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Photosynthesis and Calcification at Cellular, Organismal and Community Levels in Coral Reefs: A Review on Interactions and Control by Carbonate Chemistry[1]

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SYNOPSIS. **Photosynthesis and calcification in zooxanthellate scleractinian corals and coral reefs are reviewed at several scales: cellular (pathways and transport mechanisms of inorganic carbon and calcium), organismal (interaction between photosynthesis and calcification, effect of light) and ecosystemic (community primary production and calcification, and air-sea CO_2 exchanges).**

The coral host plays a major role in supplying carbon for the photosynthesis by the algal symbionts through a system similar to the carbon-concentrating mechanism described in free living algal cells. The details of carbon supply to the calcification process are almost unknown, but metabolic CO_2 seems to be a significant source. Calcium supply for calcification is diffusional through oral layers, and active membrane transport only occurs between the calicoblastic cells and the site of calcification. Photosynthesis and calcification are tightly coupled in zooxanthellate scleractinian corals and coral reef communities. Calcification is, on average, three times higher in light than in darkness. The recent suggestion that calcification is dark-repressed rather than light-enhanced is not supported by the literature. There is a very strong correlation between photosynthesis and calcification at both the organism and community levels, but the ratios of calcification to gross photosynthesis (0.6 in corals and 0.2 in reef communities) differ from unity, and from each other as a function of level.

The potential effect of global climatic changes (pCO_2 and temperature) on the rate of calcification is also reviewed. In various calcifying photosynthetic organisms and communities, the rate of calcification decreases as a function of increasing pCO_2 and decreasing calcium carbonate saturation state. The calculated decrease in $CaCO_3$ production, estimated using the scenarios considered by the International Panel on Climate Change (IPCC), is 10% between 1880 and 1990, and 9–30% (mid estimate: 22%) from 1990 to 2100. Inadequate understanding of the mechanism of calcification and its interaction with photosynthesis severely limits the ability to provide an accurate prediction of future changes in the rate of calcification.

INTRODUCTION

Coral reefs are the most striking example of benthic, photosynthetic and calcifying ecosystems. They display the greatest abundance and diversity of $CaCO_3$-depositing organisms that carry out photosynthesis (calcareous algae) or harbor photosynthetic symbionts (scleractinian corals, foraminiferans and mollusks). The photosynthetic fixation of carbon dioxide (CO_2) and precipitation of $CaCO_3$ are intimately linked both at spatial (cell to ecosystem) and temporal (day-night) scales. Large fluxes of carbon and calcium carbonate occur at the cell and community levels on reefs. Transepithelial calcium transport in scleractinian corals can reach 1,700 nmol cm^{-2} h^{-1} (Wilbur and Simkiss, 1979), which would be equivalent to 149 mol Ca m^{-2} yr^{-1}, while the rates of community gross primary pro-

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duction and respiration of coral reef flats range, respectively, from 79 to 584 and from 76 to 538 mol C m^2 yr^{-1}, the rate of net calcification ranges from 5 to 126 mol C m^{-2} yr^{-1} (Gattuso *et al.,* 1998*b*).

The modern study of coral calcification began more than 40 years ago with the pioneering works of Goreau and collaborators (Goreau and Bowen, 1955; Goreau, 1959; Goreau and Goreau, 1959; Goreau, 1963) but many aspects, such as the transport mechanisms of calcium and inorganic carbon from the surrounding seawater to the sites of photosynthesis and skeletogenesis, and their environmental controls, remain poorly known. Likewise, the concentrations and transport of secondary products (OH^- and H^+), as well as the interaction between photosynthesis and calcification, are poorly understood; the latter is a matter of recent controversy (Carlon, 1996; Goreau *et al.,* 1996; Marshall, 1996*a, b*).

Photosynthetic CO_2 fixation and CO_2 release by calcification are relatively minor components of the present global carbon cycle (Ware *et al.,* 1992; Smith, 1995) but may have contributed to the control of atmospheric pCO_2 during glacial-interglacial cycles (Opdyke and Walker, 1992). Global climatic changes, such as the predicted increases in temperature and pCO_2 (Houghton *et al.,* 1996), and changes in related parameters, such as pH and aragonite saturation state, are likely to have significant effects on the cycling of carbon and carbonate in coral reefs. These effects are discussed using the limited information available about scleractinian corals and coral communities, as well as some data for temperate coralline algae.

The aim of our paper is to review the cycles of carbon and carbonate in zooxanthellate scleractinian corals and coral reefs. We first provide background information on processes (photosynthesis, respiration and calcification) and carbonate chemistry. We then consider several scales: molecular and cellular (pathways and transport mechanisms of inorganic carbon and calcium), organismal (interaction between photosynthesis and calcification, effect of light), and ecosystemic (community production and calcification, and air-sea CO_2 fluxes). We also review the effect of changes in the seawater carbonate chemistry and provide a tentative prediction of the effect of increased pCO_2 and temperature on the rate of calcification.

Background Information on Chemistry and Processes

Calcium and inorganic carbon are the two major substrates of photosynthesis and calcification. Calcium chemistry is relatively simple because there is only one primary ionic species of this element, although various neutral and charged complexes of the divalent ion are known to exist in seawater (Kennish, 1994). In contrast, carbonate chemistry is much more complex because it involves a gaseous form and both ionic and neutral species as well as complexed forms in seawater.

Carbonate chemistry

Dissolved inorganic carbon (DIC) comprises 3 species: dissolved CO_2 (CO_2 + H_2CO_3) as well as bicarbonate (HCO_3^-) and carbonate ions (CO_3^{2-}):

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+ \leftrightarrow CO_3^{2-} + 2H^+ \quad (1)$$

The distribution of these species is set by the two equilibrium constants that describe the acid/base reactions of inorganic carbon in seawater:

$$K_1 = \frac{[HCO_3^-][H^+]}{[CO_2]} \quad \text{and} \quad (2a)$$

$$K_2 = \frac{[CO_3^{2-}][H^+]}{[HCO_3^-]} \quad (2b)$$

Where [X] is the total (free + complexed) concentration of component X in seawater, and K_1 and K_2 are the equilibrium constants which depend on temperature, salinity and pressure (Dickson and Millero, 1987; Roy *et al.,* 1993). These two equations have several implications for chemical dynamics. First, any change in temperature induces a change in K_1 and K_2 and therefore modifies the chemical speciation. Second, the speciation strongly depends on pH (pH = $-\log_{10}$ $[H^+]$). For 'standard' surface seawater pH condition (*ca.* 8 to 8.25), the re-

spective contributions of HCO_3^-, CO_3^{-2} and CO_2 are approximately 90%, 10%, and <1%. Third, any biological or chemical process that consumes or releases one of the three inorganic carbon species changes the speciation as a result of ultimate control by these thermodynamic equilibrium constants.

In addition to its dynamics in solution, inorganic carbon also interacts with both the gaseous and solid phases according to the following thermodynamic constants:

$$K_0 = \frac{pCO_2}{[CO_2]} \quad \text{and} \tag{3}$$

$$K_s = [M^{2+}][CO_3^{2-}] \tag{4}$$

Where K_0 is the solubility constant of dissolved CO_2, K_s is the solubility constant of the carbonate mineral considered, and M^{2+} is the metal involved (*e.g.*, Ca^{2+} or Mg^{2+}). These constants also depend on temperature, salinity and pressure. In contrast with the dynamics in solution, equilibrium with the gas and solid phases is seldom achieved in seawater. Surface seawater is often under- or super-saturated with respect to atmospheric CO_2 on short time scales (*e.g.*, diel) and local spatial scales, because processes that modify the dissolved CO_2 concentration are faster than the time required to restore equilibrium through air-sea CO_2 exchange. However, at larger scales of both space and time, the surface mixed layer remains close to equilibrium with the atmospheric CO_2 concentration; this equilibrium permits estimation of the overall effects of atmospheric concentration change on marine biomineralization. Also, surface seawater is super-saturated with respect to both calcite and aragonite, the two major forms of calcium carbonate, down to a depth of a few thousand meters (reviewed in Morse and Mackenzie [1990]). Each solid carbonate is characterized by a saturation state (Ω) defined as:

$$\Omega = \frac{[M^{2+}][CO_3^{2-}]}{K_s} \tag{5}$$

where a value of unity means saturation equilibrium (100% saturation). Values greater than 1 indicate supersaturation. The typical present-day oceanic aragonite and calcite saturation states are *ca.* 4 and 6.1 for 'standard' surface seawater at 25°C (salinity = 35, pCO_2 = 360 µatm, and total alkalinity = 2,350 µeq kg^{-1}).

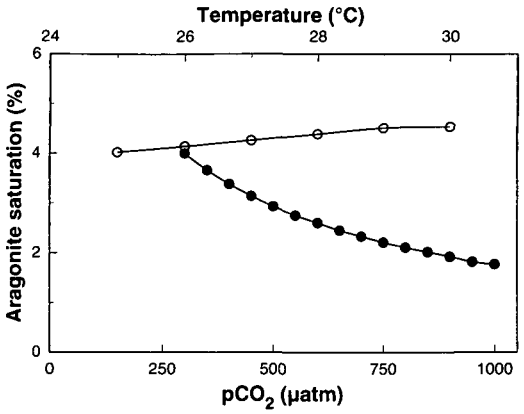

FIG. 1. Aragonite saturation state as a function of pCO_2 (salinity = 35; total alkalinity = 2,350 µeq kg^{-1}; temperature = 25°C; dark symbols) and temperature (S = 35; TA = 2,350 meq kg^{-1}; pCO_2 = 600 µatm; white symbols).

