



Contribution to Special Issue: 'Towards a Broader Perspective on Ocean Acidification Research Part 2' Original Article

Environmental sensitivity of *Neogoniolithon brassica-florida* associated with vermetid reefs in the Mediterranean Sea

Maoz Fine^{1,2*}, Rami Tsadok^{1,3}, Dalit Meron^{1,2}, Stephanie Cohen^{1,2}, and Marco Milazzo⁴

¹The Mina and Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat Gan 52900, Israel

²The Interuniversity Institute for Marine Sciences, P.O. Box 469, Eilat 88103, Israel

³Leon Charney School of Marine Sciences, University of Haifa, Mt. Carmel 31905, Israel

⁴Department of Earth and Marine Sciences, CoNISMa, University of Palermo, Via Archirafi 28, Palermo I-90123, Italy

*Corresponding author: tel: 972-8-6360123; fax: 972-8-6374329; e-mail: Maoz.fine@biu.ac.il

Fine, M., Tsadok, R., Meron, D., Cohen, S., and Milazzo, M. Environmental sensitivity of *Neogoniolithon brassica-florida* associated with vermetid reefs in the Mediterranean Sea. – ICES Journal of Marine Science, 74: 1074–1082.

Received 29 February 2016; revised 28 August 2016; accepted 5 September 2016; advance access publication 6 October 2016.

Vermetid reefs in the Mediterranean Sea are increasingly affected by both anthropogenic actions and global climate change, which are putting this coastal ecosystem at risk. The main species involved in building these reefs are two species of intertidal vermetid gastropods and the crustose calcareous alga, *Neogoniolithon brassica-florida*, which cements the gastropod shells and thus solidifying the reef edges. In the present study, we examined the pattern of distribution in the field and the environmental sensitivity (thermal tolerance, resilience to low pH, high light intensity and desiccation) of *N. brassica-florida* along the coasts of Sicily and Israel by means of chlorophyll fluorescence and total alkalinity measurements in situ and in the laboratory. Tidal regimes did not affect photosynthesis of *N. brassica-florida* but light intensity in the intertidal did. Sensitivity to increased light intensity was amplified by elevated temperature and reduced pH. Winter temperature above 16 °C caused a decrease in the photosynthetic performance of photo-system II. Similarly, a decrease in pH resulted in decreased maximum photosynthetic yield and electron transport rate. Calcification was significantly lower at pH 7.9 as compared with ambient (8.1) pH. In fact, dissolution at pH 7.9 at night was higher than net calcification during the day, suggesting that *N. brassica-florida* may not be able to contribute to reef accretion under the levels of seawater warming and ocean acidification projected by the end of this century.

Keywords: climate change, Mediterranean Sea, *Neogoniolithon*, ocean acidification, Vermetid reefs.

Introduction

Vermetid reefs in the Mediterranean Sea are the temperate and subtropical equivalent of coral reefs in terms of their bioengineering function and support of biodiversity. Furthermore, these reefs are important for coastal protection and prevention of beach erosion (Ben-Eliahu, 1975; Fishelson and Haran, 1987; Lipkin and Safriel, 1971; Safriel, 1974). The framework builders of vermetid reefs are two species of vermetid gastropods: *Vermetus triqueter* inhabiting the inner shallow basins of the reef flat and the densely aggregated *Dendropoma petraeum*, a filter-feeder, protected by a thick shell and a close fitting operculum, inhabiting the surf-beaten edge of the reef where it creates raised rims (Safriel, 1974).

A recent study (Templado *et al.* 2016) distinguished between 4 cryptic species within the *D. petraeum* complex. Although morphologically almost undistinguishable, the genetic divergence of these genotypes is clear (Templado *et al.* 2016). We now know that different basins of the Mediterranean Sea are dominated by a different species of the genus *Dendropoma*. Relevant vermetid species for the present study are *Dendropoma cristatum* along the shores of Sicily and *Dendropoma anguliferum* along the coast of Israel.

The four Mediterranean species of *Dendropoma* are closely associated with the crustose coralline red algae (CCA) *Neogoniolithon brassica-florida* Harvey (Setchell and Mason,

1943), which is responsible for cementation of the *Dendropoma* spp. shells creating a strong conglomerate at the abrasion platforms at the lower intertidal zone.

The CCA is also important for *Dendropoma* settlement (Spotorno-Oliveira, 2015). Living *Dendropoma* spp. reefs have been described for rocky shores in the southern Mediterranean from the Strait of Gibraltar and southeastern Spain to Sicily and the Levantine Basin (Calvo *et al.*, 1998).

The two associated rim builders *Dendropoma* spp. and *N. brassica-florida* live in what is considered an obligatory association, particularly in the rim surrounding the abrasion platform.

The framework offered by these reef builders is critical to the whole ecosystem functioning in the intertidal zone (Safriel and Ben-Eliahu, 1991); therefore, *D. petraeum* has been recognized as a species in need of protection (Scotti and Chemello, 2000).

In recent years, wide mortalities of *Dendropoma* spp. were observed Mediterranean-wide, in particular along the southeastern (Levant basin) coast (Usyatsov and Galil, 2012). The cause for vermetid reefs demise is still unknown although anthropogenic activities (mainly pollution) were speculated as the main cause of mortality. Di Franco *et al.* (2011) demonstrated that the presence of a small marina in NW Sicily heavily affected the density of *D. petraeum* and the cover of *N. brassica-florida*, when compared with reference locations. Although not causing local extinction of the reef builder species, these reductions were specifically recorded in the outer rim of the vermetid reef, likely as a result of alteration of water flow regimes, water turbidity, nutrient enrichment and the accumulation of toxic compounds inside the marina (Di Franco *et al.*, 2011).

Bioconstruction is counteracted by biological and physical erosion. To maintain a positive accretion of the whole bioconstruction, the synergistic calcification rate of the gastropod *Dendropoma* spp. and the alga *N. brassica-florida* must be higher than the biological and physical erosion rate. While the gastropod is heterotrophic, the CCA is autotrophic and therefore its calcification rate is dependent on light availability, intensity and quality. In the intertidal zone, *N. brassica-florida* is exposed to considerable oscillations in abiotic conditions such as irradiance, temperature and humidity (Ganning, 1971; Truchot and Duhamel-Jouve, 1980; Morris and Taylor, 1983) however it is unknown how this affects its photosynthesis and calcification rates, during low tide when it is exposed to air. During extreme low tide events it may also be exposed to extreme high/low temperature, salinity and radiation. Some factors such as tides and temperature are more predictable whereas local weather (wave wash, cloud cover) is less predictable.

High irradiances and desiccation during low tide presents the algae with significant photo-stress (Davison and Pearson, 1996; Häder *et al.*, 2003; Franklin and Forster, 1997). Reactive oxygen species (ROS) as a result of increased photosynthesis under high irradiance may cause damage to the photosynthetic apparatus and eventually lead to bleaching and death (Muller *et al.*, 2001). Temperature is often interacting with irradiance (Lobban and Harrison, 1994) to affect metabolic pathways such as photosynthesis, diffusion rates, carbonic anhydrase (CA) activity, and active transport of CO_2 and HCO_3^- .

