

# Temperature and salinity: two climate change stressors affecting early development of the New Zealand sea urchin *Evechinus chloroticus*

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**Abstract** Temperature and salinity are important environmental factors affecting the normal functioning of marine animals, particularly animals such as sea urchins living in shallow waters and tide pools. Here, we evaluated the effect of different combinations of temperature and salinity on early embryos of the endemic New Zealand sea urchin *Evechinus chloroticus*. Animals were collected at Matheson's Bay (36°18'17"S; 174°47'51"E) in north-eastern New Zealand in February 2013. Embryos were exposed to five salinities (29, 31, 34, 35 and 37 ppt) and two temperatures (18 and 21 °C) during the first 24 h of development. Low salinity (29 ppt) affected all parameters (fertilization, development rate, gastrulation and normal development), with ca. 50 % of embryos surviving at 29 ppt, whereas seawater temperature only affected development rate and gastrulation. An increase in temperature from 18 to 21 °C minimized the negative effect of low salinity ( $\leq 31$  ppt) on development rate and gastrulation of *E. chloroticus*. Overall, the results of this study suggest that early embryos of *E. chloroticus* have developmental plasticity to withstand reductions in salinity up to 29 ppt; however, it is still unknown whether the surviving embryos will be able to complete larval development at low salinities, particularly whether the embryos and larvae are carried into extreme environments such as estuaries where salinity is even lower.

Multistressor studies are very important for climate change research as multiple environmental factors will act together in the wild, having major consequences for development and recruitment of marine invertebrates.

## Introduction

Environmental conditions play an important role in the normal functioning of all animals (Willmer 1999), with temperature and salinity of greatest importance for marine animals living in intertidal and shallow subtidal environments (Castro and Huber 2005). Extreme differences in seawater temperature can occur daily or seasonally in relation to the intensity of solar radiation that reaches the surface of the oceans (Lalli and Parsons 1997). In addition, changes in salinity occur naturally, either in shallow waters influenced by river discharge, in tide pools after heavy rain events (Lawrence 1975; Russell 2013) or by evaporation when ambient air temperatures are high (Trowbridge 1994).

In addition, at a regional scale, seawater temperature and salinity are also projected to vary globally due to anthropogenic climate change (IPCC 2014). In general, a rise in seawater temperature is projected due to a global increase in atmospheric heat, while salinity of the water is projected to vary regionally due to changes in rainfall patterns, ice melt and evaporation (IPCC 2014). In the specific case of New Zealand, an increase in seawater temperature of 0.7–2.3 °C is projected by 2090, whereas salinity levels are most likely to be affected by changes in precipitation patterns (IPCC 2014), and decreased rainfall/increased salinity for the East coast of the North Island and the opposite pattern on the West coast (Nottage et al. 2010). In addition, the number of extreme rainfall events is projected to increase about 32 %

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around New Zealand by the next century (Renwick et al. 2013).

Echinoderms are generally affected by changes in temperature and salinity (Moore 1966; Russell 2013), with important effects on fertilization success and cleavage (Greenwood and Bennett 1981; Cameron et al. 1985; Sewell and Young 1999; Kashenko 2006; Byrne et al. 2009; Allen and Pechenik 2010; Delorme and Sewell 2013), survival (Roller and Stickle 1993; Metaxas 1998; Sewell and Young 1999; Li et al. 2011), larval morphology and metamorphosis (George and Walker 2007; Diaz-Perez and Carpizo-Ituarte 2011; Pia et al. 2012; Armstrong et al. 2013; Byrne et al. 2013). In general, temperature and salinity have opposing effects during early development of echinoderms. For example, an increase in temperature (within optimal ranges) results in an increase in development rate (Fujisawa 1995; Sewell and Young 1999; Azad et al. 2010; Li et al. 2011; Delorme and Sewell 2013; Hardy et al. 2014), whereas a decrease in salinity results in a delay of development (Roller and Stickle 1985, 1993; Metaxas 1998; Cowart et al. 2009; Allen and Pechenik 2010; Li et al. 2011; Armstrong et al. 2013). Although there is little research on the combined effect of temperature and salinity on the development of echinoderms (Azad et al. 2010), previous studies suggest that at optimal temperatures, lower salinities delay development. However, when the temperature increase is within the optimal range, the effect of low salinities is diminished (Roller and Stickle 1993; Bressan et al. 1995; Kashenko 2006; Armstrong et al. 2013).

The echinometrid sea urchin *Evechinus chloroticus* (Valenciennes 1846) is an endemic New Zealand species, distributed from the Three Kings Island in the north (34°10'S) to the Snares Islands in the subantarctic (47°60'S) (Fenwick and Horning 1980; Schiel et al. 1986). Individuals are commonly found in shallow waters (<14 m) in a variety of habitats, including tide pools and fiords (Dix 1970; Barker 2007). As a result of the broad distribution of *E. chloroticus*, individuals around New Zealand can be exposed to a variation of seawater temperatures (9.5–21 °C) and salinities (34–35 ppt) depending on the latitude and time of the year (Garner 1969). In waters of the Hauraki Gulf, northern New Zealand, seawater temperature varies seasonally between 14 and 21 °C (Leigh Marine Laboratory temperature records 1967–2011), whereas salinity can vary between 34.3 and 35.5 ppt during early spring and late summer (Zeldis 2004; Zeldis et al. 2004). However, lower salinities (>25 psu, Walker and Vaughan 2013) can be encountered in shallow waters during winter and spring due to increased river flow, while higher salinities are present in summer due to reduced freshwater input (Zeldis and Smith 1999). In tide pools of the Hauraki Gulf, temperature and salinity can undergo more extreme seasonal changes, ranging between 10 and 37 °C, and 31 and

60 ppt (Trowbridge 1994). Additionally, in the fiords of the South Island, surface salinity undergoes more extreme changes due to the combination of natural variation of the input of freshwater through precipitation and/or input from hydroelectric power stations. In Doubtful Sound, for example, this results in surface seawater (to 5 m depth) having salinities between 5 and 30 ppt, overlaying more oceanic salinities of 34–35 ppt at depths >10 m (Peake et al. 2001).

