Coral Reefs
DOI 10.1007/s00338-013-1066-5

REPORT

Direct and indirect effects of high pCO_2 on algal grazing by coral reef herbivores from the Gulf of Aqaba (Red Sea)

E. M. Borell · M. Steinke · M. Fine

Received: 3 November 2012 / Accepted: 12 July 2013

Abstract Grazing on marine macroalgae is a key structuring process for coral reef communities. However, ocean acidification from rising atmospheric CO_2 concentrations is predicted to adversely affect many marine animals, while seaweed communities may benefit and prosper. We tested how exposure to different pCO_2 (400, 1,800 and 4,000 µatm) may affect grazing on the green alga *Ulva lactuca* by herbivorous fish and sea urchins from the coral reefs in the northern Gulf of Aqaba (Red Sea), either directly, by changing herbivore behaviour, or indirectly via changes in algal palatability. We also determined the effects of pCO_2 on algal tissue concentrations of protein and the grazing-deterrent secondary metabolite dimethylsulfoniopropionate (DMSP). Grazing preferences and overall consumption were tested in a series of multiple-choice feeding experiments in the laboratory and in situ following exposure for 14 d (algae) and 28 d (herbivores). 4,000 µatm had a significant effect on the biochemical composition and palatability of *U. lactuca*. No effects were observed at 1,800 relative to 400 µatm (control). Exposure of *U. lactuca* to 4,000 µatm resulted in a significant decrease in protein and increase in DMSP concentration. This coincided with a reduced preference for these algae by the sea urchin *Tripneustes gratilla* and different herbivorous fish species in situ (Acanthuridae, Siganidae and Pomacanthidae). No feeding preferences were observed for the rabbitfish *Siganus rivulatus* under laboratory conditions. Exposure to elevated pCO_2 had no direct effect on the overall algal consumption by *T. gratilla* and *S. rivulatus*. Our results show that CO_2 has the potential to alter algal palatability to different herbivores which could have important implications for algal abundance and coral community structure. The fact that pCO_2 effects were observed only at a pCO_2 of 4,000 µatm, however, indicates that algal-grazer interactions may be resistant to predicted pCO_2 concentrations in the near future.

Keywords Ocean acidification · Grazing · DMSP · Protein · Fish · Sea urchins

Communicated by Ecology Editor Dr. Stuart Sandin

E. M. Borell (✉) · M. Fine
The Interuniversity Institute for Marine Sciences,
88000 Elat, Israel
e-mail: estherborell@yahoo.co.uk

M. Steinke
Coral Reef Research Unit, School of Biological Sciences,
University of Essex, Colchester CO4 3SQ, UK

M. Fine
The Mina and Everard Goodman Faculty of Life Sciences,
Bar-Ilan University, 52900 Ramat Gan, Israel

Introduction

Plant-herbivore interactions are important structuring processes in diverse marine environments (Lubchenco and Gaines 1981; Hay 1997). On tropical coral reefs, grazing by herbivorous fish and sea urchins is a key component for maintaining high-coral and low-algal cover (Hughes et al. 1987, 2007; Ferrari et al. 2012). Consumption by many marine herbivores is, however, strongly affected by the nutritional quality of the algae (Duffy and Paul 1992; Cruz-Rivera and Hay 2003). Especially, nitrogen, but also carbohydrates and lipids can play a critical role in the food selection of herbivorous organisms (e.g. Duffy and Paul 1992; Hemmi and Jormalainen 2002; Cruz-Rivera and Hay 2003). Herbivores may preferentially graze on algae with high nutritional value (Duffy and Paul 1992) or increase their consumption (compensatory feeding) of poor quality

Published online: 26 July 2013

food (Cruz-Rivera and Hay 2001) in order to satisfy their nutritional requirements. On the other hand, many algae (and seagrasses) produce a variety of secondary metabolites including phenolics, terpenes and amino acid-derived substances, which affect algal palatability, acting as feeding deterrents to many grazers (McClintock and Baker 2001). Any changes in the interactions between primary producers and their consumers will affect grazing pressure and herbivore densities, and this is likely to have significant ramifications for food-web interactions and the stability of community structures (Hay 2009).

Approximately, one-third of the anthropogenically produced CO_2 released into the atmosphere in the past 200 years has been taken up by the oceans (Zeebe et al. 2008). This has led and continues to lead not only to an increase in available CO_2 in the water but also to a decrease in seawater pH, making the oceans more acidic (Caldeira and Wickett 2005). As such, CO_2 could become an important resource-mediated (bottom-up) catalyst of enhanced seaweed growth; either due to increased vegetative growth (Zou 2005) and enhanced competitive abilities (Diaz-Pulido et al. 2011), or via reduced competitive interactions with calcareous algae (Connell and Russell 2012; Kroeker et al. 2013). At the same time, grazing rates by herbivores may be altered, either directly through changes in metabolic rate (Michaelidis et al. 2007; Wood et al. 2008) or indirectly via changes in the biochemical composition of the primary producers.

High CO_2/low pH is predicted to impact survival, development and physiology of a wide range of species, especially calcifying ones (Hendriks et al. 2010; Kroeker et al. 2010). Yet, our understanding of how effects beyond the individual organism level, especially of non-calcifying organisms, may influence functional diversity and community structure is still very limited (Hall-Spencer et al. 2008; Wernberg et al. 2012).

In an extensive meta-analysis, Stiling and Cornelissen (2007) found that in terrestrial plants elevated levels of atmospheric CO_2 generally lead to a decrease in plant protein, an increase in secondary metabolites and a decrease in herbivore abundance. In contrast, high levels of pCO_2 in seawater resulted in lower concentrations of phenols in seagrasses and concurrent rates of increased grazing (Arnold et al. 2012), but data on the effect of pCO_2 on algal-grazer interactions are scarce.

A reduction in nitrogen in response to elevated pCO_2 has been reported for a few algal species (Kübler et al. 1999; Gordillo et al. 2001a). Rossoll et al. (2012) have shown a positive correlation between decreased levels of fatty acids in diatoms following exposure to pCO_2 of 750 µatm and reduced fitness of copepods, while Swanson and Fox (2007) found an increase in phlorotannins in the kelp species *Saccharina latissima* following exposure to high levels of pCO_2 (3,000 µatm) only in conjunction with increased UV radiation. No changes in phlorotannins were detected in the kelp *Laminaria hyperborea* exposed to 700 µatm (Olischlaeger et al. 2012).

Here we tested the direct and indirect effects of elevated pCO_2 on algal grazing by sea urchins and herbivorous fish from the Gulf of Aqaba using the opportunistic chlorophyte *Ulva lactuca* as a model alga. Although *U. lactuca* may generally not be considered a typical reef alga, this species and a variety of other *Ulva* spp. (including former *Enteromorpha* spp.) frequently colonize the coral reefs along the northern tip of the Gulf (Lundberg and Golani 1995; Lundberg and Popper 1999) and occasionally smother large patches of reefs following deep water mixing and upwelling of nutrients (Genin et al. 1995). The *Ulva* blooms coincide with the recruitment of juvenile rabbitfish (*Siganus* spp.) which are prominent grazers on the reef (Bouchon-Navaro and Harmelin-Vivien 1981) that utilize *Ulva* spp. as an important dietary component (Lundberg and Golani 1995; Khait 2009). Due to the high abundance of herbivorous fish (Brokovich et al. 2010) and the general oligotrophic nature of the Gulf, algal cover is typically sparse. Perhaps, for this reason and the high nutrient content of *U. lactuca* (Caceres et al. 1994), all major macroherbivores appear to operate as generalists, which have been shown to preferentially graze on *U. lactuca* when available (Khait 2009; Einbinder et al. 2006).

