

# Eutrophication offsets increased sea urchin grazing on seagrass caused by ocean warming and acidification

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**ABSTRACT:** The accumulation of atmospheric [CO<sub>2</sub>] continues to warm and acidify oceans concomitant with local disturbances, such as eutrophication. These changes can modify plant–herbivore grazing interactions by affecting the physiology of grazers and by altering the nutritional value of plants. However, such environmental changes are often studied in isolation, providing little understanding of their combined effects. We tested how ocean warming and acidification affect the per capita grazing by the sea urchin *Amblypneustes pallidus* on the seagrass *Amphibolis antarctica* and how such effects may differ between ambient and eutrophic nutrient conditions. Consistent with metabolic theory, grazing increased with warming, but in contrast to our expectations, acidification also increased grazing. While nutrient enrichment reduced grazing, it did not fully counterbalance the increase associated with warming and acidification. Collectively, these results suggest that ocean warming and acidification may combine to strengthen top-down pressure by herbivores. Localised nutrient enrichment could ameliorate some of the increased per capita grazing effect caused by warming and acidification, provided other common negative effects of eutrophication on seagrass, including overgrowth by epiphytes and herbivore aggregation, are not overwhelming. There is value in assessing how global and local environmental change will combine, often in non-intuitive ways, to modify biological interactions that shape habitats.

**KEY WORDS:** Temperature · Carbon dioxide · Herbivory · Consumption · Nutrient enrichment · Climate change · *Amblypneustes pallidus* · *Amphibolis antarctica*

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## INTRODUCTION

The unprecedented magnitude of anthropogenic impacts on natural environments continues to challenge our theoretical and applied understanding of ecosystem dynamics (Vitousek et al. 1997, Jackson 2001). In recent years, there has been considerable interest regarding the effects of ocean warming and acidification (i.e. reduced pH) generated by increasing atmospheric [CO<sub>2</sub>] (Meehl et al. 2007). These global-scale changes occur concomitantly with local

disturbances, such as eutrophication (Gorman et al. 2009, Russell et al. 2009), raising new questions regarding the cumulative effects of global and local disturbances on ecological processes and species interactions (Hoegh-Guldberg & Bruno 2010, Russell et al. 2012).

Understanding whether abiotic change can modify the outcome of species interactions that subsequently scale up to the community level is of central importance to ecologists, particularly for species that have disproportionately large effects on community and

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habitat structure (Sanford 1999, Harley et al. 2006, Gooding et al. 2009, Kordas et al. 2011). To date, research has primarily focused on how changing abiotic conditions directly affect individual organisms (e.g. Orr et al. 2005, Parker et al. 2009, Brennand et al. 2010), with little insight into how changing conditions may indirectly alter whole ecosystems by modifying fundamental ecological processes, such as grazing (Kordas et al. 2011, Russell et al. 2012).

Sea urchins can play a pivotal role in controlling the abundance of habitat-forming species via their grazing (Hughes 1994, Eklöf et al. 2008). Perhaps the world's best known example is the kelp forest–sea urchin–sea otter interaction chain in the north-east Pacific Ocean, which has long been held as a classic example of top-down control on habitat abundance depending on the local control of urchin herbivores by otters (Estes et al. 1998, Steneck et al. 2002). Strong effects of urchins certainly extend to other habitats, particularly seagrass meadows where overgrazing can damage habitats on both tropical and temperate coasts (Rose et al. 1999, Valentine et al. 2000, Eklöf et al. 2008, Valentine & Edgar 2010).

Abiotic change can modify plant–herbivore interactions both directly by affecting the physiology of grazers and indirectly by altering the nutritional value and chemistry of plants (Cruz-Rivera & Hay 2000, Hillebrand et al. 2009, O'Connor 2009). Warming has a predictable effect on the metabolic rate of ectotherms, which subsequently drives proportional increases in consumption (Hillebrand et al. 2009, O'Connor 2009). Near physiological limits, this relationship often deteriorates as physiological stress reduces consumption or grazing (Sanford 2002, Lemoine & Burkepile 2012). In contrast, the relative infancy of research into ocean acidification means its effects on grazing have rarely been quantified. While emerging research suggests acidification will disrupt biological processes such that feeding ability and opportunities may be reduced (e.g. through exoskeletal structural damage, increased predator avoidance and damage to feeding parts) (Bibby et al. 2007, Wood et al. 2008, Marchant et al. 2010, Stumpp et al. 2012), acidification can elevate grazing rates (Li & Gao 2012). While urchins can decrease consumption in response to large declines in pH (Stumpp et al. 2012), little is known regarding responses to more intermediate acidification scenarios. Increased grazing could occur because acidification boosts the metabolic rate of ectotherms through disruption of growth and physiological regulation, which are both energy-dependent processes (Brown et al. 2004, Cummings et al. 2011, Catarino et al. 2012).

