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ORIGINAL ARTICLE

## Effects of ocean acidification and high temperatures on the bryozoan *Myriapora truncata* at natural CO<sub>2</sub> vents

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### Keywords

Bryozoan; calcification; global warming; Mediterranean Sea; *Myriapora truncata*; ocean acidification; volcanic CO<sub>2</sub> vents.

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### Abstract

There are serious concerns that ocean acidification will combine with the effects of global warming to cause major shifts in marine ecosystems, but there is a lack of field data on the combined ecological effects of these changes due to the difficulty of creating large-scale, long-term exposures to elevated CO<sub>2</sub> and temperature. Here we report the first coastal transplant experiment designed to investigate the effects of naturally acidified seawater on the rates of net calcification and dissolution of the branched calcitic bryozoan *Myriapora truncata* (Pallas, 1766). Colonies were transplanted to normal (pH 8.1), high (mean pH 7.66, minimum value 7.33) and extremely high CO<sub>2</sub> conditions (mean pH 7.43, minimum value 6.83) at gas vents off Ischia Island (Tyrrhenian Sea, Italy). The net calcification rates of live colonies and the dissolution rates of dead colonies were estimated by weighing after 45 days (May–June 2008) and after 128 days (July–October) to examine the hypothesis that high CO<sub>2</sub> levels affect bryozoan growth and survival differently during moderate and warm water conditions. In the first observation period, seawater temperatures ranged from 19 to 24 °C; dead *M. truncata* colonies dissolved at high CO<sub>2</sub> levels (pH 7.66), whereas live specimens maintained the same net calcification rate as those growing at normal pH. In extremely high CO<sub>2</sub> conditions (mean pH 7.43), the live bryozoans calcified significantly less than those at normal pH. Therefore, established colonies of *M. truncata* seem well able to withstand the levels of ocean acidification predicted in the next 200 years, possibly because the soft tissues protect the skeleton from an external decrease in pH. However, during the second period of observation a prolonged period of high seawater temperatures (25–28 °C) halted calcification both in controls and at high CO<sub>2</sub>, and all transplants died when high temperatures were combined with extremely high CO<sub>2</sub> levels. Clearly, attempts to predict the future response of organisms to ocean acidification need to consider the effects of concurrent changes such as the Mediterranean trend for increased summer temperatures in surface waters. Although *M. truncata* was resilient to short-term exposure to high levels of ocean acidification at normal temperatures, our field transplants showed that its ability to calcify at higher temperatures was compromised, adding it to the growing list of species now potentially threatened by global warming.

## Problem

Increasing human CO<sub>2</sub> emissions threaten marine biodiversity due to the consequent effects of ocean acidification, a term used to describe the 30% increase in hydrogen ions that has occurred since pre-industrial times, measured as a decrease in 0.1 pH units for sea surface waters globally (Doney *et al.* 2009). A further fall of 0.3–0.4 pH units is predicted by 2100 (Caldeira & Wickett 2003), which will lower the amount of calcium carbonate available in seawater and may disrupt calcification in a range of ecologically important organisms such as coralline algae (Kuffner *et al.* 2008; Martin *et al.* 2008), foraminiferans (*e.g.* Moy *et al.* 2009), corals (*e.g.* Silverman *et al.* 2009), echinoderms (*e.g.* Michaelidis *et al.* 2005) and molluscs (Gazeau *et al.* 2007). Among these organisms, rates of calcification have been predicted to fall by up to 60% within this century, depending on the physiology of the species and their mineralogy (Kleypas *et al.* 2006). Shells can dissolve when exposed to seawater with low carbonate saturation states such as in estuaries (Marshall *et al.* 2008), in upwelling areas (Feely *et al.* 2008) and around volcanic CO<sub>2</sub> vents (Hall-Spencer *et al.* 2008). Shells and/or skeletons made of high Mg-calcite are highly susceptible to dissolution as carbonate saturation states fall, followed by aragonitic skeletons and finally low Mg-calcite skeletons. Because CO<sub>2</sub> dissolves more readily in cold water, shallow water dissolution of marine carbonates is expected to be noted first at high latitudes (Orr *et al.* 2005), whereas deeper water dissolution will occur as the interface between saturated and unsaturated waters shoals throughout the world's oceans (Fabry *et al.* 2008; Feely *et al.* 2008). Laboratory and mesocosm experiments show that many organisms lose their ability to lay down carbonate at increased CO<sub>2</sub> levels. Most corals are expected to decrease their calcification rates drastically (Hoegh-Guldberg *et al.* 2007) and may start to dissolve by the end of 2100 (Silverman *et al.* 2009), although some can maintain normal calcification rates (Rodolfo-Metalpa *et al.* 2009) or even increase their calcification rates (Jury *et al.* 2009; Ries *et al.* 2009), and others can survive without their skeletons (Fine & Tchernov 2007) when exposed to high pCO<sub>2</sub> concentrations. High CO<sub>2</sub> levels may even increase calcification rates in fish otoliths (Checkley *et al.* 2009) and in certain species of coccolithophore (Iglesias-Rodríguez *et al.* 2008), echinoderm (Wood *et al.* 2008; Gooding *et al.* 2009) and barnacle (McDonald *et al.* 2009). Ries *et al.* (2009) found that calcification rates increased in nine of 18 species exposed to moderate (560 µatm) or high (840 µatm) pCO<sub>2</sub> levels, including the temperate coral *Oculina arbuscula*, a mussel and some crustaceans. Similarly, Findlay *et al.* (2009) found that the calcification rates in four of six benthic