Furthermore, *U. lactuca*, like many other chlorophytes, synthesizes and accumulates high concentrations of the secondary metabolite dimethylsulfoniopropionate (DMSP; Steinke et al. 1996), which is thought to be part of an activated anti-grazing defence mechanism in macroalgae (Van Alstyne et al. 2001; Van Alstyne and Houser 2003). This makes it an ideal model alga to test potential effects of CO_2 on herbivore grazing pressure in the Gulf of Aqaba.

Although the environmental factors affecting the production and accumulation of DMSP are fairly well understood (Stefels 2000), knowledge on the possible influence of increased pCO_2 on the synthesis of DMSP is mixed. So far, only one study has reported a direct effect of elevated pCO_2 on the cellular production of DMSP in algae. Spielmeyer and Pohnert (2012) found that increased levels of pCO_2 in conjunction with elevated temperature resulted in an increase in cellular DMSP concentrations in the phytoplankton *Emiliania huxleyi,* whereas DMSP concentrations in several other species decreased. Other studies found no effects of pCO_2 on DMSP concentrations in either phytoplankton (Lee et al. 2009) or macroalgae (Kerrison et al. 2012; Olischläger et al. 2012).

The objectives of this study were (1) to determine the effects of elevated pCO_2 on algal protein and DMSP concentrations in *U. lactuca*, (2) to use multiple-choice feeding assays in situ and under controlled laboratory

conditions to assess CO_2 effects on algal palatability to a natural community of herbivorous adult fish (Acanthuridae, Siganidae and Pomacanthidae), the rabbitfish *S. rivulatus* and the sea urchin *T. gratilla* and (3) to examine the direct effects of pCO_2 on the grazing behaviour of *S. rivulatus* and *T. gratilla.*

Materials and methods

Our experiments were placed in a regionally relevant setting for the northern Gulf of Aqaba (29°30′N, 34°55′E), which is a highly oligotrophic and hypersaline (salinity of 41 on the Practical Salinity Scale) extension from the Red Sea (Genin 2008).

Organism collection

Since the presence of naturally occurring *U. lactuca* in the Gulf of Aqaba is seasonal, the algal material for our experiments was obtained from the National Center for Mariculture in Eilat, Israel. The thalli of the cultured algal material were generally smaller compared to the thalli of the algae from the field. Preliminary tissue analyses showed no significant difference in protein or chlorophyll concentrations between field and cultured *U. lactuca*, and all herbivores readily grazed both types. As model herbivores, we chose the juvenile rabbitfish *S. rivulatus* and the collector urchin *T. gratilla* as these species are among the most abundant grazers in the northern Gulf of Aqaba and occupy both reef- and seagrass-dominated areas. *S. rivulatus* (5–6 cm length) and *T. gratilla* (2–3 cm diameter) were collected at night by scuba diving from the North Beach of the Gulf of Aqaba, in August 2010 and were transported directly to the Interuniversity Institute for Marine Sciences (IUI) in Eilat, Israel.

A total of 50 fish and sea urchins were collected and kept as stock in separate 100-L outdoor plastic containers with running seawater to be used in our experiments. Fish were fed daily and sea urchins were fed every other day with a fixed amount of non-assay *U. lactuca* which was either obtained from the National Center of Mariculture or freshly collected from the sea near the IUI.

Seawater enrichment with CO_2

Herbivores and algae were exposed to either control or CO_2-enriched seawater at three different pCO_2 levels.

The aim of this study was to test a general mechanism that could underlie future effects of increased CO_2 absorption by the oceans on herbivore–algae interactions in reefs. In order to amplify potential physiological response thresholds of animals and algae to ocean acidification conditions (Pörtner 2008), we chose pCO_2 levels which lie at the far end of predicted future values (Barry et al. 2010), following the 'Logistic' emission pathways for the year 2300 based on estimates of the use of global fossil fuel resources (Caldeira and Wickett 2005): (1) 400 µatm (control), (2) 1,800 µatm and (3) 4,000 µatm.

The experiments were carried out using an indoor CO_2 system for *T. gratilla* and *U. lactuca* and an outdoor system for *S. rivulatus*. Both systems employed the same principle to achieve and maintain different pCO_2 treatment conditions. Seawater was pumped from a depth of 30 m into three 1,000 L storage tanks (per system). The storage tank for the control received unmodified seawater, whereas the two other storage tanks received CO_2-enriched seawater. The desired pCO_2 levels were adjusted via pH, following standard techniques for ocean acidification research, as outlined by the Best Practices Guides for Ocean Acidification Research (Gattuso et al. 2010). The tanks were fitted with pH electrodes (Aqua Medic, Germany) and connected to a pH controller (AT-Control, Aqua Medic, Germany). Whenever the pH of the seawater increased above the set value for pH, CO_2 was injected via a solenoid valve into a water pump located in the centre of the storage tank, which rapidly dissolved CO_2 in the seawater by turbulent mixing. An additional large water pump at the bottom of each tank ensured rapid mixing of the water.

Data on salinity and nutrients were obtained once a month from the Gulf of Aqaba National Monitoring program. Total alkalinity (TA) in the aquaria, seawater tables and storage tanks was measured weekly using a Metrohm 862 compact titrosampler (Cohen 2011). Temperature and pH_{NBS} were measured daily (CyberScan pH 11; Eutech Instruments Pte Ltd, Singapore). The experimental pCO_2 levels were calculated from salinity, TA and pH_{NBS} measurements using the program CO_2SYS (Pierrot et al. 2006), applying the constants of Mehrbach et al. (1973). Experimental seawater parameters are shown in Table 1.

Protein and DMSP

Ulva lactuca was placed into one of three round indoor seawater tables ($\sim$ 200 g per table, 120 L) that received running seawater (1.7 L min^{-1}) from each of the respective pCO_2 treatments and a light intensity of $\sim$270 µmol m^{-2} s^{-1} (Li-COR 1000) for 14 d. Seawater circulation was enhanced by water pumps. Three thalli from each pCO_2 treatment were frozen at −80 °C and then freeze-dried for the analyses of proteins. The experiment was run in three replicate trials, which were treated as replicates later in the data analyses ($n = 3$). For the analysis of DMSP, $\sim$30 g of *U. lactuca* were allocated to one of 15 glass aquaria (15 L), each of which was supplied with a continuous flow of seawater at 1.7 L min^{-1} from one of the three pCO_2 treatments ($n = 5$). Algae were incubated for 14 d, after which one piece of thallus was sampled from

Table 1 Mean seawater parameters in the experimental system

pH_{NBS}	Temperature (°C)	Salinity	TA (μmol kg^{-1} SW)	pCO_2 (μatm)
U. lactuca				
8.18 (0.05)	26.98 (0.56)	41	2,496.51 (6.73)	411 (53)
7.62 (0.08)	26.75 (0.76)	41	2,504.23 (10.83)	1,763 (107)
7.31 (0.08)	26.91 (0.54)	41	2,503.76 (11.21)	3,973 (112)
S. rivulatus				
8.20 (0.04)	26.66 (0.88)	41	2,500.01 (8.92)	387 (67)
7.65 (0.06)	26.87 (0.59)	41	2,504.23 (12.01)	1,721 (86)
7.31 (0.07)	26.54 (0.79)	41	2,503.98 (9.76)	3,958 (104)
T. gratilla				
8.22 (0.07)	26.74 (0.83)	41	2,510.73 (14.13)	367 (84)
7.66 (0.06)	26.65 (0.67)	41	2,502.54 (10.11)	1,673 (68)
7.33 (0.03)	26.69 (0.63)	41	2,502.41 (13.88)	3,775 (104)

Data shown are the average (±SD) experimental seawater parameters from three pCO_2 treatments (400, 1,800 and 4,000 μatm) of *U. lactuca* (112 d), *S. rivulatus* (84 d) and *T. gratilla* (84 d)

each aquarium, blotted dry, weighed (Shinko Denshi Co., LTD, Japan, accuracy 0.001 g) and processed immediately for DMSP extraction. After each trial, the water tables and pCO_2 treatment were switched to avoid confounding effects of experimental location.