Increased atmospheric pCO_2 and temperature, both of which are expected as global climatic changes, have opposite effects on the saturation state (Fig. 1). Increased concentration of dissolved CO_2 results in a decreased carbonate concentration and, therefore, a decreased saturation state. Increased temperature results in a decreased K_s, and an increase in Ω. The chemical forcing (pCO_2 change) is far more important than the physical forcing (temperature change). According to the mid-range estimates of the International Panel on Climate Change (Houghton *et al.*, 1996), the aragonite and calcite saturation states of tropical surface seawater will decrease by 39% from 1880 to 2100 (Table 1).

Photosynthesis and calcification

Dissolved inorganic carbon is used by the animal host to deposit skeletal $CaCO_3$ and by the endosymbiont for its photosynthesis. Photosynthesis, respiration (of the animal and algal components) and calcification can take place simultaneously according to the following simplified equations:

TABLE 1. *Carbonate chemistry of tropical surface seawater in glacial and interglacial periods.**

	Glacial	Pre-industrial	Present	Year 2065	Year 2100
Temperature (°C)	**25**	**27**	**27**	**28.2**	**29**
Salinity	**35**	**35**	**35**	**35**	**35**
pH (SWS)	8.29	8.16	8.08	7.92	7.83
TA (μeq kg^{-1})	**2,457**	**2,350**	**2,350**	**2,350**	**2,350**
pCO_2 (μatm)	**200**	**280**	**360**	**560**	**706**
HCO_3^- (μmol kg^{-1})	1,566	1,613	1,708	1,845	1,908
CO_3^{2-} (μmol kg^{-1})	370	305	266	210	184
DIC (μmol kg^{-1})	1,942	1,925	1,983	2,070	2,110
Ω aragonite	5.87	4.88	4.26	3.38	2.98
Ω calcite	8.91	7.36	6.42	5.08	4.46

* The parameters shown in bold were used to compute the parameters shown in standard fonts. TA during the glacial time is from Broecker and Peng (1982). Values for pCO_2 was well as future increase of temperature in 2065 and 2100 are the mid-range estimates (IS95a) of the International Panel for Climate Change (Houghton *et al.*, 1996). We assumed that surface seawater is fully equilibrated with atmospheric CO_2 and that the increase in temperature will be identical in air and seawater. TA was held constant at its pre-industrial value from the late 1800s onward. The CO_2 speciation and pH, on the seawater pH scale, were computed using the constants of Roy *et al.* (1993). The aragonite and calcite saturation states were calculated according to Mucci (1983).

$$CO_2 + H_2O \rightarrow CH_2O + O_2 \text{ (photosynthesis)} \quad (6)$$

$$CH_2O + O_2 \rightarrow CO_2 + H_2O \text{ (respiration)} \quad (7)$$

$$Ca^{2+} + 2HCO_3^- \rightarrow CaCO_3 + CO_2 + H_2O \text{ (calcification)} \quad (8)$$

Photosynthesis and calcification both consume inorganic carbon (eqs. 6 and 8) but the combined processes can also be viewed as mutually supporting because CO_2 generated by calcification can be used for photosynthetic carbon fixation.

Photosynthesis is higher than respiration during most of the daylight period; the resulting sum of eqs. 6–8 is then:

$$Ca^{2+} + 2HCO_3^- \rightarrow CaCO_3 + CH_2O + O_2 \quad (9)$$

Whereas at night the equations combine to:

$$Ca^{2+} + 2HCO_3^- + CH_2O + O_2 \rightarrow CaCO_3 + 2CO_2 + 2H_2O \quad (10)$$

Eqs. 9 and 10 hold in freshwater only because the ratio of CO_2 released/$CaCO_3$ precipitated (Ψ), which is close to 1 in freshwater, is approximately 0.6 in standard seawater due to its buffering capacity (Frankignoulle *et al.*, 1994). The amount of CO_2 generated by marine calcification that can potentially be used by photosynthesis is therefore lower than suggested by the stoichiometry of eq. 9.

CELLULAR PATHWAYS OF CALCIUM AND CARBON

Coral polyps are organisms whose anatomy can be simply compared to a "bag" enclosing a coelenteric (=gastrovascular) cavity open to the surrounding seawater by the mouth. The coelenteric cavities of neighboring polyps are connected. In actual fact, the "bag" is far from simple; its shape conforms to the complex skeletal structure of the calyx, it is partially compartmentalized by mesenteries, and tentacles, and it contains cilia that are capable of inducing water movement. The walls of the polyp are made of two single-cell-thick epithelial layers, the ectoderm (epidermis) and the endoderm (gastrodermis), separated by a thin connective layer, the mesoglea. The oral ectoderm (which includes the body wall at the oral, as opposed to basal, end of the organism as well as the tissues of the mouth itself) is in contact with the external seawater and the aboral ectoderm is in contact with the calcium carbonate skeleton. Both the oral and the aboral endoderm are in contact with the fluid in the coelenteric cavity. These relationships are illustrated for the

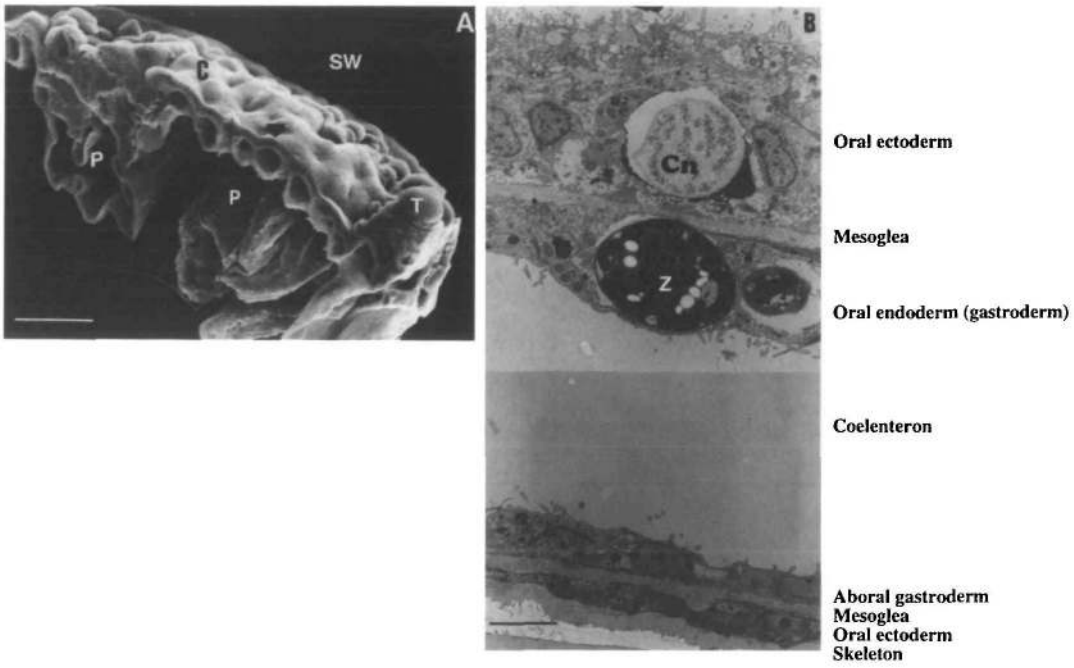

FIG. 2. Tissue morphology of specimens of the zooxanthellate scleractinian coral *Stylophora pistillata* grown on a microscope slide (photographs: D. A. and É. Tambutté). A: Scanning electron micrograph of a demineralized specimen; C, coenosteal tissue; P, polyp; SW, sea water; T, tentacle; scale bar = 200 μm. B: Transmission electron micrograph of transverse section through the coenosteal tissue, as indicated by the arrow in Figure 2A; Cn, cnidocyte; Z, zooxanthella; scale bar = 5 μm.

coenosarc, the region located between the polyps, in Figure 2.

The processes of calcification and photosynthesis are spatially separated (Vandermeulen and Muscatine, 1974). Skeletogenesis is performed by the ectodermal cells of the aboral layers, the calicoblastic epithelium, whereas photosynthesis is carried out by zooxanthellae which are mainly located in the endodermal cells of the oral layers. The distance separating the sites of photosynthesis and calcification is at least 25 μm. The calicoblastic cells are long (10 to 100 μm) in the direction parallel to the skeletal surface, thin (0.5 to 3 μm) in the dimension normal to that surface, highly digitate (Johnston, 1980; É. Tambutté and D. A., unpublished data), and attached to the skeleton by desmocytes (Muscatine *et al.,* 1997).