calcifying species increased in acidified seawater. To predict the likely impacts of ocean acidification on marine species, and therefore the likely structure and function of future benthic communities, more studies are needed to determine the metabolic, physiological and ecological mechanisms by which hypercapnia affects survival across a range of taxonomic groups (Pörtner 2008; Findlay *et al.* 2009; Widdicombe *et al.* 2009). To address this, we undertake the first examination of the response of bryozoans to ocean acidification, as they play an important ecological role as they can increase habitat heterogeneity and species diversity (Cocito 2004; Ballesteros 2006).

In the present study we used natural volcanic CO<sub>2</sub> vents where marine communities tolerate long-term reductions in seawater pH (Hall-Spencer *et al.* 2008). We experimented on bryozoans, as they are major calcifiers about which little is known in relation to the effects of ocean acidification (Martin *et al.* 2008). Bryozoans occur on most rocky shores; they are often abundant in shallow sublittoral habitats and form a significant component of carbonate sediments in cool-water areas of the planet (Zabala 1986; Ballesteros 2006; Smith *et al.* 2006). Erect, branching bryozoans form long-lived three-dimensional structures that provide attachment surfaces for other epifauna and they provide protection and trap sediment and food for a variety of infauna (Cocito 2004). We investigated rates of calcification and dissolution of the robust, branched bryozoan *Myriapora truncata* (Pallas 1766). Although 15% of the species of bryozoan are aragonitic and 17% are bimineralic (Smith *et al.* 2006), *M. truncata* is typical of most Bryozoa in that it has a calcitic skeleton. This species occurs in sciaphilous rocky habitats from the shallow subtidal in sheltered sites down to 60 m depth (Zabala 1986; Ballesteros 2006); it is widespread in the Mediterranean and occurs from Northern Morocco to Southern Spain on Atlantic coasts (López de la Cuadra & García-Gómez 1994).

The aim of our study was to investigate the effects of 4-month *in situ* exposure to different pH conditions on the calcification and dissolution of *M. truncata* using *in situ* transplantation experiments at natural volcanic CO<sub>2</sub> vent sites. We test the hypothesis that temperature affects the degree to which ocean acidification alters calcification and dissolution in these bryozoans.