Proteins were extracted in deionized water and 0.05 % (v/v) mercaptoethanol and quantified using a commercial Protein Assay (Bio-Rad) following a modified method described by Zor and Selinger (1996). DMSP was quantified from 100 to 200 mg fresh weight of *U. lactuca* by gas chromatography (Steinke et al. 2011). First, the tissue was placed in 4.92 mL gas-tight vials capped with Teflon-silicone septa and containing 3 mL of 0.5 M NaOH to hydrolyse the DMSP to DMS in the dark at 30 °C for at least 24 h. Second, 8 μL of headspace was injected into a gas chromatograph (GC-2010, Shimadzu, Milton Keynes, UK) equipped with a 30 m × 0.53 mm × 5 μm HP-1 capillary column (Agilent, Wokingham, UK) and a flame photometric detector. The oven was operated isothermally at 120 °C. Carrier gas (He) was supplied at 10.56 mL min^{-1} (linear velocity 80 cm s^{-1}), and the flame gases compressed air, and H_2 were set to 70 and 60 mL min^{-1}, respectively. DMS eluted after ~1 min. The instrument was calibrated with a series of DMSP standards (1–472 μM) that were hydrolysed in 3 mL 0.5 M NaOH using the same glass vials as for tissue samples. Linear regression of DMS concentration versus the square root of peak area yielded an $r^2 > 0.99$. The detection limit in 8 μL of headspace sample was ≤1 μM DMS (lowest concentration used in headspace calibrations).

Effects of pCO_2 on algal palatability—in situ feeding assays

Experiments on the effects of pCO_2 on the palatability of algae to different herbivorous fish in situ were carried out in the Gulf of Aqaba close to the IUI in May 2010. Prior to the feeding assays, *U. lactuca* was freshly obtained from the Mariculture Center and incubated for 14 d under three pCO_2 concentrations as described above. Eighteen bricks (3 rows × 6 bricks, 15 cm apart from each other) were placed on an empty gravel patch next to the reef with high herbivore abundance at a depth of 3 m, and pieces of *U. lactuca* [1.5–2 g fresh weight (FW)] from each treatment were haphazardly allocated to one of the bricks ($n = 6$) and fixed with clothes pegs. Fish feeding was recorded with a stationary underwater video camera, which was placed at a distance of 1.5 m from the site of the assay allowing the fish to feed undisturbed.

The natural grazing activity of herbivorous fish follows a distinct diel pattern, i.e. grazing activity is lowest in the morning and increases gradually over the day with maximum foraging activity in the afternoon (e.g. Zemke-White et al. 2002). In order to capture morning and afternoon feeding activity of the fish and to minimize a bias of feeding activity on potential algal preferences, feeding assays were carried out at three time points over the course of the day at 0800, 1200 and 1530 hrs and also served as replicates ($n = 3$). To quantify the relative removal rates of each of the three algal types by the fish, the video footage was analysed and the total number of bites taken by individuals from each fish family was scored over 10 min, after which more than half of the algae had been consumed. Algal preference was then calculated for each time point as the percentage bites per fish family per algal treatment over the total number of bites across algal treatments. A 'bite' was recorded only if the fish clearly ingested the algae. Rapid, consecutive bites were counted as a single bite. The experiment was repeated in two trials at an interval of 2 weeks. Herbivorous fish in both trials of the feeding assay comprised adults (sizes ranged between 15 and 25 cm) of three families including surgeonfish (Acanthuridae, *Acanthurus nigrofuscus*, *Zebrasoma desjardinii*, *Z. xanthurum*), rabbitfish (Siganidae, *S. rivulatus, S. luridus*) and to a lesser extent, the Emperor Angelfish (Pomacanthidae, *Pomacanthus imperator*). Even though parrotfish (Scaridae) are considered as major herbivores in this area, in preliminary feeding trials, we found that they were not interested in the *Ulva*. Some individuals took an occasional bite but never ingested the algae and were therefore not considered in our experimental trials.

Effects of pCO_2 on algae palatability and herbivore consumption—laboratory feeding assays

To test the simultaneous effects of pCO_2 on both algal palatability and overall consumption of *S. rivulatus* and *T. gratilla*, multiple-choice feeding assays were carried out in three repeat trials for each species in September 2010.

S. rivulatus and *T. gratilla* were exposed to three different pCO_2 levels for 28 d, and *U. lactuca* was exposed to different pCO_2 levels for 14 d as described above. Although behavioural effects of elevated pCO_2 are thought to manifest after only a few days (Munday et al. 2010), due to the lack of a gradual acclimation period to high pCO_2 in our study, we chose an incubation duration of 1 month. For each trial, *S. rivulatus* was placed into one of three white plastic containers (62 L, $n = 5$) which were supplied with running seawater (1.2 L min^{-1}) at three different pCO_2 levels and received natural sunlight at a maximum water temperature of 26 ± 0.5 °C. Plastic netting (1 mm mesh) was used to attain a 50 % reduction in ambient light intensity (maximum intensity of about 700 µmol m^{-2} s^{-1} at noon, Li-COR 1000). Fifteen *T. gratilla* were transferred to one of three indoor seawater tables (~150 L, $n = 5$) and were supplied with running seawater (1.7 L min^{-1}) at three different pCO_2 levels. All holding containers and seawater tables contained a 1-cm gravel substrate to trap faeces and other debris and were cleaned once a week. Water circulation was ensured using small aquarium water pumps. After each trial, the combination between holding containers, aquaria and pCO_2 treatment level were rearranged to minimize a bias treatment response due to experimental location. For the feeding assay, the fish or sea urchins were transferred to one of 15 individual glass aquaria (15 L, $n = 5$), which received running seawater of the respective pCO_2 treatment to which the animals were conditioned to. The animals were allowed to acclimate to the experimental conditions for 2 d and were fed daily with non-assay *U. lactuca.* To test the effects of pCO_2 on algal palatability, each individual was simultaneously offered a choice of three similar-sized pieces of *U. lactuca* (0.6–0.7 g FW for *S. rivulatus* and 0.7–0.8 g for *T. gratilla*) from one of the three pCO_2 treatments using the approach described by Hay et al. (1988). The three pieces of algae were positioned randomly on the bottom of each aquarium using clothes pegs. *S. rivulatus* was allowed to feed for 5 h and *T. gratilla* for 4 h. An additional three aquaria contained algae without animals in order to control for autogenic changes in mass (see below). The quantity of algal material and 'foraging' time had been determined in preliminary experiments to assure that algal material was supplied in excess. The sum of the changes in tissue biomass for each of the three pieces of algae was calculated to give the total amount eaten for each individual.