The composition of the coelenteric fluid is influenced by photosynthesis, by calcification, by advective exchange of seawater through the mouth and/or by transepithelial transport mechanisms (Fig. 3). Wright and Marshall (1991) have argued that water exchange through the mouth is probably too small to supply the amounts of calcium and bicarbonate ions required for coral calcification and photosynthesis, but there are no data to confirm this hypothesis, and the results of Tambutté *et al.* (1996) indicate rapid coelenteron equilibration with the external seawater that would be consistent with both passive transport through oral layers and advective exchange. The fact that such equilibration is not dependent on cellular energy (cyanide insensitive) argues in favor of a passive pathway. Whether or not such exchange occurs with the coelenteron, transepithelial ion fluxes through transcellular and/or paracellular pathways, are required to provide material fluxes to and from the sites of photosynthesis and calcification. The transcellular pathway involves membrane protein (carriers) and at least one energy-dependent step, either uptake by or efflux from the cell, depending on the elec-

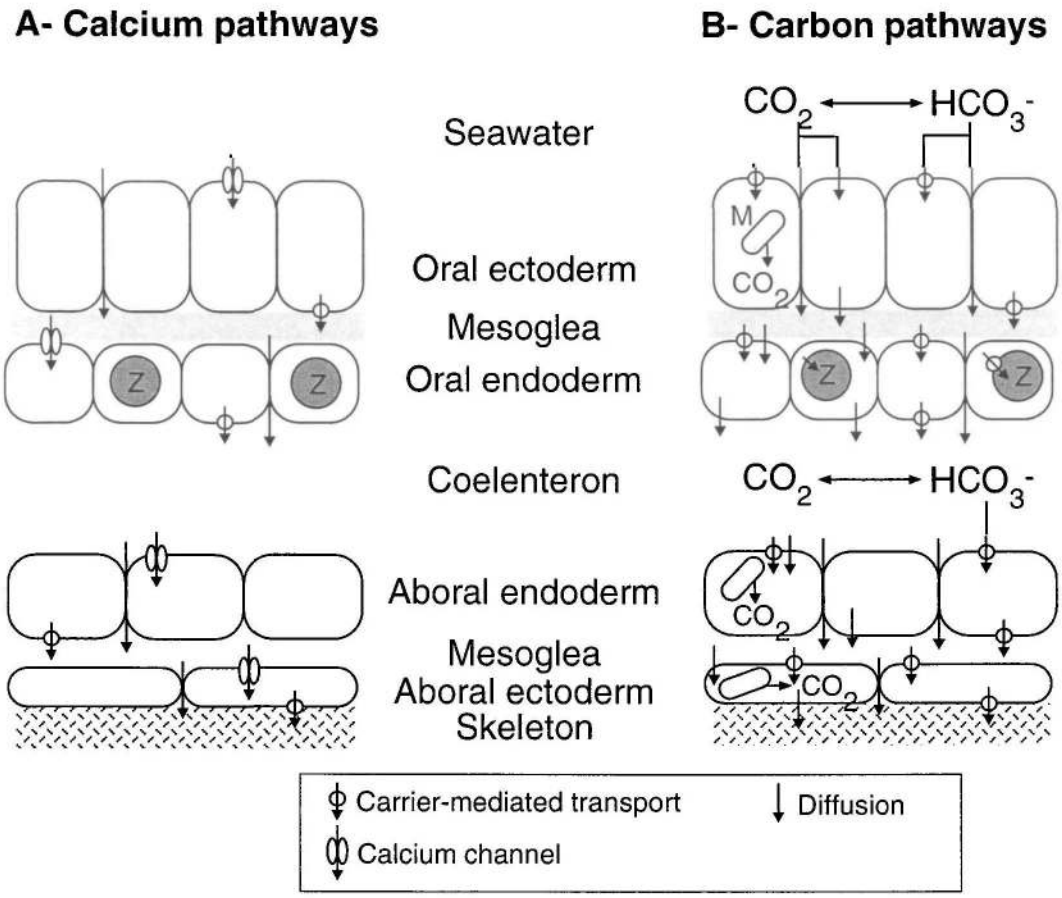

FIG. 3. Calcium (A) and carbon (B) pathways involved in calcification and photosynthesis in scleractinian corals. Anatomy is greatly simplified; see Figure 2 for details. The extracytoplasmic calcifying fluid is located between the aboral ectoderm and the skeleton. Every possible pathway is shown; the actual transport mechanisms for each of the four cell layers can be transcellular or paracellular, or a combination of both (see text). M, mitochondria; Z, zooxanthella.

trochemical potential of the transported molecule. The paracellular pathway is driven by molecular diffusion through the lateral cell junctions that enable attachment of cells among themselves, although there is some evidence that suggests the possibility of advective transport (discussed below). Different septate desmosomes (Green and Flower, 1980; Holley, 1985) and tight junctions (Vandermeulen, 1975; Kinchington, 1980) have been described for the two layers of actinians, suggesting that they exhibit different permeability characteristics.

Bénazet-Tambutté *et al.* (1996*b*) demonstrated that the oral epithelial layers of the coral *Heliofungia actiniformis* and the sea anemone *Anemonia viridis* are "leaky," *i.e.,* transepithelial transport is essentially achieved by a diffusional paracellular pathway. High permeability is restricted to small ions such as Na^+, Cl^- and Ca^{2+}. Larger molecules such as amino-acids do not cross the oral epithelial layers. Low water permeability (Bénazet-Tambutté and Allemand, 1997) is probably an adaptation to maintain a positive intracoelenteric hydrostatic pressure (Batham and Pantin, 1950).

The pathways of calcium and inorganic carbon molecules are reviewed in the next two sub-sections. There are relatively few studies of ion transport in zooxanthellate scleractinian corals. We will use additional

data collected in taxonomically-related groups such as octocorals and actinians, bearing in mind that these groups have different evolutionary lineages and environmental preferences. The calcium pathways, which are better known and simpler in many ways than the carbon pathways, are reviewed first. Because of the limited number of relevant studies, the findings reviewed relate to a variety of organisms—different taxa, both calcifying and non-calcifying, with a wide range of phyologenetic relationships. The assumption inherent in this approach is that the mechanisms and structures involved are the same or very similar across the wide range of organisms considered. This review process is thus a mechanism for hypothesis development; to the extent that contradictions among studies appear, the assumption is questionable and new hypotheses are needed.

A specific example of this hypothesis-oriented approach is the fact that the review of laboratory experiments suggests that the concentration of calcium, and possibly of other ions as well, in the extracytoplasmic calcifying fluid (ECF) is controlled by the calicoblastic epithelium, and depends on active, transcellular transport mechanisms. Such control is not in agreement with the fact that corals from a wide variety of taxa and locations have been shown to produce skeletal records of factors affecting seawater Sr, Cd, Pb, Mn, Ba, and U, suggesting relatively free passage of elements between seawater and the ECF (reviewed by Dunbar and Cole, 1993).

Strontium is the minor skeletal component that has been most studied but conflicting results were obtained. Chalker (1981) concluded that Sr is transported via a transcellular, carrier-mediated pathway, in *Acropora cervicornis*. He also showed that a competition for the transport exists between Sr^{2+} and Ca^{2+}, suggesting that skeletal Sr and Ca are not in equilibrium with seawater. Ip and Krishnaveni (1991) found opposite results in *Galaxea fascicularis* and suggested a paracellular transport pathway for Sr^{2+}. This differs from the transport mechanisms of Ca^{2+} discussed below, which might help to explain the discrepancies observed between skeletal Sr/Ca and $\delta^{18}O$, two proxies of temperature (Boiseau *et al.*, 1997). More information on the transport of trace elements and stable isotopes is therefore required in order to reconcile the laboratory experiments with the field observations.

Calcium pathways

The mechanisms of calcium transport for calcification in corals are poorly known, as is the case in other invertebrates (Simkiss and Wilbur, 1989). Potential pathways of calcium from the surrounding seawater to the coelenteron and to the site of skeletogenesis are shown in Figure 3A. Calcium ions can reach the ECF by transcellular transport (energy-dependent), by paracellular diffusion and possibly by advection (both are energy-independent), or a combination of all processes.

An active process is involved in the incorporation of calcium into the coral skeleton (Chalker and Taylor, 1975; Chalker, 1976; Tambutté *et al.*, 1996). It follows a saturable kinetics with respect to external calcium concentration, implying an enzyme-mediated step (Chalker, 1976; Krishnaveni *et al.*, 1989; Tambutté *et al.*, 1996). At ambient seawater Ca^{2+} concentration (*ca.* 10 mM), the rate of calcification is saturated in some species (Chalker, 1976; Tambutté *et al.*, 1996) but not in others (Chalker, 1976; Krishnaveni *et al.*, 1989). Calcium limitation of skeletogenesis, as was proposed by Chapman (1974), can therefore occur.

The Ca^{2+} transepithelial pathway involves at least one transcellular mechanism (*e.g.*, Marshall, 1996*a*; Tambutté *et al.*, 1996) but there are conflicting results on the location of the active calcium transport. Wright and Marshall (1991) suggested it occurs across both the oral and aboral epithelia. Active transport across oral layers is not consistent with the results of $^{45}Ca^{2+}$ efflux experiments which showed that equilibrium of the coelenteron of *Stylophora pistillata* with external seawater results from passive transport (Tambutté *et al.*, 1995*a*). Also, oral epithelia of the coral *Heliofungia actiniformis* are leaky with respect to divalent ions (Bénazet-Tambutté *et al.*, 1996*b*) and fluxes into the coelenteron does not appear to limit the incorporation $^{45}Ca^{2+}$

in the skeleton (Bénazet-Tambutté *et al.*, 1996*b*; Tambutté *et al.*, 1996). Tambutté *et al.* (1996) showed, using a compartmental approach, that $^{45}Ca^{2+}$ is incorporated into the skeleton of the coral *Stylophora pistillata* after a lag of less than 2 min from which they infered a very fast equilibration with seawater calcium and the absence of a large calcium pool for calcification. Uptake and efflux experiments indicate that there is only one transcellular, energy-dependent step of calcium transport, located in the calicoblastic epithelium. The other steps result from rapid paracellular pathways (Tambutté *et al.*, 1996).

