The Journal of Experimental Biology 205, 2107–2113 (2002)
Printed in Great Britain © The Company of Biologists Limited 2002
JEB4037

Effect of increased calcium concentration in sea water on calcification and photosynthesis in the scleractinian coral *Galaxea fascicularis*

Alan T. Marshall* and Peta L. Clode

Analytical Electron Microscopy Laboratory, Department of Zoology, La Trobe University, Bundoora (Melbourne), Victoria 3083, Australia

*Author for correspondence (e-mail: zooam@zoo.latrobe.edu.au)

Accepted 30 April

Summary

The relationship between calcification and photosynthesis in coral was investigated using standard sea water with enhanced calcium concentration. In standard sea water at 23 °C with the calcium concentration increased by 2.5 mmol l^{-1}, incorporation of calcium into the skeleton increased by 30–61 %, depending on the method of data normalisation, and photosynthesis, measured as ^{14}C incorporation into the tissues, also increased by 87 %. At 29 °C, calcium incorporation into the skeleton increased by 54–84 % and ^{14}C incorporation increased by 32 % when sea water calcium concentration was increased by 5 mmol l^{-1}. However, photosynthesis measured as net photosynthetic oxygen production did not increase. Similarly there was no change in respiration rate when coral polyps were incubated in high-calcium sea water. It is conjectured that an increase in photorespiration may be responsible for the latter observations. Bisphosphonate has been considered to inhibit calcification but not photosynthesis in corals. We show that bisphosphonate may not inhibit formation of amorphous calcium carbonate and that the inhibition of calcification is possibly illusory. The data are consistent with the *trans*-calcification model, which suggests that calcification is a source of CO_2 for photosynthesis in corals.

Key words: coral, *Galaxea fascicularis*, calcification, photosynthesis, zooxanthellae, bisphosphonate.

Introduction

In zooxanthellate scleractinian corals the processes of calcification and photosynthesis are coupled in some unknown way (reviewed by Barnes and Chalker, 1990; Gattuso et al., 1999). It has been proposed that calcification in these corals is 'light enhanced' (Kawaguti and Sakumoto, 1948; Goreau, 1959), i.e. the calcification rate in light conditions is greater than in the dark. This difference in rate is attributed to the photosynthetic activities of symbiotic algae or zooxanthellae. Photosynthesis, in some unknown manner, is believed to stimulate calcification. A number of speculative ideas have been advanced to account for this stimulation (reviewed by Barnes and Chalker, 1990). Most recently it has been suggested that OH^- ions secreted during photosynthesis facilitate calcification by buffering the H^+ produced during calcification (Allemand et al., 1998).

Alternatively it has been suggested that calcification enhances photosynthesis by providing a source of protons that convert sea water HCO_3^- to CO_2 and H_2O, thereby supplying some of the CO_2 used in photosynthesis (McConnaughey, 1989; McConnaughey and Whelan, 1997). This has been described as the *trans*-calcification model. Conceptually, this model is attractive because it explains why an organism only two cell layers thick produces a massive external skeleton of calcium carbonate (McConnaughey, 1994).

Whilst it can be readily demonstrated that the prevention of photosynthesis results in a marked reduction or cessation of calcification (Vandermeulen et al., 1972; Barnes, 1985), it is only recently that attempts have been made to determine whether a reduction in calcification in corals also leads to a reduction in photosynthesis. Yamashiro (1995) showed that bisphosphonate reduced ^{14}C incorporation into the skeleton (i.e. calcium carbonate deposition) but not into the tissues (i.e. photosynthesis) of a zooxanthellate coral and concluded that calcification is not necessary for photosynthesis. Bisphosphonate, however, may prevent the formation of calcium carbonate crystals but not necessarily the formation of amorphous calcium carbonate; it is known to prevent calcium phosphate crystallization but not the formation of amorphous calcium phosphate (Francis et al., 1969). Gattuso et al. (2000) showed that artificial sea water with a low calcium concentration lowered calcification rate but did not reduce the production of photosynthetic oxygen and concluded that '*calcification is not a significant source of photosynthetic CO_2*'.

The experimental procedures used so far to investigate the relationship between calcification and photosynthesis have relied upon low or zero concentrations of calcium in artificial sea water. A possible problem with this approach is that low-

calcium artificial sea water may have a profoundly deleterious effect on some aspects of coral physiology (A. T. Marshall and P. L. Clode, manuscript submitted for publication). We have reinvestigated the concept of calcification enhancing photosynthesis, therefore, by studying the relationship between calcification and photosynthesis when these processes take place in standard sea water that has an increased calcium concentration.

Materials and methods

Colonies of the scleractinian zooxanthellate coral *Galaxea fascicularis* L. were collected from the reef flat at Heron Island Reef, Great Barrier Reef, Queensland. Corals were collected for separate sets of experiments at different times of year when sea water temperatures were different. The colonies were transferred in sea water in plastic buckets to outdoor aquaria, provided with a continuous flow of fresh sea water, at Heron Island Research Station. The colonies were allowed to recover for a minimum of 3 days. In *G. fascicularis* the large (5–10 mm diameter), well-spaced corallites are joined by a cellular coenosteum, which is easily broken. The corallites were readily separated, without damaging the tissues of the polyps, by teasing them apart with forceps. The individual, intact, polyps were kept in trays, with constantly flowing sea water, for a further 2 days before being used in experiments. When used for a particular experiment, polyps from a single colony were carefully selected to match in size.