## Material and Methods

### Species collection and preparation

In May 2008, *Myriapora truncata* colonies (2–4 cm high) were carefully removed from rock surfaces in a shaded crevice at 14 m depth off the S. Angelo cliff (Ischia

Island; 40°041.31' N; 13°53.36' N). They were transported in temperature controlled tanks ( $19 \pm 1$  °C) to the laboratory where they were maintained in flow-through aquaria. Turnover rate of seawater in the 20-l aquaria was  $50\% \text{ h}^{-1}$  and temperature was maintained constant at the *in situ* value of 18 °C. The aquaria were shaded to provide low-light conditions ( $<10 \mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ). After a few days, bryozoans were carefully cleaned of epibionts, associated fauna, and sediment. Thirty-two live *M. truncata* fragments were prepared for the experiment; these were weighed using the buoyant weight technique (Davies 1989) before and after attachment to tagged plastic plates using epoxy glue (HoldFast®, Holdfast Technologies, Newton, Hamilton, New Zealand). Another 20 fragments of *M. truncata* were killed by dissolving their tissues in  $\text{H}_2\text{O}_2$  (30% by volume; 12-h immersion). Skeletons were then washed later for 24-h in running seawater for subsequent measurements of skeletal dissolution rates in acidified conditions. Skeletal fragments were then weighed before and after being glued to plastic plates. The difference (plate and glue weight) was recorded and was subtracted from the total weight. Live and dead fragments (eight and five replicates for each treatment, respectively) were randomly assigned to one of four cages and were mounted ~10 cm apart on PVC plates measuring  $30 \times 50$  cm. Bryozoan colonies were attached to the cover plate of the cage to mimic their natural orientation and to reduce irradiance.

### Field transplantation

On 16 May 2008, two cages were positioned 2 m apart at 2–3 m depth on the south side of Castello Aragonese (40°043.84' N; 013° 57.08' E) near  $\text{CO}_2$  vents (B1 and B2) where the pH varied around 7.2–7.9 (Hall-Spencer *et al.* 2008), and two control cages were placed 100–150 m away from the vents (C1 and C2), at 3–4 m depth in normal pH (8.1–8.2) conditions. The controls C1 and C2 experienced normal seawater conditions (Table 1) and whereas are true replicates, B1 and B2 experienced different carbonate saturation levels during the experiment; B1 had the lowest carbonate saturation levels as it lay closest

to the  $\text{CO}_2$  vents. The bryozoan transplants were ~10 cm apart in each cage, therefore sufficiently spaced to allow water to circulate, transplants at B1 and B2 are strictly speaking pseudo-replicates (*sensu* Hurlbert 1984), as it is logistically impossible to replicate these low pH treatments. The cages were attached to 30-kg concrete blocks fitted with Hobo Onset loggers to monitor seawater temperatures at 15-min intervals for the duration of the experiment. Cages were collected after 45 days (Period 1) and again after a further 83 days (Period 2) (recovered on 10 October 2008). In the laboratory, bryozoan colonies were carefully cleaned with a brush and scalpel to detach epibionts. This procedure was particularly laborious on dead colonies maintained at normal pH which were heavily covered by epibionts and were fragile. Colonies were then photographed, weighed and reattached to the respective cages before being replaced in the field. This procedure lasted 2–3 days, during which fragments were maintained in aquaria with running seawater at pH 8.1.

### Net calcification and $\text{CaCO}_3$ dissolution rates

Net calcification rate was measured by weighing each colony fragment before transplantation, after 45 days (Period 1) and again after 83 days (Period 2), giving a total of 128 days.  $\text{CaCO}_3$  dissolution rates were only measured during Period 1. Fragments, both live and dead, were weighed in seawater using the buoyant weight technique (Davies 1989). Bryozoan net buoyant weight (total weight – the weight of each plate) was converted into dry weight according to the equation:

$$\text{Dry weight} = \text{Buoyant weight} / (1 - D_{\text{water}} / D_{\text{skeleton}})$$

where  $D_{\text{water}}$  is the density of the water in which the sample was weighed (calculated from the water temperature and salinity) and  $D_{\text{skeleton}}$  the density of calcite ( $2.71 \text{ g}\cdot\text{cm}^{-3}$ ). Calcification rates were calculated as the change in dry weight between two periods of measurement and normalized to the initial weight per month ( $\text{mg}\cdot\text{g}^{-1} \text{ 30 days}^{-1}$ ). The buoyant weight technique is

**Table 1.** Mean  $\pm$  SD seawater carbonate chemistry between 10 May and 17 September 2008 at  $\text{CO}_2$  vents off Ischia, Italy. Salinity was 38 at all stations; total alkalinity (TA) is  $\text{mEq}\cdot\text{kg}^{-1}$ ;  $\text{pH}_T$  is in total scale;  $\text{pCO}_2$  is  $\mu\text{atm}$ ;  $\text{CO}_2$ ,  $\text{HCO}_3^-$ , and  $\text{CO}_3^{2-}$  are in  $\text{nmol}\cdot\text{kg}^{-1}$ . See all data set in Electronic Supplementary Information.