*Evechinus chloroticus* has an annual breeding cycle, spawning during the austral summer when temperatures are highest, 13 °C for the South and 21 °C for the North Island (Garner 1969; Barker 2007). During the reproductive season, embryos may also experience acute exposure to either lower or higher salinities due to sporadic storm events resulting in a high input of freshwater, or by evaporation in shallow coastal waters. Previous research has shown that the early developmental stages of a north-eastern population of *E. chloroticus* are very sensitive to a temperature increase of 3 °C above ambient (Delorme and Sewell 2013); however, no studies have been published on how the interaction of temperature with other variables such as salinity will affect early life stages. Therefore, the aim of the present study was to evaluate the capacity of the early embryonic stages to develop under a combination of five salinity levels and two temperatures within the optimal range for development of the study population of this species in northern New Zealand (Delorme and Sewell 2013). Overall, this study found that low salinities (<31 ppt) had a negative effect on fertilization and early development of *E. chloroticus*, and that an increase in temperature from 18 to 21 °C helps to minimize the effects of low salinity.

## Materials and methods

### Animal collection and laboratory maintenance

Mature adult animals were collected from shallow depths (2 m) at Matheson's Bay (36°18'17"S; 174°47'51"E) in north-eastern New Zealand in February 2013. Seawater temperature and salinity at the time of collection were 21 °C and 34 ppt, measured with a submersible mercury thermometer and a refractometer, respectively. Average seawater temperature during the previous weeks of collection was 20.7 ± 0.5 °C (Leigh Marine Laboratory temperature dataset). After collection, the animals were transported ca. 5 km to the Leigh Marine Laboratory (36°16'09"S; 174°47'54"E) and maintained in ambient flow through for 2 weeks in a 460-L tank and fed with fresh *Ecklonia radiata*.

### Temperature and salinity effects on early development

The complete experiment was run in three separate experimental trials using multiple males and females with no

differences in sea urchin size (test diameter) between trials (average size  $\pm$  SD: 6.2 cm  $\pm$  0.4 cm,  $N = 15$  ANOVA,  $F_{(2,12)} = 0.113$ ,  $P = 0.894$ ). In each trial, eggs from 3 females and sperm from 2 males were pooled to reduce inter-individual variation from single male–female crosses (Byrne et al. 2010, 2011). Sea urchins were induced to spawn by intracoelomic injection of 5 mL of KCl (0.55 M) and gentle agitation. Eggs were collected in 250-mL plastic containers with 1- $\mu$ m filtered and UV-treated fresh seawater (FSW), whereas sperm were collected “dry” from the gonopore of the males and stored in 1.5-mL Eppendorf tubes at 4 °C until use. Eggs were washed 3  $\times$  with FSW and filtered through a 200- $\mu$ m mesh to remove extraneous material such as feces or spines. Egg quality was checked by observing the eggs under a microscope to check for roundness; additionally, eggs were also checked for fertilization success in a test assay, and only eggs with >95 % fertilization success were used for pooling and experimentation. Eggs were pooled in a 1-L container with FSW, and then, the concentration was determined based on three subsamples of 100  $\mu$ L. Sperm quality was checked by observing the motility of diluted sperm under the compound microscope at 40  $\times$  magnification. Suitable dry sperm were pooled in equal amounts in a petri dish, and then, 20  $\mu$ L of the pooled sperm was diluted in 60 mL of FSW to obtain a sperm concentration suitable to ensure fertilization and avoid polyspermy (50,000–100,000 sperm mL<sup>-1</sup>, Franke 2005). The final sperm concentration determined from hemocytometer counts was 85,556  $\pm$  11,706 sperm mL<sup>-1</sup> ( $\pm$ SD,  $n = 3$ ).

### Experimental design

A combination of five salinities (29, 31, 34, 35 and 37 ppt) and two temperatures (18 and 21 °C) was used for experiments on early developmental stages. The salinity levels used included the ambient salinity for coastal and ocean waters in New Zealand (34 and 35 ppt, respectively, Garner 1969), with two salinities below and one salinity above the ambient levels. The temperatures used correspond to optimal temperatures for development of this species in north-eastern New Zealand, with 18 °C being a temperature which results in a high percent of normal development (>90 %), and 21 °C being the upper pejus temperature for development of the study population of *E. chloroticus* (Delorme and Sewell 2013). Thus, the control treatments for this study were considered to be 34 and 35 ppt and 18 °C.

Eggs were fertilized, and embryos cultured in 35-mL plastic containers with FSW at the different salinities (three replicates per salinity). The containers were held by a polypropylene rack inside a 14-L tank with flowing seawater at the desired temperature controlled by a heating

unit. Salinity treatments were prepared using deionized water and sea salt (Red Sea Salt) according to the package instructions and based on refractometer measurements. Temperature and salinity were checked before and after the experiment with a submersible mercury thermometer and a refractometer, respectively. In addition, measurements of dissolved oxygen and pH were measured at the end of the experiment in containers with embryos at the same experimental conditions and with FSW-only using a Hach IntelliCAL™ LDO101 probe and a pH tester with spear electrode, respectively.