Although the concentration of free calcium is at least 100,000 times lower in cells than in seawater (10–100 nM *vs.* 10 mM), calcium does not diffuse freely into the cell, and specialized transport proteins, Ca^{2+} channel and/or cation antiporter, are required to mediate Ca^{2+} entry across the lipid bilayer of the aboral ectoderm. The pharmacological properties of the coral Ca^{2+} channel are typical of an L-type of voltage-dependent Ca^{2+} channel (Tambutté *et al.*, 1996). The $\alpha 1$ subunit of this protein was cloned and immunolocalized on the calicoblastic epithelium (Zoccola and Allemand, 1996; Zoccola *et al.*, in press). This subunit cannot be assigned to one of the known L-type subfamilies but it has an 86% similarity in the amino-acid sequence with the rabbit $\alpha 1C$ subunit.

Once inside the calicoblastic cells, Ca^{2+} ions must be sequestered in vesicles or organelles and/or bound to Ca^{2+}-binding proteins in order to maintain a low free intracellular concentration (Bronner, 1990). Ca^{2+}-binding substances have been isolated from the skeletal organic matrix (Isa, 1986; Isa and Okazaki, 1987) but the role of such compounds remains to be investigated in calicoblastic cells. Vesicles within the calicoblastic cells do not contain calcium (Kinchington, 1980), in spite of previous reports of vesicles containing electron dense material (Kawaguti and Sato, 1968), "crystals" (Hayes and Goreau, 1977; Goreau and Hayes, 1977) or of Ca^{2+}-rich cytoplasm (Vandermeulen, 1975). Transcellular Ca^{2+} transport through vesicles is suggested by the sensitivity of calcification to inhibitors of cytoskeleton polymerization (Kinchington, 1980; Tambutté *et al.*, 1996). However, it has been shown recently that the cytoskeletal-dependent step of calcification is the exocytosis of organic matrix-containing vesicles rather than the intracellular Ca^{2+} transport (Allemand *et al.*, 1998*b*).

Calcium exit from the calicoblastic cells may occur by two mechanisms: Na^+/Ca^{2+} exchanger (Marshall, 1996*a*) and Ca^{2+}-ATPase (Isa *et al.*, 1980). The Ca^{2+} affinity of the Ca^{2+}-ATPase investigated by Isa *et al.* (1980; 700 μM) is too high to account for a plasma-membrane Ca^{2+}-ATPase, whose affinity is generally well below 1 μM (Carafoli, 1987). More recently, Ip *et al.* (1991) isolated two components of a Ca^{2+}-sensitive ATPase in the coral *Galaxea fascicularis.* These two components have distinct affinities (150 and 2.1 μM). The low affinity component is likely a plasma-membrane Ca^{2+}-ATPase but it is unknown whether it is the one that accounts for the exit of Ca^{2+} from the calicoblastic cells. One intriguing point, which has never been explored, is the coupling mechanism between Ca^{2+} uptake and Ca^{2+} efflux that is required to maintain a low Ca^{2+} concentration in calicoblastic cells despite large transepithelial Ca^{2+} fluxes.

McConnaughey (1991, 1995; see also McConnaughey and Whelan, 1997) suggested that the H^+ generated by $CaCO_3$ precipitation was removed by a Ca^{2+}-ATPase-mediated $1Ca^{2+}/2H^+$ exchanger. A constant pumping of H^+ from the site of skeletogenesis to the coelenteron must occur but how such a large flux is achieved in the cytoplasm of the aboral epithelial cells without disrupting the intracellular pH remains unknown.

Carbon pathways

The preferred DIC substrate for coral photosynthesis is external HCO_3^- (Land *et al.*, 1975; Goreau, 1977; Al-Moghrabi *et al.*, 1996; Goiran *et al.*, 1996), although it has been suggested that CO_2 is a major source (Taylor, 1983). DIC transport for photosynthesis of zooxanthellate Anthozoa is reviewed by Allemand *et al.* (1998*a*). The DIC reservoir used by free-living algae is seawater, while the immediate source

used by zooxanthellae is that in the coral host cell, which is derived to a significant extent from the external seawater. Possible transepithelial pathways are shown in Figure 3B. Carbon supply is saturated at ambient DIC concentrations in corals (*ca.* 2.2 mM; Burris *et al.,* 1983; Goiran *et al.,* 1996). The relatively high affinity (*i.e.,* concentration at which half-saturation is achieved) of photosynthetic O_2 release for inorganic carbon (Burris *et al.,* 1983; Goiran *et al.,* 1996) strongly suggests that a carbon concentrating mechanism (CCM)-like system, which actively absorbs HCO_3^- to sustain photosynthesis, operates in the animal host, as previously hypothesized by Raven (1992). Ribulose biphosphate carboxylase-oxygenase (Rubisco) is an enzyme that catalizes net photosynthetic carbon fixation that also exhibits a high affinity for oxygen, which decreases its photosynthetic efficiency. Rubisco is a form II enzyme in dinoflagellates (Morse *et al.,* 1995; Whitney *et al.,* 1995). This form, usually restricted to anaerobic proteobacteria, has a high affinity for O_2 that prevents net fixation of carbon in an aerobic environment. The presumably high CO_2 concentration near Rubisco due to a CCM-like activity could favor the carboxylase function of the enzyme at the expense of its oxygenase function (Rowan *et al.,* 1996).

Al-Moghrabi *et al.* (1996) and Goiran *et al.* (1996) demonstrated that the uptake of HCO_3^- by the host cell involves two anion carriers that are sensitive to DIDS, an inhibitor of anion transport. One of them is either a Na^+-dependent Cl^-/HCO_3^- exchanger or a Na^+/HCO_3^- cotransporter. The nature of the second carrier is not clear. DIC is supplied to zooxanthellae by a transepithelial active mechanism present in both endodermal and ectodermal cells of the oral layers of the temperate zooxanthellate sea anemone *Anemonia viridis* (Bénazet-Tambutté *et al.,* 1996*a*; Furla *et al.,* 1998*a, b*). Within the endodermal cell, HCO_3^- is dehydrated into CO_2 the substrate of Rubisco. The transepithelial transport of HCO_3^- generates, under light conditions, a net efflux of OH^- (or net H^+ uptake) into (or from) the coelenteric cavity resulting in a pH gradient of about 0.8 unit across the tentacle of the anemone (Furla *et al.,* 1998*a, b*). If this process occurs in corals, it could represent a portion of the mechanism by which H^+ ions produced by $CaCO_3$ precipitation are buffered, maintaining the high levels of aragonite saturation state that are needed to sustain calcification.

There are very limited data on the source and transport mechanisms of the inorganic carbon used for coral calcification. Radioisotopic tracer experiments demonstrate that DIC from seawater can be incorporated into the skeleton (Goreau 1961, 1963; Taylor, 1983). However, the observation that the skeletal $\delta^{13}C$ isotopic ratio is different from the one in seawater (*e.g.,* Keith and Weber, 1965) suggests that a DIC source other than external seawater can also be used. Feeding with ^{14}C-labelled food provided direct evidence that some CO_2 generated by host respiration is deposited in the skeleton as carbonate (Pearse, 1970, 1971). Goreau (1977) estimated, from stable isotope data, that approximately 40% of the carbon supply is from seawater DIC and 60% from recycled metabolic CO_2. Erez (1978) showed, using double labelling experiments (^{14}C and ^{45}Ca), that the skeletal ratio of $^{14}C/^{45}Ca$ ranges from 0.1 to 0.5 in the light suggesting that the major part (50 to 90%) of the skeletal carbon originates from metabolic CO_2 in the species (*S. pistillata* and *A. variabilis*) and under the conditions studied. Lucas and Knapp (1997) obtained similar results with the spicules of the non-zooxanthellate octocoral *Leptogorgia virgulata.* Goreau (1961) suggested that DIC supply may be rate-limiting in calcification. Such limitation has been observed in the temperate non-symbiotic octocoral, *Corallium rubrum* (Allemand and Grillo, 1992) but there are no data available for scleractinian corals. However, the discussion of saturation state effects (above) indicates that carbonate ion concentration can be limiting; if pH is held constant, this translates into a DIC limitation.

More than one HCO_3^- transport mechanism is probably involved in calcification. Tambutté *et al.* (1996) showed that DIDS inhibits the rate of calcification in the scleractinian coral, *Stylophora pistillata* by up to 95%, both in the light and in the dark.

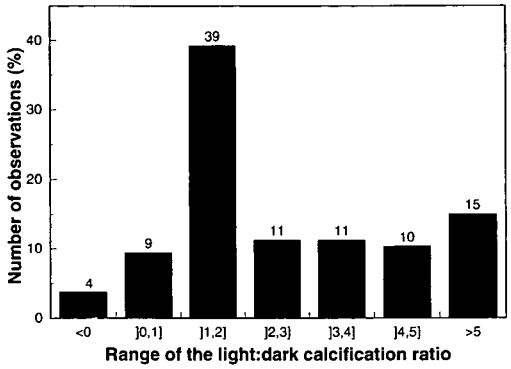

FIG. 4. Frequency distribution of the light to dark calcification ratio of in zooxanthellate scleractinian corals. Data were compiled from 26 publications (list available from JPG). The median value is 3.0.

This indicates that the anion-carrier mechanism is the rate-controlling process, but does not prove the absence of other pathways. Lucas and Knapp (1997) found that DIDS does not completely inhibit calcification in the non-zooxanthellate octocoral *L. virgulata,* suggesting that CO_2 can reach the site of skeletogenesis by a passive transport pathway and be used as a substrate for calcification. Carbonic anhydrase, an enzyme located in the calicoblastic epithelium (Isa and Yamazato, 1984), catalyzes the conversion of respiratory CO_2 into HCO_3^-, as demonstrated by inhibition experiments (Goreau, 1959; Tambutté *et al.,* 1996).