Site	TA	$\text{pH}_T$	$\text{pCO}_2$	$\text{CO}_2$	$\text{HCO}_3^-$	$\text{CO}_3^{2-}$	$\Omega_{\text{calcite}}$
B1	$2.58 \pm 0.02$	$7.43 \pm 0.31$	$2918.8 \pm 2470.2$	$0.085 \pm 0.068$	$2.366 \pm 0.11$	$0.26 \pm 0.04$	$1.99 \pm 1.09$
B2	$2.58 \pm 0.03$	$7.66 \pm 0.22$	$1420.3 \pm 752.8$	$0.042 \pm 0.023$	$2.261 \pm 0.14$	$0.26 \pm 0.03$	$3.08 \pm 1.47$
C1	$2.59 \pm 0.03$	$8.06 \pm 0.07$	$426.2 \pm 98.9$	$0.013 \pm 0.003$	$1.596 \pm 0.807$	$0.08 \pm 0.04$	$6.12 \pm 1.00$
C2	$2.59 \pm 0.02$	$8.07 \pm 0.10$	$425.4 \pm 117.1$	$0.012 \pm 0.002$	$1.957 \pm 0.062$	$0.13 \pm 0.06$	$6.11 \pm 0.63$

normally used to measure weight gain as the result of  $\text{CaCO}_3$  deposition (*i.e.* gross calcification) but here we used this technique to examine the net calcification and dissolution because acidified seawater may dissolve existing  $\text{CaCO}_3$  skeletons.

#### pH measurements and carbonate system characterization

During the experiment, pH in total scale ( $\text{pH}_T$ ) and total alkalinity (TA) were measured seven times between 10 and 20 May, five times between 23 and 29 June and then at the end of the experiment. Water samples were collected in glass bottles next to the cages, and the  $\text{pH}_T$  was measured immediately using a meter accurate to 0.01 pH units (Metrohm 826 pH mobile) calibrated using TRIS/HCl and 2-aminopyridine/HCl buffer solutions (DOE 1994). Seawater samples were then passed through 0.45- $\mu\text{m}$  pore size filters (GF/F Whatman), poisoned with 0.05 ml of 50%  $\text{HgCl}_2$  (Merck, Analar) to avoid biological alteration, and stored in the dark at 4 °C. Three replicate 20-ml sub-samples were analyzed at 25 °C using a titration system composed of a pH meter with an ORION pH electrode (calibrated using NBS standard solutions) and a 1-ml automatic burette (METHROM). The pH (in NBS scale) was measured at 0.02-ml increments of 0.1 N HCl. Total alkalinity was calculated from the Gran function applied to pH variations from 4.2 to 3.0, as  $\text{mEq l}^{-1}$  from the slope of the curve HCl volume *versus* pH. Parameters of the carbonate system [ $\text{pCO}_2$ ,  $\text{CO}_3^{2-}$ ,  $\text{HCO}_3^-$ , and saturation state of calcite ( $\Omega_{\text{calcite}}$ )] were calculated from  $\text{pH}_T$ , TA, temperature and salinity (38) using the free-access  $\text{CO}_2$  SYSTAT package.