Eutrophication can modify plant–herbivore interactions, as changes in water quality affect the uptake and storage of nutrients by primary producers (McGlathery 1995, Valentine & Heck 2001), but can also be impacted by temperature and [CO<sub>2</sub>] (Touquette & Burkholder 2002, Jiang et al. 2010, Falkenberg et al. 2013). However, the effect of the nutritional balance of plants on grazing is difficult to forecast because organisms can display both avoidance and compensatory responses to changes in perceived food quality (Cruz-Rivera & Hay 2000, Hillebrand et al. 2009, Tomas et al. 2011, de los Santos et al. 2012). Urchins can reduce per capita grazing when nutrient content in primary food sources are enriched, thus providing more rapid satiation (Lares & McClintock 1991, Valentine & Heck 2001), or when epiphytes and phytoplankton offer a nutrient-rich alternative food (Tewfik et al. 2005).

The short-spined sea urchin *Amblypneustes pallidus* has the potential to cause overgrazing of the seagrass *Amphibolis antarctica* to a point where virtually all of the above-ground biomass is lost (O. W. Burnell & A. D. Irving pers. obs.). To understand how this plant–herbivore interaction may be modified by changing environmental conditions, we tested the independent and cumulative effects of warming, acidification and nutrient enrichment on the grazing of seagrass by fixed densities of sea urchins. We tested the hypothesis that increased grazing due to warming would be counteracted by decreases associated with ocean acidification and nutrient enrichment.

## MATERIALS AND METHODS

Urchins *Amblypneustes pallidus* and seagrasses *Amphibolis antarctica* were maintained in experimental microcosms to test the effects of warming, acidification and nutrient enrichment on grazing. Two temperature levels (15 and 20°C), 2 pH levels (current and forecasted) and 2 nutrient levels (ambient and enriched) were manipulated in a fully crossed design, making a total of 8 treatment combinations.

Levels of temperature and pH were selected to identify realistic future grazing responses, rather than inducing stress-related responses, which would likely affect the survival or physiological ability of urchins to function (i.e. below pH 7.5 or outside the thermal tolerance range) (Sanford 2002, Miles et al. 2007). Temperature and pH were manipulated to induce grazing effects likely under future projection

scenario A1B for the year 2100 (i.e. 3°C and 0.2 pH units) (Meehl et al. 2007). For many marine invertebrates, a disproportionate increase in consumption is observed per 1°C increase at the higher end of the thermal tolerance range (Hanks 1957, Garton & Stickle 1980), particularly for small-bodied sea urchins (Siikavuopio et al. 2008). Therefore, a 5°C temperature differential from within the natural range of *Amblypneustes pallidus* (~12 to 25°C) was used to produce changes in consumption likely to be similar in magnitude to those that would be observed for a 3°C increase in summer maximum temperatures, as this is the period when seagrasses appear most susceptible to intense grazing (O. W. Burnell & A. D. Irving pers. obs.). This approach ensured that any stress-related reduction in grazing would not confound more realistic responses. The enriched concentration of nutrients was chosen to mimic peak levels encountered on the Adelaide metropolitan coastline based on Gorman et al. (2009).

Urchins were collected from beds of *Amphibolis antarctica* and *Posidonia* spp. from Lady Bay, South Australia (35.47645° S, 138.27570° E), while juvenile *A. antarctica* recruits were collected at Grange, South Australia (34.90415° S, 138.47079° E). Experiments were conducted in 22 l microcosms, with water constantly recirculating (60 l h<sup>-1</sup>) from a 180 l reservoir attached to each microcosm. Six replicate microcosms for each treatment were divided into 2 compartments with 10 seagrass recruits in each compartment (mean seagrass weight 9.07 ± 0.03 g microcosm<sup>-1</sup>). One compartment in each microcosm contained 2 urchins (mean urchin weight 15.63 ± 0.28 g). As seagrass and any epibiota growing upon leaves were able to continue growing while being consumed, it was necessary to account for any autogenic change by maintaining seagrass in the absence of urchins. Urchins and seagrass were maintained separately under the experimental conditions for 6 d prior to the grazing experiment, which lasted for 5 d, until >60 % of biomass was lost in the treatment with the highest grazing loss.