In each experimental trial, ~1,000 pooled eggs were placed in each of the three containers (per temperature–salinity treatment), and after 30 min of egg acclimation, 90  $\mu$ L of pooled diluted sperm was added (without previous acclimation of the sperm to the different salinity treatments). Embryo samples (500  $\mu$ L) were taken from each container at different time points after fertilization [1, 2, 4, 8 and 24 h post-fertilization (hpf)]. Pre-hatched embryos (1, 2 and 4 hpf) were sampled from the bottom of the container; for post-hatch (8 and 24 hpf), the containers were gently agitated to make an even suspension of the embryos before sampling. Each sample was placed in a 1.5-mL Eppendorf tube and preserved with 5 % formalin in seawater for later analysis. One hundred and fifty samples were collected in each of the three experimental trials (5 salinities, 2 temperatures, 5 times after fertilization, 3 replicates per treatment), with a total of 450 samples collected during the complete experiment (3 separate trials of 3 populations of embryos). Each sample was placed in a Sedgewick Rafter counting cell, and the cell was placed under the microscope for scoring of embryos. The first 100 embryos observed in a random transect across the cell were scored for developmental stage as unfertilized eggs, fertilized eggs, 2-cell, 4-cell, 8-cell and 16-cell embryos, blastula, gastrula and abnormal embryos (degenerating eggs/embryos, asymmetric divisions). Fertilization success was evaluated at 1 hpf, and the sum of all normally developing embryos was used to calculate the percent normal development at each time after fertilization for each treatment. The percent of gastrula stage embryos was determined at 24 hpf in each treatment and graphed as lines to show the pejus points, which were considered to be when gastrulation/performance fell below 75 % (Delorme and Sewell 2013). The average from the three experimental trials was used for all illustrations.

The rate of early development was calculated for each treatment based on the number of divisions of the embryos at each time after fertilization up to 4 hpf. The number of divisions was determined based on the number of cells of each developmental stage, with 1 division = 2-cells, 2 divisions = 4-cells, 3 divisions = 8-cells, and 4 divisions = 16-cells or more. The logarithm of the number of divisions in each treatment was plotted against the

logarithm of the time after fertilization in order to linearize the data, with the regression slope being the development rate in each treatment (Cowart et al. 2009). In order to obtain a mean development rate per treatment, a single slope was calculated for each replicate (each population of embryos) allowing further statistical analyses of the development rate in each treatment.

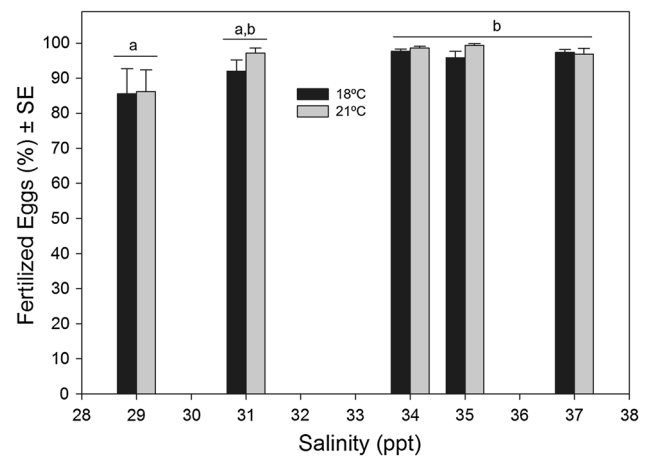
### Statistical analyses

Dissolved oxygen and pH data from containers with embryos and with FSW-only were analyzed for each temperature treatment. Dissolved oxygen data met the assumptions of normality and homoscedasticity (Shapiro–Wilk and Levene’s tests, respectively), and a one-way ANOVA was performed. In contrast, pH data did not meet the assumptions and a Kruskal–Wallis nonparametric test was performed.

The percent of embryos in each developmental stage and time after fertilization was calculated from the frequency data obtained from each sample and arcsine transformed for statistical analyses. Statistical analyses were performed on the data resulting from the average of the three experimental trials from fertilization success (1 hpf), number of divisions, early development rate, gastrulation and normal development. Normal development was calculated at each time after fertilization until 24 hpf. As all the data sets met the assumptions of normality and homoscedasticity, they were analyzed with two-way ANOVA with temperature and salinity as factors, and the percent of fertilization, number of divisions, rate of early development, gastrulation or normal development as the dependent variables. The number of divisions and the percent of normal development were analyzed separately for each time point after fertilization. Comparisons between groups were analyzed using the Tukey a posteriori test ( $\alpha = 0.05$ ). All statistical analyses were run using the statistical software included within Sigma Plot 11.0. (SYSTAT Software, Inc.).

## Results

Average temperatures and salinities ( $\pm$ SD) during experimentation across the three experimental trials were  $18.0 \pm 0.2$  °C and  $20.8 \pm 0.1$  °C, and  $29 \pm 0.3$ ,  $31 \pm 0.1$ ,  $34 \pm 0.3$ ,  $35 \pm 0.1$ ,  $37 \pm 0.3$  ppt. At 24 hpf, oxygen saturation and pH in the containers were  $>90$  % and  $8.1 \pm 0.1$  pH units, respectively, with no significant differences between containers with embryos and with FSW-only (oxygen: ANOVA,  $F_{(1,23)} = 3.5$ ,  $P = 0.076$  and  $F_{(1,23)} = 3.8$ ,  $P = 0.065$  for 18 and 21 °C, respectively; pH: Kruskal–Wallis test,  $H_1 = 2.323$ ,  $P = 0.128$  and  $H_1 = 0.397$ ,  $P = 0.529$  for 18 and 21 °C, respectively).