In this study, we examined the overlooked algal partner of *Dendropoma*, *N. brassica-florida* performance under different environmental conditions, both *in situ* and in laboratory-based experiments, in an effort to understand its response to varying environmental parameters.

This study aims to answer two main questions raised in view of the observed vermetid reef rapid mortality: (i) Does the recently observed vermetid reef regression involve both the gastropod and calcareous algae? and (ii) What are the environmental sensitivities of *N. brassica-florida* and what are the consequences of expected environmental change?

Material and methods

Survey

A field survey of *N. brassica-florida* living in association with the reef-building *Dendropoma petraeum* (Figure 1) was conducted in 13 sites along ~350 km of the northwestern coast of Sicily (Figure 2a) and 6 sites along ~140 km of the Mediterranean coast of Israel (Figure 2b). These sites were selected for their extensive vermetid platforms and available information from earlier studies (Safriel, 1974; Scotti and Chemello, 2000). Surveys were performed over 6 days in June 2011 in Sicily and February–June 2012 in Israel.

Percent cover of erect macroalgae, algal turfs, and of the calcareous reef building alga *N. brassica-florida* and the density of the gastropod *Dendropoma* spp. were assessed by a standard photo-sampling method using a high-resolution digital camera (Terlizzi *et al.*, 2005). Replicated photos on 10×10 cm plots were taken between +0.2 to -0.2 m mean sea level both on the inner and the outer rims of the reef. In each site, 4 photos were taken in the seaward side of the reef where *Dendropoma* spp. typically has the highest density (Lipkin and Safriel 1971). The abundance of living individuals of *D. cristatum* and *D. anguliferum* was assessed by counting the number of chitinous operculum closing each tubular shell on a standard 10×10 cm² sampling surface (Di Franco *et al.*, 2011).

Chlorophyll fluorescence

A portable underwater fluorometer Diving-PAM (Walz GmbH, Germany) was used to examine the photosynthetic capacity of *N. brassica-florida* in the surveyed sites. The PAM light meter was pre-calibrated against a quantum sensor (Li-Cor, USA). To generate rapid light curves (of photosynthesis vs. irradiance—RLCs),

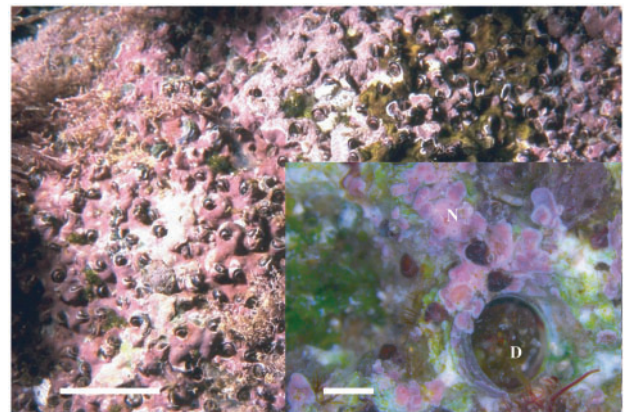


Figure 1. A close up on a vermetid rim showing an aggregate of the reef building vermetid gastropod *D. anguliferum* and the red coralline algae *N. brassica-florida* filling the gaps between its shells. Scale bar = 5 cm. At a higher magnification (inset) an operculum of a single *D. cristatum* (D) surrounded by the encrusting coralline *N. brassica-florida* (N) cementing the vermetid reef rim. Note the newly settled *D. petraeum* juveniles on *N. brassica-florida* above the adult operculum. Scale bar = 5 mm.



Figure 2. (a) Thirteen study sites along the Sicilian coast and location of the CO₂ vent site in Vulcano Island where the transplantation experiments were performed (star). (b) 6 study sites along the Israeli coast. In Sicily: 1. Capo Milazzo; 2. Capo Grosso; 3. Capo Zafferano; 4. Addaura; 5. Mondello; 6. Capo Gallo; 7. Zingaro; 8. Isulidda; 9. Cala Mancina; 10. Cornino; 11. Lido Valderice; 12. Bonagia; 13. Favignana; Along the Israeli coast: 1. Rosh Hanikra; 2. Achziv; 3. Shiqmona; 4. Habonim; 5. Sdot-Yam; 6. Bat-Yam.

the “coral clip” was positioned on the *Dendropoma-Neogoniolithon* conglomerate surface with the tip of the PAM main optical fibre placed 5 mm from the reef surface. RLCs were generated by illuminating the *N. brassica-florida* for 10 s at each one of a series of 8 irradiances. At each irradiance, the quantum yield of photosystem II was derived as $Y = (F_m' - F) / F_m'$ according to (Genty *et al.* 1989). The photosynthetic yield, non-photochemical quenching (NPQ) and electron transport rate (ETR) were plotted against irradiance. RLCs are strongly influenced by the ambient irradiance at the time the measurements are performed. In order to neutralize such effects, we performed the RLCs after a darkening period of 5 min to completely dark-adapt the calcareous algae and obtain Y_{max} values $[(F_m - F_0) / F_m]$. Due to the harsh work conditions in the intertidal (waves and splashes) it was impossible to dark adapt the surface for a longer time.

In a similar manner, the diving PAM was used to measure the photosynthetic performance of *N. brassica-florida* on the surface of vermetid reef cores (13 cm in diameter, $n = 3$ per site) that were collected in Palermo (Sicily) and transplanted in four intertidal sites off Vulcano Island [$38^{\circ}25'08.52''N$, $14^{\circ}57'39.13''E$, Sicily, Italy] along a pH gradient: Low pH 7.4 ± 0.09 , Medium pH 7.82 ± 0.07 , Normal pH 8.07 ± 0.04 and a reference site with pH 8.15 ± 0.03 (Arnold *et al.*, 2012; Milazzo *et al.* 2014). Three cores were left in the collection site in Palermo as reference for transplanted cores. The collection site in Palermo, a relatively undisturbed site, has a high density of living *D. cristatum* and cover of *N. brassica-florida*. Cores were transplanted to the same tidal level and were allowed to grow in the various sites along the pH gradient for 6 months before the measurements were performed.

A diel cycle was performed by measuring the effective quantum yield ($Y(ii) = (F_m' - F) / F_m'$) on each core in each of the vent sites every 4 h from 06:00 to 20:00.