INTERACTIONS BETWEEN PHOTOSYNTHESIS AND CALCIFICATION

The first indication of a possible link between zooxanthellar photosynthesis and coral calcification is probably the finding by Kawaguti and Sakumoto (1948) that calcification is higher in the light than in darkness. The analysis of 108 data compiled from 26 publications provides overwhelming evidence that calcification in the light is significantly higher than calcification in the dark. The ratio of light:dark calcification ranges from negative values ($CaCO_3$ dissolution has been observed in 4% of the observations) to 127 (Fig. 4). Skeletal dissolution, which is not related to calcification, has not been found in corals since the early measurements of the 1940s. Only 9% of the observations are in the range 0–1 (calcification higher in the dark than in the light), 71% are in the range 1–5, and 15% of the ratios are higher than 5. The median ratio is 3.0. These data were collected using various techniques (*e.g.,* ^{14}C and ^{45}Ca fixation, buoyant weight and alkalinity anomaly techniques), under a wide range of environmental (*e.g.,* light, temperature, pH, pO_2 and pCO_2) and biological (*e.g.,* feeding) conditions, both *in situ* and in the field, which probably accounts for the wide range of variation. The site of $CaCO_3$ deposition is also different between light and dark periods (Marshall and Wright, 1998), but it is likely that the same transport mechanisms are used (Marshall, 1996*a*; Marshall and Wright, 1998).

Mechanisms invoked to explain the higher rate of calcification in light than in darkness include the uptake by zooxanthellae of animal metabolic wastes (Yonge, 1968; Crossland and Barnes, 1974) or of substances interfering with $CaCO_3$ precipitation (Simkiss, 1964); the translocation of photosynthate to fuel active transport mechanisms (Chalker and Taylor, 1975) or to synthesize the organic matrix (Wainwright, 1963); the increase of $CaCO_3$ saturation by photosynthetic CO_2 uptake (Goreau, 1959); and the maintenance of an oxic environment (Rinkevich and Loya, 1984; Rands *et al.,* 1992). It remains unclear as to which of these mechanisms (or set of thereof) is involved. It was nevertheless generally accepted, until recently, that calcification was light-enhanced during the day (see Barnes and Chalker, 1990). However, Marshall (1996*a*) measured similar rates of calcification in a non-zooxanthellate coral and in a zooxanthellate coral incubated in the light and argued that calcification is not light enhanced in zooxanthellate corals but, rather, that it is dark-repressed. This view has been subsequently challenged by several authors who pointed out some limitations in Marshall's results, especially with regard to the normalization of the data (Carlon, 1996; Goreau *et al.,* 1996). There is, additionally, valuable information on the rate of calcification of colonies of the same species harboring a normal or a reduced density of zooxanthellate, whether naturally occurring or artificially obtained (for convenience the

latter colonies will be referred to as non-zooxanthellate hereafter). Calcification in light is higher in zooxanthellate than in non-zooxanthellate specimens (Goreau, 1959; Goreau and Goreau, 1959; Jacques *et al.,* 1977; Jacques and Pilson, 1980; Jacques *et al.,* 1983; Kajiwara *et al.,* 1995), with a ratio from 1.1 to 19 (median = 1.9), observations that cannot be explained by a dark-repression mechanism. Rates of dark $CaCO_3$ precipitation are more equivocal as the zooxanthellate:non-zooxanthellate calcification ratio ranges from 0.9 to 3.1. Therefore, at least in some species and/or under some experimental conditions, the presence of zooxanthellae slightly enhances, not repress, calcification in darkness.

Interaction at the cellular level

There are two major hypothesis regarding the interactions between photosynthesis and calcification at the cellular level. In the first one, photosynthetic CO_2 uptake lowers the extracellular CO_2 partial pressure in the coral tissue, which increases carbonate saturation and favors precipitation of $CaCO_3$ (Goreau, 1959, 1961). In the second model (*trans* calcification; McConnaughey, 1991, 1995; McConnaughey and Whelan, 1997), Ca^{2+}-ATPase supplies Ca^{2+} to and removes H^+ from the site of calcification (stoichiometry: $1Ca^{2+}/2H^+$). In the coelenteron, protons may generate CO_2 by dehydrating HCO_3^-. This mechanism favors (1) $CaCO_3$ precipitation by maintaining an elevated pH of the extracytoplasmic calcifying fluid and (2) photosynthesis by increasing the coelentric CO_2 reservoir. These two models imply a high concentration of bicarbonate in the coelenteron to buffer calcification-induced H^+. The coelenteric HCO_3^- pool has never been measured in corals but it becomes quickly depleted during the day in the sea anemone *A. viridis* (Bénazet-Tambutté *et al.,* 1996*a*; Furla *et al.,* 1998*a, b*).

The validity of the *trans* calcification mechanism was tested using various approaches. Decreased calcium seawater concentration inhibits photosynthesis in corals (McConnaughey, 1994 and personal communication; Al-Moghrabi *et al.,* 1996), foraminiferan (Kuile *et al.,* 1989*a*), coccolithophorids (Brownlee *et al.,* 1994), and calcareous macroalgae (McConnaughey 1991; McConnaughey and Falk, 1991). A similar result was obtained in the coral *Galaxea fascicularis* using a calcium-channel inhibitor (Al-Moghrabi *et al.,* 1996). In contrast, an inhibitor of mineral deposition (HEBP) inhibits coral calcification by 99% without any effect on the rate of photosynthesis (Yamashiro, 1995). Similarly, protein-synthesis inhibitors decrease coral calcification by 60 to 85% without disturbing photosynthesis (Allemand *et al.,* 1998*b*). In coccolithophorids, the decrease of photosynthesis in low-calcium seawater could result from a direct inhibition of HCO_3^- transport rather than from an effect mediated by the inhibition of calcification (Brownlee *et al.,* 1994). The inhibition of coral photosynthesis by verapamil increases linearly for concentrations ranging from 0 to 250 μM (Al-Moghrabi *et al.,* 1996), whereas calcification is almost totally inhibited at a concentration of 100 μM (Tambutté *et al.,* 1996). This suggests that photosynthesis does not depend on calcification for carbon supply.

Photosynthesis and calcification could also compete for a single DIC pool as shown in a symbiont-bearing foraminiferan (Kuile *et al.,* 1989*b*). The inhibition of calcification should make more inorganic carbon available for photosynthesis and stimulate photosynthesis. As mentioned above, such response as not been observed (Yamashiro, 1995; Al-Moghrabi *et al.,* 1996). This supports the concept of complementarity rather than competition for carbon between photosynthesis and calcification (Taylor, 1983).

The endodermal cell layer of *Anemonia viridis* secretes OH^- in the light and generates a pH gradient of about 0.8 units across the epithelial layers, with the endodermal side (*i.e.,* the coelenteric cavity) being alkaline (Furla *et al.,* 1998*b*; Allemand *et al.,* 1998*a*). Such secretion of OH^- could neutralize the H^+ generated by coral calcification, providing an alternative explanation to Goreau's hypothesis that photosynthesis stimulates calcification by removing by-products.

Interactions at the organism level

It has been suggested, on the basis of eq. 9, that photosynthetic and calcifying plants,

symbiotic animals and ecosystem often display ratios of calcification to photosynthesis (*G/P*) close to 1 (*e.g.*, McConnaughey and Whelan, 1997). In the literature, it is not always reported whether P_g, P_n, or an undefined production value (when photosynthesis is measured using the ^{14}C fixation technique) is considered. Furthermore, these production estimates are sometimes examined together (*e.g.*, McConnaughey, 1994; McConnaughey and Whelan, 1997). G/P_n relates to the amount of CO_2 that can potentially be supplied by calcification to the CO_2 required by net photosynthesis (*i.e.*, taking into account the CO_2 supplied by respiratory processes). The G/P_g ratio is difficult to analyze because P_g is not known accurately for either corals or reef communities, as it is derived from P_n and R_n the night-time respiration. Such a procedure underestimates P_g and overestimates G/P_g because respiration has been shown, at least in corals, to be significantly higher during the day than at night (*e.g.*, Kühl *et al.*, 1995).

Yamashiro (1995) measured the rates of photosynthesis (^{14}C fixation) and calcification at three irradiances and showed that the *G/P* ratio decreases as a function of increasing irradiance. There are, however, very few data available on the relationship between photosynthesis and calcification over a diel cycle in zooxanthellate corals. Data obtained on a colony of *Stylophora pistillata* (S. Romaine-Lioud and J.-P.G., unpublished data) can be used to study the relationship between *G* and *P* during the course of 24-hr. These data are limited to one species under certain conditions, but they provide insight into the interaction between *P* and *G* at the organism level. Peak net photosynthetic CO_2 fixation and net $CaCO_3$ precipitation are, respectively, 160 and 176 μmol CO_2 (mg Chl-a)$^{-1}$ hr^{-1}. CO_2 uptake by net photosynthesis under saturating irradiance is significantly higher than the concurrent CO_2 release by calcification (*ca.* 160 *vs.* 106 μmol CO_2 (mg Chl-a)$^{-1}$ hr^{1}; Fig. 5). An additional source of inorganic carbon (from external seawater) is therefore required to sustain photosynthetic CO_2 fixation.