**Fig. 1** Effect of salinity and temperature on fertilization success in *Evechinus chloroticus* measured by the percent of fertilized eggs observed at 1 h after addition of sperm. Data represent the mean percent of fertilized eggs  $\pm$  standard error ( $\pm$ SE,  $n = 3$ ) at each salinity (29, 31, 34, 35 and 37 ppt) and temperature (18 and 21 °C). Significant differences between groups are denoted by lower case letters above bars and in Table 1

### Fertilization success

Fertilization of *E. chloroticus* 1 h after sperm addition was over 80 % in all treatments (Fig. 1). Statistically, fertilization was affected by salinity (ANOVA,  $F_{(4,20)} = 5.313$ ,  $P = 0.004$ ), with no significant effect of temperature (ANOVA,  $F_{(1,20)} = 2.649$ ,  $P = 0.119$ ) and no significant interaction between factors (ANOVA,  $F_{(4,20)} = 0.502$ ,  $P = 0.735$ ). Within salinity treatments, fertilization was lower at 29 ppt compared to 34, 35 and 37 ppt (Table 1; Fig. 1).

### Early development

The rate of development of *E. chloroticus* increased with an increase in temperature from 18 to 21 °C; however, lower salinities (29 and 31 ppt) resulted in a decrease in development rate compared to high salinities at the same temperature (Fig. 2). At 1 hpf, most embryos were fertilized at 18 °C in all salinity treatments, whereas at 21 °C embryos were undergoing cleavage. At 2 and 4 hpf, embryos had more advanced development at higher temperature, but again embryos cleaved fewer times at lower salinities (29 and 31 ppt) compared to high salinities in both temperatures (Fig. 2). At 8 hpf, in all salinities, most embryos were at the 16-cell stage at 18 °C, whereas at 21 °C embryos had reached the blastula stage; however, the percent of 16-cell and blastula was lower at 29 ppt than at higher salinities in both temperatures, reaching only 31 and 45 % at 18 and 21 °C, respectively (Fig. 2).

**Table 1** Summary of statistical analyses performed on fertilization success, the percent of normal embryos at each time after fertilization (hpf) and the percent of gastrulation at different salinities and temperatures in *Evechinus chloroticus*. ANOVA statistic ( $F$ ), mean square ( $MS$ ) and  $P$  value ( $P$ ) are shown for each time using salinity and temperature as factors. Significant results ( $P < 0.05$ ) given by the multiple comparison Tukey test are also shown

hpf	Factor	df	$MS$	$F$	$P$
1	Temperature (T)	1	100.423	2.649	0.119
	Salinity (S)	4	201.390	5.313	<b>0.004</b>
	T x S	4	19.033	0.502	0.735
	Residual	20	37.908		
29 31 34 35 37 ppt					
2	Temperature (T)	1	14.981	0.358	0.556
	Salinity (S)	4	182.608	4.364	<b>0.011</b>
	T x S	4	18.005	0.430	0.785
	Residual	20	41.847		
29 31 37 34 35 ppt					
4	Temperature (T)	1	9.209	0.481	0.496
	Salinity (S)	4	1016.361	53.135	<b>&lt;0.001</b>
	T x S	4	71.379	3.732	<b>0.020</b>
	Residual	20	19.128		
(29-18°) (29-21°) (31-18°) (31-21°) (34-18°) (34-21°) (35-18°) (35-21°) (37-18°) (37-21°)					
8	Temperature (T)	1	38.946	2.507	0.129
	Salinity (S)	4	1119.786	72.089	<b>&lt;0.001</b>
	T x S	4	23.018	1.482	0.245
	Residual	20	15.533		
29 31 34 35 37 ppt					
24	Temperature (T)	1	76.034	3.038	0.097
	Salinity (S)	4	1125.028	44.955	<b>&lt;0.001</b>
	T x S	4	9.447	0.377	0.822
	Residual	20	25.026		
29 31 34 35 37 ppt					
Gastrulation	Temperature (T)	1	1625.401	58.934	<b>&lt;0.001</b>
	Salinity (S)	4	2036.094	73.825	<b>&lt;0.001</b>
	T x S	4	223.676	8.110	<b>&lt;0.001</b>
	Residual	20	27.580		
(29-18°) (29-21°) (31-21°) (31-18°) (37-18°) (34-18°) (35-18°) (34-21°) (35-21°) (37-21°)					

