiment

In the final experiment we used deionized water to estimate the maximum hyposalinity tolerance level of *P. regularis*. Four sets of replicate ($n=5$ for each set) *P. regularis* were placed in 500 ml containers of deionized water. These sets were held in a

water bath at ambient seawater temperature (14 °C). After 24, 48, 72 and 96 h, a set of seastars was placed oral side up in full salinity running seawater. Survival (righted seastar exhibiting normal movement and morphology) was determined for each of the 24 hour periods of exposure to extreme hyposalinity.

2.10. Statistics

Tests are two-tail and $p < 0.05$ was considered significant. A χ^2 with a Yates correction factor was used to compare geographic and bathymetric distributions. A Mann–Whitney test was used to compare mean depths between species, and AC between depths within species in the reciprocal transplant experiment. Simple linear regression was used to assess vertical displacement changed as a function of salinity in the time-lapse video observations. A Kruskal–Wallis test was used to compare AC within each species in the salinity-dilution series. A factorial ANOVA was used to compare body fluid osmolarities and ANOVA was used to compare righting times in the 5‰ extended hyposalinity exposures.

3. Results

3.1. Distribution

Densities (mean abundance m^{-2} for all depths) of *P. regularis* and *P. mortenseni* are shown in Table 1. In Milford Sound both species were only found at sites 1 and 4 at the head and entrance to the Sound however at Doubtful Sound they are distributed throughout the Sound with no obvious pattern of abundance. *P. regularis* was generally more abundant than *P. mortenseni* and both species are less common towards the entrance of Doubtful Sound.

The depth-specific counts of the two species of seastars were combined within each fiord across the different replicate transects (Fig. 2). The mean depths for *P. regularis* were 4.8 ± 2.3 m and 6.9 ± 1.5 m, and for *P. mortenseni* 9.8 ± 3.3 m and 10.2 ± 3.4 m, in Milford and Doubtful Sounds respectively (these and all other \pm variance estimates are sd). In both Milford ($\chi^2=47.3$, $p < 0.001$) and Doubtful ($\chi^2=169.4$, $p < 0.001$) Sounds there were significant differences in the depth distributions of the two species. Although there was some overlap in distribution, in both fiords *P. mortenseni* occurred below *P. regularis*. There was no significant difference in the depth distribution of *P. mortenseni* between the two fiords ($\chi^2=3.3$, $p=0.92$). *P. regularis* were however, significantly deeper in Doubtful than in Milford Sound and ($\chi^2=131.0$, $p < 0.001$).

3.2. Time-lapse video

The surface area of the rock wall where *P. regularis* were observed with the time-lapse video was approximately $1.2 m^2$. Seastars as small as 1.5 cm (arm length) were both distinguishable and traceable on the recording. The positions of a minimum of 20 individuals were plotted every 30 min; however, the recording was stopped at 12:45 to change the battery and this time interval was 19 min (Fig. 3, eight individuals were found

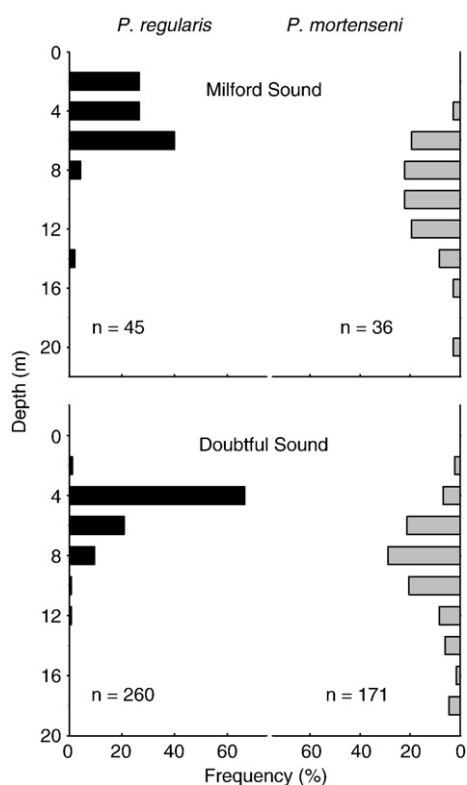


Fig. 2. Bathymetric frequency distributions. The black bars represent *P. regularis* (left axes) and the shaded bars *P. mortensenii* (right axes). Counts of seastars in photographs of 0.25 m⁻² quadrants were from 10 sites in Doubtful Sound and 4 sites in Milford Sound. Each site had 7 bathymetric transects with quadrants placed at every 2 m depth.

moving during this interval). There was a gap of 15 min for the battery change and the positions of four individuals that could be traced moving from 12:45 were plotted at 13:01 – a