An analysis of data from the literature

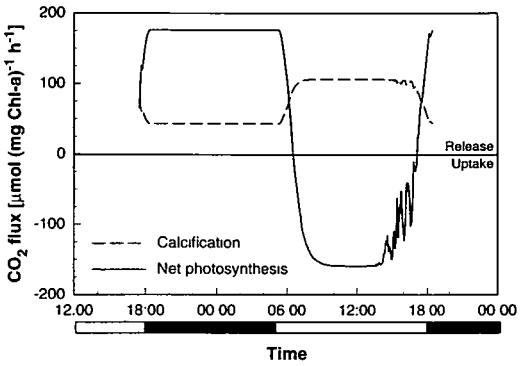

FIG. 5. CO_2 fluxes generated by calcification and net photosynthesis of the scleractinian coral *Stylophora pistillata* (S. Romaine-Lioud and J.-P. G., unpublished data). Net photosynthesis (using pH and alkalinity) and net calcification (by the alkalinity anomaly technique) were measured in the laboratory under various light conditions. Exponential functions [$y = a\,(1 - \exp(-x/b) + c$] were fitted to the light saturation curves. The fitting parameters for net photosynthesis are: a = 337 μmol CO_2 (mg Chl-a)$^{-1}$ h^{-1}; b = 144.6 μmol photons m^{-2} s^{-1}; c = 176.1 μmol CO_2 (mg Chl-a)$^{-1}$ h^{-1}. The fitting parameters for net calcification are: a = 104 μmol $CaCO_3$ (mg Chl-a)$^{-1}$ h^{-1}; b = 72.3 μmol photons m^{-2} s^{-1}; c = 71.8 μmol CO_2 (mg Chl-a)$^{-1}$ h^{-1}. CO_2 fluxes were estimated using light data measured at 10 m in the Northern Red Sea (personal communication, M. Marchioretti). The theoretical CO_2 flux generated by calcification was derived assuming a constant Ψ ratio (CO_2 released/$CaCO_3$ precipitated) of 0.6 (Frankignoulle *et al.*, 1994).

confirms the wide range of variation of the molar G/P_g (0.2 to 1.5; median = 0.6) and G/P_n (−8 to 17; median = 1.3) ratios (Fig. 6). The median G/P_n ratio of 1.3 indicates that CO_2 generated by $CaCO_3$ deposition (0.6 times net calcification) could *potentially* supply 78% of the inorganic carbon required for zooxanthellar photosynthesis. As discussed earlier, the actual significance of this DIC source remains unclear.

Interactions at the community level

Reef metabolic data have recently been reviewed by Gattuso *et al.* (1998*b*). Coral/algal reef flats exhibit wide ranges of community gross primary production (P_g: 79–584 mol C m^{-2} yr^{-1}), respiration (R: 76–538 mol C m^{-2} yr^{-1}), and net calcification (G: 5–126 mol $CaCO_3$ m^{-2} yr^{-1}). Such variability is mostly due to differences in the community structure of the sites investigated (*e.g.*, Pichon, 1997). For example, it is

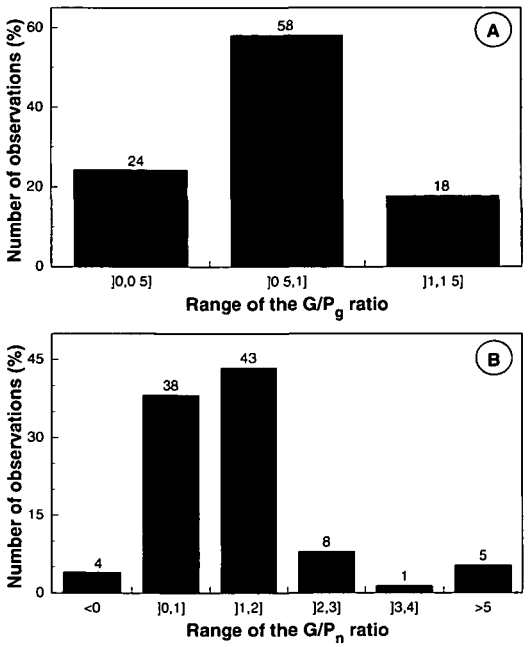

FIG. 6. Frequency distribution of the calcification to photosynthesis ratios in zooxanthellate scleractinian corals (A:G/P_g; B:G/P_n). Data, expressed in various molar units, were compiled from the literature (list available from JPG). The rates of photosynthesis estimated by the ^{14}C fixation technique were not included as it is unclear whether they relate to net or gross photosynthesis, or to some intermediate value (see Peterson, 1980). The medians of the G/P_g and G/P_n ratios are 0.60 and 1.30, respectively.

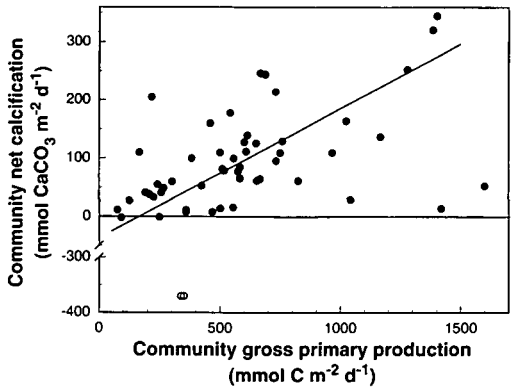

FIG. 7. Net community calcification as a function of community gross primary production. All sites shown in Table 1 were pooled. The line of the geometric regression is shown: $y = -37.1 + 0.22x$ ($N = 52$, $r = 0.49$, $P = 0.0002$). Open circles are two data points not included in the regression analysis.

well established that community primary production increases and community calcification decreases with increasing surface cover of fleshy algae (*e.g.*, Smith, 1973). Community metabolism data have been compiled from the literature and distributed in the categories defined by Kinsey (1985). Overall, G and P_g are significantly correlated and the G/P_g estimated as the slope of the geometric regression, is 0.2 (Fig. 7 and Table 2). However, no significant correlation is found when the various categories are examined separately, except for algal-dominated areas. An opposite trend (*i.e.*, decrease in G with increasing P_g) is observed in three of the systems examined (lagoons, algal pavements, and whole reefs). Nevertheless, most daily G/P_g ratios are distributed in the range 0.1–0.4 in the various communities and ecosystems, and they rarely exceed 0.5 (data not shown). The suggestion that $G/P_g = 1$ in photosynthetic

TABLE 2. *Correlation between the rates of community gross primary production and calcification of coral reef ecosystems and communities, and median value of the* G/P_g *ratio.**

Community	Correlation coefficient	N	P	Median of G/P_g
Overall	0.49	52	0.0002	0.2
Algal-dominated zones	0.98	4	0.02	0.0
Whole reefs	0.67	5	0.09	0.2
Lagoons	0.82	4	0.18	0.1
Sediments	0.63	4	0.21	0.1
High activity areas	0.43	4	0.18	0.2
Reef flats	0.10	29	0.61	0.1
Algal pavements	–	2	–	0.4

* Categories of communities defined by Kinsey (1985). N = sample number; P = probability. The data set is available from JPG.

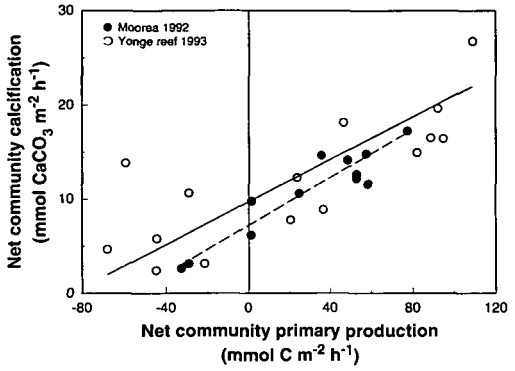

FIG. 8. Net community calcification as a function of net community primary production measured using a Lagrangian technique at Moorea, French Polynesia (solid line), and Yonge Reef, Great Barrier Reef (dashed line). Several transects were carried out at night and during the day; each data point represents one transect. The lines of the geometric regressions are shown: Moorea, $y = 0.13 - 7.05x$ ($N = 12$, $r = 0.87$, $P = <0.0001$); Yonge reef $y = 0.11 - 9.69x$ ($N = 16$, $r = 0.63$, $P = 0.0002$).

and calcifying communities (*e.g.*, McConnaughey, 1994) is therefore not supported by the relevant literature.

Daily calcification and net community production at various sites are not significantly correlated, but short-term data obtained at the same site are strongly correlated. For example, G and P_n have recently been estimated on two Pacific reef flats using a Lagrangian technique (Gattuso *et al.*, 1996*b*). Both processes are significantly correlated, with a G/P_n ratio of about 0.1 at both sites (Fig. 8). The ratios estimated at the same sites using an Eulerian technique are higher (0.37 and 0.24; Frankignoulle *et al.*, 1996). Both estimates clearly show that the CO_2 generated by community calcification is not the major source of inorganic carbon sustaining net community production when the water residence time is short. The large decrease in pCO_2 measured in such systems during the day (down to *ca.* 250 μatm; Frankignoulle *et al.*, 1996) demonstrates that DIC is drawn from the seawater reservoir and, to a much lesser extent, from the invasion of atmospheric CO_2. It has been proposed, based on both theoretical considerations (Smith, 1985) and data from non-reefal systems (*e.g.*, Smith and Veeh, 1989), that organic production tightly controls calcification when the residence time is longer (*ca.* 1 year). pH and $CaCO_3$ saturation rise when the net community production exceeds the invasion of atmospheric CO_2. Calcification is therefore stimulated, increases pCO_2 and reduces the CO_2 invasion induced by the net organic carbon metabolism. The net air-sea CO_2 flux is close to zero in such systems as a result of this tight interaction between the organic and inorganic carbon metabolism.