To calculate grazing, a modified version of the equation  $\{[H_0 \times (C_f/C_0)] - H_f\}$  from Taylor et al. (2002) was used, where  $H_0$  is the fresh weight pre-assay,  $H_f$  is the fresh weight post-assay of seagrass exposed to urchin grazing,  $C_0$  is the fresh weight pre-assay, and  $C_f$  is the fresh weight post-assay of seagrass in control treatments. One additional variable was used to control for seagrass decline throughout the experiment in the grazing treatments. As seagrass was continually removed by the urchins, its autogenic change in grazing treatments is reduced relative to

control treatments; therefore, the modified equation  $\{[H_0 \times (C_f/C_0)] - H_f\} - \{(C_f - C_0)[1 - (H_a/100)]\}$  was used, where  $H_a$  is the average percentage of seagrass shoots surviving when exposed to urchin grazing. The percentage of surviving shoots was counted daily and averaged over the 5 d period to calculate  $H_a$ . Grazing was divided by initial urchin fresh weight to remove treatment differences as this can act as a covariate for grazing (Underwood 1997). Seagrass and urchin fresh weight were determined by placing samples on absorbent paper for 10 s and blotting dry any excess water before weighing. At the conclusion of the experimental period, seagrasses in control treatments were frozen, and 2 individuals from each treatment were later scraped with a razor blade to estimate the percentage contribution of any epibiota to seagrass fresh weight. The ingestion of epibiota by urchins was then calculated for each experimental replicate using the equation  $(C_e/C_f) \times G$ , where  $C_e$  is the control fresh weight of the epibiota post-assay,  $C_f$  is the control seagrass fresh weight post-assay, and  $G$  is the grazing.

### Temperature, pH and nutrient manipulation

Temperatures were maintained by recirculating water through heater/chiller units (TECO), while pH was controlled using pH probes and automatic solenoid controllers (Sera). The temperature, pH, total alkalinity and salinity were measured daily so carbon chemistry components could be calculated (Table 1). Seawater carbon parameters were calculated using dissociation constants K1 and K2 from Roy et al. (1993) using the program CO<sub>2</sub>Calc v. 1.0.30315.

Elemental ratios were quantified in seagrass tissue to determine changes in their nutritional value. Two seagrass individuals from each microcosm were dried for 48 h at 60°C, acid washed in 5% HCl and scraped with a razor blade to remove epibiota, then re-dried for 48 h at 60°C. Seagrass tissue was then ground with a mortar and pestle and analysed for carbon and nitrogen (%N and %C) on an isotope ratio mass spectrometer (Hydra 2020 ANCA-GSL Version 4.0, Sercon).

Nutrient enrichment was controlled by supplying 1 g of Osmocote Plus® (Scotts) controlled release fertilizer per 15 l seawater (N:P:K = 17:4.3:8.2). Fertiliser pellets were placed in nylon mesh bags (1 mm mesh size) and submerged in the experimental reservoirs. Water samples were taken on 3 separate occasions throughout the experimental period on Days 1, 3 and 5. Water was sampled using sterile 25 ml syringes, fil-

Table 1. Carbonate chemistry in microcosms at different nutrient (ambient vs. enriched), pH (current vs. forecasted) and temperature (15 vs. 20°C) levels (mean ± SE). CpH: current pH treatment; FpH: forecasted pH treatment; AN: ambient nutrients; EN: enriched nutrients. Seawater carbon parameters were calculated using dissociation constants K1 and K2 from Roy et al. (1993) using the program CO<sub>2</sub>Calc v. 1.0.30315. TA: total alkalinity; CO<sub>3</sub>: carbonate; HCO<sub>3</sub>: bicarbonate; pCO<sub>2</sub>: CO<sub>2</sub> partial pressure; TCO<sub>2</sub>: total carbon dioxide