#### Statistical analysis

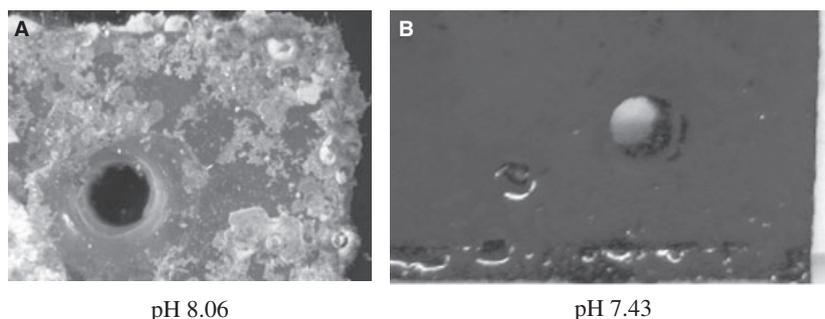
Student's *t*-test was used to test for differences in pH and bryozoan net calcification and dissolution rates in the two control cages. After verification of the homogeneity of variances (Cochran test,  $P < 0.05$ ), one-way ANOVAs were used to compare the pooled control data (C) with the two treatments (B1 and B2) using STATISTICA®

(Statsoft, USA). When ANOVAs revealed significant differences ( $P < 0.05$ ) the Tukey HSD test for unequal numbers (Spjøtvoll/Stoline test) was used.

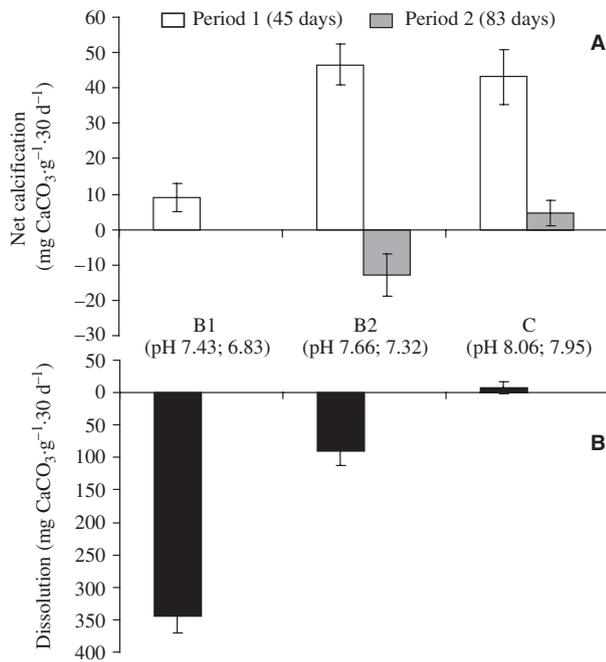
#### Results and Discussion

Environmental conditions at the  $\text{CO}_2$  vents clearly affected coralline algae, serpulids and encrusting bryozoans, as they heavily colonized plates in the control cages after 45 and 128 days (Fig. 1A) but were never found on plates at mean  $\text{pH} < 7.7$  (Fig. 1B), adding to a growing suite of evidence that high seawater  $\text{CO}_2$  levels have a profound impact on settlement and survival of calcifiers (Hall-Spencer *et al.* 2008; Jokiel *et al.* 2008; Kuffner *et al.* 2008; Martin *et al.* 2008).

Our *Myriapora truncata* transplants all grew well in control cages ( $\text{pH} > 8.0$ , Table 1 and data in ESI) with no differences in their net calcification rates between cages C1 and C2 (*t*-test,  $P > 0.05$ ), so these data were pooled and termed treatment C (Fig. 2). Although the low pH treatments were not replicated, most environmental parameters that could affect the comparison between treatments were monitored. Seawater temperature, salinity (mean:  $38.35 \pm 0.20$ ,  $n = 34$ ), and irradiance did not differ between treatments and the same number of bryozoans were randomly assigned to one of four cages. They were transplanted at the same time, and after the same preparation treatment. We have no evidence to suspect a difference in the bryozoans' food availability between sites because the controls (C1 and C2) were only 100–150 m away from the vents (B1 and B2), and at the same depth. We therefore conclude that differences in seawater carbonate chemistry provide the most likely explanation for the differences we observed in survival and calcification of the bryozoans. During Period 1, 45-day exposure to high  $\text{CO}_2$  significantly affected rates of net calcification (Fig. 2A; ANOVA:  $F_{2,26} = 7.78$ ,  $P = 0.022$ ) and colonies gained significantly less weight in B1 (Tukey test:  $B1 < B2 = C$ ,  $P < 0.01$ ) at mean  $\text{pH} 7.43$  (Table 1, min.  $\text{pH} 6.83$ ) compared to normal pH. Colonies in cage B2 were surprisingly resilient to the acidified



**Fig. 1.** PVC plates (credits: E. Tambutté) maintained 45 days at mean pH of 8.06 (A), and 7.43 (B), in C and B1, respectively. Note the lack of calcifying epibionts at low pH.