Despite the drawdown of CO_2 during the day, most reef flats are sources of CO_2 to the atmosphere, on a 24-hr basis, due to their low net fixation of CO_2 via photosynthetic processes (excess [=net] production close to 0) and rather large release of CO_2 by precipitation of calcium carbonate (Ware *et al.*, 1992; Gattuso *et al.*, 1993; Gattuso *et al.*, 1995; Smith, 1995; Frankignoulle *et al.*, 1996; Gattuso *et al.*, 1996*b*). There is one notable exception: algal-dominated reef communities, which exhibit a larger community excess production and/or a lower community calcification, are sinks for atmospheric CO_2 (*e.g.*, Kayanne *et al.*, 1995; Gattuso *et al.*, 1996*a*; Gattuso *et al.*, 1997).

EFFECT OF GLOBAL ENVIRONMENTAL CHANGE: CARBONATE CHEMISTRY

The signs and magnitudes of many environmental changes that are expected in the next decades (Pittock, 1999) are similar to the current range of climatic variation within which reefs exist (Done, 1999; Kleypas *et al.*, 1999), and are considerably less extreme than those experienced by reefs during geological history (Buddemeier and Smith, 1999; Benzie, 1999 issue; Pandolfi, 1999). This suggests that existence of efficient adaptative mechanisms in reef organisms and the potential for the persistence of reef ecosystems. Reef ecosystems may respond to environmental change through alteration in their physical and ecological structure and through changes in rate constants of accretion and biogeochemical cycling (Gates and Edmunds, 1999; B. G. Hatcher, personal communication). However, the potential for adaptation of reef organisms may be overwhelmed, as present and predicted rates of change of some global climatic parameters and local non-cli-

matic variables (*e.g.*, nutrients) are unprecedented in the geological record.

Smith and Buddemeier (1992) identified the following major parameters that may affect the structure and function of coral reefs: sea level, temperature, pCO_2, ultraviolet (UV) radiation, hydrodynamics, sedimentation, salinity and nutrients. They also pointed out that some of these variables will change at a global scale (sea level rise and pCO_2), some at a regional scale (temperature), and some at a local scale (nutrients). This section is deliberately focused on the response of the carbon and carbonate metabolism to changes in the carbonate chemistry driven by increasing pCO_2 and temperature. Reef response to other environmental variables have recently been reviewed by Smith and Buddemeier (1992), whereas the acclimation of scleractinian corals to environmental changes has been addressed by Brown (1997*a, b*) and Gates and Edmunds (1999). Additionally, a recent special issue of *Global Change Biology* (1996, 2(6)) is dedicated to global change issues in coral reefs, other specific information is available on UV (Dunlap and Shick, 1998) as well as on temperature and coral bleaching (Glynn, 1993, 1996; Brown, 1997*c*)

Response at the organism and community levels

Photosynthesis of marine phototrophs is generally not considered as carbon-limited due to the large pool of total inorganic carbon in the form of bicarbonate (Raven, 1997). This is only valid for phototrophs able to use bicarbonate effectively, *i.e.*, species having a carbon-concentrating mechanism (CCM). Photosynthesis can be stimulated by CO_2 enrichment in species lacking a CCM or when the CCM is not operating. For example, short-term or long-term exposure of seagrasses to elevated CO_2 leads to a 3-fold increase of photosynthesis (Zimmerman *et al.*, 1997). Similarly, some diatoms (Riebesell *et al.*, 1993), macroalgae (Borowitzka and Larkum, 1976; Gao *et al.*, 1993*b*), and microalgae (Nimer and Merrett, 1993) exhibit higher rates of photosynthesis under CO_2 enrichment. A decrease of pH at constant DIC concentration stimulates the rate of photosynthesis of CO_2-users, whereas it inhibits that of HCO_3^--users (Munoz and Merrett, 1989). Consequently, CO_2 enrichment should enable CO_2-users to compete more effectively with HCO_3^--users.

The available evidence indicates that coral symbiotic units as well as isolated zooxanthellae are HCO_3^--users (Burris *et al.*, 1983; Al-Moghrabi *et al.*, 1996; Goiran *et al.*, 1996). During short-term experiments, the rate of photosynthesis of the coral *Galaxea fascicularis* is not significantly different at pH 7.5 and 8.0. It is inhibited when pH is lower than 7.5, even when DIC is maintained at a constant concentration (Goiran *et al.*, 1996); this is presumably due to pH effects on physiological and biological processes (Madshus, 1988) rather than on the DIC system. There is no information on the response of coral photosynthesis to long-term increase in DIC and pCO_2.

Until recently, carbonate chemistry was generally not considered to be an important parameter controlling calcification (see Smith and Buddemeier, 1992; Buddemeier, 1994) and there are limited data on the effect of the calcium carbonate saturation state (Ω) on $CaCO_3$ deposition of photosynthetic and calcifying marine organisms and communities. There are four data sets on temperate and tropical coralline algae (Smith and Roth, 1979; Borowitzka, 1981; Agegian, 1985; MacKenzie and Agegian, 1989; Gao *et al.*, 1993*a*), two data sets on scleractinian corals (Gattuso *et al.*, 1998*a*; F. Marubini and M. J. Atkinson, personal communication) and one on a coral reef community (Langdon *et al.*, submitted). Techniques used to manipulate Ω were: changes in pH, pCO_2, DIC and calcium concentrations. Six out of 7 data sets clearly show a linear or curvilinear decrease in the rate of calcification as a function of decreasing Ω (over the range 0 to 6.2; Table 3). These data confirm that carbonate chemistry has a significant role in the control of calcification (see Gattuso *et al.*, 1998*a* and references therein).

The curves described by the equations in Table 3 were used to predict the response of calcification to expected changes in Ω (Fig. 9) calculated from the updated IPCC estimates of temperature and pCO_2 increas-

TABLE 3. *Relationship between the relative rate of calcification (Y, % of the maximum rate measured) as a function of the aragonite saturation state (Ω) of photosynthetic and calcifying marine organisms and communities.**

System	*a*	*b*	*c*	*N*	r^2	Source
Bossiela orbigniana (temperate coralline alga)	77.2	0.24	16.8	6	0.95	Smith and Roth (1979)
Amphiroa foliacea (tropical coralline alga)	17.0	–0.99	–	5	0.99	Borowitzka (1981)
Porolithon gardineri (tropical coralline alga)	14.5	28.5	–	15	0.87	Agegian (1985) and Mackenzie and Agegian (1989)
Corallina pilulifera (temperate coralline alga)	44.5	–37.1	–	2	1	Gao *et al.* (1993b) and K. Gao (personal communication)
Stylophora pistillata (scleractinian coral)	226.5	0.69	127	5	0.99	Gattuso *et al.* (1998a)
Biosphere 2 reef mesocosm	21	–41.1	–	26	0.81 1	Langdon *et al.* (submitted)
Porites compressa (scleractinian coral)	29.1	41.9	–	2	1	F. Marubini and M. J. Atkinson (personal communication)

* In some instances the data were visually interpolated from figures. Ω was calculated according to Mucci (1983) using two parameters among the following: pH, TA, DIC and pCO_2. The data were fitted, depending on the distribution of the data points, to a linear ($Y = b + a\Omega$) or an exponential ($Y = a\ (1 - \exp(-\Omega/b) + c)$ function using Ω values ranging from 0 to 6.2. *N*, number of data; r^2, coefficient of determination.

es (Houghton *et al.*, 1996; Pittock, 1999). The decrease of calcification in response to the predicted change of Ω was estimated for each individual relationship and averaged. CO_2 emission scenarios considered were: high (IS92e), mid (IS92a), and low (IS92c).

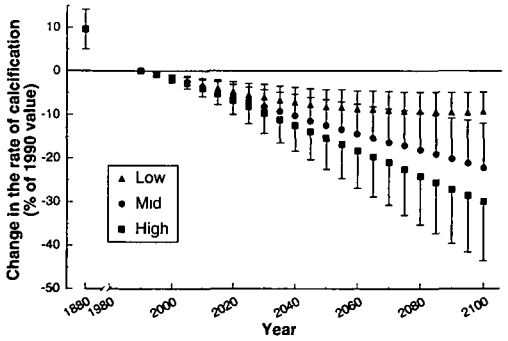

FIG. 9. Past and future changes in rate of $CaCO_3$ deposition (relative to 1990) of various marine photosynthetic and calcifying organisms and communities. Ω was calculated as described for Table 3. The following revised IPCC scenarios were considered (Houghton *et al.*, 1996): high (IS92e), mid (IS92a) and low (IS92c). Five of the data sets shown in Table 3 were used; the remaining two (Gao *et al.*, 1993*a*; F. Marubini and M. J. Atkinson, personal communication) could not be used because they did not cover the entire range of Ω encountered during the period 1880–2100. Mean I standard error of the mean; N = S.

It was assumed that the surface seawater and the atmosphere are in equilibrium with respect to CO_2 and that the increase in seawater temperature will be identical to the predicted increase in atmospheric temperature. According to these calculations, the rate of $CaCO_3$ deposition of photosynthetic and calcifying marine organisms and communities may have been 10% higher in 1880 than in 1990, and may decrease by 9 to 30% (mid estimate: 22%) between 1990 and 2100 (Fig. 9). The rate of calcification can be expected to decrease by 15% by 2065, the year during which pCO_2 would be twice its pre-industrial value under the IS92a scenario.