Treatment	pH	TA ( $\mu\text{mol kg}^{-1}$ )	Salinity (ppt)	Temp. (°C)	CO <sub>2</sub> ( $\mu\text{mol kg}^{-1}$ )	CO <sub>3</sub> ( $\mu\text{mol kg}^{-1}$ )	HCO <sub>3</sub> ( $\mu\text{mol kg}^{-1}$ )	pCO <sub>2</sub> ( $\mu\text{atm}$ )	TCO <sub>2</sub> ( $\mu\text{mol kg}^{-1}$ )
15°C, CpH, AN	8.115 ± 0.003	2682.64 ± 26.64	38.92 ± 0.17	15.06 ± 0.01	14.125 ± 0.177	236.83 ± 3.13	2109.61 ± 20.66	387.57 ± 5.20	2360.57 ± 23.50
15°C, CpH, EN	8.106 ± 0.001	2686 ± 6.93	39.25 ± 0.14	15.1 ± 0.02	14.429 ± 0.059	234.72 ± 0.34	2117.58 ± 6.61	397.08 ± 1.66	2366.73 ± 6.97
15°C, FpH, AN	7.933 ± 0.001	2698.67 ± 25.30	38.58 ± 0.17	15.03 ± 0.02	23.381 ± 0.328	168.17 ± 1.11	2292.97 ± 23.43	639.71 ± 8.94	2484.52 ± 24.82
15°C, FpH, EN	7.925 ± 0.001	2682.33 ± 20.33	39 ± 0.01	15.02 ± 0.02	23.662 ± 0.171	165.46 ± 1.35	2282.18 ± 17.61	648.63 ± 4.57	2471.3 ± 19.13
20°C, CpH, AN	8.109 ± 0.001	2686.33 ± 25.22	39.5 ± 0.14	19.85 ± 0.01	12.312 ± 0.126	275.1 ± 2.79	2023.27 ± 19.41	389.03 ± 4.11	2310.68 ± 22.30
20°C, CpH, EN	8.115 ± 0.001	2706.67 ± 10.90	39.25 ± 0.01	19.88 ± 0.01	12.204 ± 0.061	279.71 ± 1.10	2033.97 ± 8.79	385.38 ± 1.91	2325.88 ± 9.92
20°C, FpH, AN	7.926 ± 0.001	2699 ± 12.00	39.33 ± 0.08	19.88 ± 0.01	20.604 ± 0.103	198.12 ± 0.86	2223.01 ± 10.58	650.91 ± 3.21	2441.73 ± 11.47
20°C, FpH, EN	7.921 ± 0.001	2686.67 ± 12.33	39.25 ± 0.14	19.94 ± 0.02	20.781 ± 0.112	195.4 ± 1.04	2217.04 ± 10.07	657.38 ± 3.83	2433.21 ± 11.19

tered through 0.45  $\mu\text{m}$  and frozen. Analysis was later done using a Lachat Quickchem 8200 Flow Injection Analyser (Hach) for nitrate and nitrite (NO<sub>x</sub>), ammonia (NH<sub>4</sub>) and phosphate (PO<sub>4</sub>).

Light was provided in a 12 h light:12 h dark cycle by pairs of fluorescent lights at an average irradiance of  $62.71 \pm 1.51 \mu\text{mol m}^{-2} \text{s}^{-1}$ .

### Statistical analyses

Statistical analysis of grazing was performed using seagrass tissue chemistry (i.e. C:N ratio) and epibiota percentage as potential covariates in a fully orthogonal ANCOVA design, using the program PERMANOVA+ for Primer. To further investigate any indirect contribution of food source nutritional value to seagrass grazing loss, the C:N ratio and epibiota percentage from treatment means were used as predictor variables in a distance-based linear model (DISTLM) using the program PERMANOVA+ for Primer. Tissue chemistry, epibiota percentage, epibiota ingestion and seagrass autogenic change were also analysed separately using ANOVA with 2 fixed levels of temperature, pH and nutrients, in a fully orthogonal design. Average values from individual microcosms were treated as replicate samples. Pair-wise comparisons were used when significant interaction terms were detected. The water chemistry was analysed using ANOVA with 2 fixed levels of temperature, pH and nutrients, in a fully orthogonal design. Average daily values from 3 sampling dates were used as replicate samples.

