Effects of acidified seawater on early life stages of scleractinian corals (Genus *Acropora*)

**Ryota Suwa · Masako Nakamura · Masaya Morita ·
Kazuaki Shimada · Akira Iguchi · Kazuhiko Sakai ·
Atsushi Suzuki**

Abstract Ocean acidification, caused by increased atmospheric carbon dioxide (CO_2) concentrations, is currently an important environmental problem. It is therefore necessary to investigate the effects of ocean acidification on all life stages of a wide range of marine organisms. However, few studies have examined the effects of increased CO_2 on early life stages of organisms, including corals. Using a range of pH values (pH 7.3, 7.6, and 8.0) in manipulative duplicate aquarium experiments, we have evaluated the effects of increased CO_2 on early life stages (larval and polyp stages) of *Acropora* spp. with the aim of estimating CO_2 tolerance thresholds at these stages. Larval survival rates did not differ significantly between the reduced pH and control conditions. In contrast, polyp growth and algal infection rates were significantly decreased at reduced pH levels compared to control conditions. These results suggest that future ocean acidification may lead to reduced primary polyp growth and delayed establishment of symbiosis. Stress exposure experiments using longer experimental time scales and lower levels of CO_2 concentrations than those used in this study are needed to establish the threshold of CO_2 emissions required to sustain coral reef ecosystems.

Keywords Carbon dioxide · Coral · Early life stages · Ocean acidification

R. Suwa and M. Nakamura contributed equally to this work.

R. Suwa · M. Nakamura (✉) · M. Morita · A. Iguchi · K. Sakai
Sesoko Station, Tropical Biosphere Research Center,
University of the Ryukyus, Okinawa 905-0227, Japan
e-mail: m_coral29@yahoo.co.jp

K. Shimada
Ocean Research Institute, University of Tokyo, Tokyo
164-8639, Japan

A. Suzuki
Geological Survey of Japan, National Institute of Advanced
Industrial Science and Technology (AIST),
Tsukuba 305-8567, Japan

Present Address:
R. Suwa
Seto Marine Biological Laboratory, Field Science Education
and Research Center, Kyoto University,
Wakayama 649-2211, Japan

Introduction

Ocean acidification and climate change caused by increasing atmospheric carbon dioxide (CO_2) concentrations threaten marine organisms, including corals [1]. Previous studies have suggested that ocean pH could be decreased by 0.3–0.4 units by the end of the century [2, 3]. Decreases in calcification rates and decalcification caused by lowered pH levels have been reported in several marine calcifying organisms, such as corals [4, 5], foraminiferans [6, 7], and coralline algae [8–10]. However, other physiological responses to ocean acidification remain elusive [11–13]. In addition, the range of pH tolerance of various life stages must be evaluated, as the effects of acidified seawater almost certainly differ across life stages [14]. The early life stages of many marine organisms appear to be particularly vulnerable to acidified seawater [11, 12]. For example, the sperm flagellar motility of several marine organisms has been found to be seriously impaired in acidified seawater [15, 16]. Several studies have recently addressed the impacts of CO_2-driven acidification on the early life stages of marine invertebrates, such as echinoderms [17, 18], crustaceans [19], and mollusks [20–22].

However, more information on the effects of acidification on the early life history of marine organisms is still required in order to be able to estimate the tolerance levels of early life stages, which are essential for recruitment success, species abundance, and distribution and population maintenance [23].

Scleractinian corals play important roles in maintaining coral reef ecosystems, which are biologically diverse and economically important marine ecosystems. Studies examining the effects of acidified seawater on scleractinian corals have mainly focused on decreases in calcification rates in adult corals (reviewed in [11, 24]), while the effects of acidified seawater on the early life stages of corals remain largely unexplored [12, 25]. The genus *Acropora* is one of the most widespread, abundant, and species rich (113–180 species) of coral genera on Pacific coral reefs [26, 27]. Previous studies have demonstrated that acidified seawater has negative effects on adult growth of *Acropora* species [9, 28], but the detailed effects of increased CO_2 on early life stages of this genus remain unknown (but see [12, 16]). In the study reported here, we examined the effects of increased CO_2 on early life stages of *Acropora* species to estimate their tolerance threshold for CO_2 concentrations by using a range of pH values (pH 7.3, 7.6, and 8.0). We focused on the two main early life stages: (1) planula (survival rate) and (2) primary polyps [polyp size and symbiosis establishment (algal infection rate)].