These estimates must be considered with caution. First, the database used in small ($N = 5$) and comprises a very limited number of taxa and communities. Second, although the effect of increased temperature on Ω has been considered, its effect on metabolic processes has not and the synergistic effect of both changes remains to be investigated. Third, most studies were carried out in the short term (typically hours to weeks), and very little is known on the acclimation processes that may enable these organisms and communities to overcome the adverse effect

TABLE 4. *Calcification, ratio of CO_2 released to $CaCO_3$ precipitated (Ψ), and predicted estimate of CO_2 release in seawater by $CaCO_3$ precipitation.**

	Pre-industrial	Present	Year 2065	Year 2100
Calcification	1	0.90	0.76	0.7
Ψ	0.52	0.57	0.65	0.69
CO_2 release	0.52	0.51	0.49	0.48

* The rate of calcification is arbitrarily set at 1 in 1880 and decreases as predicted in Figure 9 using the revised IPCC IS92a CO_2 emission estimate. Ψ was calculated as described by Frankignoulle *et al.* (1994) using the parameters shown in Table 1.

of decreased Ω. Of special interest is the response of photosynthesis to CO_2 fertilization as it may partly offset the inhibition due to the decreased $CaCO_3$ saturation state. Even though corals are bicarbonate users, a CCM-like system is costly in terms of energy; it might be possible that CO_2 use will increase, at it becomes more available, at the expense of HCO_3^- use (Beardall *et al.*, 1998). Increased pCO_2 could have other adverse effects such as to cause bleaching of corals and other symbiont-bearing reef invertebrates (Pêcheux, 1993).

Corals are being increasingly used as sources of environmental information (Barnes and Lough, 1996), and data obtained from coral cores might be used to check whether calcification has decreased over the past century. Unfortunately, only one paper provides information on past coral calcification. Lough and Barnes (1997) reported that the annual rate of calcification of 35 cores of the massive coral *Porites* declined significantly over the period 1934–1982 but other, sometimes larger, declines occurred prior to that period in a subset of 10 cores. The calcification record is strongly correlated with temperature and no information is available on the contribution of other environmental factors to the observed changes in calcification.

What is the implication of changes in coral calcification in terms of the global carbon cycle? Calcification is known to be a source of dissolved CO_2 in the surrounding water due to chemical equilibria involved in the precipitation process (Wollast *et al.*, 1980; Ware *et al.*,1992; Frankignoulle *et al.*, 1995). The ratio of released CO_2 to precipitated carbonate (Ψ) displays a positive feedback response to increasing pCO_2 (Frankignoulle *et al.*, 1994). It will increase from its present value of 0.57 (for pCO_2 = 360 µatm) to 0.69 for a pCO_2 of 706 µatm (Tables 1 and 4). The increase of pCO_2 in surface seawater resulting from anthropogenic carbon release in the atmosphere has therefore two antagonistic effects on air-sea CO_2 fluxes. The pCO_2 of coral reef waters will generally be equilibrated with the higher atmospheric CO_2 levels, but non-atmospheric inputs will (1) diminish as a consequence of the decreased rate of calcification, and (2) increase as a result of the increased Ψ value. Present available evidence suggest that calcification may be 30% lower in 2100 than it was in 1880 but changes in the carbonate equilibrium during the same period will increase the amount of CO_2 generated per mole $CaCO_3$ precipitated by 33% (Table 4). It is therefore predicted that the release of CO_2 to the atmosphere due to calcification may not change significantly in the future.

Cellular mechanisms

The cellular and molecular mechanisms involved in the response of calcification to increased pCO_2 observed at the organism and community levels are difficult to estimate due to the paucity of data. It is also difficult to predict the long-term response from short term experimental data.

It is predicted that seawater pH may decrease by 0.25 unit by 2100 (Table 1). There is no information available on the value of intracellular pH (pH_i) in coral cells and its control by seawater pH but it is well established that changes of external pH alter pH_i and regulate numerous cellular process (*e.g.*, Busa and Nucitelli, 1984). For example, a decrease of external pH as small as 0.07 unit induces a decrease of pH_i of 0.06 unit, which can trigger tyrosine phos-

phorylation and gene activation in renal epithelial cells (Yamaji *et al.,* 1994, 1997). It is also well established that the activity of a large number of intracellular enzymes is pH-sensitive and displays a pH optimum around the physiological range (Madshus, 1988). For example, the activity of phosphofructokinase, a key enzyme of the glycolytic pathway, exhibits a 10- to 20-fold reduction when pH decreases by as little as 0.1 unit below the physiological pH optimum (Trivedi and Danforth, 1966). Membrane permeability and conductance can also change greatly over a small pH interval. A decrease in external pH increase anionic permeability (reviewed by Madshus, 1988). Cell acidification can also increase the intracellular calcium concentration (*e.g.,* Neglescu and Machen, 1990), which can, in turn, trigger numerous events such as exocytosis, kinase activation, and stimulation of cell membrane transport (Carafoli and Penniston, 1985).

Despite the significant effect of pH_i on cellular processes, the questions are whether (1) the expected decrease of seawater pH is large enough to trigger a cellular response, and (2) the cellular machinery will be able to compensate for those changes in the long term. Bown (1985) calculated that pH_i would decrease by only 0.008 units in plant cells in response to an increase of pCO_2 of 330 μatm. Additionally, coral cells undergo daily changes in external pH larger than those expected in the next century: the pH of the coral surface can change within 5 min from 7.5 in the dark to 8.5 in the light (Kühl *et al.,* 1995). Changes measured in the coelenteric cavity of sea anemones are even more dramatic, with pH ranging from *ca.* 7 to 9 (Furla *et al.,* 1998*b*).

The increase in pCO_2 will not only affect pH_i but will also increase the total DIC concentration and change the proportion of its various species (Table 1). Zooxanthellae display considerable ability to change their mechanism of carbon supply depending on their environment. *In hospite,* the species of inorganic carbon transported across the algal membrane is CO_2 (produced by dehydration of bicarbonate in the host cell) whereas it is HCO_3^- in cultured zooxanthellae (Al-Moghrabi *et al.,* 1996; Allemand *et al.,* 1998*a*). As mentioned earlier, coral photosynthesis is saturated at ambient DIC concentration, so the predicted increase in HCO_3^- may have no effect on photosynthesis. However, the increased concentration of dissolved CO_2 together with the increase in uncatalyzed rate of CO_2 generation by HCO_3^- dehydration, may favor the diffusional carbon supply at the expense of the CCM-like carbon supply (Beardall *et al.,* 1998).

The effect of the predicted decrease of CO_3^{2-} is more difficult to analyze. It can be transported by the Cl^-/HCO_3^- exchanger (Boron, 1985). Its role as a carbon source for photosynthesis is poorly documented but seems minor (Smith, 1988). Goiran *et al.* (1996) found no evidence for CO_3^{2-} transport in the coral *Galaxea fascicularis.*

The physico-chemical characteristics of the extracytoplasmic calcifying fluid are unknown but if the carbonate saturation state is largely biologically-controlled, it is difficult to determine the cellular processes involved in the decrease of calcification as a function of decreasing $CaCO_3$ saturation state. When the $CaCO_3$ saturation state is altered by manipulating the seawater calcium concentration, the decrease in calcification can be due to Ca^{2+} limitation. The interpretation is less straightforward when the carbonate concentration is manipulated because seawater DIC may not dominate the carbon supply for the skeletal carbonate (see above) and there is likely no CO_3^{2-} carrier in the membrane of ectodermal cells (Goiran *et al.,* 1996). This, in combination with the near-equilibrium of many coral skeletons with seawater isotopes and chemistry may suggest that at least some taxa have a significant component of advective or rapid diffusional control over the composition of the ECF.

We suggest that the different calcification mechanisms used by corals and coralline algae could explain the higher sensitivity to changes in Ω of an algal-dominated coral reef community (Langdon *et al.,* submitted) and coralline algae (Smith and Roth, 1979; Borowitzka, 1981; Agegian, 1985; Mackenzie and Agegian, 1989) compared to zooxanthellate scleractinian corals (Gattuso *et al.,* 1998*a*). The site of skeletogenesis is ex-

ternal in calcareous algae (Borowitzka, 1984), which are therefore extremely sensitive to changes in seawater Ω. Conversely, $CaCO_3$ deposition occurs between the calicoblastic epithelium and the skeleton in corals, a much more isolated site.

CONCLUSION

Short-term experiments show that the rate of calcification of photosynthetic and calcifying organisms and communities decreases, sometimes dramatically, in response to increased pCO_2. However, inadequate understanding of the mechanisms of coral calcification and its interactions with photosynthesis, as well as the response of both processes to environmental variables severely limits our ability to provide an accurate prediction of future changes.

The responses of scleractinian corals to short-term changes in a single environmental parameter are reasonably well known through experimental and ecological observations, but synergistic effects are extremely difficult to predict. Furthermore, responses in experimental tanks, over periods of days to months, have been studied, but there has been no attempt to investigate the long-term (several years) response of reef communities to large-scale changes in environmental variables. FACE (Free Air CO_2 Enrichment; Hendrey, 1993) and FATI (Free Air Temperature Increase; Nijs *et al.*, 1996) have been used successfully to investigate the effect of elevated pCO_2 and temperature on terrestrial communities in open field conditions. The use of such approach in marine communities would be technically difficult, although not impossible, but probably too expensive to implement. The use of experimental mesocosms would certainly be easier due to recent advances in their design and maintenance (Adey, 1983; Jaubert, 1989).

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