Response to elevated temperature and pCO₂

To study the sensitivity of *N. brassica-florida* to elevated temperature and pCO₂, equal size cores from vermetid reef rims in Israel

(5 cm in diameter, $n = 15$) were sampled using a steel corer and a hammer, tagged, brought to the laboratory and kept in a temperature-controlled system and light intensity of $400 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$. Following an acclimation period of 1 week at a temperature of 26 °C (temperature in the collection site was 25 °C), the temperature was reduced to 16 °C over two days. After 24 h at that temperature, the temperature was increased by 2 °C every 24 h and sampling points (Chlorophyll fluorescence) were performed at 4 temperature regimes, reflecting winter (16 °C) spring (24 °C), summer-peak (31 °C), and summer-peak +2 °C (33 °C). By the end of each incubation period the cores were darkened for 15 min and a rapid light curve (RLC) was performed using an Imaging PAM (Walz, GmbH, Germany). The Imaging PAM allows measurement of the entire core surface but also allows examination of spatial heterogeneity (e.g. on the operculum and in between *Dendropoma* shells) of the calcareous algae with respect to chlorophyll fluorescence. The variables we analysed were (i) Electron transfer rate ($\text{ETR} = F_v / F_m' \times \text{PAR} \times \text{ETR-factor}$), where the ETR-factor is the product of the amount of total PAR absorbed and its allocation to photosystem II (PSII) (Genty *et al.*, 1989; Schreiber *et al.*, 1995). Since the ETR-factor was not determined for each fragment, it was set to 1. Thus, all presented ETR are relative values (rETR). (ii) Maximum quantum yield of PSII (F_v / F_m) was derived from the first yield measurement of the RLC. rETR_{max} were derived from computational curve-fitting using SigmaPlot12.3, following previously published methods (Platt *et al.*, 1980; Ralph and Gademann, 2005). (iii) NPQ is a mechanism employed by the algae to protect themselves from the adverse effects of high light intensity. NPQ is measured by the quenching of chlorophyll fluorescence (Ralph and Gademann, 2005).

Response to elevated pCO₂

Similarly, to study the effect of increased pCO₂ on photosynthesis and compare laboratory-controlled measurements to our field observations in the CO₂ vents, *N. brassica-florida* cores were maintained as above in pH 8.1_{nbs} (ambient) and 7.9_{nbs}, pH expected by the end of the 21st century (Caldeira and Wickett 2005) at a temperature of 25 °C for 7 days. Then the cores were darkened for 15 min and a light curve was performed using an Imaging PAM.

Calcification

To study Net calcification rates of *N. brassica-florida* under elevated pCO₂, four incubation chambers (300 ml, one core in each) were used for each pH treatment (8.1_{nbs}, pCO₂ = 387 μatm and 7.9_{nbs}, pCO₂ = 668 μatm). *N. brassica-florida* cores were incubated for 2 h under light ($400 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$) and dark conditions at 25 °C with a magnetic stirrer. Water samples (100 ml) were collected from each treatment for total alkalinity (TA) measurement at the beginning and end of the incubation (light and dark) to determine changes in carbonate chemistry. Water samples were stored in the dark at 4 °C until analysed the next day. Calcification rates were calculated from the difference between TA measured at the beginning and the end of each incubation period. TA values were measured using an automatic potentiometric titration to the second end point (Almgren *et al.* 1983). It was then computed using the first derivative of the curve for the evaluation of the exact end point. Components of the carbonate system (pCO₂, CO₃⁻², HCO₃⁻, and DIC concentrations) were

calculated from TA along with pH values, temperature and salinity using the CO2SYS program, version 01.03 (Lewis and Wallace, 1998).

Surface area was calculated by photography of the cores and image analysis using ImageJ software.

Statistical analyses

Correlation coefficient between *Dendropoma* density, *Neogoniolithon* cover and macroalgae cover were computed using Spearman's correlation test on SPSS ver. 16 software.

One or two-way ANOVA was conducted on SPSS ver. 16 software to determine differences between sets of measurements of photosynthetic efficiency. Tukey's *post hoc* multiple comparison tests were performed to detect significant differences over data points and between treatments (SPSS).

Results

Neogoniolithon brassica-florida live cover

Thirteen sites in Sicily and 5 in Israel were inspected for *Dendropoma* spp. and its associated calcareous algae *N. brassica-florida* live cover. Vermetid reef sites along the Sicilian coast were overall healthy (high *Dendropoma cristatum* live cover, Table 1) with continuous rims characterizing a high percentage of the reefs, except for polluted/eutrophicated sites dominated by green algae (data not shown).

In vermetid reefs of Sicily (Table 1), no correlation was found between *D. cristatum* density and *N. brassica-florida* percent cover. A negative correlation was found between *D. cristatum* density and macroalgal cover (Spearman's Correlation coefficient -0.72 , $p < 0.01$, $n = 72$) and between macroalgae and *N. brassica-florida* cover (Spearman's Correlation coefficient -0.89 , $p < 0.01$, $n = 72$). Areas of the Vermetid reef rim where partial mortality of the *Dendropoma* gastropod occurred had a higher cover of *N. brassica-florida*. This demonstrates that, while under pristine conditions, the two rim

builders coexist on limited space, *N. brassica-florida* can persist and take over space previously occupied by *Dendropoma*. In Israel, almost no living *D. anguliferum* was found (see also Galil 2013). Patches of rims that died more recently (according to our previous surveys) were covered by nearly 100% *N. brassica-florida*. A crust of the calcareous alga covered the rim that was built over tens to hundreds of years by the gastropod and its counterpart algae (Sisma-Ventura et al. 2009). Patches of rims which died several years ago (Lipkin and Safriel 1971) were covered by turf or macroalgae (Table 1).

N. brassica-florida was photosynthetically insensitive to the natural range of tides during the study period as indicated by chlorophyll fluorescence measurements (Figure 3) in low and high tide. No significant difference (1-way ANOVA, $p > 0.05$) in dark-adapted Y_{max} (maximum photosynthetic yield) was found between sites and between tides (note that the test above refers only to high/low tide data for sun-exposed areas). The emersion period was not taken as a parameter but it is expected that following long periods of emersion during spring tides there will be an effect on physiological performance of the CCA. Light intensity had a greater effect on Y_{max} . At low tide, *N. brassica-florida* in shaded niches (Figure 3) had significantly higher Y_{max} than that exposed to sunlit areas (1-way ANOVA $p < 0.001$).

Temperature

A significant difference was recorded between dark-adapted Fv/Fm of *N. brassica-florida* cores incubated at temperatures of 16 and 24°C and those incubated at 31 and 33°C (2-way ANOVA $p < 0.01$, with temperature and PAR as independent treatments, $n = 15$, Figure 4a). No significant difference was found between *N. brassica-florida* incubated at 16 and 24°C and between 31 and 33°C ($p > 0.05$). Rapid light curves performed on the cores revealed increased sensitivity to light with increasing temperature (Figure 4a–c). *N. brassica-florida* incubated at 16°C

Table 1. Sites in Sicily and Israel where vermetid reefs were examined.