High-calcium sea water

High-calcium sea water was prepared by adding either 100 mg l^{-1} or 200 mg l^{-1} calcium (added as $CaCl_2.2H_2O$) (Swart, 1980) to filtered (0.2 μm Millipore filter) sea water (FSW), which resulted in an increased calcium concentration of 2.5 mmol l^{-1} (FSW+100) and 5 mmol l^{-1} (FSW+200), respectively. All solutions were made freshly as required, to pH 8.11 (standard FSW) and pH 8.12 (high-calcium FSW). Standard artificial sea water (ASW) was prepared according to Benazet-Tambutte et al. (1996). The pH of all incubation media was monitored before and after each incubation and was observed to be constant to within 0.1 pH units.

Scintillation counting of ^{45}Ca and ^{14}C in skeleton and tissues

Polyps were incubated in glass jars. The jars were immersed in a large fibreglass outdoor aquarium through which sea water flowed at a rate sufficient to maintain a constant temperature. The aquarium was not shaded and corals were exposed to light levels similar to those they would have naturally experienced on the reef flat at low water. The incubation sea water was vigorously stirred and aerated by a diaphragm-operated aquarium pump, except when $NaH^{14}CO_3$ was present in the sea water, when it was necessary to prevent exchange with atmospheric CO_2. Samples (200 μl) of incubation medium were taken before and after incubation for determination of specific activities of radioisotopes.

Incubation times and post-incubation processing depended upon the experiment being done. In all experiments polyps were washed after incubation for 1.5–2 h in 2 l of vigorously aerated, fresh incubation medium to remove coelenteric ^{45}Ca (Tambutte et al., 1996). This procedure was carried out at the incubation temperature and in low light levels [photosynthetic photon flux density (PPFD) 7–45 μmol photons m^{-2} s^{-1}]. Following the wash, bare corallite that would have been a site for isotopic exchange was removed and discarded. Depending on the experiment, polyps were cut into pieces with wire cutters and tissue was removed by digestion with 5 mol l^{-1} NaOH at 58 °C for periods of 20 min to 1 h. Where appropriate the digest was neutralised with HCl and samples taken for scintillation counting and protein determination. After rinsing in tap water, the skeleton was dried overnight at 60 °C, weighed and dissolved in HCl. Samples of the acid digest were taken for scintillation counting.

(a) Ten polyps were preincubated in 200 ml aerated FSW and ten polyps in 200 ml FSW+100 for 1 h, under laboratory conditions (PPFD, 8 μmol photons m^{-2} s^{-1}, 24 °C). Following preincubation, the polyps were transferred to jars containing 200 ml of fresh aerated FSW or FSW+100, prior to the addition of 2 μCi (74 kBq) ml^{-1} of $^{45}CaCl_2$ and incubated in the outdoor aquarium (PPFD, 50–1600 μmol photons m^{-2} s^{-1}, 23 °C) for 4 h. A second experiment was carried out exactly as the first but with three sets of five polyps incubated in each of FSW, ASW and FSW+200 in the outdoor aquarium (PPFD, 1200–1950 μmol photons m^{-2} s^{-1}, 29 °C) after preincubation in the laboratory (PPFD, 7 μmol photons m^{-2} s^{-1}, 27 °C).

(b) Five polyps were preincubated in glass jars containing 200 ml aerated FSW and five polyps in 200 ml aerated FSW+100 for 1 h, under laboratory conditions (PPFD, 3 μmol photons m^{-2} s^{-1}, 23 °C). Following preincubation, the polyps were transferred to jars containing 100 ml of fresh FSW or FSW+100 prior to the addition of 2 μCi (74 kBq) ml^{-1} of $NaH^{14}CO_3$, and incubated in the outdoor aquarium (PPFD, 1600 μmol photons m^{-2} s^{-1}, 23 °C) for 2 h. A second experiment was carried out exactly as the first but with five polyps incubated in each of FSW, ASW and FSW+200 in the outdoor aquarium (PPFD, 500–1950 μmol photons m^{-2} s^{-1}, 29 °C) after preincubation in the laboratory (PPFD, 5.5 μmol photons m^{-2} s^{-1}, 27.5 °C).

(c) Two series each of five experimental and five control polyps were preincubated in glass jars containing 200 ml of FSW either with or without 0.5 mmol l^{-1} 1-hydroxyethylidene-diphosphonic acid (HEBP) (Fluka Chemika) for 15 min prior to the addition of 1 μCi (37 kBq) ml^{-1} of $^{45}CaCl_2$. Following the addition of radioisotope the polyps were then incubated for a further 2.2 h in the outdoor aquarium (PPFD, 1900 μmol photons m^{-2} s^{-1}, 27.5 °C). One series of polyps was treated after incubation in the standard way, i.e. tissue was removed followed by dissolution in acid. The other series was treated with HCl, after washing, drying and weighing, without prior removal of the tissue. After dissolving the skeleton in acid the remaining tissue was filtered, dried and weighed to facilitate calculation of skeletal mass.