The relative palatability of a piece of alga from one of the three pCO_2 treatments (here after referred to as pCO_2*Ulva*) was determined via the proportional consumption of each pCO_2*Ulva* piece relative to the total consumption and calculated using the following equation modified after Lockwood III (1998):

$$\frac{\left[(U_O - U_F)_i - (U_{AO} - U_{AF})_i\right]}{\sum_{j=1}^{3}\left[(U_O - U_F)_j - (U_{AO} - U_{AF})_j\right]}$$

where i represents a piece of alga from each of the three pCO_2 treatments j; U_O and U_F are pre- and post-assay FW of the three pieces of *Ulva* tissue that were offered to the herbivores; and U_{AO} and U_{AF} are pre- and post-assay FW of controls for autogenic changes in mass.

Effects of pCO_2 on the overall consumption of herbivores (hereafter referred to as pCO_2*Grazer*) were determined via the sum of algal tissue eaten from each of the three different pCO_2 *Ulva* pieces by each individual:

$$\sum_{j=1}^{3}\left[(U_O - U_F)_j - (U_{AO} - U_{AF})_j\right]$$

where j is one of the three different pCO_2 treatments in which the grazers were cultured.

Data analyses

Protein and DMSP concentrations were analysed by one-way ANOVA. For the protein analysis, concentrations of the three thalli from each treatment were averaged. Each trial was treated as a replicate unit. Algal palatability and effects of pCO_2 on animal consumption were analysed by ANOVA using a nested design. Algal preferences of each fish family in situ for *U. lactuca* exposed to one of the three different pCO_2 treatments were analysed as the percentage bites of each pCO_2*Ulva* relative to all bites during 10 min of each time point using three-way ANOVA with pCO_2*Ulva* and fish family as fixed factors and trial as a random factor which was nested in pCO_2*Ulva*. Each time point was treated as a replicate unit. Relative palatability of different pCO_2*Ulva* to *S. rivulatus* and *T. gratilla* in the laboratory was analysed by three-way ANOVA with pCO_2*Ulva* and pCO_2*Grazer* as fixed factors and trial as random factor nested in pCO_2*Ulva* and pCO_2*Grazer*. To assure that the consumption of algal biomass was not confounded by initial algal mass, algal loss for each piece of algae for each of the respective pCO_2 aquaria over the three trials were regressed to its initial mass. The direct effect of pCO_2 on the overall consumption of *U. lactuca* by *S. rivulatus* and *T. gratilla* was analysed by two-way ANOVA with pCO_2*Grazer* as fixed and trial as random nested factors.

All data were checked for homogeneity of variances using Cochran's C-test prior to each analysis and, if necessary, ln(x) transformed to stabilize variances. Student–Newman–Keuls (SNK) tests were used for post hoc multiple comparisons. ANOVAs were performed using WinGMAV (EICC, University of Sydney, Australia).

Results

Protein and DMSP

Exposure to different concentrations of pCO_2 for 14 d had a significant effect on the concentrations of tissue protein ($F_{2,18} = 13.45$, $p < 0.001$) and DMSP ($F_{2,12} = 7.67$, $p < 0.001$) for *U. lactuca* exposed to a pCO_2 of 4,000 µatm. High pCO_2 resulted in a 60 % decrease in tissue proteins (SNK, $p < 0.01$) compared to concentrations in *Ulva* exposed to 1,800 and 400 µatm (Fig. 1a). DMSP concentrations by contrast significantly increased in *U. lactuca* at 4,000 µatm (SNK, $p < 0.01$) relative to the other two pCO_2 treatments (Fig. 1b).

Effects of pCO_2 on algae palatability—in situ feeding assays

Overall, *U. lactuca* was consumed rapidly at each sampling event, and grazing activity (i.e. average number of bites) did not follow a natural diet pattern as is often observed for herbivorous fish (e.g. Zemke-White et al. 2002).

Interestingly, fish from all three families clearly discriminated between *Ulva* grown at 4,000 µatm and the other two pCO_2 levels. In both trials, bites from *U. lactuca* exposed to 4,000 µatm were significantly lower ($F_{2,36} = 7.48$, $p < 0.01$) than bites from *U. lactuca* cultured at 1,800 and 400 µatm (SNK, $p < 001$; Fig. 2). Fish usually probed the algae by taking a bite and then either stayed and took more bites, or in case of the *Ulva* pieces that were cultured at a pCO_2 of 4,000, moved on to 'test' neighbouring algal pieces or swam away. In both trials, there were no significant differences in consumption between *Ulva* exposed to 1,800 and 400 µatm. A significant variation in overall bites between species ($F_{2,36} = 12.35$, $p < 0.001$) reflected the relative abundance of each group during the experiment; Trial one: Acanthuridae > Siganidae > Pomacanthidae (SNK, $p < 0.001$); Trial two: Acanthuridae = Siganidae > Pomacanthidae (SNK, $p < 0.001$).

Effects of pCO_2 on food consumption and algal palatability

The results of the feeding assays in the laboratory differed from our observations in situ. Juvenile *S. rivulatus* displayed no clear feeding preference for any of the three pCO_2 *Ulva* (Fig. 3a). ANOVA revealed a significant $pCO_2Ulva \times pCO_2Fish$ interaction ($F_{4,108} = 5.22$, $p < 0.01$). This was due to the significantly lower consumption of *Ulva* cultured in 4,000 µatm relative to *Ulva* from the other two treatments by fish incubated at 1,800 µatm (SNK, $p < 0.01$). In contrast, exposure to different pCO_2 had a significant effect on algal preference of *T. gratilla* ($F_{2,108} = 28.48$, $p < 0.001$). Sea urchins

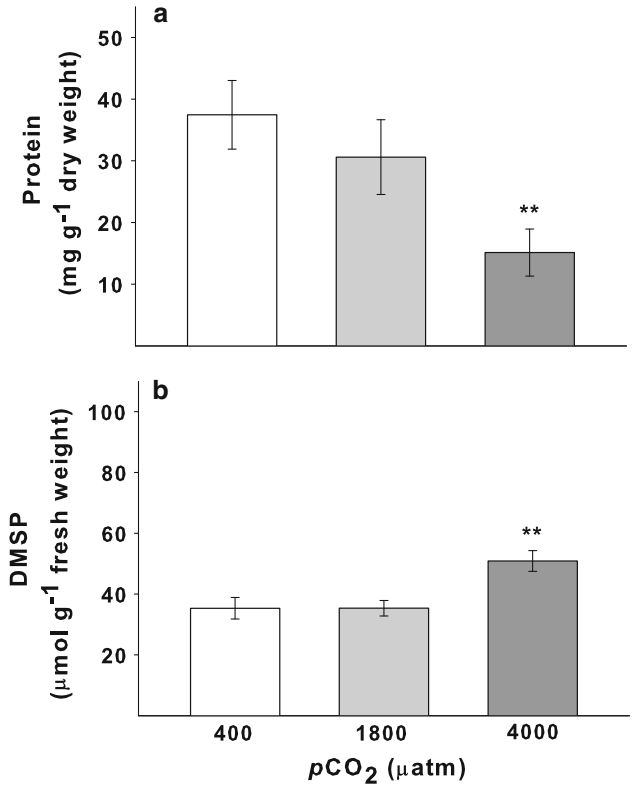

Fig. 1 Mean concentrations of (**a**) protein ($n = 3$, ±SEM) and (**b**) DMSP ($n = 5$, ±SEM) in *U. lactuca* after 14 d of exposure to three different pCO_2 levels (400, 1,800 and 4,000 µatm). *Asterisks* denote significant differences in concentrations (SNK: $^{**}p < 0.01$)