Latitude, longitude	Location	Reef morphology	Exposition	<i>Neogoniolithon</i> % cover	<i>D. petraeum</i> density in 100 cm ⁻²	Macroalgae and turf % cover
Italy						
38° 15' 59.88" N, 15° 14' 0.26" E	Capo Milazzo	Platform	N-NW	60.9	306	29.3
38° 5' 16.44" N, 13° 32' 25.18" E	Capo Grosso	Platform	N-NW	41.3	31	53.6
38° 6' 41.52" N, 13° 32' 2.03" E	Capo Zafferano	Ledge	N-NW	49.2	256	42.1
38° 11' 36.94" N, 3° 21' 18.85" E	Addaura	Ledge	N-NE	49.6	300	46.7
38° 12' 31.63" N, 13° 19' 46.73" E	Mondello	Ledge	N-NE	38.3	335	52.5
38° 13' 7.73" N, 13° 19' 20.84" E	Capo Gallo	Platform	N-NW	64.7	421	20.6
38° 10' 49.22" N, 12° 46' 1.89" E	Zingaro	Platform	E-NE	77.3	426	14.4
38° 10' 43.85" N, 12° 44' 58.10" E	Isulidda	Platform	W	59.3	381	24.8
38° 11' 2.26" N, 12° 43' 58.44" E	Cala Mancina	Platform	W-NW	58.5	308	25.0
38° 5' 25.02" N, 12° 39' 27.58" E	Cornino	Ledge	W-NW	40.8	340	43.3
38° 4' 23.70" N, 12° 37' 56.89" E	Lido Valderice	Platform	W-NW	47.1	256	43.3
38° 4' 2.68" N, 12° 35' 37.79" E	Bonagia	Platform	N-NW	71.7	412	7.9
37° 56' 11.61" N, 12° 20' 17.75" E	Favignana	Platform	N-NW	59.6	529	19.2
Israel						
33° 5' 25.87" N, 35° 6' 19.02" E	Rosh Hanikra	Platform	W-SW	3.5	0	96.0
33° 3' 19.60" N, 35° 6' 7.24" E	Achziv	Platform	W	5.3	0	92.5
32° 49' 31.79" N, 34° 57' 18.98" E	Shiqmona	Platform	W-NW	8.0	0	91.2
32° 38' 44.69" N, 34° 55' 20.02" E	Habonim	Platform	W	34.5	0	63.0
32° 29' 28.03" N, 34° 53' 10.76" E	Sdot-Yam	Platform	SW-NW	35.2	0	59.0
32° 1' 22.77" N, 34° 44' 13.01" E	Bat-Yam	Platform	W	13.4	0	86.0

The relative contribution to space occupation (% live cover and density measured in 100 cm⁻²) by the vermetid rim builders *Dendropoma* spp and *N. brassica-florida* is presented as well as the combined space occupation of turf and macro algae.

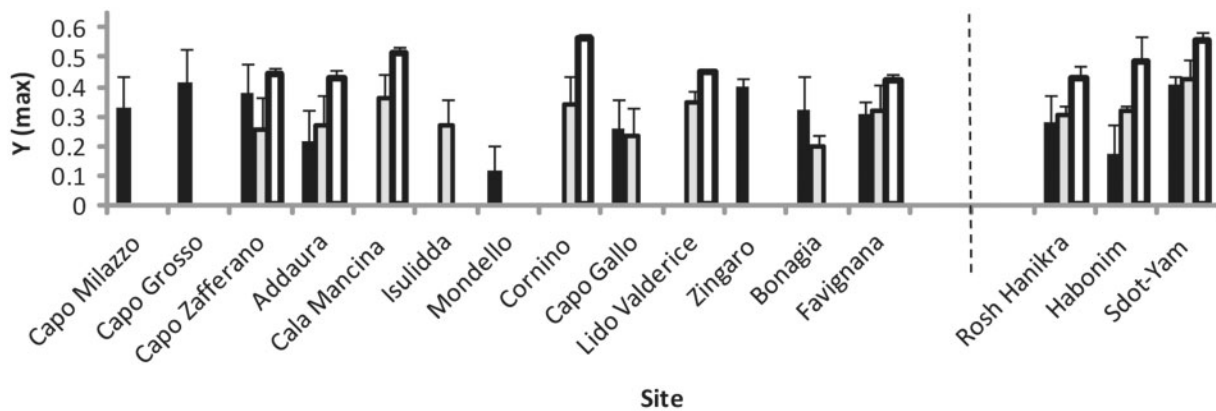


Figure 3. Dark adapted photosynthetic yield (Y_{\max} , mean \pm SD) of *N. brassica-florida* measured in their natural habitat at high (black bars) or low (grey bars) tide (covered by water or exposed to air) and shaded (overhangs, north-facing walls) during low tide (dark grey bars).

dropped to half of its maximal photosynthetic yield at a light intensity of $450 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$ while at 33°C it dropped to half of its maximal yield at $50 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$, a relatively low light intensity as compared with its natural light experience.

No significant difference (2 way ANOVA $p > 0.05$) was found in non-photochemical quenching between temperature treatments (Figure 4b) despite the 33°C curve showed consistently lower NPQ. Beyond light intensity of $200 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$, electron transport rate was significantly higher at a temperature of 16°C than at higher temperature treatments (2-way ANOVA $p < 0.01$, $n = 15$, Figure 4c)

Following 1 week incubation at 33°C , *N. brassica-florida* cores that were returned to temperatures of 24°C recovered almost instantly with respect to their response to light intensity.

pH

Sensitivity of *N. brassica-florida* to pH was first examined along a natural pH gradient in the CO_2 vents of Vulcano Island on vermetid reef rim transplants. Rapid light curves (Figure 4d–f) performed on these transplants revealed significantly lower Y_{\max} of PSII closer to the low pH site (mid and low pH, 2-way ANOVA $p < 0.01$ $n = 4$) following dark acclimation. No significant difference in the quantum yield was found at light intensities higher than $100 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$, suggesting similar sensitivity to light of the CCA in all pH sites despite the chronic lower Y_{\max} . A diel cycle of photosynthetic yield in each of the pH sites along the Vulcano gradient (Figure 5) showed no significant difference between pH sites, except for mid-day ($1500 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$) when the effective quantum yield of *N. brassica-florida* at the low pH site was significantly lower than the medium and ambient pH (2-way ANOVA $p < 0.01$ $n = 4$). No significant difference in non-photochemical quenching of *N. brassica-florida* was detected between the various sites although average NPQ of *N. brassica-florida* at the low and mid pH site was consistently greater than at higher pH and the reference sites. Electron transport rate of *N. brassica-florida* at the high and reference sites was significantly higher than these measured at mid and low pH sites (2-way ANOVA $p < 0.01$ $n = 4$)

Calcification rate of *N. brassica-florida*

Calcification rate was measured using the TA method (Figure 6) on cores composed of *N. brassica-florida* growing over dead

Dendropoma shells. In the light ($400 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$), cores ($n = 4$) incubated at pH 7.9 calcified at a rate half that of the cores incubated at ambient pH 8.1 (0.045 ± 0.019 and $0.164 \pm 0.03 \mu\text{mol CaCO}_3 \text{cm}^{-2} \text{h}^{-1}$, respectively). In the dark, cores dissolved with a much higher dissolution rate at the lower pH treatment (-0.043 ± 0.004 and $-0.207 \pm 0.009 \mu\text{mol CaCO}_3 \text{cm}^{-2} \text{h}^{-1}$ for pH 8.1 and 7.9, respectively).

Discussion

Vermetid reefs in the Mediterranean Sea are hot-spots of biodiversity (Goren and Galil, 2001) and have a socio-economic and cultural importance but are rapidly deteriorating with local extinction of the main reef builders in some areas (Galil, 2013). The causes for mortality are hitherto unknown and in order to recommend a strategy for their protection, adequate management and conservation policy, it is crucial to identify sensitivities of these engineering species to changes in environmental conditions.