Measurement of photosynthesis and respiration rates

Measurements of oxygen concentration were carried out in small custom-made Perspex chambers holding 20 ml of FSW (assumed to be free of bacteria), which was vigorously stirred with a magnetic stirrer. Three polyps, closely matched for size, were placed in each chamber. Light was provided to each chamber by a double, fibre optic, light source delivering a PPFD of approximately 1200 µmol photons m^{-2} s^{-1}. Oxygen concentrations were measured at intervals of 1 min over a period of 15 min by means of a Clarke-type electrode (accurate to 0.01 mg l^{-1}) inserted into each chamber. Readings were made using a YSI Model 58 oxygen meter attached to each electrode. The temperature of the laboratory, and therefore the chambers, was maintained at the same temperature as the prevailing inter-reefal sea water temperature. Following oxygen measurements in the light, the chambers were shrouded in aluminium foil and oxygen concentrations were recorded as before to obtain respiratory oxygen consumption. Polyps were then removed from the chambers and each chamber's complement was incubated separately in 200 ml of aerated FSW+100 for 2 h. Following this incubation, the polyps were reintroduced into the chambers, filled with FSW+100, and oxygen concentrations were measured using the same protocol and conditions as previously. At the termination of the experiment the polyps were frozen and held at –70 °C until processed by heating at 58 °C in 5 mol l^{-1} NaOH for 1 h. The digest was neutralised with a known volume of hydrochloric acid and samples taken for protein determination.

Rates of gross photosynthesis and respiration were obtained from regression equations and net photosynthesis was calculated from the algebraic sum of gross photosynthesis and respiration.

Oxygen measurements on sets of three polyps were also made with essentially the same equipment except that the recorder output of the YSI oxygen meters was connected to a Macintosh Powerbook *via* a MacLab interface (AD Instruments). In this way oxygen concentrations were continuously recorded whilst solutions of HEBP were injected (with pressure compensation) into the measuring chambers. The amount of HEBP injected was adjusted to give a final concentration of 0.5 mmol l^{-1}. Rates of change of oxygen concentration were determined directly from the computer traces using the MacLab software.

Protein determination

Protein content in tissue digests was determined without precipitation using a Protein Assay Kit (Lowry method, procedure no. 5656, Sigma Diagnostics). A standard curve was constructed from bovine serum albumin concentrations ranging from 0 to 400 mg ml^{-1} protein and absorbances were read using a spectrophotometer (UV-2401PC) at a wavelength of 750 nm. For polyp samples, the neutralised tissue digest was diluted tenfold to produce a concentration within the range of the standard curve. Following the addition of Folin and Ciocalteu's reagent, all samples were centrifuged for 30 min at 1900 ***g***. Absorbances of the supernatant were then obtained as before, with concentrations (uncorrected for dilution) automatically calculated from the standard curve.

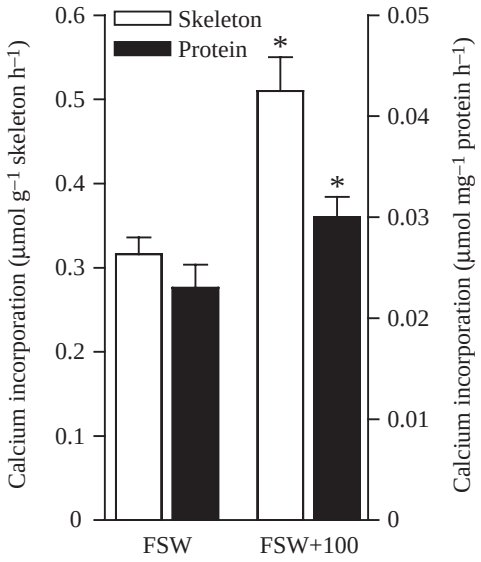

Fig. 1. Skeletal calcium incorporation in *Galaxea fascicularis* incubated at 23 °C in standard filtered sea water (FSW) and filtered sea water plus 100 mg l^{-1} calcium as $CaCl_2$ (FSW+100). The rate of calcium incorporation is normalised to skeletal mass and tissue protein content. Values are means ± S.E.M., N=10. *Significant difference from FSW values for skeleton and protein (P<0.05).

Statistical analysis

Statistical analyses were carried out using the computer program JMP 3.1.6 (SAS Institute, Cary, North Carolina). Results are expressed as mean ± standard error of the mean (S.E.M.), N is the sample size. Values of P<0.05 were taken to indicate significant differences between means.

Results

Effect of high-calcium sea water on calcium incorporation by the skeleton

After incubation in high-calcium sea water (FSW+100) at 23 °C the rate of incorporation of calcium into the skeleton of *G. fascicularis* polyps was significantly higher than after incubation in standard sea water (FSW), regardless of whether the results were normalised in terms of skeletal mass or tissue protein content (Fig. 1). Incubation of polyps in high-calcium sea water (FSW+200) at 29 °C also resulted in a significant increase in calcium incorporation, compared with polyps incubated in FSW and ASW, when normalised to both skeletal mass and protein content (Fig. 2). Calcium incorporation in polyps incubated in FSW and ASW was not significantly different.