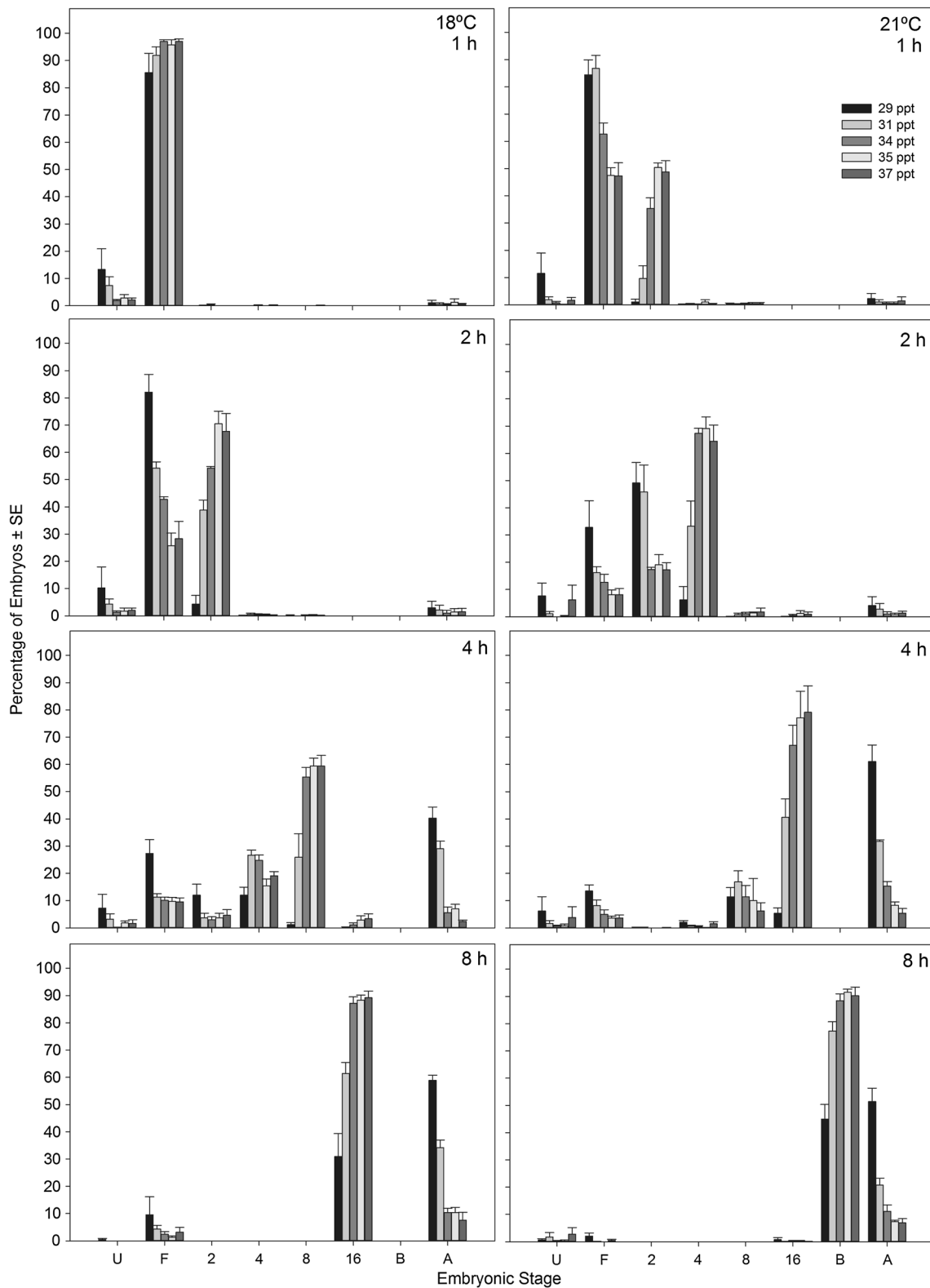
The mean number of divisions was affected by salinity and temperature at all times after fertilization (Table 2; Fig. 3). At 1 hpf, temperature had a significant effect on cleavage as the number of divisions were higher at 21 °C in 31, 34, 35 and 37 ppt (Table 2; Fig. 3). Additionally, at salinities of 29, 31 and 34 ppt, the number of divisions was lower compared to the other salinity treatments (Table 2; Fig. 3). At 2 hpf, the number of divisions was lower at 18 °C and 29 and 31 ppt (Table 2; Fig. 3), whereas at 4 hpf the number of divisions was also lower at 18 °C but only at a salinity of 29 ppt (Table 2; Fig. 3). Development rate was significantly lower at 18 °C in all salinities compared to 21 °C, whereas within temperature treatments development rate at 29 was ca. 1/4 and ca. 3/4 of the rate at salinities greater than 34 ppt at 18 and 21 °C (Table 2; Fig. 4).

Gastrulation in *E. chloroticus* was affected by salinity (ANOVA,  $F_{(4,20)} = 73.825$ ,  $P < 0.001$ ) and temperature (ANOVA,  $F_{(4,20)} = 58.934$ ,  $P < 0.001$ ), with a significant interaction between factors (ANOVA,  $F_{(4,20)} = 8.110$ ,  $P < 0.001$ ) (Table 1; Fig. 5). Lower numbers of

gastrulae were observed at 29 and 31 ppt in both temperatures (Table 1; Fig. 5), whereas the highest percent of gastrulae was observed at 34 and 35 ppt with 79 and 91 % of gastrula observed at 18 and 21 °C, respectively (Table 1; Fig. 5).

#### Normal development

Normal development decreased with time in all treatments (Fig. 6). At 2 hpf, the percent of normal embryos was only affected by salinity, with no interaction between factors (Table 1). At this time, normal embryos were >80 % in all salinity/temperature treatments (Fig. 6), with a significant ~10 % reduction in normal embryos at 29 ppt compared to 34 and 35 ppt at both temperatures (Table 1; Fig. 6). At 4 hpf, normal development was also only affected by salinity; however, a significant interaction between salinity and temperature occurred (Table 1). At 18 °C, the percent of normal embryos at 29 and 31 ppt (53–68 %, respectively) was significantly lower compared



**Fig. 2** Effect of salinity and temperature on early development in *Evechinus chloroticus*. Data represent the mean percent of each stage  $\pm$  standard error ( $\pm$ SE,  $n = 3$ ) at each salinity (29, 31, 34, 35

and 37 ppt) and temperature (18 and 21 °C). U: unfertilized eggs, F: fertilized eggs, 2: 2-cell, 4: 4-cell, 8: 8-cell, 16: 16-cell or more, B: blastula, and A: abnormal embryos