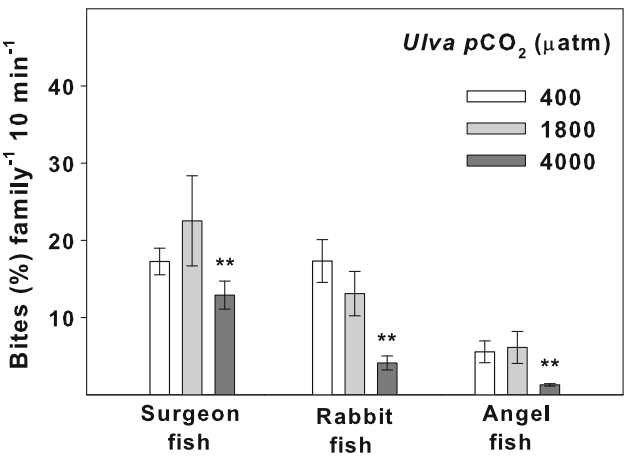

Fig. 2 Percentage bites of *U. lactuca* cultured under three different pCO_2 concentrations (400, 1,800 and 4,000 µatm) for 14 d by surgeonfish, rabbitfish and the emperor angelfish. Shown are means of percentage bites of each pCO_2 *Ulva* for each family over 10 min and averaged over three time points and two trials ($n = 3$, ±SEM). *Asterisks* indicate significant differences in bite rates between different pCO_2Ulva (SNK: $^{**}p < 0.01$)

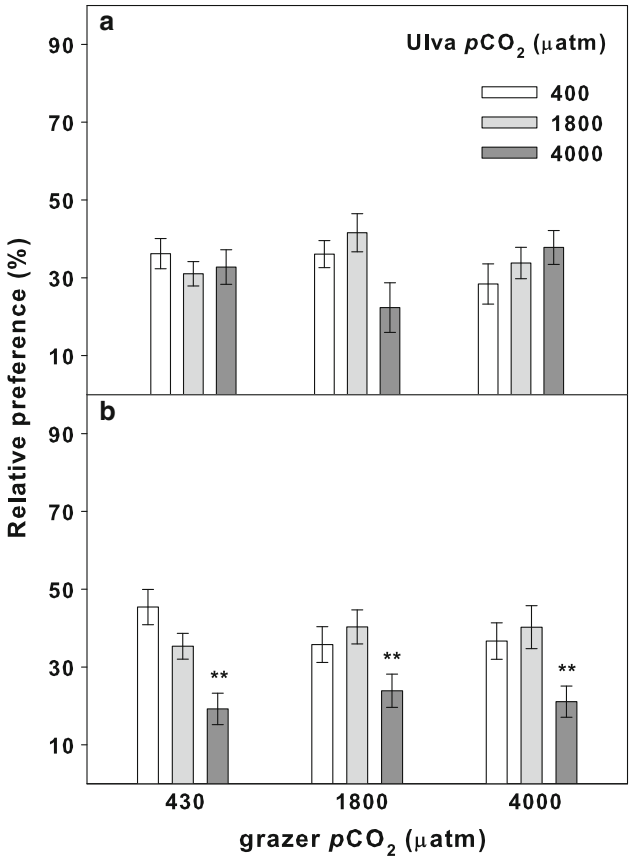

Fig. 3 Consumption of *U. lactuca* by (**a**) *S. rivulatus* and (**b**) *T. gratilla* following 28 d of exposure to one of three different pCO_2 concentrations (400, 1,800 and 4,000 µatm) over three repeat trials ($n = 3$, ±SEM). Data are the average total consumption of three pieces of *Ulva* from different pCO_2 concentrations (400, 1,800 and 4,000 µatm). *Asterisks* indicate significant differences in *Ulva* consumption (SNK: $^{**}p < 0.01$)

consistently displayed a significant preference for *U. lactuca* cultured at 400 and 1,800 µatm over *U. lactuca* which was grown at 4,000 µatm (SNK, $p < 0.01$; Fig. 3b). There was no significant difference between consumption of *Ulva* exposed to 1,800 and 400 µatm. pCO_2 had no significant direct effect on the overall consumption of either species. Irrespective of pCO_2 concentrations, feeding of both *S. rivulatus* and *T. gratilla* was observed to be constant (0.3–0.5 g) over a period of 4 and 5 h, respectively. Linear regression analyses showed that there was no significant relationship between macroalgal mass loss and initial mass of macroalgae for either fish or sea urchins ($p < 0.01$ for all levels).

Discussion

Grazing pressure on marine macroalgae is strongly influenced by the nutritional quality of the algae and the production of a variety of secondary metabolites, which may affect algal palatability or could function as herbivore deterrents (McClintock and Baker 2001; Cruz-Rivera and Hay 2003).

Our results show that sea urchins in the laboratory and fish in situ displayed a significant dislike for *Ulva* cultured at 4,000 µatm pCO_2. This coincided with a decrease in tissue protein and an increase in DMSP in these algae.

Previous studies investigating the effects of CO_2 on macroalgal secondary metabolites are consistent with our results and found a change in concentrations of algal secondary metabolites only at very high pCO_2 (>3,000 µatm), whereas lower levels of pCO_2 had no effect (Swanson and Fox 2007; Kerrison et al. 2012; Olischläger et al. 2012). For example, Kerrison et al. (2012) found that DMSP tissue concentrations in *U. lactuca* and *U. clathrata* remained unchanged following exposure to 1,514 µatm pCO_2 for 7 and 14 d, respectively. However, changes in DMSP in response to fluctuation in carbonate chemistry were reported recently by Burdett et al. (2013) who measured DMSP concentrations in tropical macroalgae and the overlying water column in response to natural changes in the reefal carbonate chemistry. Their results showed that DMSP concentrations were highest at night when the carbonate saturation state was lowest coinciding with the highest pCO_2 and lowest pH.

A possible explanation for the apparent lack of response of secondary metabolism in many species to elevated CO_2 could be that these are well adapted to utilize HCO_3^- as their inorganic carbon source (e.g. Johnston 1991), suggesting that physiological and metabolic processes would generally not be carbon limited and as such not readily affected by 'moderate' levels of CO_2 enrichment (e.g. Israel and Hophy 2002; Swanson and Fox 2007). It is also possible that DMSP furnishes different metabolic functions in different algal species (Stefels 2000). In addition, the response of algal biochemical composition to CO_2 enrichment is likely to be influenced by a series of other factors such as light (Johnston et al. 1992), life history strategy (Swanson and Fox 2007) and nutrient availability (Gordillo et al. 2001a, b). Kerrison et al. (2012) used artificial seawater with high nutrient concentrations, especially nitrate (549 µM), compared to the oligotrophic seawater in the Gulf of Aqaba (<1.5 µM nitrate during our experiment). Hence, the combination of high pCO_2 and low nutrients may to some extent account for the observed differences in DMSP concentrations between our experiment and the data presented by Kerrison et al. (2012). Indeed, a proposed physiological function of DMSP is that of an overflow mechanism facilitating the dissipation of excess carbon and simultaneous assimilation of sulphate under nitrogen deficient conditions, thereby ensuring the continuation of metabolic pathways and the maintenance of

low cysteine and methionine concentrations (Stefels 2000). Likewise, Gordillo et al. (2001a, b) showed that CO_2 enrichment strongly interacted with nitrogen availability. A decrease in tissue protein and changes in fatty acid composition only occurred under nitrogen limitation when *U. rigida* was exposed to a pCO_2 of 10,000 µatm. Thus, the combined effects of low nutrient concentrations and high pCO_2 may have caused a decrease in protein and increase in DMSP concentration in our experiment.