Effect of high-calcium sea water on ^{14}C incorporation into tissues

Incubation of polyps in high-calcium sea water (FSW+100) at 23 °C resulted in a significant increase in the rate of ^{14}C

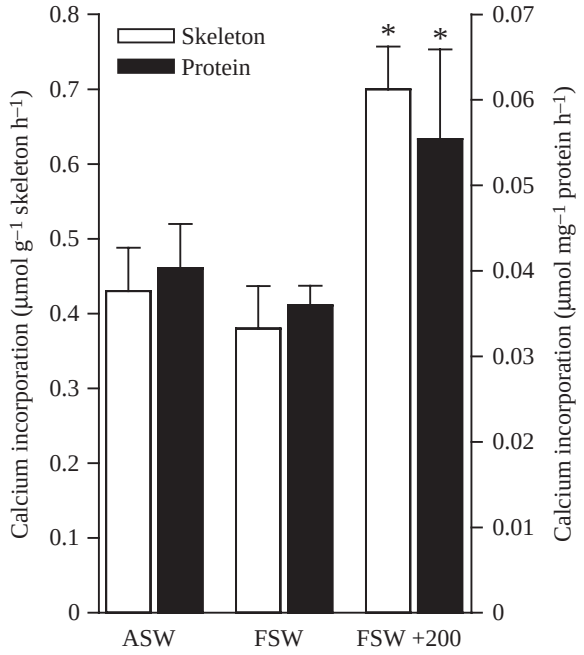

Fig. 2. Skeletal calcium incorporation in *Galaxea fascicularis* incubated at 29 °C in standard artificial sea water (ASW), standard filtered sea water (FSW) and filtered sea water plus 200 mg l^{-1} calcium as $CaCl_2$ (FSW+200). Calcium incorporation is normalised to skeletal mass and tissue protein content. Values are means ± S.E.M., *N*=5. *Significantly different from ASW and FSW values (*P*<0.05).

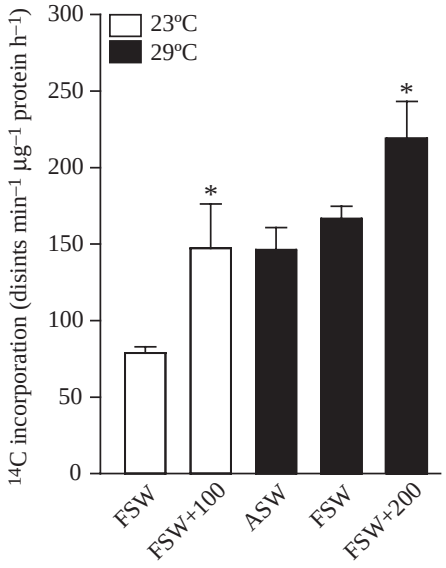

Fig. 3. ^{14}C incorporation into tissues of *Galaxea fascicularis* incubated at 23 °C and 29 °C in standard filtered sea water (FSW), standard filtered sea water plus 100 mg l^{-1} (FSW+100) or 200 mg l^{-1} (FSW+200) calcium as $CaCl_2$ and artificial sea water (ASW). Values are means ± S.E.M., *N*=5. *Significant difference from FSW and ASW values (*P*<0.05) at 23 °C and 29 °C.

incorporation into the tissues compared with polyps incubated in standard sea water (FSW) (Fig. 3). At 29 °C there was also a significant increase in ^{14}C incorporation into the tissues in polyps incubated in FSW+200 compared with polyps incubated in FSW and ASW (Fig. 3). The rate of ^{14}C incorporation in standard sea water was higher at 29 °C than at 23 °C.

Effect of high-calcium sea water on photosynthetic and respiration rates

Rates of net photosynthesis (4.2±0.39 μg O_2 mg^{-1} protein min^{-1}) and respiration (–1.9±0.09 μg O_2 mg^{-1} protein min^{-1}) (means ± S.E.M., *N*=8) were unaffected (*P*>0.05) by incubation in high-calcium sea water (FSW+100) when compared to rates obtained during incubation in standard sea water (FSW) (4.9±0.4 and –2.1±0.12 μg O_2 mg^{-1} protein min^{-1} for photosynthesis and respiration, respectively). It is noticeable that rates in high-calcium sea water tended to be slightly lower than in standard sea water. However, pilot experiments, in which polyps were incubated in standard sea water in place of high-calcium sea water, showed similar variation.

Effect of HEBP on calcium uptake by the skeleton

When the effect of HEBP on calcium incorporation into the skeleton was measured, using the standard protocol of digesting the tissues prior to dissolving the skeleton, the rate of incorporation was dramatically reduced compared to the control (Fig. 4). However, when the processing protocol was changed so that the skeleton was dissolved prior to digesting the tissues then the reduction in calcium incorporation was substantially less than with the former protocol (Fig. 4). The amount of calcium incorporated was significantly higher when the skeleton was dissolved first than when the tissues were digested first. We interpret this to indicate that HEBP does not prevent the formation of amorphous calcium carbonate, which is lost during the standard processing procedure, but is retained to a much greater extent by dissolving the skeleton prior to digesting the tissue.

Effect of HEBP on photosynthetic and respiration rates

Recordings of oxygen concentration were made from four sets of three polyps in the light, and a further four sets in the dark, before and after exposure to 0.5 mmol l^{-1} HEBP. The gross photosynthetic rate after HEBP treatment increased by 26±6 % and the respiration rate decreased by 42.5±5.5 %, thus the net photosynthetic rate was reduced by 16.5 % (data not shown).