**Fig. 2.** Net calcification (A) measured during Period 1 and Period 2, and dissolution rates (B) measured during Period 1, respectively, on live and dead transplanted colonies of *Myriapora truncata* at extremely low (B1), low (B2) and normal pH (C) near and outside CO<sub>2</sub> vents at Ischia. Data are mean  $\pm$  SE ( $n = 8$  and  $5$ , respectively). Mean and minimum pH measured during the whole experiment are reported in brackets.

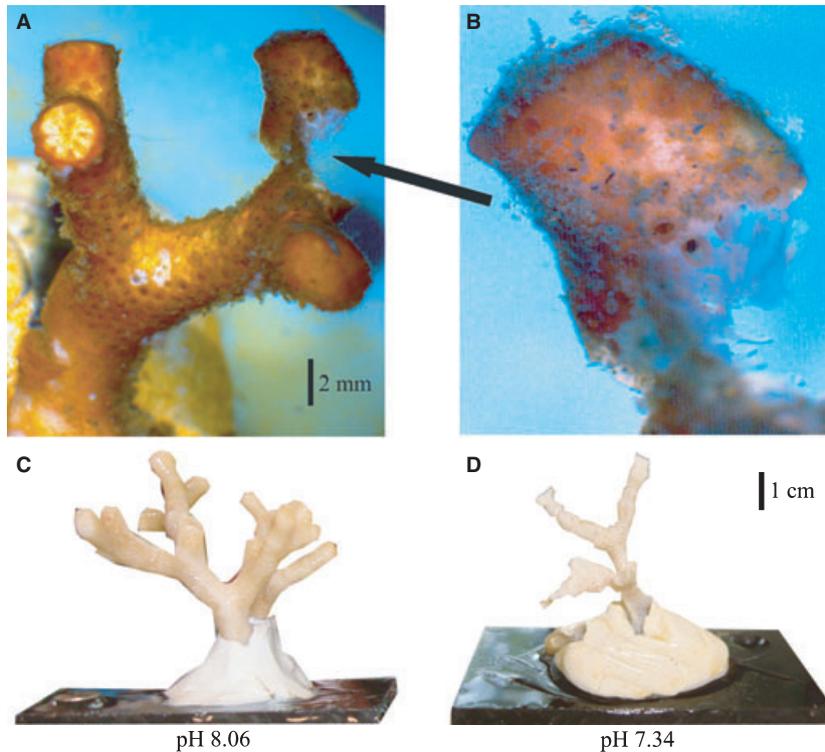
conditions (mean pH 7.66, min. pH 7.32), as their net calcification rates did not differ significantly from the controls. The decrease in calcification measured at B1 is consistent with laboratory and mesocosm observations of reduced calcification rates in other calcitic groups such as coralline algae (Kuffner *et al.* 2008; Martin & Gattuso 2009), foraminifera (Spero *et al.* 1997) and coccolithophores (Riebesell *et al.* 2000; Delille *et al.* 2005). The lack of any difference in colony weight change at B2 was unexpected and indicated that the calcification ability of *M. truncata*, and potentially other calcitic bryozoans, might not be negatively affected by ocean acidification in the next 200 years, according to IPCC (2007) CO<sub>2</sub> emission scenarios.

During this initial 45-day period, dead *M. truncata* skeletons did not dissolve and were heavily colonized by epibionts at normal pH (C), therefore increasing their weight (Fig. 2B), whereas they were dissolving both in B2 (90 mg·g<sup>-1</sup>·30 days<sup>-1</sup>) and at a very high rate in B1 (344 mg·g<sup>-1</sup>·30 days<sup>-1</sup>; Fig. 1B). Dissolution at B1 and B2 occurred even though these treatments normally had saturated  $\Omega_{\text{calcite}}$  levels (Table 1). This is likely due to periods of carbonate undersaturation, which occur at the site when the sea state is particularly calm (Hall-Spencer