One of the most intriguing findings of this study is that *N. brassica-florida* lives and prospers after the vermetid gastropod *Dendropoma* spp. died. Given reports of vermetid reefs degradation (Klerman et al., 2004) and recent vast mortality of *Dendropoma anguliferum* in the eastern Mediterranean (Usvyatsov and Galil, 2012), the resilience of its counterpart calcareous algae, *N. brassica-florida*, is of great interest and importance to the future of vermetid reefs structure and function. We show that vermetid reef rims in Israel where *D. anguliferum* has gone extinct are totally overtaken by *N. brassica-florida*. Rims that were dead for decades were covered by turf and macroalgae, suggesting a succession following the collapse of the *Dendropoma-Neogoniolithon* community that eventually climaxed in a community of turfs and macroalgae. This also suggests different resilience of the 2 rim builders to changes in ambient conditions, or higher susceptibility of *Dendropoma* spp. to the causative agent of these vast mortalities. It appears that while the formation of the rim structure requires a close association between the two organisms (*Dendropoma* spp. and *N. brassica-florida*) there is no obligatory relationship between them for survival. Geological findings of *N. brassica-florida* covering Porites coral skeletons in the Miocene Mediterranean Sea (Braga et al., 2009), also implies that *N. brassica-florida* takes over space once the main reef builder dies off. Recent *N. brassica-florida* reefs where this rhodophyte is the primary bio-builder were reported from the Mediterranean coast of

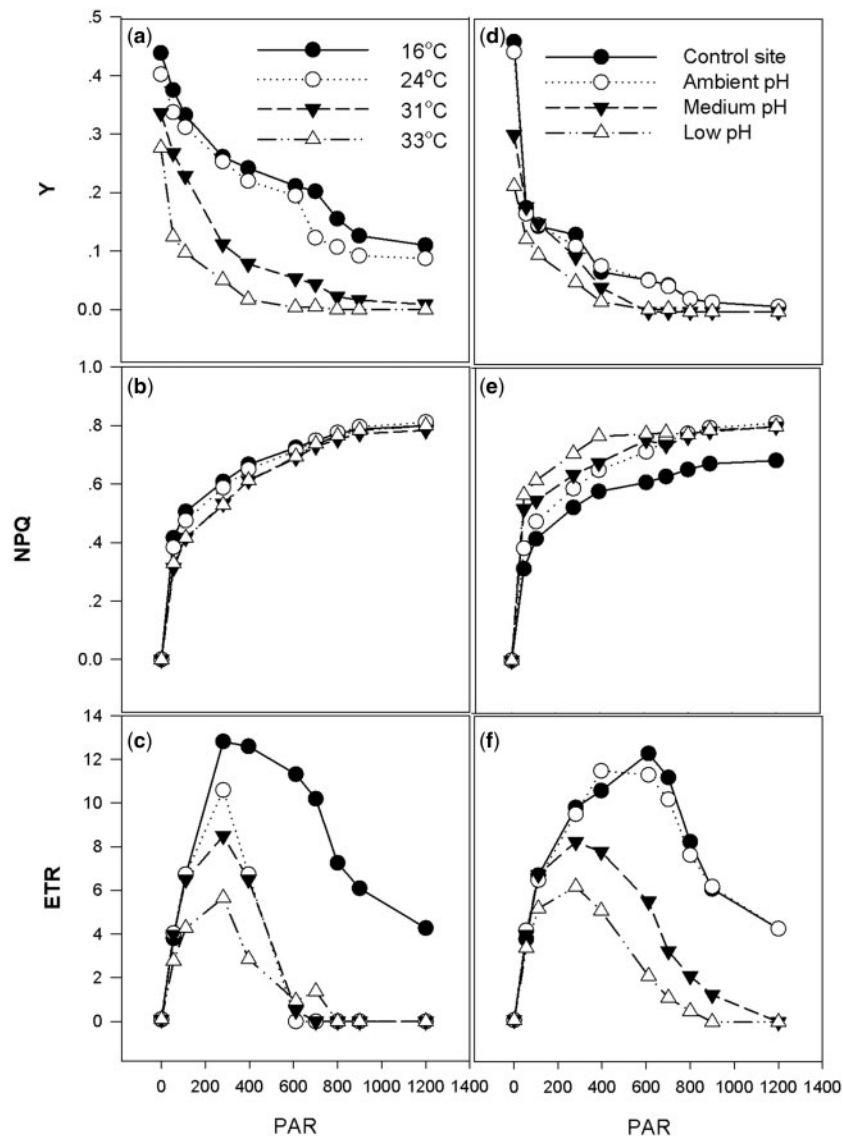


Figure 4. Rapid light curve performed on *N. brassica-florida* exposed to four temperature treatments in the laboratory (a–c) and four pH treatments (d–f) along a pH gradient near a CO₂ vent in Vulcano Island (Sicily).

Tunisia (Langar *et al.*, 2011), demonstrating the species ability to be a significant builder even in the absence of *Dendropoma*. However, even these Tunisian *N. brassica-florida* reefs, previously extending over 30 km in length, degraded by half over the last 30 years (Langar *et al.*, 2011).

The extinction of *Dendropoma* along the Israeli coast is a recent one. In previous surveys we performed, there were still living *D. anguliferum* rims although most of the reefs had only patches of active rims. Less than a decade ago (2003–2004), *D. anguliferum* was collected monthly for a study on reproduction of the species (Usvyatsov and Galil, 2012) in the vermetid reefs of Shiqmona, Atlit and Habonim (Israel) but none have been found there since 2010. Sicilian vermetid reefs still have a high percentage of living *D. cristatum* cover. Cover of *N. brassica-florida* was not correlated with densities of *Dendropoma* spp. but macroalgae cover was. This may be explained by the close relationship between *Dendropoma* and *Neogoniolithon*, which share the same space (rim) regardless of the density of the gastropod, whereas

macroalgae can settle and grow only where there is sufficient low density of *Dendropoma* individuals.

Given the recent realization of two separate species of *Dendropoma* in Sicily (*D. cristatum*) and Israel (*D. anguliferum*) it will be important to examine if their physiological performance and response to environmental change is similar (not the scope of the present study). This may explain, in part, the present state of the two populations. Furthermore, it will be interesting to study the association between the algae (*Neogoniolithon*) and the complex of species of *Dendropoma*.

Photophysiology

The use of chlorophyll fluorescence was found to be suitable as an indicative method for the state and function of PSII in *N. brassica-florida* both in the field and in the laboratory.

Tide (exposure to air or submergence in water) had little effect on the photosynthetic capacity of *N. brassica-florida*, as

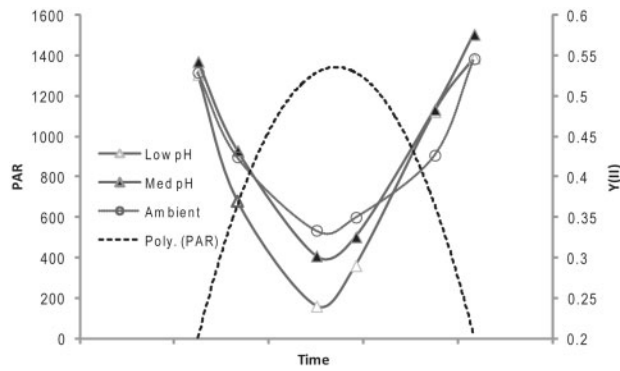


Figure 5. Diel photosynthetic yield of *N. brassica-florida* measured in 3 sites along a pH gradient near a CO₂ vent in Vulcano Island (Sicily). A greater sensitivity to light in the low pH site is reflected as decreased photosynthetic yield compared with mid and ambient pH sites at mid day.