Discussion

Artificial sea water with zero or low calcium concentrations has been used by several investigators for experiments on aspects of coral physiology (Chalker, 1976; Krishnaveni et al., 1989; Ip and Krishnaveni, 1991; Al-Moghrabi et al., 1996; Tambutte et al., 1996; Gattuso et al., 2000). The possibility of undesirable effects resulting from exposure to such media do

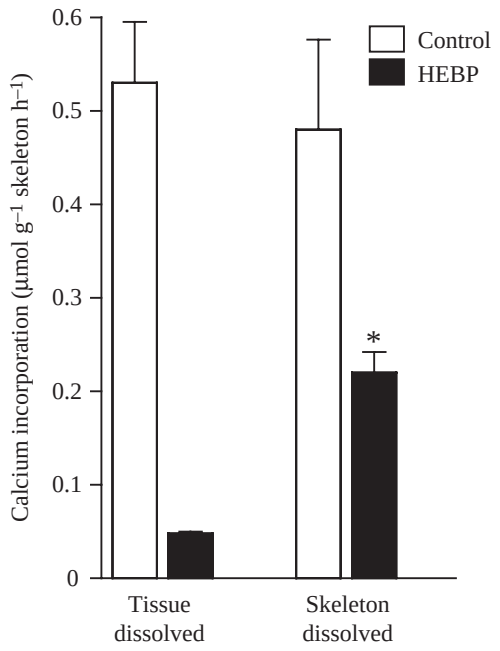

Fig. 4. Skeletal calcium incorporation in *Galaxea fascicularis* incubated in standard filtered sea water with and without HEBP (0.5 mmol l^{-1}). Following incubation in HEBP, when the skeleton was dissolved before the tissues, the incorporation of calcium into the skeleton was significantly higher (*; P<0.05), than when the skeleton was dissolved after tissue removal. There was no significant difference in calcium incorporation in polyps incubated in standard sea water alone (P>0.05), regardless of whether the skeleton was dissolved before or after tissue removal. Values are means ± S.E.M., N=5.

not appear to have been considered in detail. Exposure to artificial sea water with zero or low concentrations of calcium had a profound effect on *G. fascicularis* polyps (A. T. Marshall and P. L. Clode, manuscript submitted for publication). The polyps were anaesthetised and extruded enormous numbers of mucocytes. This inevitably must affect the transport of Ca^{2+} across the epithelia and compromise any experiments in which Ca^{2+} transport is a factor. As a consequence of the effects of low-calcium sea water, we have chosen, in the present investigation, to examine the relationship between calcification and photosynthesis by using high-calcium sea water.

Increasing calcium concentration in sea water by 2.5 mmol l^{-1} resulted in a marked increase in calcium incorporation into the skeleton of *G. fascicularis*. The increase at 23 °C was 61 % and 30 % when normalised to skeletal mass or to tissue protein content, respectively. The method of normalising physiological data in corals is a contentious one (Marshall, 1996). However, regardless of the method used there was undoubtedly a statistically significant increase in calcification rate. At 29 °C there was also a significant increase in calcium incorporation when polyps were incubated in sea water in which the calcium concentration had been increased by 5 mmol l^{-1}, regardless of the method of normalisation. The increase, compared to polyps incubated in standard sea water, was 84 % and 54 % when normalised to skeletal mass or tissue protein content, respectively. Calcification rates in standard sea water are very similar at these two temperatures, which represent the extremes of a Gaussian distribution of calcification rate *versus* temperature (A. T. Marshall and P. L. Clode, manuscript submitted for publication).

In previous studies, as artificial sea water calcium concentration increased, calcification rate tended to plateau at about 10 mmol l^{-1} (Chalker, 1976; Ip and Krishnaveni, 1991; Tambutte et al., 1996). Although a continuing increase was recorded (Chalker, 1976) in *Acropora cervicornis* and in *G. fascicularis* (Krishnaveni et al., 1989), the increase at a concentration comparable to that in the present investigation (13.5 mmol l^{-1}) was approximately 5 and 8 %, respectively, compared with 61 % in the present investigation. At a calcium concentration of 16 mmol l^{-1} the increase in calcification rate in *G. fascicularis* in the present investigation was even higher, at 84 % when normalised to skeletal mass and 54 % when normalised to protein content. The investigations of Chalker (1976), Krishnaveni et al. (1989), Ip and Krishnaveni (1991) and Tambutte et al. (1996) were carried out using artificial sea water under laboratory conditions, with relatively low light intensities, whereas the present experiments were carried out using natural sea water and in the open air where light conditions were much higher and comparable with those on the reef flat. The difference in the experimental conditions could be presumed to be causal factors in the observed differences in calcification rate.

The calcium carbonate saturation state of sea water has been suggested to influence the distribution of zooxanthellate corals by virtue of its effect on calcium carbonate precipitation and hence the calcification rate of corals (Buddemeier and Fautin, 1996; Kleypas et al., 1999). Experimental evidence to support this was provided by Gattuso et al. (1998), who showed that increasing the calcium carbonate saturation state, by adding calcium to artificial sea water, increased the calcification rate in *Stylophora pistillata*. The rate increased up to contemporary sea water saturation levels, but showed no further increase at saturation values above this threshold. These experiments were again conducted with artificial sea water and Gattuso et al. (1998) note that the calcification rate in *S. pistillata* measured in standard sea water was almost twice that measured in artificial sea water with the same calcium carbonate saturation state. It should be noted that in the present investigation, exposure of *G. fascicularis* polyps to the 'standard' artificial sea water formulation of Gattusso et al. (1998) in pilot experiments had obviously deleterious effects. These effects were not evident in polyps exposed to artificial sea water formulated according to Benazet-Tambutte et al. (1996).