**Table 2** Summary of statistical analyses performed on the number of divisions at each time after fertilization (hpf) and on development rate (DR) at different salinities and temperatures in *Evechinus chloroticus*. ANOVA statistic ( $F$ ), mean square ( $MS$ ) and  $P$  value ( $P$ ) are shown for each time using salinity and temperature as factors. Significant results ( $P < 0.05$ ) given by the multiple comparison Tukey test are also shown

hpf/DR	Factor	df	$MS$	$F$	$P$
1	Temperature (T)	1	0.717	296.319	<0.001
	Salinity (S)	4	0.084	34.817	<0.001
	T x S	4	0.081	33.643	<0.001
	Residual	20	0.002		
				(29-18°) (31-18°) (34-18°) (35-18°) (37-18°) (29-21°) (31-21°) (34-21°) (35-21°) (37-21°)	
2	Temperature (T)	1	5.254	264.654	<0.001
	Salinity (S)	4	0.739	37.237	<0.001
	T x S	4	0.045	2.242	0.101
	Residual	20	0.020		
				29 31 34 35 37 ppt	
4	Temperature (T)	1	14.533	182.092	<0.001
	Salinity (S)	4	2.488	31.175	<0.001
	T x S	4	0.064	0.805	0.537
	Residual	20	0.080		
				29 31 34 35 37 ppt	
DR	Temperature (T)	1	1.014	199.350	<0.001
	Salinity (S)	4	0.208	40.926	<0.001
	T x S	4	0.015	3.012	0.043
	Residual	20	0.005		
				(29-18°) (29-21°) (31-18°) (31-21°) (34-18°) (34-21°) (35-18°) (35-21°) (37-18°) (37-21°)	

to the normal development observed at 34, 35 and 37 ppt (Table 1; Fig. 6). At 21 °C, the percent of normal embryos at 29 ppt was ~37 % lower than that observed at 31, 34, 35 and 37 ppt (Table 1; Fig. 6). In addition, the significant interaction between factors at 4 hpf showed that at 31 ppt, there were ~19 % less normal embryos at 18 °C than at 21 °C (Table 1; Fig. 6).

At 8 and 24 hpf, there was a significant further reduction in normal development at 29 and 31 ppt at both temperatures, with no interaction between factors (Table 1; Fig. 6). At 8 hpf, normal development at 29 ppt was 49 and 25 % lower than the other treatments at 18 and 21 °C, respectively (Table 1; Fig. 6), whereas at 31 ppt normal development was 44 and 24 % lower than the other salinity treatments at 18 and 21 °C, respectively (Table 1; Fig. 6). At 24 hpf, normal development at 29 and 31 ppt was even lower, reaching less than 45 and 65 %, respectively, in both temperature treatments. At 24 hpf, the highest percent of normal development was observed at 34 and 35 ppt with 89 and 92 % at 18 and 21 °C, respectively (Fig. 6).

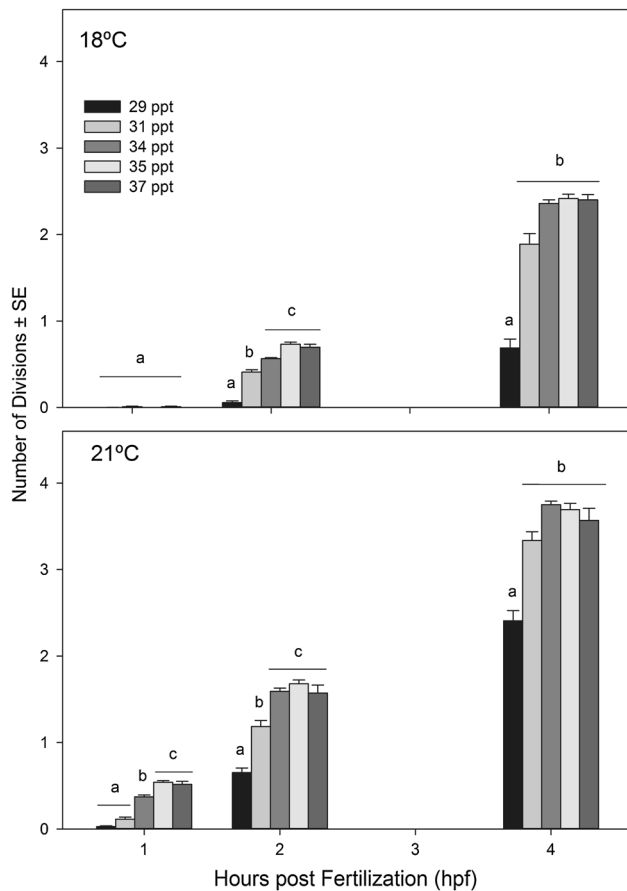
## Discussion

In the present study, both fertilization and early development of the sea urchin *E. chloroticus* were affected by lower salinities, whereas the seawater temperatures used had an effect only on development rate and gastrulation. In multistressor studies, the tested environmental stressors can

interact to have synergistic, antagonistic or additive effects on performance (Folt et al. 1999). Only an antagonistic effect was observed in this study, where an increase in seawater temperature of 3 °C (within optimal range of the study population in northern New Zealand) minimized the negative effect of low salinities, as previously observed in early development of other sea urchins (Roller and Stickle 1993; Bressan et al. 1995; Kashenko 2006; Armstrong et al. 2013).

As a single stressor, seawater temperature is known to negatively affect optimum performance and survival of ectothermic animals (Willmer 1999). In *E. chloroticus*, seawater temperature has been reported to affect early development, decreasing normal development at temperatures of 24 °C, i.e., 3 °C above upper pejus temperature (Delorme and Sewell 2013). As the seawater temperatures used in the present study were within the optimal thermal window for early development of this species (Delorme and Sewell 2013), seawater temperature had a positive effect on early development. In contrast, salinity had a negative effect on early development of *E. chloroticus*, from fertilization success to normal development.