### RESULTS

Both warming and acidification increased grazing, with the greatest change observed when they were elevated concurrently (Fig. 1, Table 2). A partially additive effect was found in which warming and acidification increased grazing to a greater extent than when either was elevated in isolation. This was considered a partially additive effect because the combined effect was smaller than the combination of the 2 independent effects added together, but larger than either of them independently (Fig. 2). For a detailed summary of partially additive and additive effects, see Brook et al. (2008). Nutrient enrichment offset the effect of warming and acidification by reducing grazing, though it did not fully counter the increase caused by warming and acidification (Fig. 1, Table 2). No pair-wise tests are reported for grazing

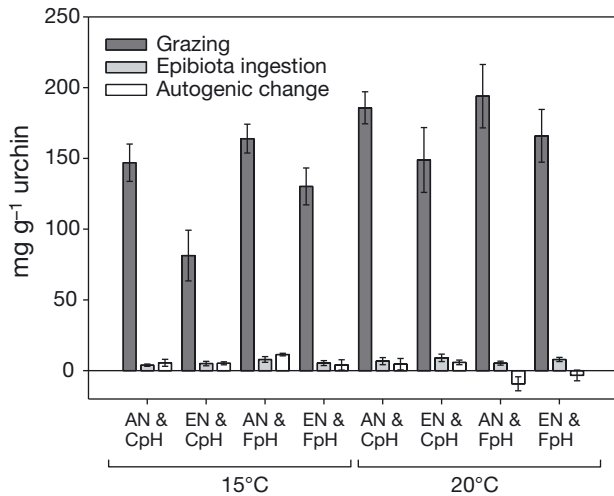


Fig. 1. Effect of nutrients (ambient vs. enriched), pH (current vs. forecasted) and temperature (15 vs. 20°C) on the grazing of seagrass, epibiota ingestion by urchins and autogenic change of seagrass. Error bars indicate the mean  $\pm$  SE for grazing, epibiota ingestion and autogenic change in seagrass fresh weight. CpH: current pH; FpH: forecasted pH; AN: ambient nutrients; EN: enriched nutrients

Table 2. ANCOVA comparing the effect of nutrients (ambient vs. enriched), pH (current vs. forecasted) and temperature (15 vs. 20°C) on the grazing of seagrass *Amphibolis antarctica* in the presence of fixed densities of a sea urchin *Amblypneustes pallidus*. The C:N ratio and epibiota percentage of seagrass were included in the analysis as covariates (CV) of grazing. Significant values in **bold**

Source	df	MS	F	p
<b>Grazing</b>				
CV	2	0.004	1.39	0.244
Temperature	1	0.046	15.46	<b>0.001</b>
pH	1	0.014	4.66	<b>0.038</b>
Nutrients	1	0.036	11.29	<b>0.001</b>
Temperature $\times$ pH	1	0.004	1.16	0.255
Temperature $\times$ Nutrients	1	0.001	0.27	0.606
pH $\times$ Nutrients	1	0.002	0.80	0.351
Temperature $\times$ pH $\times$ Nutrients	1	0.003	1.06	0.318
Residual	38	0.003		

as the ANCOVA only detected main effects. The contribution of seagrass autogenic change to grazing was small (between 2 and 7%). However, a significant interaction was detected, whereby the elevation of both temperature and acidification reduced autogenic change, an effect that was not evident when either was elevated in isolation. Nutrient enrichment had no effect on autogenic change (Fig. 1, Tables 3 & 4).

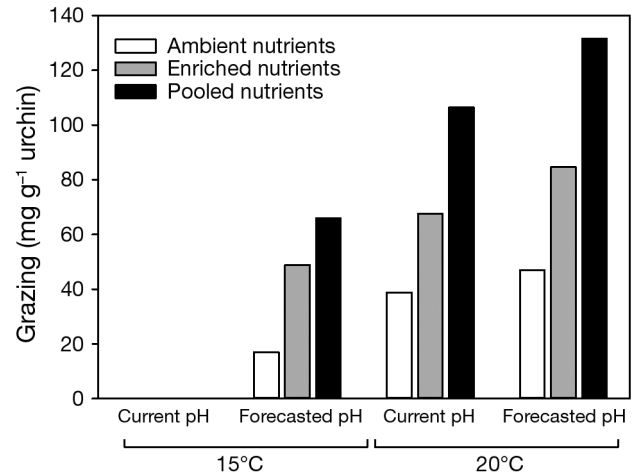


Fig. 2. Additive effect of pH (current vs. forecasted) and temperature (15 vs. 20°C) on the grazing of seagrass by urchins at ambient nutrients, enriched nutrients and pooled nutrients

Nutrient enrichment decreased the C:N ratio of seagrass, whilst warming and ocean acidification had no overall effect on the C:N ratio (Tables 3 & 4). Nutrient enrichment also increased the epibiota percentage of seagrass fresh weight, whilst temperature and ocean acidification had no overall effect on the epibiota percentage (Tables 3 & 4). The epibiota percentage was higher when nutrients were enriched, but when this was multiplied by grazing, the total ingestion of epibiota by urchins was not different between the experimental treatments (Fig. 1, Tables 3 & 4). Seagrass tissue chemistry (i.e. C:N ratio) and epibiota percentage were non-significant covariates of grazing (Table 2). However, when treatment means were used in a distance-based linear model, epibiota percentage was a significant predictor of grazing ( $p = 0.049$ ,  $R^2 = 0.512$ ), whereas tissue chemistry had no effect ( $p = 0.374$ ,  $R^2 = 0.147$ ).