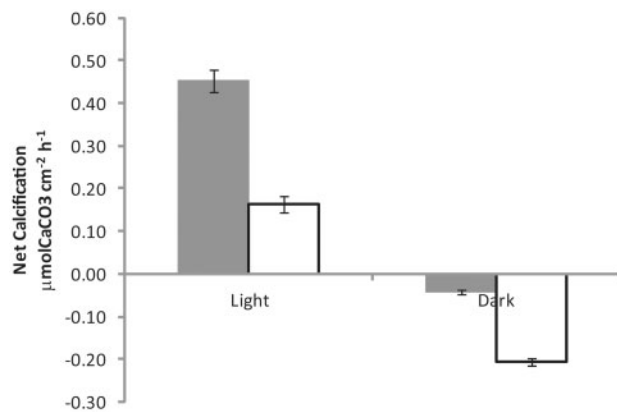


Figure 6. Light and dark calcification rate ($\mu\text{mol CaCO}_3 \text{ cm}^{-2} \text{ h}^{-1}$) by *N. brassica-florida* at 25 °C in ambient pH 8.1, (grey bars) and pH 7.9 (blank bars). Calcification is performed only by the algae (*Dendropoma* is dead) while dissolution occurs of both aragonitic (*Dendropoma*) and high magnesium calcite (*Neogoniolithon*).

reflected from maximal photosynthetic yield measurements. *N. brassica-florida* has an inter-meshed thallus making it resistant to wave action as well as assisting in retaining moisture when exposed at low tide (Nelson, 2009). With that said, mid-day low tide during prolonged exposures to air and desiccation during spring-tide events are likely to affect its photosynthetic performance.

High light intensity imposes light stress on the photosynthetic apparatus of *N. brassica-florida* as evident from higher Y_{max} of shaded algae on north-facing walls and in crevices (Figure 3), under high light intensity in rapid light curve (Figure 4) and about 50% reduction in Y_{max} at mid-day (Figure 5). It has been demonstrated that CCA sensitivity to temperature was greater in winter than in summer. In summer, only a combination of elevated temperature and high $p\text{CO}_2$ resulted in a reduction in photosynthesis. This study was performed in summer yet temperature alone resulted in decreased photosynthesis as measured by chlorophyll fluorescence.

Sensitivity to light was amplified by elevated temperature. In fact, winter temperature (16 °C) had the highest resilience to

increased light intensity as apparent from rapid light curves (Figure 4a). Spring temperatures above 24 °C caused a dramatic decrease in quantum yield of PSII. The initial slope of the RLC (α) was steeper with an increase in temperature. Increased temperature negatively affected the electron transport rate (ETR) of PSII (Figure 4c).

Decreased pH negatively affected photosynthesis of *N. brassica-florida*. Although there were no visual signs of stress in cores translocated to the low pH site, lower photosynthetic potential (dark-adapted Fv/Fm differences) was associated with decreasing pH. No significant difference was found between cores from the high (normal) and reference sites. Diel cycle of the effective quantum yield showed that soon after sunrise and just before sunset there were only minor differences between pH sites (Figure 5). Only at mid-day there were significant changes between the low pH site, showing a lower photosynthetic yield, and the medium and ambient pH sites which did not differ. This may suggest increased sensitivity to light of the CCA in the low pH site. This is also evident from the rapid light curve performed on these cores. This finding is supported by other studies, demonstrating reduced photosynthetic capacity of a coralline alga species at high irradiance when exposed to high $p\text{CO}_2$ (Hofmann et al., 2012). A decrease in photosynthesis at elevated $p\text{CO}_2$ was also reported for the CCA *Lithophyllum cabiochae* (Martin, 2013) and the authors suggested that a higher NPQ may be the reason. Indeed, in this study, reduced pH led to a higher NPQ. Although a higher NPQ was measured at low pH sites, elevated temperature resulted in decreased NPQ. This may be related to different cellular mechanisms involved or differences in the experimental setup. The pH gradient experiment was conducted in an open system (Vulcano CO₂ vents) and relatively long exposure time (i.e. 9 months) whereas the temperature perturbation experiment was performed in the lab under controlled conditions and a relatively short exposure time. ETR was significantly lower in the medium and low pH sites as compared with the ambient and low pH sites.

Elevated temperature caused a decrease in the maximum yield of PSII and affected the sensitivity to irradiance; a decrease in pH showed a similar pattern. It is likely that under combined elevated temperature and calcification the effect of reduced pH will be exacerbated by warming, as reported in previous studies (Anthony et al., 2008).

Calcification

N. brassica-florida thalli are made of high-magnesium calcite, the most soluble form of the common CaCO₃ minerals (Martin and Gattuso, 2009). Calcification of *N. brassica-florida* was greatly affected by acidification. This is in agreement with several studies examining the sensitivity of CCA to ocean acidification conditions (Anthony et al., 2008; Jokiel et al., 2008; Kuffner et al., 2008; Martin and Gattuso, 2009; Ries, 2011; Semesi et al., 2009) yet in contrast to other studies demonstrating no or positive response (higher calcification rate) at higher $p\text{CO}_2$ (Smith and Roth, 1979; Ries et al., 2009; Martin et al., 2013). Reports of increased photosynthesis under high $p\text{CO}_2$ imply amelioration of calcification, but reports (Semesi et al., 2009) of reduced calcification in CO₂-enriched seawater in spite of increased photosynthetic rates suggest a direct pH-effect on the calcification process (Semesi et al., 2009).

In this study, we found that in the absence of living *Dendropoma* individuals, as is the case along the Israeli coast, the rim accretes high magnesium calcite in the day and dissolves at night. At present day pH level (8.1), the dissolution rate in the dark is an order of magnitude lower than the calcification rate in the light. This suggests that calcification in *N. brassica-florida* is photosynthesis dependent as shown for other species (Martin *et al.*, 2013; Smith and Roth, 1979). At pH 7.9, the dissolution rate in the dark is higher than calcification in the light, suggesting that under these conditions the CCA may not be able to compete for space (Kuffner *et al.*, 2008) and even be competitively excluded (by turf and macroalgae e.g.) from this ecosystem by the end of the century. However, it is important to note that pH increases within the canopies of macroalgal assemblage in the light and decreases in the dark due to macroalgal photosynthesis and respiration, respectively (Cornwall *et al.*, 2013). This may have an ameliorating effect on *N. brassica-florida* growth in a macroalgae-dominated landscape.