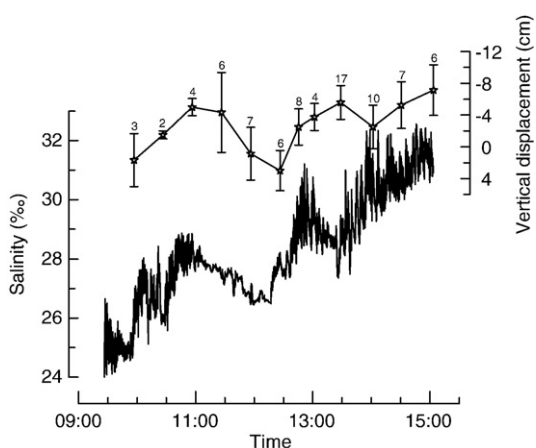


Fig. 3. Salinity fluctuation at Espinosa Point on July 26, 2005 on an incoming tide (left y-axis, bottom trace) and the simultaneous mean vertical displacement of *P. regularis* observed with the time-lapse video camera (right y-axis, top line). The conductivity sensor was deployed at 5.5 m depth in the field of view of the video recorder. The sensor recorded salinity $1 \times s^{-1}$. The equipment was set up, in final position, and functional at 09:26, and continuously recorded for approximately 5.5 h until 15:04. The number of individual seastars observed changing their vertical position during a time interval appears above the mean (± 1 standard error) of their displacement.

16 minute interval (Fig. 3). The remaining intervals to the end of the tape were 30 min.

The number of seastars observed moving during an interval varied from 2 out of 21 (09:56–10:26) to 17 out of 24 (13:01–13:28) with an average of $31\% \pm 16\%$ for all intervals. Particularly during the morning, low-salinity periods, few individuals were found changing positions (3 of 20, 2 of 21, and 4 of 23 respectively for the first three intervals). It was not possible to always keep track of the same individuals between all intervals. For example, some seastars started out within the frame of view but moved out of it during the course of the filming. Vertical displacement data were only gathered on individuals that were observable within at least two consecutive periods. Three individuals did not move during the 5.5 hour period.

In addition to the overall increase in salinity with the rising tide, there are fine-scale fluctuations (Fig. 3). The minimum salinity reading was 24.07‰ at 9:30:24, and the maximum 32.56‰ at 14:47:35 (range=8.49‰, a 35% change over the time course). In the video mixing of low-salinity and high-salinity water was evident as blurred clips due to the different refractive properties of the water which sometimes interfered with tracking individuals. A regression of the mean salinity during an interval (independent variable) and mean vertical displacement was significant ($F_{1,10}=13.55, p=0.004, r=0.76$).

3.3. Reciprocal transplant experiment

Salinity varied (with the rising and falling tide) at the 6 m site over the course of the reciprocal transplant experiment and was 22‰ at the conclusion. The salinity at the 12 m site remained constant (34‰). Separate analyses on each species showed that there was no difference in righting response for *P. regularis* held at the two different depth-salinity regimes ($Z=1.72; p=0.09$) whereas *P. mortensenii* showed significantly lower AC levels ($Z=2.94; p=0.03$) at the lower-salinity shallow depth (Fig. 4).

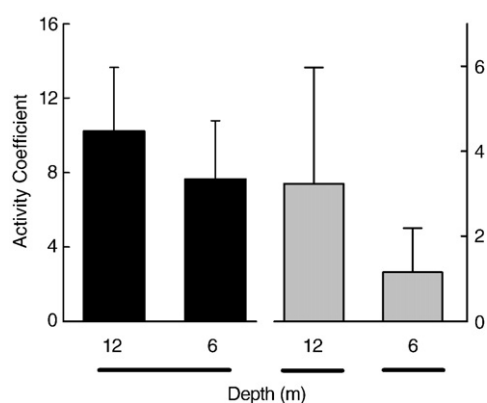


Fig. 4. Reciprocal transplant experiment. Means \pm sd of AC after 24 h ($n=15$ for each group). Black bars represent *P. regularis* (left axis) and shaded bars *P. mortensenii* (right axis, note different scale). Horizontal lines under the x-axis underscore groups that are not significantly different. Separate analyses on each species showed no difference in AC between control (6 m) and transplanted (12 m) *P. regularis* whereas there was a significant difference between control (12 m) and transplanted (6 m) *P. mortensenii*.