$\text{NO}_x$  (nitrate + nitrite) concentrations were greater (by  $\sim 7.4$ -fold) in enriched ( $0.094 \pm 0.007 \text{ mg l}^{-1}$ ) than ambient ( $0.013 \pm 0.000 \text{ mg l}^{-1}$ ) treatments ( $F_{1,16} = 6.78$ ,  $p = 0.027$ ). Neither temperature nor ocean acidification had detectable effects on  $\text{NO}_x$  concentration ( $F_{1,16} = 0.80$ ,  $p = 0.417$  and  $F_{1,16} = 1.27$ ,  $p = 0.889$ , respectively).  $\text{NH}_4$  concentrations were greater (by  $\sim 2.3$ -fold) in enriched ( $0.087 \pm 0.015 \text{ mg l}^{-1}$ ) than ambient ( $0.038 \pm 0.002 \text{ mg l}^{-1}$ ) treatments ( $F_{1,16} = 10.64$ ,  $p = 0.003$ ). Neither temperature nor ocean acidification had detectable effects on  $\text{NH}_4$  concentration ( $F_{1,16} = 4.07$ ,  $p = 0.059$  and  $F_{1,16} = 2.74$ ,  $p = 0.850$ , respectively).  $\text{PO}_4$  concentrations were greater (by  $\sim 1.9$ -fold) in enriched ( $0.054 \pm 0.003 \text{ mg l}^{-1}$ ) than ambient ( $0.029 \pm 0.004 \text{ mg l}^{-1}$ ) treatments ( $F_{1,16} =$



Table 3. Mean  $\pm$  SE of C:N ratio, epibiota biomass, epibiota ingestion and autogenic change of seagrass *A. antarctica* in the presence of fixed densities of a sea urchin *Amblypneustes pallidus* exposed to nutrients (ambient vs. enriched), pH (current vs. forecasted) and temperature (15 vs. 20°C). CpH: current pH treatment; FpH: forecasted pH treatment; AN: ambient nutrients; EN: enriched nutrients

Treatment	C:N	Epibiota biomass (%)	Epibiota ingestion (mg g <sup>-1</sup> urchin)	Autogenic change (mg g <sup>-1</sup> urchin)
15°C, CpH, AN	16.35 $\pm$ 1.18	2.53 $\pm$ 0.35	3.84 $\pm$ 0.78	5.54 $\pm$ 2.47
15°C, CpH, EN	14.73 $\pm$ 0.21	6.63 $\pm$ 1.87	5.01 $\pm$ 1.59	5.21 $\pm$ 1.06
15°C, FpH, AN	15.65 $\pm$ 0.51	4.71 $\pm$ 1.19	7.89 $\pm$ 2.03	11.34 $\pm$ 0.91
15°C, FpH, EN	14.03 $\pm$ 0.37	4.17 $\pm$ 1.20	5.35 $\pm$ 1.75	4.02 $\pm$ 3.64
20°C, CpH, AN	15.71 $\pm$ 0.15	3.37 $\pm$ 1.08	6.72 $\pm$ 2.49	4.59 $\pm$ 4.06
20°C, CpH, EN	14.30 $\pm$ 0.40	5.40 $\pm$ 1.10	9.07 $\pm$ 2.63	5.82 $\pm$ 1.64
20°C, FpH, AN	15.30 $\pm$ 1.02	2.58 $\pm$ 0.62	5.29 $\pm$ 1.39	-9.29 $\pm$ 4.93
20°C, FpH, EN	14.67 $\pm$ 0.45	4.69 $\pm$ 0.80	7.86 $\pm$ 1.48	-3.38 $\pm$ 3.75