Inorganic calcification rates can be calculated from the aragonite precipitation equation of Burton and Walter (1990):

$$\log R = 1.61 + 1.63\log(\Omega - 1), \quad (1)$$

where R is precipitation rate and Ω is saturation state, and

$$\Omega = \mathrm{mCa} \times \mathrm{mCO_3}/K_{\mathrm{aragonite}}, \quad (2)$$

where mCa and mCO_3 are molal concentrations and $K_{aragonite}$

is the apparent solubility constant of aragonite. Assuming $mCa=0.01028\,mol\,kg^{-1}$, $mCO_3=0.000244\,mol\,kg^{-1}$ and $K_{aragonite}=10^{-6.19}$ (Millero, 1996) then $\Omega=3.88$ and $R=228\,\mu mol\,m^{-2}\,h^{-1}$. If calcium concentration is increased by $2.5\,mmol\,l^{-1}$ and $5\,mmol\,l^{-1}$ then Ω and R increase to approximately 4.83 and $364\,\mu mol\,m^{-2}\,h^{-1}$ and 5.77 and $520\,\mu mol\,m^{-2}\,h^{-1}$, respectively. This suggests that the addition of calcium would increase aragonite precipitation rate by factors of 1.6 and 2.3. In *G. fascicularis* the calcification rate, although increased, was somewhat lower than predicted by the Burton and Walter (1990) equation. Depending on the method of normalisation the increase in calcification rate was a factor of 1.3 or 1.6 for a $2.5\,mmol\,l^{-1}$ increase in calcium and 1.5 or 1.8 for a $5\,mmol\,l^{-1}$ increase. These data suggest that whilst sea water aragonite saturation state may directly influence aragonite precipitation rate at the skeletal interface, the calicoblastic epithelium is not entirely permeable and must control the saturation state of the subskeletal fluids.

Consistent with our measurements showing substantial increases in calcification rates with increases in the calcium concentration of standard sea water are the measurements obtained by Swart (1979, 1980). Swart (1980) recorded high growth rates in several species of corals when exposed to standard sea water with elevated calcium concentrations and the growth rates peaked at the concentrations used in the present investigation. Increases in growth rate ranged from 70 % to 820 %. Swart (1980) also found that response was variable, depending on the locality on the reef flat from which specimens of a particular species had been collected. This phenomenon may also have been operating in the present investigation, since the *G. fascicularis* polyps used in experiments carried out at 23 °C came from a locality where this species is prolific, whereas the polyps used at 29 °C were obtained (due to collecting permit restrictions) from a locality on the reef flat where this species is uncommon. Yamazato (1966) also obtained a twofold increase in calcium uptake in *Fungia scutaria* following the addition of $200\,mg\,l^{-1}$ of calcium to sea water.

The amount of fixed carbon in coral tissues is generally regarded as a measure of photosynthetic activity of the zooxanthellae. The effect of high-calcium sea water was to increase ^{14}C incorporation, and therefore photosynthesis, by 87 %, after incubation in sea water at 23 °C with calcium concentration increased by $2.5\,mmol\,l^{-1}$, and by 32 % after incubation in sea water at 29 °C with calcium concentration increased by $5\,mmol\,l^{-1}$. It should be noted that the control rate of ^{14}C incorporation was markedly higher at 29 °C than at 23 °C. Paradoxically, incubation in high-calcium sea water had no effect on net photosynthetic oxygen production or on respiratory oxygen consumption. A full explanation of this is not possible without further investigation. Since zooxanthellae possess the C-3 carbon-fixation pathway (Streamer et al., 1993) it may be conjectured that photorespiration, which is considered to be likely in zooxanthellae (Black et al., 1976; Randall, 1976), is a possible reason for an apparent lack of increase in photosynthetic oxygen production. However, an increase in photorespiration would generally lead to a decline in CO_2 fixation (Tolbert and Osmond, 1976).

Yamashiro (1995) presented evidence that appears to be counter to the *trans*-calcification model. It was shown that the bisphosphonate (1-hydroxyethylidene-1, 1-biphosphonic acid, HEBP) reduced calcification, measured by ^{14}C incorporation into the skeleton, and did not affect photosynthesis, measured by ^{14}C incorporation into the tissues. Whilst HEBP is known to prevent the growth of calcium phosphate crystals, it does not prevent the formation of amorphous calcium phosphate (Francis et al., 1969). Therefore, it may not prevent the formation of amorphous calcium carbonate. If this is so then the standard processing technique used by Yamashiro would not retain the amorphous compound. In this procedure the tissue is digested in NaOH prior to dissolving the skeleton. Digestion in NaOH would remove amorphous calcium carbonate and the labeled carbon would be lost as CO_2 during subsequent acid treatment of the digest. In our experiments, using ^{45}Ca to measure calcium incorporation, we attempted to prevent losses of amorphous calcium carbonate by dissolving the skeleton prior to digesting the tissue. The polyps were first well washed to remove as much ^{45}Ca from the tissue and coelenteron as possible (Tambutte et al., 1995, 1996). The amount of ^{45}Ca associated with tissue and coelenteric sea water relative to the skeleton has been shown to be very small (Marshall and Wright, 1998). There was no significant difference, in calcium incorporation into the skeleton, between polyps in which tissue was removed first and polyps in which skeleton was removed first when they were incubated in standard sea water. However, when incubated in sea water containing HEBP, calcium incorporation was reduced by 91 % in polyps with tissue removed first, compared with control polyps but the reduction was only 54 % in polyps with skeleton removed first. The difference between the two treatments was significant. These results suggest that amorphous calcium carbonate may be formed during treatment with HEBP and that the loss of amorphous calcium carbonate may have been undetected in Yamashiro's (1995) experiments, thereby invalidating the assumption that a reduction in calcification has no effect on photosynthetic rate.