Grazing-induced enzymatic cleavage of DMSP to acrylate, and DMS has been shown to be a potent feeding deterrent to the temperate sea urchins *Strongylocentrotus droebachiensis* and *S. purpuratus* (Van Alstyne et al. 2001; Van Alstyne and Houser 2003). Low protein and high cellular DMSP content may have been associated with the low preference for high pCO_2*Ulva* by sea urchins in the laboratory and fish in situ. Interestingly, while adult fish in situ clearly discriminated between *Ulva* from different pCO_2 treatments, juvenile rabbitfish in the laboratory showed no preferences for any of the different pCO_2*Ulva*. It is possible that food preferences and susceptibilities to secondary metabolites differed between adults and juveniles. A difference in dietary preference between juveniles and adults was reported for several species of rabbitfish in the western Pacific and the Red Sea (e.g. von Westernhagen 1973; Lundberg and Lipkin 1979; Hay et al. 1990), likely due to a greater sensitivity to secondary metabolites in adults than in juveniles (Paul et al. 1990). Alternatively, recognizing secondary metabolites may be a cognitive process and juvenile fish may only become deterred after repetitive encounters with these compounds. Thacker et al. (1997), for example, showed that the magnitude of deterrence to some secondary metabolites by juvenile rabbit and parrotfish increased over time.

In the northern Gulf of Aqaba, high herbivore biomass and low nutrient concentrations restrict primary production, rendering food availability a major limiting factor for herbivores. *U. lactuca* generally features a high nutrient content and can substantially increase herbivore fitness (Caceres et al. 1994). It is therefore not surprising that algal pieces offered in situ were consumed rapidly during each assay and that a diel feeding pattern was absent. Our observations are in agreement with previous studies which demonstrated that coral reef herbivorous fish were able to distinguish between different food qualities among patches of algae (Hay et al. 1987; Burkepile and Hay 2009). The precise chemical basis by which fish and urchins discriminated between the different pCO_2*Ulva*, however, requires further investigation. Equally, it should be considered that pCO_2 may affect structural toughness of the algal thallus which in turn could affect feeding preference (Watson and Norton 1985; Prado and Heck 2011). Although we did not investigate the structural characteristics of the algae, superficial assessment of the tearing properties of the thalli indicated that there were no differences between treatments. This, however, would merit further tests.

High concentrations of pCO_2 had no direct effect on the overall algal consumption of sea urchins and rabbitfish. This is in contrast to previous observations that comparatively moderate increases in pCO_2 (up to ~700 µatm) can alter the qualitative and quantitative consumption of different reef fish species (Cripps et al. 2011; Ferrari et al. 2011a). Similarly, Stumpp et al. (2012) found a 30 % decrease in food intake by the green sea urchin *S. droebachiensis,* following exposure to 2,840 µatm pCO_2 for 45 d.

CO_2 tolerance is likely to vary considerably between species, age (Wood et al. 2008; Melzner et al. 2009; Dupont et al. 2010; Ferrari et al. 2011b) and exposure times (Dupont et al. 2012). Exposure times by Cripps et al. (2011) and Ferrari et al. (2011a) ranged between 4 and 7 d, while CO_2 exposure of the animals in our experiments was about 4 times longer. This may have allowed *S. rivulatus* to more fully acclimate to ambient pCO_2 conditions. Conversely, a 28 d exposure may have been too short to affect *T. gratilla*. The challenge of exposure time versus relative pCO_2 effects and acclimation of marine organisms is well illustrated in a recent paper by Dupont et al. (2012): Exposure of the green sea urchin *S. droebachiensis* to 1,200 µatm for 4 months resulted in decreased female fecundity, reduced larval settlement and larval survival rates. However, when the exposure time was extended to 16 months, there were no significant differences between control and treated animals. This clearly demonstrates that it will be challenging to quantify the effects of ocean acidification on marine organisms and ecological processes using short-term studies (i.e. several days to weeks). There is a risk to either underestimate an organism's ability to adapt or to fail to capture adverse cumulative effects which will only begin to manifest after a prolonged period of exposure time.

While our data show that CO_2 has the potential to alter algal palatability to different herbivores, the fact that pCO_2 effects were observed only at high pCO_2 indicates that algal—herbivore interactions may be resistant to predicted pCO_2 concentrations in the near future. Concern over the rapid decline in coral reefs due to direct anthropogenic activities and global climate change has risen sharply in recent years. Thermal stress, ocean acidification and nutrient loads are reducing coral health and render corals more vulnerable to algal overgrowth (Fabricus 2005; Diaz-Pulido et al. 2011; Anthony et al. 2011; Edwards et al. 2011). Identifying the changes in algal-herbivore dynamics is thus critical to understand the direction of community development of reefs in the future. To better predict global change scenarios, more research will be necessary examining not only isolated but also interacting

(e.g. CO_2 × temperature, CO_2 × nutrients, CO_2 × temperature × nutrients) effects on trophic interactions and cascades on coral reefs.

Acknowledgments Many thanks to the Israel National Center for Mariculture for providing the algal material. We thank R. Farage and B. Linden for their support with the in situ experiment and the collection of fish and sea urchins. We are grateful to P. Munday and R. Geider whose comments greatly improved earlier versions of the manuscript and would like to thank M. Hay and two anonymous reviewers for their constructive comments on the current version of the manuscript. E.M.B. was funded by the German National Academy of Sciences Leopoldina Fellowship programme (LPDS 2009-17), M.S. was supported by the UK Natural Environment Research Council (NERC; NE/H009485/1) and MF by the Israeli Science Foundation (328/09).

References

Anthony KRN, Maynard J, Diaz-Pulido G, Mumby PJ, Marshall P, Long C, Hoegh-Guldberg O (2011) Ocean acidification and warming will lower coral reef resilience. Global Change Biol. doi:10.1111/j.1365-2486.2010.02364.x

Arnold T, Mealey C, Leahey H, Miller AW, Hall-Spencer JM, Milazzo M, Maers K (2012) Ocean acidification and the loss of phenolic substances in marine plants. PLoS ONE 7:e35107. doi:101371/journalpone0035107

Barry JP, Tyrrell T, Hansson L, Plattner G-K, Gattuso JP (2010) Atmospheric CO_2 targets for ocean acidification perturbation experiments. In: Riebesell U, Fabry VJ, Hansson L, Gattuso J-P (eds) Guide to best practices for ocean acidification research and data reporting. Publications Office of the European Union, Luxembourg, p 260

Bouchon-Navaro Y, Harmelin-Vivien ML (1981) Quantitative distribution of herbivorous reef fishes in the Gulf of Aqaba (Red Sea). Mar Biol 63:79–86

Brokovich E, Ayalon I, Einbinder S, Segev N, Shaked Y, Genin A, Kark S, Kiflawi M (2010) Grazing pressure on coral reefs decreases across a wide depth gradient in the Gulf of Aqaba, Red Sea. Mar Ecol Prog Ser 399:69–80

Burdett HL, Donohue PJC, Hatton AD, Alwany MA, Kamenos NA (2013) Spatiotemporal variability of dimethylsulphoniopropionate on a fringing coral reef: The role of reefal carbonate chemistry and environmental variability. PLoS ONE 8: e64651. doi:10.1371/journal.pone.0064651

Burkepile DE, Hay M (2009) Nutrient versus herbivore control of macroalgal community development and coral growth on a Caribbean reef. Mar Ecol Prog Ser 389:71–84

Caceres CW, Fuentes LS, Ojeda FP (1994) Optimal feeding strategy of the temperate herbivorous fish *Aplodactylus punctatus*: the effects of food availability on digestive and reproductive patterns. Oecologia 99:118–123