Table 4. p-values from ANOVAs comparing the effect of nutrients (ambient vs. enriched), pH (current vs. forecasted) and temperature (15 vs. 20°C) on the C:N ratio, epibiota biomass, autogenic change of seagrass *Amphibolis antarctica* and epibiota ingestion by urchins *Amblypneustes pallidus*. Autogenic change interaction term: Temperature  $\times$  pH; at 15°C, current pH = forecasted pH; at 20°C, current pH > forecasted pH; at current pH, 15°C = 20°C; at forecasted pH, 15°C > 20°C

Source	C:N	Epibiota biomass (%)	Epibiota ingestion (mg g <sup>-1</sup> urchin)	Autogenic change (mg g <sup>-1</sup> urchin)
Temperature	0.663	0.527	0.199	0.003
pH	0.419	0.579	0.738	0.044
Nutrients	<b>0.005</b>	<b>0.019</b>	0.502	0.955
Temperature $\times$ pH	0.450	0.702	0.188	<b>0.003</b>
Temperature $\times$ Nutrients	0.515	0.854	0.237	0.104
pH $\times$ Nutrients	0.677	0.155	0.510	0.797
Temperature $\times$ pH $\times$ Nutrients	0.651	0.142	0.459	0.197

66.41,  $p = 0.001$ ). A significant interaction was detected between temperature and nutrients on PO<sub>4</sub> ( $F_{1,16} = 10.99$ ,  $p = 0.003$ ), where in ambient nutrients low temperature treatment > high temperature treatment, whereas in enriched nutrients, low temperature treatment = high temperature treatment. CO<sub>2</sub> had no effect ( $F_{1,16} = 2.66$ ,  $p = 0.131$ ). Ocean acidification had no detectable effect on the PO<sub>4</sub> concentration.

## DISCUSSION

Predicting the indirect effects of environmental change on habitat-forming plants requires knowledge of how their interactions with other species may be modified (Ives 1995). The complexity inherent in

such tests means that indirect effects often remain unresolved relative to more conspicuous direct effects (Kordas et al. 2011). Altered species interactions can, however, have profound ecological impacts by disturbing trophic relationships within natural ecosystems (Benoit & Swain 2008, Barton et al. 2009, Connell et al. 2011). We identify the potential combination of ocean warming and acidification may combine to indirectly increasing seagrass loss due to increased grazing by urchins.

Warming commonly increases herbivore grazing due to increases in metabolic demand (Hillebrand et al. 2009, O'Connor 2009). We hypothesised that the strong positive effect of warming could be ameliorated by potentially negative effects associated with acidification (Orr et al. 2005, Miles et al. 2007, Byrne et al. 2011, Cummings et al. 2011), which may indirectly reduce feeding ability or opportunities (Bibby et al. 2007, Wood et al. 2008, Marchant et al. 2010, Stumpp et al. 2012). Contrary to this hypothesis, however, we detected a positive effect of acidification on urchin grazing. Ocean acidification has been found to increase metabolic rate and in

some instances grazing activity, as organisms partition more energetic resources to maintenance and repair of physiological processes (Wood et al. 2008, Cummings et al. 2011, Catarino et al. 2012, Li & Gao 2012).

Herbivores typically adjust their consumption relative to the nutritional content of their food source, which can be modified by environmental conditions (Ritchie et al. 1998, Russell & Connell 2007, Bressendorff & Toft 2011, Tomas et al. 2011). Although not ubiquitous to all macro-invertebrate groups (Hillebrand et al. 2009), we identified reduced urchin grazing when nutrients were enriched (e.g. Lares & McClintock 1991, Valentine & Heck 2001), supporting the hypothesis that nutrient enrichment could counteract increases in grazing associated with warming. Any nutrient-driven reduction in grazing

pressure, however, should be interpreted cautiously given the widely documented detrimental effects of nutrient enrichment on seagrass, such as overgrowth by epiphytic algae and grazer aggregation (Silberstein et al. 1986, Ruiz et al. 2001, Orth et al. 2006, Ralph et al. 2006).