The 54 % reduction in calcium incorporation seen after HEBP treatment in polyps in which the skeleton was dissolved first may be attributable in part to a direct effect of HEBP on the physiology of *G. fascicularis* polyps. That such an effect occurred was revealed by the reduction in respiration and net photosynthetic rates. It was also observed that polyps exposed to HEBP then placed in fresh running sea water invariably bleached within a few hours. Consistent with these observations are reports that bisphosphonates may inhibit intracellular ATP-dependent enzymes or have physiological effects on intracellular second messenger pathways (Rogers et al., 1994; Russell et al., 1999). These effects are distinct from the inhibition of mineralisation due to the binding of bisphosphonates to mineral surfaces in organisms. Some bisphosphonates also appear to have a herbicidal action (Kafarski et al., 2000).

We conclude that the evidence showing that calcification does not stimulate photosynthesis, based on the use of low-

calcium artificial sea water and inhibitors of crystal formation such as bisphosphonates, should be interpreted with caution. Artificial sea water containing low concentrations of calcium caused a marked stress response (A. T. Marshall and P. L. Clode, manuscript submitted for publication) that inevitably must compromise experiments carried out in this medium. Standard sea water in which calcium concentration was increased resulted in higher rates of calcification and photosynthesis. The increase in calcification rate was greater than that previously observed in experiments conducted with artificial sea waters in which calcium concentration was increased above standard sea water concentration. Although it is not possible to say from these data that increased calcification promotes an increase in photosynthesis according to the *trans*-calcification model, since calcium may act to independently potentiate both processes, it is possible to say that the data are consistent with the model.

We are most grateful to Dr Ted McConnaughey for valuable comments on this research, reading early drafts of this paper and also for assistance with the calculation of aragonite precipitation rates. We thank the Director and Staff of Heron Island Research Station for provision of facilities. This work was carried out under collecting permits from the Great Barrier Reef Marine Parks Authority to A.T.M. and with the aid of a grant from the Australian Research Council to A.T.M.

References

Al-Moghrabi, S., Goiran, C., Allemand, D., Speziale, N. and Jaubert, J. (1996). Inorganic carbon uptake for photosynthesis by the symbiotic coral-dinoflagellate association. 2. Mechanisms for bicarbonate uptake. *J. exp. mar. Biol. Ecol.* **199**, 227–248.

Allemand, D., Furla, P. and Benazet-Tambutte, S. (1998). Mechanisms of carbon acquisition for endosymbiont photosynthesis in Anthozoa. *Can. J. Bot.* **76**, 925–941.

Barnes, D. J. (1985). The effect of photosynthetic and respiratory inhibitors upon calcification in the staghorn coral, *Acropora formosa*. In *Proceedings of the Fifth International Coral Reef Congress, Tahiti.* pp. 161–165.

Barnes, D. J. and Chalker, B. E. (1990). Calcification and photosynthesis in reef-building corals and algae. In *Coral Reefs* (ed. Z. Dubinsky), pp. 109–131. Amsterdam: Elsevier.

Benazet-Tambutte, S., Allemande, D. and Jaubert, J. (1996). Permeability of the oral epithelial layers in Cnidarians. *Mar. Biol.* **126**, 43–53.

Black, C. C., Burris, J. E. and Everson, R. G. (1976). Influence of oxygen concentration on photosynthesis in marine plants. *Aust. J. Plant Physiol.* **3**, 81–86.

Buddemeier, R. W. and Fautin, D. G. (1996). Saturation state and the evolution and biogeography of symbiotic calcification. *Bull. Inst. Oceanogr., Monaco* **14**, 23–32.

Burton, E. A. and Walter, L. M. (1990). The role of pH in phosphate inhibition of calcite and aragonite precipitation rates in sea water. *Geochim. Cosmochim. Acta* **54**, 797–808.

Chalker, B. E. (1976). Calcium transport during skeletogenesis in hermatypic corals. *Comp. Biochem. Physiol.* **54A**, 455–459.

Francis, M. D., Graham, R., Russell, G. and Fleisch, H. (1969). Diphosphonates inhibit formation of calcium phosphate crystals in vitro and pathological calcification in vivo. *Science* **165**, 1264–1266.

Gattuso, J.-P., Allemand, D. and Frankignoulle, M. (1999). Photosynthesis and calcification at cellular, organismal and community levels in coral reefs: a review on interactions and control by carbonate chemistry. *Amer. Zool.* **39**, 160–183.

Gattuso, J.-P., Frankignoulle, M., Bourge, I., Romaine, S. and Buddemeier, R. W. (1998). Effect of calcium carbonate saturation of sea water on coral calcification. *Global and Planetary Change* **18**, 37–46.

Gattuso, J.-P., Reynaud-Vaganay, S., Furla, P., Romaine-Lioud, S., Jaubert, J., Bourge, I. and Frankignoulle, M. (2000). Calcification does not stimulate photosynthesis in the zooxanthellate scleractinian coral *Stylophora pistillata*. *Limnol. Oceanogr.* **45**, 246–250.