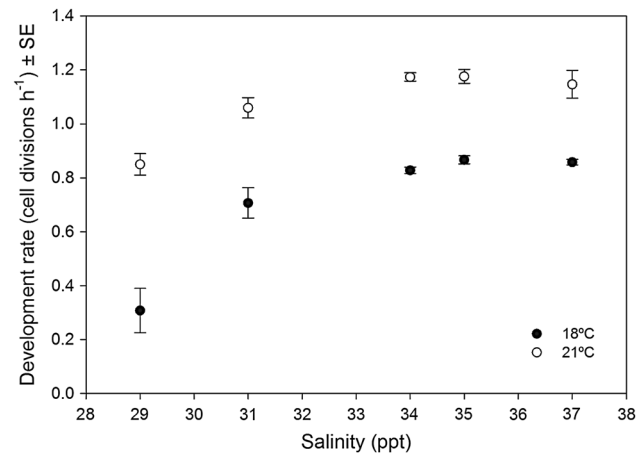
Fertilization success was significantly affected by low salinity [effect size of  $-0.13$  calculated as the LnRR: natural log of the ratio between the treatment response and the control response (Nakagawa and Cuthill 2007)]; however, it had greater effects on early development (effect size of  $-0.83$ ). Low salinity in *E. chloroticus* affected cleavage directly (i.e., through mitosis) and increased the occurrence



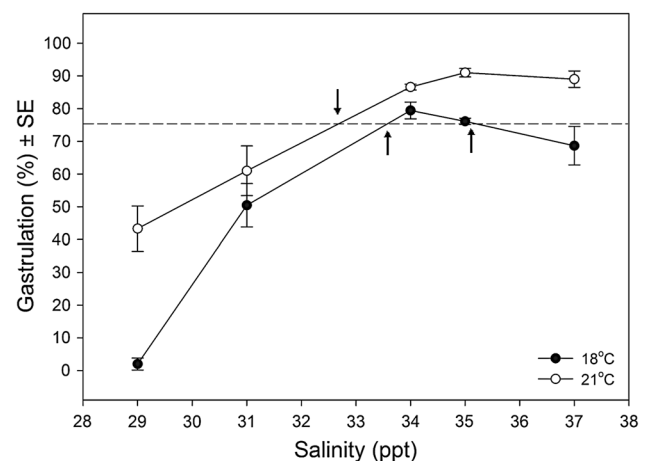
**Fig. 3** The effect of salinity and temperature on the number of divisions observed at different hours post-fertilization (hpf) in *Evechinus chloroticus*. Data represent the mean number of divisions  $\pm$  standard error ( $\pm$ SE,  $n = 3$ ) at each salinity (29, 31, 34, 35 and 37 ppt) and temperature (18 and 21 °C). Significant differences in the number of divisions at each time after fertilization are denoted by lower case letters above bars and in Table 2

of abnormalities in embryos (i.e., asymmetric cleavage patterns and degenerating embryos; Roller and Stickle 1985, 1993, 1994; Metaxas 1998; Kashenko 2006, 2007, 2009; Allen and Pechenik 2010; Carballeira et al. 2011; Li et al. 2011). In other echinoids, low salinity has been shown to have a greater effect on embryonic development (before hatching) than in the swimming stages (Kashenko 2007, 2009) and may completely inhibit development (Roller and Stickle 1994; Cowart et al. 2009). This sensitivity during early development has also been observed in holothurians (Li et al. 2011) and asteroids (Kashenko 2006; Pia et al. 2012). However, developmental stages of asteroids may be more resilient to decreasing salinities when compared to echinoids with a similar geographical distribution (Sameoto and Metaxas 2008).

Although the mechanism by which salinity affects cleavage is still unknown, it has been suggested that survival



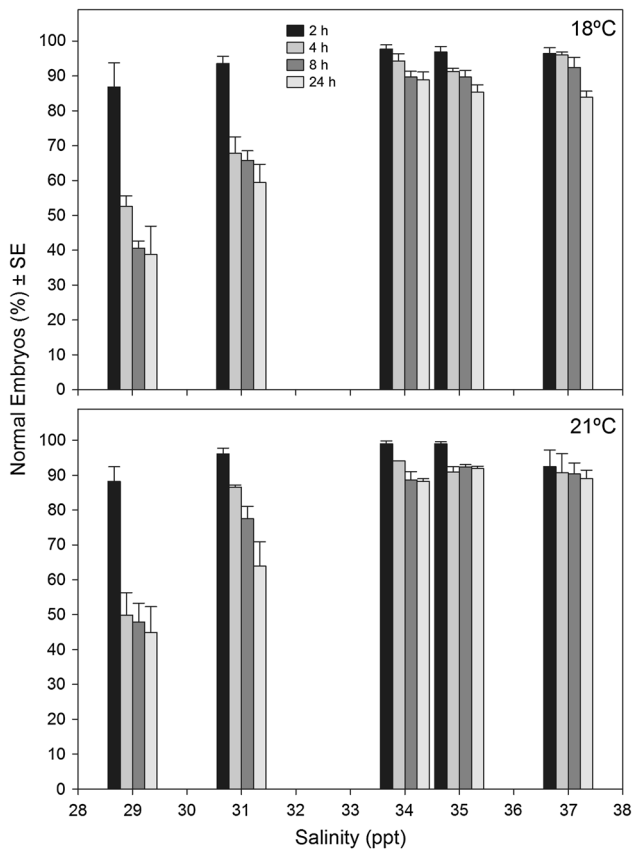
**Fig. 4** Effect of salinity and temperature on development rate in *Evechinus chloroticus*. Data represent the mean development rate  $\pm$  standard error ( $\pm$ SE,  $n = 3$ ) at each salinity (29, 31, 34, 35 and 37 ppt) and temperature (18 and 21 °C) obtained from the slope of the regression line between the number of divisions and time after fertilization at each salinity and temperature. Significant differences in development rate are shown in Table 2