Caldeira K, Wickett ME (2005) Ocean model predictions of chemistry changes from carbon dioxide emissions to the atmosphere and ocean. J Geophys Res 110:C09S04

Cohen S (2011) Measuring gross and net calcification of a reef coral under ocean acidification conditions: methodological considerations. MSc thesis, Bar Ilan University, Tel Aviv

Connell SD, Russell BD (2012) The direct effects of increasing CO_2 and temperature on non-calcifying organisms: increasing the potential for phase shifts in kelp forests. Proc R Soc B 277:1409–1415

Cripps IL, Munday PL, McCormick MI (2011) Ocean acidification affects prey detection by a predatory reef fish. PLoS ONE 6:e22736

Cruz-Rivera E, Hay M (2001) Effects of macroalgal traits on the feeding and fitness of an herbivorous amphipod: the relative importance of selectivity, mixing, and compensation. Mar Ecol Prog Ser 218:249–266

Cruz-Rivera E, Hay ME (2003) Prey nutritional quality interacts with chemical defences to affect consumer feeding and fitness. Ecol Monogr 73:483–506

Diaz-Pulido G, Gouezo M, Tilbrook B, Dove S, Anthony KRN (2011) High CO_2 enhances the competitive strength of seaweeds over corals. Ecol Lett 14:156–162

Duffy JE, Paul VJ (1992) Prey nutritional quality and the effectiveness of chemical defenses against tropical reef fishes. Oecologia 90:333–339

Dupont S, Ortega-Martínez O, Thorndyke M (2010) Impact of near-future ocean acidification on echinoderms. Ecotoxicology 19:449–462

Dupont S, Dorey N, Stumpp M, Melzner F, Thorndyke M (2012) Long-term and trans-life-cycle effects of exposure to ocean acidification in the green sea urchin *Strongylocentrotus droebachiensis*. Mar Biol. doi: 10.1007/s00227-00012-01921-x

Edwards HJ, Elliott IA, Eakin CM, Irikawa A, Madin JS, MCField M, Morgan JA, Van Woesik R, Mumby PJ (2011) How much time can herbivore protection buy for coral reefs under realistic regimes of hurricanes and coral bleaching? Global Change Biol 17:2033–2048

Einbinder S, Perelberg A, Ben-Shaprut O, Foucart MH, Shashar N (2006) Effects of artificial reefs on fish grazing in their vicinity: Evidence from algae presentation experiments. Mar Environ Res 61:110–119

Fabricus KE (2005) Effects of terrestrial runoff on the ecology of corals and coral reefs: review and synthesis. Mar Pollut Bull 50:125–146

Ferrari MCO, McCormick MI, Munday PL, Meekan MG, Dixson DL, Lonnstedt Ö, Chivers DP (2011a) Putting prey and predator into the CO_2 equation – qualitative and quantitative effects of ocean acidification on predator–prey interactions. Ecol Lett 14: 1143–1148

Ferrari MCO, Dixon DL, Munday PL, McCormick MI, Meekan MG, Sih A, Chivers DP (2011b) Intrageneric variation in antipredator responses of coral reef fishes affected by ocean acidification: implications for climate change projections on marine communities. Global Change Biol 17:2980–2986

Ferrari R, Gonzalez-Rivero M, Ortiz JC, Mumby PJ (2012) Interaction of herbivory and seasonality on the dynamics of Caribbean macroalgae. Coral Reefs 31:683–692

Gattuso J-P, Kunshan G, Lee K, Rost B, Schulz KG (2010) Approaches and tools to manipulate the carbonate chemistry. In: Riesebell U, Fabry VJ, Hansson L, Gattuso JP (eds) Guide to best practices for ocean acidification research and data reporting. Publications Office of the European Union Luxembourg, Luxembourg, pp 41–52

Genin A (2008) The physical setting of the Gulf of Aqaba: an explanation for a unique occurence of tropical communities in the subtropics. In: Por FD (ed) Aqaba-Eilat, the improbable Gulf. The Hebrew University Magnes Press, Jerusalem, p 463

Genin A, Lazar B, Brenner S (1995) Vertical mixing and coral death in the Red Sea following the eruption of Mount Pinatubo. Nature 377:507–510

Gordillo FJL, Niell FX, Figueroa FL (2001a) Non-photosynthetic enhancement of growth by high CO_2 level in the nitrophilic seaweed *Ulva rigida* C. Agardh (Chlorophyta). Planta 213:64–70

Gordillo FJL, Jiménez C, Goutx M, Niell X (2001b) Effects of CO_2 and nitrogen supply on the biochemical composition of *Ulva rigida* with especial emphasis on lipid class analysis. J Plant Physiol 158:367–373

Hall-Spencer JM, Rodolfo-Metalpa R, Martin S, Ransome E, Fine M, Turner SM, Rowley SJ, Tedesco D, Buia M-C (2008) Volcanic carbon dioxide vents show ecosystem effects of ocean acidification. Nature 454:96–99

Hay ME (1997) The ecology and evolution of seaweed-herbivore interactions on coral reefs. Coral Reefs 16:S67–S76

Hay ME (2009) Marine chemical ecology: Chemical signals and cues structure marine populations, communities, and ecosystems. Annu Rev Mar Sci 1:193–212

Hay ME, Fenical W, Gustafson K (1987) Chemical defense against diverse coral-reef herbivores. Ecology 68:1581–1591

Hay ME, Renaud PE, Fenical W (1988) Large mobile versus small sedentary herbivores and their resistance to seaweed chemical defenses. Oecologia 75:246–252

Hay ME, Duffy JE, Fenical W (1990) Seaweed chemical defenses: among-compound and among-herbivore variance. Proc 6th Int Coral Reef Symp 3:43–48

Hemmi A, Jormalainen V (2002) Nutrient enhancement increases performance of a marine herbivore via quality of its food alga. Ecology 83:1052–1064

Hendriks IE, Duarte CM, Álvarez M (2010) Vulnerability of marine biodiversity to ocean acidification: A meta-analysis. Estuar Coast Shelf Sci 86:157–164

Hughes TP, Reed DC, Boyle M-J (1987) Herbivory on coral reefs: community structure following mass mortalities of sea urchins. J Exp Mar Biol Ecol 113:39–59

Hughes TP, Rodrigues MJ, Bellwood DR, Ceccarelli D, Hoegh-Guldberg O, McCook L, Moltschaniwskyj N, Pratchett M, Steneck R, Willis B (2007) Phase shifts, herbivory and the resilience of coral reefs to climate change. Curr Biol 17:360–365

Israel A, Hophy M (2002) Growth, photosynthetic properties and Rubisco activities and amounts of marine macroalgae grown under current and elevated seawater CO2 concentrations. Global Change Biol 8:831–840

Johnston AM (1991) The acquisition of inorganic carbon by marine macroalgae. Can J Bot 69:1123–1132

Johnston AM, Maberly SC, Raven JA (1992) The acquisition of inorganic carbon by four red macroalgae. Oecologia 92:317–326

Kerrison P, Suggett DJ, Hepburn LJ, Steinke M (2012) Effect of elevated pCO_2 on the production of dimethylsulphoniopropionate (DMSP) and dimethyl sulphide (DMS) in two species of *Ulva* (Chlorophyceae). Biogeochemistry 110:5–16

Khait R (2009) Resolving the enigma of delayed feeding in herbivorous coral-reef fishes. MSc. Thesis, The Hebrew University, Jerusalem

Kroeker KJ, Kordas RL, Crim RN, Singh GG (2010) Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms. Ecol Lett 13:1419–1434