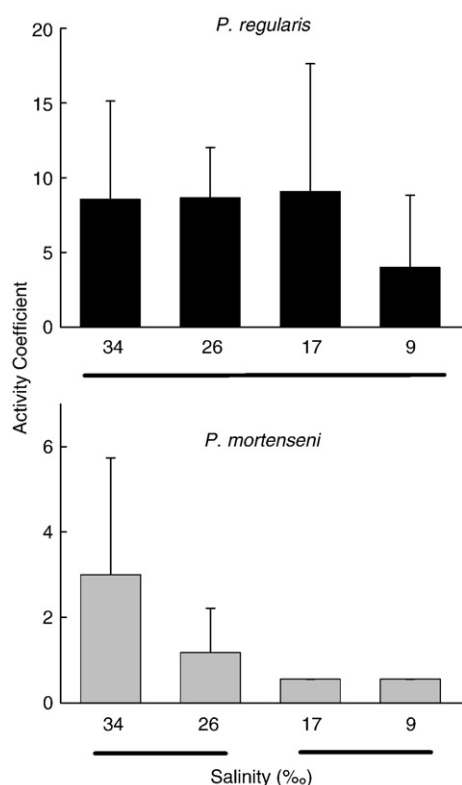


Fig. 5. Seawater dilution series. Means \pm sd ($n=11$ for each group) of AC under different salinities for *P. regularis* (upper graph) and *P. mortenseni* (lower graph). Horizontal lines under the x-axis underscore groups that are not significantly different. There were no differences in AC for *P. regularis* among any of the salinity dilutions. There were differences in AC for *P. mortenseni* between the 34‰–26‰ treatments and the 17‰–9‰ treatments. None of the individuals in the later two treatments righted after 30 min (all values=the minimum, hence no variation or error bars).

3.4. Seawater dilution series

In the salinity-dilution series experiment there was a different response between the two species. There was no significant difference ($F_{3,40}=2.28$, $p=0.09$) in AC levels among salinity treatments for *P. regularis* (Fig. 5), however there was a sharp separation in the AC levels for *P. mortenseni* between 26‰ and 17‰ (Fig. 5). There was no difference between the 34‰ and 26‰ treatment levels, but both of these levels were significantly different from the 17‰ and 9‰ levels ($F_{3,40}=9.84$, $p<0.0001$). None of the seastars at the lower two treatments successfully righted.

3.5. Osmolarity measures

After the exposure to all the hyposalinity levels in the osmolarity experiment, all *P. regularis* were alive whereas only the *P. mortenseni* in the >15 ‰ treatments survived. There was a significant interaction ($F_{4,40}=16.21$, $p<0.001$) between species and salinity level. In all salinities with levels less than full-strength seawater, there were no significant differences in the osmolarity of the body fluid between species (Fig. 6). At all salinity levels except deionized water (0‰) and 5‰ there were

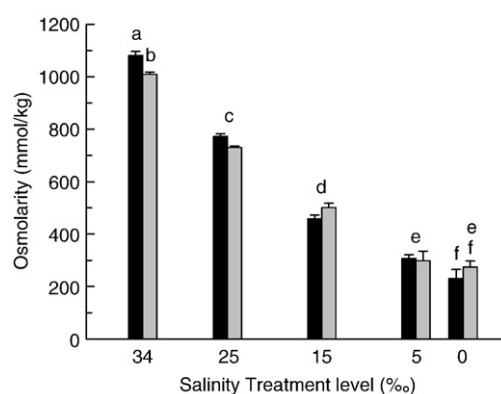


Fig. 6. Body fluid osmolarity 2 \times 5 ANOVA. Black bars=*P. regularis* and shaded bars=*P. mortenseni* (means \pm sd, $n=5$ for each combination of species and salinity level). There was a significant interaction and letters indicate groups that are significantly different.

significant differences in body fluid osmolarity among salinity treatments.

3.6. Extended exposure experiment

All *P. regularis* survived all levels of exposure to 5‰ in the extended-hyposalinity experiment, even the 133 hour level (6 days). There was a pronounced inhibition of coordination indicated by the positive correlation with exposure and time to right (Fig. 7). There was no significant difference in time to right among the control group ($F_{4,20}=1.87$, $p=0.16$) whereas there was a significant difference in righting time among the 5‰ exposure time groups ($F_{6,28}=38.7$, $p<0.0001$).