*et al.* 2008). Findlay *et al.* (2009) found calcium ion concentration loss (*i.e.* CaCO<sub>3</sub> dissolution) on dead *Amphiura filiformis* arms, *Patella vulgata* and *Mytilus edulis* shells maintained in saturated carbonate conditions at pH 7.7. Martin & Gattuso (2009) reported dissolution of the coralline alga *Lithophyllum cabiochae* maintained at mean pH 7.8. These studies suggest dissolution may also take place at saturation states  $>1$ . Microbes are likely to have been abundant in the highly porous dead skeletons of *M. truncata* and may accelerate skeletal dissolution.

Therefore, in B2, *M. truncata* skeletons dissolved when exposed directly to the seawater but live specimens were able to maintain the same net calcification rates as occurred in normal pH conditions. In contrast, extreme hypercapnic conditions experienced at B1 damaged dead skeletons and significantly decreased the net calcification in live specimens. However, live specimens were still able to calcify in these hypercapnic conditions. This suggests that the zooidal soft tissues that cover the skeleton of each zooid confer protection from acidified seawater. At high levels of acidification (B2) this skeletal protection seems to allow calcification to continue at a normal rate, whereas at extreme pH levels (B1) this protective role appears to decrease, resulting in lower calcification rate.

Our results suggest that *M. truncata* is able to increase its calcification rate under acidified conditions. We calculated gross calcification by adding CaCO<sub>3</sub> dissolution to the net calcification rates measured on dead and live fragments, respectively. At B1 the very high dissolution rates caused colonies to break apart (Figs 2B and 3), whereas at B2, colonies did not break, allowing more accurate calculation of dissolution rates (90 mg·g<sup>-1</sup>·30 days<sup>-1</sup>). The calculated gross calcification rate at B2 was 136 mg·g<sup>-1</sup>·30 days<sup>-1</sup>, three times higher than the net calcification rates measured under normal conditions. Increases in calcification under acidified conditions have recently reported for several species (Wood *et al.* 2008; Findlay *et al.* 2009; Ries *et al.* 2009). However, before firm conclusions are made about the ability of *M. truncata* to increase its calcification rate under high CO<sub>2</sub> conditions, more experiments are necessary using accurate methods able to discriminate gross calcification and dissolution. Our transplants were adult, robust colonies, which may underestimate the vulnerability of this species, as in other phyla it is the embryonic stage of development that seems most vulnerable to the effects of ocean acidification (*e.g.* Dupont *et al.* 2009; Ellis *et al.* 2009; Widdicombe *et al.* 2009). Only by understanding the trade-offs between different physiological (*e.g.* calcification, respiration, growth, mobilization of energy stores) and ecological (feeding rates, movement) responses, can we fully appreciate the consequences on organism success and survival of changing environmental conditions (Finlay *et al.* 2009). It is likely

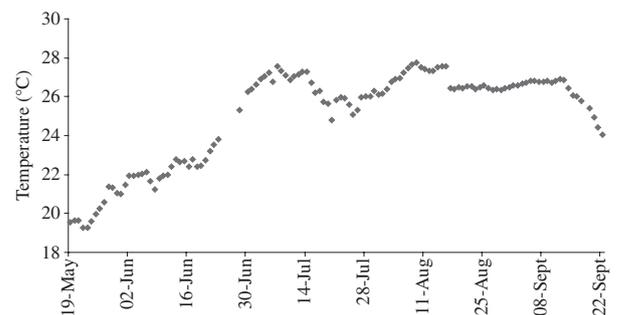


**Fig. 3.** *Myriapora truncata* maintained 128 days at mean pH 7.43 in B1 (A, B) showing the breakage of the zooidal soft tissues and the dissolution of the skeleton; dead fragments after 45 days at mean pH of 8.06 (C), and 7.43 (D) in C and B1, respectively.

that the surprising ability of some calcifying species to increase their calcification rates under acidified conditions corresponds to an increase in their metabolic costs. The ability of species to increase their calcification rates under acidified conditions may incur increased metabolic costs, compromising their long-term survival as shown for *Amphiura filiformis* (Wood *et al.* 2008), *Littorina littorea* (Bibby *et al.* 2007), and *Mytilus edulis* (Beesley *et al.* 2008).