Goreau, T. F. (1959). The physiology of skeleton formation in corals. I. A method for measuring the rate of calcium deposition by corals under different conditions. *Biol. Bull.* **116**, 59–75.

Ip, Y. K. and Krishnaveni, P. (1991). Incorporation of strontium ($^{90}Sr^{2+}$) into the skeleton of the hermatypic coral *Galaxea fascicularis*. *J. Exp. Zool.* **258**, 273–276.

Kafarski, P., Lejczak, B. and Forlani, G. (2000). Herbicidally active aminomethylenebisphosphonic acids. *Heteroatom Chemistry* **11**, 449–453.

Kawaguti, S. and Sakumoto, D. (1948). The effect of light on the calcium deposition of corals. *Bull. Oceanogr. Inst. Taiwan* **4**, 65–70.

Kleypas, J. A., Buddemeier, R. W., Archer, D., Gattuso, J.-P., Langdon, C. and Opdyke, B. N. (1999). Geochemical consequences of increased atmospheric carbon dioxide on coral reefs. *Science* **284**, 118–120.

Krishnaveni, P., Chou, L. M. and Ip, Y. K. (1989). Deposition of calcium ($^{45}Ca^{2+}$). in the coral *Galaxea fascicularis. Comp. Biochem. Physiol.* **94A**, 509–513.

Marshall, A. T. (1996). Calcification rates in corals. *Science* **274**, 117–118.

Marshall, A. T. and Wright, A. (1998). Coral calcification: autoradiography of a scleractinian coral *Galaxea fascicularis* after incubation in ^{45}Ca and ^{14}C. *Coral Reefs* **17**, 37–47.

McConnaughey, T. (1989). Biomineralization mechanisms. In *Origin, Evolution, and Modern Aspects of Biomineralization in Plants and Animals* (ed. R. E. Crick), pp. 57–73. New York: Plenum Press.

McConnaughey, T. A. (1994). Calcification, photosynthesis, and global carbon cycles. In *Past and Present Biomineralization Processes. Considerations about the Carbonate Cycle* (ed. F. Doumenge, D. Allemand and A. Toulemont), pp. 137–161. Monaco: Monaco Musee Oceanographique.

McConnaughey, T. A. and Whelan, J. F. (1997). Calcification generates protons for nutrient and bicarbonate uptake. *Earth Sci. Rev.* **42**, 95–117.

Millero, F. J. (1996). *Chemical Oceanography.* Boca Raton, CRC Press.

Randall, D. D. (1976). Phosphoglycollate phosphatase in marine algae: isolation and characterization from *Halimeda cylindracea. Aust. J. Plant Physiol.* **3**, 105–111.

Rogers, M. J., Watts, D. J., Russell, R. G. G., Ji, X. H., Xiong, X. J., Blackburn, G. M., Bayless, A. V. and Ebetino, F. H. (1994). Inhibitory effects of bps on growth of amoebae of the cellular slime mold *Dictyostelium discoideum. J. Bone Min. Res.* **9**, 1029–1039.

Russell, R. G., Rogers, M. J., Frith, J. C., Luckman, S. P., Coxon, F. P., Benford, H. L., Croucher, P. I., Shipman, C. and Fleisch, H. A. (1999). The pharmacology of bisphosphonates and new insights into their mechanisms of action. *J. Bone Min. Res.* **14**, 53–65.

Streamer, M., McNeil, Y. R. and Yellowlees, D. (1993). Photosynthetic carbon dioxide fixation in zooxanthellae. *Mar. Biol.* **115**, 195–198.

Swart, P. (1980). The effect of sea water chemistry on the growth of some scleractinian corals. In *Developmental and Cellular Biology of Coelenterates* (ed. P. Tardent and R. Tardent), pp. 203–208. Amsterdam: Elsevier/North Holland Biomedical Press.

Swart, P. K. (1979). The effect of sea water calcium concentrations on the growth and skeletal composition of a scleractinian coral: *Acropora squamosa. J. Sed. Petrol.* **49**, 951–954.

Tambutte, E., Allemand, D., Bourge, I. and Gattuso, J.-P. (1995). An improved ^{45}Ca protocol for investigating physiological mechanisms in coral calcification. *Mar. Biol.* **122**, 453–459.

Tambutte, E., Allemand, D., Mueller, E. and Jaubert, J. (1996). A compartmental approach to the mechanism of calcification in hermatypic corals. *J. Exp. Biol.* **199**, 1029–1041.

Tolbert, A. and Osmond, C. B. (1976). The Great Barrier Reef Photorespiration Expedition: introduction. *Aust. J. Plant Physiol.* **3**, 1–8.

Vandermeulen, J. H., Davis, N. D. and Muscatine, L. (1972). The effects of inhibitors of photosynthesis on zooxanthellae in corals and other marine invertebrates. *Mar. Biol.* **16**, 185–191.

Yamashiro, H. (1995). The effects of HEBP, an inhibitor of mineral deposition, upon photosynthesis and calcification in the scleractinian coral, *Stylophora pistillata*. *J. exp. mar. Biol. Ecol.* **191**, 57–63.

Yamazato, K. (1966). Calcification in a solitary coral, *Fungia scutaria* Lamarck in relation to environmental factors. PhD Thesis, University of Hawaii, Hawaii. 130 pp.