3.7. Deionized water experiment

When subjected to deionized water, all seastars (5 for each set) at 24, 48, and 72 h survived, however whereas the 24 and 48 hour treatments had fully recovered by 24 h, the animals held

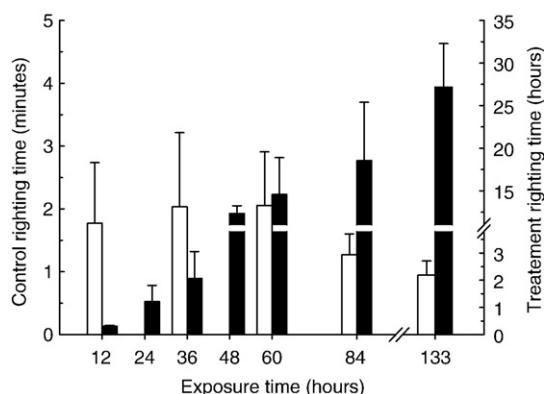


Fig. 7. *Patriella regularis* extended hyposalinity exposure. Means \pm 1 sd ($n=5$) of righting time after 5‰ exposures for control (white bars, left axis) and treatment (black bars, right axis – note differences in units and scales). The same group of controls was used throughout the 6 day long study. All seastars survived all of the treatments. There was no difference in righting times among the controls; however, there were significantly longer righting times with increased hyposalinity exposure.

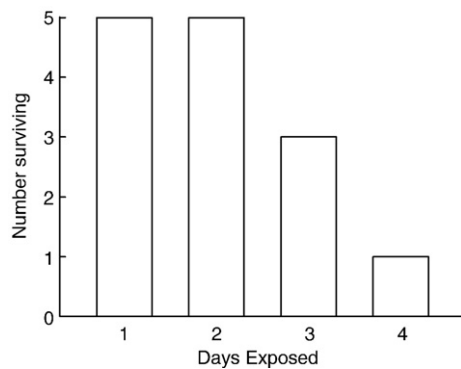


Fig. 8. Deionized water exposure. For each of the four days of exposure there were 5 *P. regularis*. All 10 seastars survived the first 3 days, and 1 survived 4 days.

for 72 h took longer to recover. Only one individual from the 4-day exposure survived (Fig. 8).

4. Discussion

The prevailing Westerlies from the Tasman Sea drive the climatological setting in Fiordland (Sansom, 1984). The combination of local wind stress and freshwater runoff from annual rainfall of $\sim 7 \text{ m yr}^{-1}$ maintains the LSL in the glacially carved, steep-sided fiords (Gibbs et al., 2000). The depth and extent of mixing of the surface LSL with the marine layer below varies with position from the head to the mouth of the fiords (Stanton and Pickard, 1981) being greater near the head and the mixing increases near the mouth. Temporal variations in precipitation also have a strong influence on the depth of the LSL. The Manapouri hydroelectric power station (in operation since 1969) augments the freshwater input into Deep Cove at the head of Doubtful Sound (Fig. 1) and results in a deeper and more stable LSL (Gibbs et al., 2000; Gibbs, 2001). The increased freshwater input has altered distribution patterns of some taxa, displacing some marine species closer to the mouth of the fiord (Boyle et al., 2001; Tallis et al., 2004).

Both *P. mortenseni* and *P. regularis* occur throughout Doubtful, Thompson and Milford Sounds. Although there is no clear association between density and position in the fiords (Table 1), at most sites *P. regularis* is more abundant than *P. mortenseni* and both species are less common towards the entrance of Doubtful Sound.

P. regularis occurs in shallower depths than *P. mortenseni* in both Milford and Doubtful Sounds. The difference in bathymetric distribution of *P. regularis* between fiords is likely the consequence of the thinner LSL in Milford Sound (Pers. Obs). In both sounds *P. regularis* is well within the depth range of reduced salinity seawater. The reported maximum depth of the LSL in Doubtful Sound is 12–14 m (Gibbs, 2001) which exceeds the bathymetric distribution of almost all *P. regularis* and most of the *P. mortenseni* populations (Fig. 2).

The deeper-dwelling seastar, *P. mortenseni*, is found below the 3–7 m depths where the LSL occurs for extended periods (Fig. 2; Gibbs, 2001). Although the bathymetric range of this species lies within the reported range of the LSL in the fiords,

we have never observed *P. mortenseni* within reduced salinity seawater. When the LSL is particularly deep, for example following prolonged periods of heavy rain, *P. mortenseni* are, however, often very close to the fresh/seawater interface.