**Fig. 5** Effect of salinity and temperature on the percent of gastrulae observed at 24 h post-fertilization in *Evechinus chloroticus*. Data represent the mean percent of gastrulae  $\pm$  standard error ( $\pm$ SE,  $n = 3$ ) at each salinity (29, 31, 34, 35 and 37 ppt) and temperature (18 and 21 °C). Broken line indicates the 75 % gastrulation point, and arrows show the pejus points in salinity for each temperature. Significant differences in gastrulation are shown in Table 1

may be affected at extreme salinities (low and high) due to a change in the osmotic balance of cells, by changes in the absorption and saturation of dissolved gases (Kinne 1964 *vide* Li et al. 2011) or by the effect of reduced osmotic pressure either on proteins associated with the microtubules or functioning of the spindles during mitosis (Sharp et al. 2000; Sharp 2002). In addition, it has been recently reported that sea urchin embryos do not develop under





**Fig. 6** Effect of salinity and temperature on normal development at different times after fertilization in *Evechinus chloroticus*. Data represent the mean percent of normal embryos  $\pm$  standard error ( $\pm$ SE,  $n = 3$ ) at each salinity (29, 31, 34, 35 and 37 ppt) and temperature (18 and 21 °C). Significant differences in normal development at each time after fertilization are shown in Table 1

absence of external  $\text{Na}^+$  due to a reduced activation of the mitosis promoting factor (MPF), which may be the cause of developmental arrest, whereas under low  $\text{Na}^+$  the cell cycle stops due to intracellular acidification, leading to cell death (Ciapa and Philippe 2013).

Low salinity also had a negative effect on development rate, which was slowed down at 29 ppt by 63 % in the 18 °C treatment and by 30 % in the 21 °C treatment, suggesting that complete PLD in *E. chloroticus* (21–30 days, Dix 1969; Walker 1984) would take 13–19 days longer at 18 °C and 6–9 days longer at 21 °C. An increase in PLD of this magnitude would likely increase mortality of the larvae due to an increase in predation pressure and the dependence on plankton as a food source (Thorson 1950; Morgan 1995; O' Connor et al. 2007), and an increased risk of being carried out by currents to areas where conditions might be more unfavourable for development (Thorson 1950; Morgan 1995; Shanks 1995).

Previous laboratory experiments have shown that echinoid species and developmental stages differ in their salinity tolerance (Cameron et al. 1985; Kashenko 2007). For

instance, the sand dollar *Echinarachnius parma* only tolerates short periods (hours) in low salinity (Allen and Pechenik 2010), whereas the heart-shaped sea urchin *Echinocardium cordatum* and sand dollar *Scaphechinus mirabilis* can tolerate long periods (days) (Kashenko 2007, 2009). In addition to the differences in tolerance, developmental stages might also avoid exposure to low salinity waters by delaying hatching, as observed in *E. cordatum* (Kashenko 2007) and *E. parma* (Armstrong et al. 2013). Small numbers of *Sterechinus neumayeri* and *E. chloroticus* embryos have also been observed to show delayed hatching in response to pH and seawater temperature, respectively (unpub. data), suggesting that delaying hatching might be a generalized response of the early developmental stages of echinoderms to stress. On the other hand, post-hatched larval stages can avoid low salinity by swimming away from vertically stratified low salinity waters (Metaxas and Young 1998; Sameoto and Metaxas 2008), increasing the likelihood of larval survival (Metaxas 1998). In the wild, Antonie (2003) monitored the fiords of the South Island showing no occurrence of larvae of *E. chloroticus* in the low salinity layer (LSL), suggesting either an ability of the larvae to avoid low salinities or, alternatively, that all the larvae that swim to the LSL die because of the extreme low salinity of the water.

The experimental salinity tolerance in this study for early developmental stages of a north-eastern population of *E. chloroticus* was 29 ppt. The results obtained for normal development at this salinity are similar to that previously reported for Doubtful Sound populations, where incomplete development was seen at salinities of 27.5 ppt (Antonie 2003). Salinities lower than 29 ppt are currently observed in New Zealand (Zeldis and Smith 1999; Peake et al. 2001), and therefore, it is likely that salinity may be currently limiting the distributional range of *E. chloroticus* due to poor embryo tolerance, which may be part of the explanation for the absence of this species from estuarine habitats, or alternatively, adult *E. chloroticus* may be avoiding spawning at low salinity. However, even considering that more extreme and frequent rainfalls are projected throughout New Zealand due to climate change by 2100 (IPCC 2014; Renwick et al. 2013), it is still premature to predict whether *E. chloroticus* will change its distributional range as a result of changes in salinity. In addition, phenotypic plasticity in embryos of *E. chloroticus* has been observed at high temperatures (Delorme and Sewell 2013), and now at low salinities with ca. 50 % embryos surviving at 29 ppt (this study), suggesting genetic capacity of this species to deal with environmental change (Byrne and Przeslawski 2013).

In conclusion, we know that seawater temperature as a single stressor can have major effects on this species, particularly for north-eastern populations, which are

developing at temperatures very close to their thermal tolerance limit (Delorme and Sewell 2013). However, here we report that the addition of low salinity as another climate change stressor could have even more dramatic effects on early development in *E. chloroticus*. These results are of particular interest considering that low seawater pH, as another climate change stressor, has also been shown to decrease survival, larval size and calcification index in *E. chloroticus* (Clark et al. 2009). Climate change stressors will act together in the wild, which may have major consequences for development and recruitment of this species and flow-on effects to the shallow subtidal ecosystem of northeast New Zealand.

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