Changes in the seagrass C:N ratio were small, perhaps because the seagrass was originally sourced from a meadow intermittently exposed to high nutrient concentrations on the Adelaide metropolitan coastline or due to the short duration of the experiment. Similarly, the effects of warming and acidification, both of which can modify C:N ratios (Touchette & Burkholder 2002, Jiang et al. 2010), had no detectable effect on the seagrass chemistry. As such, it appears that the disproportionate growth of epibiota we recorded, a common response to nutrient enrichment, may have offered a significant food resource to urchins in the enriched nutrient treatments (Tewfik et al. 2005, Bode et al. 2006, Ruiz et al. 2009). Although the epibiota biomass within each individual microcosm was not a significant covariate of grazing, the relationship between treatment means in the linear model suggests that epibiota could have contributed to nutrient-driven declines in grazing. Additional declines in grazing associated with nutrient enrichment could potentially be explained by changes in nitrogen fixation through microbial activity, which can be a source of nutrients for sea urchins (Guerinot & Patriquin 1981). While urchins appear to be able to implement fixation dependent on nutritional requirements, changes in epiphytic microflora or bacteria living on seagrass blades and ambient water quality often help aid or initiate fixation (Patriquin & Keddy 1978, Guerinot & Patriquin 1981). Alternatively to the model that urchins reduced grazing due to satiation, changes in epibiota or host plant secondary metabolites resulting from nutrient enrichment could have acted as deterrents to inhibit grazing (Wahl & Hay 1995, Weidner et al. 2004). However, this appears unlikely for the seagrass itself, given that *Amphibolis antarctica* have been found to reduce secondary phenolic compounds when nutrients are enriched (O. W. Burnell unpubl. data).

The per capita grazing we recorded does not incorporate potential population-level responses, such as consumer densities, resulting from changes in fecundity, recruitment and survival, which may occur independently under altered environments. Research in pelagic food webs suggests that warming will shift the balance of ecosystems in favour of high consumer (i.e. heterotrophs) abundance relative to producers (i.e. autotrophs), as consumption is limited primarily

by temperature, whilst production has numerous other potentially limiting factors (e.g. light, [CO<sub>2</sub>], temperature and nutrients) (Bulthuis 1987, Muren et al. 2005, O'Connor et al. 2009). A shift in favour of net heterotrophy would result in systems where overconsumption is more prevalent, further compounding the per capita effects we identified. This may be of particular concern in habitats where urchin grazing is already prolific due to over-exploitation of herbivore predators (e.g. fish and crustaceans). In such systems, there is a reduced likelihood that increasing urchin grazing will be counteracted by similar metabolically driven increases in predation at higher trophic levels, as predator populations may already be below critical levels to achieve active control (Kordas et al. 2011). Laboratory manipulations have identified that warming and ocean acidification will be important in controlling future population densities of urchins via growth and calcification (Brennand et al. 2010, Byrne et al. 2011). Similarly, nutrient enrichment and food quality contribute to urchin population dynamics, often promoting greater densities of urchins through changes in resource availability and reproduction (Tewfik et al. 2005, Lester et al. 2007, Ruiz et al. 2009). We suggest that ecological models integrating both per capita responses and population ecology are needed to accurately forecast changing grazing pressure.

Changes to seagrass productivity in response to multiple disturbances will also be key to predicting their future abundance and distribution (Orth et al. 2006, Palacios & Zimmerman 2007, Eklöf et al. 2012). Although seagrass growth was not the focus of our study, the negative interactive effect of warming and acidification on autogenic change was unexpected, and the mechanism behind this decline remains uncertain. Longer-term studies will be essential in providing more accurate predictions for changes in seagrass growth and chemistry. While acidification is likely to benefit seagrasses by reducing carbon limitation (Beer & Koch 1996), this effect can be negated by other stressors, such as continuing deterioration of water quality, light conditions (Palacios & Zimmerman 2007) and warming (Eklöf et al. 2012).

The present study is one of the first to detect the positive effects of ocean acidification on grazing by an ecologically important macro-invertebrate grazer. However, we suggest that a threshold is likely to exist beyond which further decreases in pH would eventually inhibit the ability of urchins to feed (Appelhans et al. 2012, Stumpp et al. 2012), as is common for the metabolic responses to increasing temperature (Sanford 2002). Further research to

establish realistic threshold levels of acidification will be important to better forecast organism responses to global change. Similarly, potential thresholds and compensatory responses associated with exposure time to acidification could exist, and highlight the need for studies incorporating longer acclimation times and opportunities for multi-generational acclimation (Sunday et al. 2011, Dupont et al. 2012).

The indirect effects from abiotic change can produce strong or unexpected outcomes that result in some of the largest effects known in ecology (Connell et al. 2011). We present some of the first indications that ocean warming and acidification could combine to intensify grazing pressure, which can strengthen top-down effects on habitat-forming plants, to unprecedented levels (Ling et al. 2009). Such change in global conditions will occur concomitantly with other alterations to the physical and biological environment, such as nutrient enrichment and over-fishing, potentially further modifying grazer abundance and their rates of consumption.

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