Video observations of *P. regularis* over the rising tide showed clear evidence of a significant proportion of the population of seastars moving up several centimeters in response to the increase in salinity that accompanied the rising tide although this response lagged behind increases in salinity by approximately 20 min (Fig. 3). There were also some seastars that did not move at all during the period of observations and some animals moved downwards. These observations took place during a period of relatively dry weather, and the lowest salinity to which seastars were subjected was 24–25‰. Spot checks with a salinometer subsequent to these observations, after and during periods of heavy rain at Espinosa Point have shown *P. regularis* to be bathed in water as low as 3.7‰ for periods of at least 30 min, with no observed movement out of these potentially highly stressful conditions. It is likely that under these extreme conditions many seastars have stopped moving and are waiting for higher salinity to return. It is interesting that seastars tolerate the conditions rather than move into higher salinities. As most of the population is living on vertical or near-vertical rock faces the simple response of releasing hold of the substratum would result in seastars rapidly drifting down into full salinity seawater.

Over the 4–5 day period during which these field experiments were conducted, it was only possible to make video observations over the period of a rising tide. It would have been interesting to make similar observations while the tide was falling, however this phase of the tidal cycle occurred during the very early morning or evening when light levels below the LSL were too low for video recording.

P. regularis held in mesh bags for 24 h at a depth of 6 m where the ambient salinity was 24‰, showed no obvious loss of performance of neuro-muscular coordination and behaviour when the righting response was assessed. In reciprocal treatments *P. mortenseni* showed significantly lower AC levels in hyposaline conditions (Fig. 4). The results of the salinity-dilution laboratory experiment provided further evidence that the activity of *P. regularis* was not significantly affected by salinities down to 9‰ (Fig. 5). In contrast, *P. mortenseni* showed clear evidence of stress at all hyposaline levels.

The osmolarity of perivisceral fluid of seastars held in a dilution series changed in parallel with the bathing solution (Fig. 6). Stickle and Ahokas (1974) also found that when the salinity in tanks holding *Pisaster ochraceus* was gradually reduced to 10 ppt and then raised over a 10 hour period, the osmolality of the perivisceral fluid changed in parallel to the bathing solution. Shumway (1977) obtained similar results in an experiment where 4 species of seastars were held in a seawater dilution series of 100, 90, 80 and 50%. Fluid and ion concentrations of all species followed the external medium and tissue water content varied inversely with salinity. These results indicated that there was no ionic or osmotic regulation in these species. Studies on other species suggest that there may be some level of intracellular ion regulation in response to hyposaline

conditions (Ellington and Lawrence, 1974; Stickle and De-moux, 1976; Diehl and Lawrence, 1985; Bishop et al., 1994).

As in earlier studies on seastars, our results indicate that neither *P. mortenseni* nor *P. regularis* is osmoregulating in hyposaline conditions. Thus the ability of *P. regularis* to tolerate the extreme hyposaline conditions in the laboratory and in the field is due to processes other than osmotic control of internal body fluids. The statistically significant interaction between factors (species and salinity level) in this experiment was possibly the result of cell plasmolysis in *P. mortenseni* at salinities of 15‰ and below. None of the *P. mortenseni* survived the 24 h at salinities <15‰ and massive plasmolysis could account for the slightly elevated osmolarities of the perivisceral fluid below this level (Fig. 6).

When subjected to extended periods (up to 6 days) of exposure to 5‰ seawater, all *P. regularis* survived all levels of exposure, although there was a pronounced inhibition of coordination indicated by the positive correlation with exposure and righting time. While physiologically tolerating these extreme conditions, we also observed a pattern of morphological and behavioral changes during laboratory exposures to 5‰ water. When an individual seastar is placed in hyposaline conditions, within minutes it ceases movement and retracts its arms towards the central disc while expanding the disc along the oral–aboral axis. The seastars remain motionless (in this “puffed-up” state) for days until salinity levels return to marine conditions. This response probably reflects the extreme duress levels and may serve to conserve energy and/or minimize internal osmotic changes.

We found that *P. regularis* is able to survive for up to 4 days in water (0‰). This level of hyposalinity tolerance exhibited by *P. regularis* is unprecedented for echinoderms (Stickle and Diehl, 1987). No previous studies that explored hyposalinity tolerances in this phylum used deionized water, and *P. regularis* is able to recover from exposure to this extreme treatment even after days of immersion. Shumway (1977) found that the lower lethal limit of 4 species of seastars could be lowered if the salinity was lowered slowly rather than changed rapidly. In all of our experiments we changed salinities suddenly rather than gradually acclimating animals. In the fiords the boundary separating the LSL from the marine layer is distinct and exposures change rapidly with tidal and precipitation fluctuations.

The likely physiological costs to living in such a highly stressful environment include reduced respiration and growth (Talbot and Lawrence, 2002) or reproductive output. There is likely to be some strong compensation for these costs such as access to unexploited food or refuge from a suite of deeper-dwelling predators such as another large predatory seastars *C. muricata* which occurs in shallow water immediately below the LSL (Grange et al., 1981).

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