Kroeker KJ, Micheli F, Gambi MC (2013) Ocean acidification causes ecosystem shifts via altered competitive interactions. Nature Climate Change 3:156–159

Kübler JE, Johnston AM, Raven JA (1999) The effects of reduced and elevated CO_2 and O_2 on the seaweed *Lomentaria articulata*. Plant Cell Environ 22:1303–1310

Lee PA, Rudisill JR, Neeley AR, Maucher JM, Hutchins DA, Feng YY, Hare CE, Leblanc K, Rose JM, Wilhelm SW, Rowe JM, DiTullio GR (2009) Effects of increased pCO_2 and temperature on the North Atlantic spring bloom. III. Dimethylsulfoniopropionate. Mar Ecol Prog Ser 388:41–49

Lockwood JR III (1998) On the statistical analysis of multiple-choice feeding experiments. Oecologia 116:475–481

Lubchenco J, Gaines SD (1981) A unified approach to marine plant-herbivore interactions. I Populations and communities. Annu Rev Ecol Syst 12:405–437

Lundberg B, Golani D (1995) Diet adaptations of lessepsian migrant rabbitfishes, *Siganus luridus* and *S. rivulatus*, to the algal resources of the Mediterranean coast of Israel. Mar Ecol 16:73–89

Lundberg B, Lipkin Y (1979) Natural food of the herbivorous rabbitfish (*Siganus* spp.) in Northern Red Sea. Bot Mar 22:173–181

Lundberg B, Popper N (1999) Algal vegetation in the Eilat coral reef reserve. Isr J Plant Sci 47:111–121

McClintock JB, Baker JB (eds) (2001) Marine chemical ecology. CRC Press, Boca Raton

Mehrbach C, Culberson CH, Hawley JE, Pytkowicz RM (1973) Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. Limnol Oceanogr 18:897–907

Melzner F, Gutowska MA, Langenbuch M, Dupont S, Lucassen M, Thorndyke MC, Bleich M, Pörtner H-O (2009) Physiological basis for high CO_2 tolerance in marine ectothermic animals: Pre-adaptation through lifestyle and ontogeny? Biogeosciences 6:2313–2331

Michaelidis B, Spring A, Pörtner HO (2007) Effects on long-term acclimation to environmental hypercapnia on extracellular acid-base status and metabolic capacity in Mediterranean fish *Sparus aurata*. Mar Biol 150:1417–1429

Munday PL, Dixon DL, McCormick MI, Meekan M, Ferrari MCO, Chivers DP (2010) Replenishment of fish populations is threatened by ocean acidification. Proc Natl Acad Sci USA 107:12930–12934

Olischläger M, Bartsch I, Gutow L, Wiencke C (2012) Effects of ocean acidification on different life-cycle stages of the kelp *Laminaria hyperborea* (Phaeophyceae). Bot Mar 55. doi: 10.1515/bot-2012-0163

Paul VJ, Nelson SG, Sanger HR (1990) Feeding preferences of adult and juvenile rabbitfish *Siganus argenteus* in relation to chemical defenses of tropical seaweeds. Mar Ecol Prog Ser 60:23–34

Pierrot DE, Lewis E, Wallace DWR (2006) MS Excel Program Developed for CO2 System Calculations. ORNL/CDIAC-105a, Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, U.S. Department of Energy, Oak Ridge, TN

Pörtner HO (2008) Ecosystem effects of ocean acidification in times of ocean warming: a physiologist's view. Mar Ecol Prog Ser 373:203–217

Prado P, Heck JKL (2011) Seagrass selection by omnivorous and herbivorous consumers: determining factors. Mar Ecol Prog Ser 429:45–55

Rossoll D, Bermudez R, Hauss H, Schulz KG, Riebesell U, Sommer U, Winder M (2012) Ocean acidification-induced food quality deterioration constrains trophic transfer. PLoS ONE:e34737. doi: 10.31371/journal.pone.0034737:

Spielmeyer A, Pohnert G (2012) Influence of temperature and elevated carbon dioxide on the production of dimethylsulfoniopropionate and glycine betaine by marine phytoplankton. Mar Environ Res 73:62–69

Stefels J (2000) Physiological aspects of the production and conversion of DMSP in marine algae and higher plants. J Sea Res 43:183–197

Steinke M, Daniel C, Kirst GO (1996) DMSP Lyase in marine macro- and microalgae: Intraspecific differences in cleavage activity. In: Kiene RP, Visscher PT, Keller MD, Kirst GO (eds) Biological and environmental chemistry of DMSP and related sulfonium compounds. Plenum Press, New York, pp 317–324

Steinke M, Brading P, Kerrison P, Warner ME, Suggett DJ (2011) Concentrations of dimethylsulfoniopropionate (DMSP) and dimethylsulfide (DMS) are strain-specific in symbiotic dinoflagellates (*Symbiodinium* sp., Dinophyceae). J Phycol 47:775–783

Stiling P, Cornelissen T (2007) How does elevated carbon dioxide (CO_2) affect plant-herbivore interactions? A field experiment

and meta-analysis of CO_2-mediated changes on plant chemistry and herbivore performance. Global Change Biol 13:1823–1842

Stumpp M, Trübenbach K, Brennecke D, Hu MY, Melzner F (2012) Resource allocation and extracellular acid-base status in the sea urchin *Strongylocentrotus droebachiensis* in response to CO_2 induced seawater acidification. Aquat Toxicol 110–111:194–207

Swanson AK, Fox CH (2007) Altered kelp (Laminariales) phlorotannins and growth under elevated carbon dioxide and ultraviolet-B treatments can influence associated intertidal food webs. Global Change Biol 13:1696–1709

Thacker RW, Nagle DG, Paul VJ (1997) Effects of repeated exposures to marine cyanobacterial secondary metabolites on feeding by juvenile rabbitfish and parrotfish. Mar Ecol Prog Ser 147:21–29

Van Alstyne KL, Houser LT (2003) Dimethylsulfide release during macroinvertebrate grazing and its role as an activated chemical defense. Mar Ecol Prog Ser 250:175–181

Van Alstyne KL, Wolfe GV, Freidenburg TL, Neill A, Hicken C (2001) Activated defense systems in marine macroalgae: evidence for an ecological role for DMSP cleavage. Mar Ecol Prog Ser 213:53–65

von Westernhagen H (1973) The natural food of the rabbit fish *Siganus oramin* and S. striola. Mar Biol 22:367–370

Watson DC, Norton TA (1985) The physical characteristics of seaweed thalli as deterrents to littorine grazers. Bot Mar 28:383–387

Wernberg T, Smale DA, Thomsen MS (2012) A decade of climate change experiments on marine organisms: procedures, patterns and problems. Global Change Biol 18:1491–1498

Wood HL, Spicer JI, Widdicombe S (2008) Ocean acidification may increase calcification rates, but at a cost. Proc R Soc B 275:1767–1773

Zeebe RE, Zachos JC, Caldeira K, Tyrrell T (2008) Oceans: carbon emissions and acidification. Science 321:51–52

Zemke-White WL, Choat JH, Clements KD (2002) A re-evaluation of the diel feeding hypothesis for marine herbivorous fishes. Mar Biol 141:571–579

Zor T, Selinger Z (1996) Linearization of the Bradford protein assay increases its sensitivity: theoretical and experimental studies. Anal Biochem 236:302–308

Zou D (2005) Effects of elevated atmospheric CO_2 on growth, photosynthesis and nitrogen metabolism in the economic brown seaweed, *Hizikia fusiforme* (Sargassaceae, Phaeophyta). Aquaculture 250:726–735