In this study, there was no indication for increased photosynthesis under high $p\text{CO}_2$ although this was measured indirectly by chlorophyll fluorescence. Ries (2011) reported that a species of *Neogoniolithon* showed a parabolic calcification rate when exposed to $p\text{CO}_2$ equivalent of 2, 3, and 10 times that of pre-industrial concentrations. Calcification increased beyond double pre-industrial levels but decreased at the highest CO_2 concentration.

In a survey performed near the CO_2 vents of Ischia (Italy) (Hall-Spencer *et al.*, 2008), *N. brassica-florida* showed a relatively high abundance in low pH sites. Furthermore, observations suggest reproduction of the species in the low pH sites (Porzio *et al.*, 2011). In view of our findings in this study and findings of most studies on the sensitivity of CCA to acidification and elevated temperature (Martin and Gattuso, 2009; Basso, 2012) the observations in the aforementioned studies implies acclimation/adaptation to a high CO_2 environment. Some dolomite-rich CCA species in coral reefs have 6–10 times lower rates of dissolution than Mg-calcite CCA in both high- CO_2 and present-day CO_2 environments (Nash *et al.*, 2013), suggesting that these types of CCA are likely to persist under ocean acidification scenarios. Contrary, *N. brassica-florida*, a high Mg-Calcite CCA is likely to dissolve rapidly.

N. brassica-florida has a long paleo-record in the Mediterranean Sea (Braga *et al.*, 2009). It lived with coral reefs that no longer exist in that region, “crossed” the Messinian salinity crisis and at present inhabits a vast geographical range. It appears however, that new challenges, namely global warming and ocean acidification, are putting this high Mg-calcite reef builder at risk. Although it is unclear what is the combined effect of elevated temperature, reduced pH and rising sea level on *N. brassica-florida*, our findings suggest that even reduced pH or elevated temperature in isolation threatens the integrity of this intertidal reef builder.

Funding

This study was supported by the EU FP-7 MedSeA project and an EU FP-7ASSEMBLE travel grant to MM.

Acknowledgments

The authors thank E. Borell, R. Horwitz, T. Mordechai, Y. Hazan, G. Perna and G. Lavy for assistance at sea, O. Kolesnikov for

technical assistance in the laboratory, and S. Berkowicz for editorial assistance.

References

- Almgren, T., Dyrssen, D., and Fonselius, S. 1983. Determination of alkalinity and total carbonate. *In* Methods of Seawater Analysis, pp 99–123. Ed. by K. Grasshoff, M. Ehrhardt and K. Kremling. Verlag Chemie GmbH.
- Anthony, K. R. N., Kline, D. I., Diaz-Pulido, G., Dove, S., and Hoegh-Guldberg, O. 2008. Ocean acidification causes bleaching and productivity loss in coral reef builders. Proceedings of the National Academy of Sciences of the United States of America, 105: 17442–17446.
- Arnold, T., Mealey, C., Leahey, H., Miller, A. W., Hall-Spencer, J. M., Milazzo, M., and Maers, K. 2012. Ocean Acidification and the Loss of Phenolic Substances in Marine Plants. *Plos One*, 7: e35107.
- Basso, D. 2012. Carbonate production by calcareous red algae and global change. *Geodiversitas*, 34: 13–33.
- Benelihu, M. N. 1975. Polychaete Cryptofauna from Rims of Similar Intertidal Vermetid Reefs on Mediterranean Coast of Israel and in Gulf of Elat - Nereidae Polychaeta-Errantia. *Israel Journal of Zoology*, 24: 177–191.
- Braga, J. C., Vescogni, A., Bosellini, F. R., and Aguirre, J. 2009. Coralline algae Corallinales, Rhodophyta. in western and central Mediterranean Messinian reefs. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 275: 113–128.
- Caldeira, K., and Wickett, M. E. 2005. Ocean model predictions of chemistry changes from carbon dioxide emissions to the atmosphere and ocean. *Journal of Geophysical Research-Oceans*, 110:
- Calvo, M., Templado, J., and Penchaszadeh, P. E. 1998. Reproductive biology of the gregarious Mediterranean vermetid gastropod *Dendropoma petraeum*. *Journal of Marine Biology Association United Kingdom*, 78: 525–549.
- Cornwall, C. E., Hepburn, C. D., Pilditch, C. A., and Hurd, C. L. 2013. Concentration boundary layers around complex assemblages of macroalgae: implications for the effects of ocean acidification on understory coralline algae. *Limnology and Oceanography*, 58: 121–130.
- Davison, I. R., and Pearson, G. A. 1996. Stress tolerance in intertidal seaweeds. *Journal of Phycology*, 32: 197–211.
- Di Franco, A., Graziano, M., Franzitta, G., Felling, S., Chemello, R., and Milazzo, M. 2011. Do small marinas drive habitat specific impacts? A case study from Mediterranean Sea. *Marine Pollution Bulletin*, 62: 926–933.
- Fishelson, L., and Haran, T. 1987. Epifauna of algae on a rocky platform near Mikhmoret Mediterranean Sea, Israel. composition and dynamics. *Israel Journal of Zoology*, 34: 105–123.
- Franklin, L., and Forster, R. 1997. The changing irradiance environment: consequences for marine macrophyte physiology, productivity and ecology. *European Journal of Phycology*, 32: 207–232.
- Galil, B. S. 2013. Going going gone: the loss of a reef building gastropod Mollusca: Caenogastropoda: Vermetidae. in the southeast Mediterranean Sea. *Zoology in the Middle East*, 59: 179–182.
- Ganning, B. 1971. Studies on chemical, physical and biological conditions in Swedish rockpool ecosystems. *Ophelia*, 9: 51–105.
- Genty, B., Briantais, J. M., and Baker, N. R. 1989. The relationship between the quantum yield of photosynthetic electron-transport and quenching of chlorophyll fluorescence. *Biochimica et Biophysica Acta*, 990: 87–92.
- Goren, M., and Galil, B. S. 2001. Fish biodiversity in the vermetid reef of Shiqmona Israel. *Marine Ecology-Pubblicazioni Della Stazione Zoologica Di Napoli I*, 22: 369–378.
- Häder, D. P., Lebert, M., Walter Helbling, E. 2003. Effects of Solar Radiation on the Patagonian Rhodophyte *Corallina officinatis* L. ... *Photosynthesis Research*, 78: 119–132.