Although adult *M. truncata* colonies were resilient to acidified conditions in the cooler part of our study (Period 1), all B1 specimens had died at the end of Period 2, and fragments in B2 and C, although still living, showed negligible calcification rates (Fig. 2A). The mortality of all samples maintained 128 days under severe hypercapnia was presumably due to the synergistic effect of elevated seawater temperature and prolonged exposure to low pH levels. Interaction between high CO<sub>2</sub> and elevated temperature decreased calcification in the scleractinian coral *Stylophora pistillata* (Reynaud *et al.* 2003) and killed the Mediterranean coralline alga *Lithophyllum cabiochae* (Martin & Gattuso 2009). The dramatic decreases in calcification rates measured at high CO<sub>2</sub> (B2) and also at the control, seem likely to have been caused by the prolonged exposure to high temperatures experienced during Period 2, because they had grown well during the cooler Period 1. Indeed, from 16 May 2008 to 26 June 2008 (Period 1) the water around the transplants warmed stea-

dily from 19 to 24 °C and the bryozoans grew well; then, during Period 2, the water temperature remained high at 25–28 °C for 3 months (Fig. 4) and bryozoan calcification decreased to zero. It is likely that high seawater temperatures caused such a stress to this species as to disable any calcification as well as increasing their metabolic rates such as respiration. These summer temperatures, for such long period, are particularly high for the Central Tyrrhenian Sea (Ribera d'Alcalà *et al.* 2004) and tie in with data that show an on-going warming of the Mediterranean (Coma *et al.* 2009). Concomitantly, mass mortality of benthic species has become frequent in the Western Mediterranean Sea, including our study area (*e.g.*: Cerrano



**Fig. 4.** Mean daily seawater temperatures measured in B1 every 15 min using Hobo Onset loggers.

*et al.* 2000; Rodolfo-Metalpa *et al.* 2005, 2008b; Cigliano & Gambi 2007; Garrabou *et al.* 2008; Sbrescia *et al.* 2008). Long-term exposure to relatively high temperatures causes physiological stress to benthic species, such as increased respiration (Coma *et al.* 2002; Rodolfo-Metalpa *et al.* 2006), decreased calcification (Rodolfo-Metalpa *et al.* 2008a), lowered resistance to pathogens (Bally & Garrabou 2007) and finally death due to tissue necrosis (Rodolfo-Metalpa *et al.* 2006; Garrabou *et al.* 2008). Temperature has effects on zooid size, growth rate, skeletal growth band formation, biomineral deposition and carbonate production for many bryozoan species (Smith & Key 2004; Lombardi *et al.* 2008), but the biological response of bryozoans to anomalous warming has never been tested. It would appear that *M. truncata* may be similar to certain corals in the Mediterranean which live near their thermal limits (24–26 °C, depending the length of exposure) during the summer season (Rodolfo-Metalpa *et al.* 2008b), as mortalities were reported along the coasts of Provence (France) and in the Balearic Islands in the warm summers of 1999 and 2003 (Perez *et al.* 2000; Garrabou *et al.* 2003; Coma *et al.* 2006).

Our transplant experiment shows that, at moderate temperatures, adult *M. truncata* are able to up-regulate their calcification rates and survive in areas with higher levels of  $p\text{CO}_2$  than are predicted to occur due to anthropogenic ocean acidification, although this ability broke down below mean pH 7.4. However, *M. truncata* seems particularly sensitive to high summer temperatures, decreasing calcification rates to such an extent that this in turn made the bryozoans more susceptible to the detrimental effects of ocean acidification. Determination of the interactive effects of multiple variables that affect calcification and dissolution in organisms through seasonal experimental studies is needed to identify the threshold  $p\text{CO}_2$  value where dissolution exceeds calcification and to define species sensitivity to increasing acidification. Our *in situ* transplant experiment, using natural  $p\text{CO}_2$  gradients, is the first of its kind and adds to a growing body of laboratory evidence showing that the combined warming and acidifying effects of accelerating  $\text{CO}_2$  emissions will be detrimental to important components of shallow water ecosystems.

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