- Hall-Spencer, J. M., Rodolfo-Metalpa, R., Martine, S., *et al.* 2008. Volcanic carbon dioxide vents show ecosystem effects of ocean acidification. *Nature*, 454: 96–99.
- Hofmann, L. C., Straub, S., and Bischof, K. 2012. Competition between calcifying and noncalcifying temperate marine macroalgae under elevated CO₂ levels. *Marine Ecology Progress Series*, 464: 89–105.
- Jokiel, P. L., Rodgers, K. S., Kuffner, I. B., Andersson, A. J., Cox, E. F., and Mackenzie, F. T. 2008. Ocean acidification and calcifying reef organisms: a mesocosm investigation. *Coral Reefs*, 27: 473–483.
- Klerman, A., Fine, M., and Galil, B. S. 2004. Reproductive biology of a threatened reef-building vermetid Mollusca, Gastropoda. off the coast of Israel. *Rapport du Congress de la CIESM*, 37: 530–530.
- Kuffner, I. B., Andersson, A. J., Jokiel, P. L., Rodgers, K. S., and Mackenzie, F. T. 2008. Decreased abundance of crustose coralline algae due to ocean acidification. *Nature Geoscience*, 1: 114–117.
- Langar, H., Bessibes, M., Djellouli, A., Pergent-Martini, C., and Pergent, G. 2011. The *Neogoniolithon brassica-florida* Harvey. Setchell and LR Mason 1943. Reef of Bahiret el Bibane Lagoon Southeastern Tunisia. *Journal of Coastal Research*, 27: 394–398.
- Lewis, E., and Wallace, D. W. R. 1998. Program Developed for CO₂ System Calculations. ORNL/CDIAC-105. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, U.S. Department of Energy, Oak Ridge, Tennessee.
- Lipkin, Y., and Safriel, U. 1971. Intertidal zonation on rocky shores at Mikhmoret Mediterranean, Israel. *Journal of Ecology*, 59: 1–30.
- Lobban, C. S., and Harrison, P. J. 1994. *Seaweed Ecology and Physiology*, Cambridge University Press, Cambridge.
- Martin, S., Cohu, S., Vignot, C., Zimmerman, G., and Gattuso, J. P. 2013. One-year experiment on the physiological response of the Mediterranean crustose coralline alga, *Lithophyllum cabiochae*, to elevated pCO₂ and temperature. *Ecology and Evolution*, 3: 676–693.
- Martin, S., and Gattuso, J. -P. 2009. Response of Mediterranean coralline algae to ocean acidification and elevated temperature. *Global Change Biology*, 15: 2089–2100.
- Milazzo, M., Rodolfo-Metalpa, R., Chan, V. B. S., Fine, M., Alessi, C., Thiagarajan, V., Hall-Spencer, J. M., *et al.* 2014. Ocean acidification impairs vermetid reef recruitment. *Scientific Reports*, 4
- Morris, S., and Taylor, A. C. 1983. Diurnal and seasonal variation in physico-chemical conditions within intertidal rock pools. *Estuarine, Coastal and Shelf Science*, 17: 339–355.
- Müller, P., Li, X. P., and Niyogi, K. K. 2001. Non-photochemical quenching. A response to excess light energy. *Plant Physiology*, 125: 1558–1566.
- Nash, M. C., Opdyke, B. N., Troitzsch, U., Russell, B. D., Adey, W. H., Kato, A., Diaz-Pulido, G., *et al.* 2013. Dolomite-rich coralline algae in reefs resist dissolution in acidified conditions *Nature Climate Change*, 3: 268–272.
- Nelson, W. A. 2009. Calcified macroalgae - critical to coastal ecosystems and vulnerable to change: a review. *Marine and Freshwater Research*, 60: 787–801.
- Platt, T., Gallegos, C., and Harrison, W. 1980. Photoinhibition of photosynthesis in natural assemblages of marine phytoplankton. *Journal of Marine Research*, 38: 687–701.
- Porzio, L., Buia, M. C., and Hall-Spencer, J. M. 2011. Effects of ocean acidification on macroalgal communities. *Journal of Experimental Marine Biology and Ecology*, 400: 278–287.
- Ralph, P. J., and Gademann, R. 2005. Rapid light curves: a powerful tool to assess photosynthetic activity. *Aquatic Botany*, 82: 222–237.
- Ries, J. B. 2011. Skeletal mineralogy in a high-CO₂ world. *Journal of Experimental Marine Biology and Ecology*, 403: 54–64.
- Ries, J. B., Cohen, A. L., and McCorkle, D. C. 2009. Marine calcifiers exhibit mixed responses to CO₂-induced ocean acidification. *Geology*, 37: 1131–1134.
- Safriel, U. N. 1974. Vermetid Gastropods and Intertidal Reefs in Israel and Bermuda. *Science*, 186: 1113–1115.
- Safriel, U. N., and Ben-Eliahu, M. N. 1991. The influence of habitat structure and environmental stability on the species diversity of polychaetes in vermetid reefs. *In* *Habitat Structure The Physical Arrangement of Objects in Space*. Ed. by S. S., Bell, E. D., McCoy and H. R., Mushinsky. Chapman and Hall, London. pp. 349–372.
- Schreiber, U., Bilger, W., and Neubauer, C. 1995. Chlorophyll fluorescence as a noninvasive indicator for rapid assessment of in vivo photosynthesis. *In* *Ecophysiology of photosynthesis*, pp. 49–70. Ed. by E.-D. Schulze, and M. M. Caldwell. Springer, Berlin, Heidelberg.
- Scotti, G., and Chemello, R. 2000. The Mediterranean marine mollusks that merit protection: The state of knowledge and forms of conservation. *Bollettino Malacologico*, 36: 61–70.
- Semesi, I. S., Kangwe, J., and Bjork, M. 2009. Alterations in seawater pH and CO₂ affect calcification and photosynthesis in the tropical coralline alga, *Hydrolithon* sp Rhodophyta. *Estuarine Coastal and Shelf Science*, 84: 337–341.
- Setchell, W. A., and Mason, L. R. 1943. *Goniolithon* and *Neogoniolithon*: Two Genera of Crustaceous Coralline Algae. *Proceedings of the National Academy of Sciences of the United States of America*, 29: 87–92.
- Sisma-Ventura, G., Guzner, B., Yam, R., Fine, M., and Shemesh, A. 2009. The reef builder gastropod *Dendropoma petraeum* - A proxy of short and long term climatic events in the Eastern Mediterranean. *Geochimica Et Cosmochimica Acta*, 73: 4376–4383.
- Smith, A. D., and Roth, A. A. 1979. Effect of carbon dioxide concentration on calcification in the red coralline algae *Bossiella orbigniana*. *Marine Biology*, 52: 217–225.
- Spotorno-Oliveira, P., Figueiredo, M. A. O., and Tamega, F. T. S. 2015. Coral line algae enhance the settlement of the vermetid gastropod *Dendropoma irregulare* (d'Orbigny, 1842) in the south-western Atlantic. *Journal of Experimental Marine Biology and Ecology*, 471: 137–145.
- Templado, J., Richter, A., and Calvo, M. 2016. Reef building Mediterranean vermetid gastropods: disentangling the *Dendropoma petraeum* species complex. *Mediterranean Marine Science*, 17: 13–31.
- Terlizzi, A., Scuderi, D., Frascchetti, S., and Anderson, M. J. 2005. Quantifying effects of pollution on biodiversity: a case study of highly diverse molluscan assemblages in the Mediterranean. *Marine Biology*, 148: 293–305.
- Truchot, J. P., and Duhamel-Jouve, A. 1980. Oxygen and carbon dioxide in the marine intertidal environment: Diurnal and tidal changes in rockpools. *Respiration Physiology*, 39: 241–254.
- Usvyatsov, S., and Galil, B. S. 2012. Comparison of reproductive characteristics among populations of *Dendropoma petraeum*-complex Mollusca: Caenogastropoda., an endemic Mediterranean reef-building vermetid. *Journal of Marine Biology Association United Kingdom*, 92: 163–170.

Handling editor: Joanna Norkko