Materials and methods

Coral sampling

Gravid colonies of *Acropora digitifera* and *A. tenuis* were collected from a fringing reef near Oku fishery port, Okinawa Island, Japan (26°84′N, 128°29′E). The colonies were kept in a running seawater tank under natural light conditions at Sesoko Station, Tropical Biosphere Research Center, University of the Ryukyus, Okinawa, Japan. Coral spawning took place at night around the time of the full moon in May and June 2008. Gametes were collected after spawning in accordance with Morita et al. [29].

Preparation of pH-adjusted seawater

We used a pH-stat system similar to that described by Leclercq et al. [30] (Fig. 1). Seawater was filtered using an inline filter system (0.45 μm) and then bubbled with pure CO_2 to adjust the pH of the seawater using a pH regulator connected to a pH electrode (Micro-pH; Aquabase, Kanagawa, Japan). The pH conditions of the seawater were adjusted to pH 7.3, 7.6, and 8.0, respectively, based on the total hydrogen ion concentration pH scale. These pH values
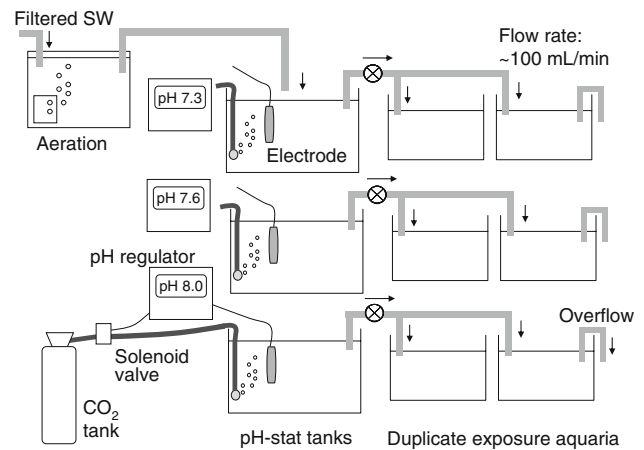
Fig. 1 Schematic of the pH-stat system used in the study. *Black arrows* direction of water flow, *SW* seawater

included the predicted values within 300 year (pH 7.3) [2] and were chosen to estimate the thresholds of tolerance for CO_2 concentrations in coral larvae and juvenile polyps. The pH regulator was adjusted using a solenoid valve that opens when pH increases to 0.01 higher than the desired level, thereby injecting pure CO_2 from the compressed CO_2 tanks. The temperature was recorded every 15 min using data loggers (Water Temp Pro, Onset, MA) and was maintained at 26.8 ± 0.5°C [mean ± standard deviation (SD)], which is the typical water temperature during the coral spawning season in Okinawa [31].

Duplicate aquaria (12 l) were filled with seawater adjusted to each pH value. Each aquarium was a flow-through system (Fig. 1). The stability of the pH in each aquarium was confirmed daily using a pH meter connected to a combined glass/reference pH electrode (713 pH Meter; Metrohm, Herisau, Switzerland), which was calibrated against the total hydrogen ion concentration pH scale buffers: TRIS and AMP [32]. The chemical and physical conditions of each treatment are summarized in Table 1. The pH and temperature were measured daily, and the aragonite saturation state was estimated from these parameter values and the mean total alkalinity of 2300 μmol/kg reported for this region [33] by using the computer program CO2SYS [34].

Planula experiment

In the planula experiment, planular larvae of two *Acropora* spp. (*A. digitifera* and *A. tenuis*) were prepared by mixing gametes from all spawned colonies to examine the responses of planular larvae to acidified seawater and differences in inherent stress responses between different coral species within same genus. Planulae were maintained in a container filled with filtered seawater until the experiment started. Water was exchanged twice per day to

Table 1 Summary of physical and chemical conditions in each experimental aquarium

pH_{tot}^{a}	Water temperature (°C)[a]	HCO_3 (µmol/kg)	CO_3 (µmol/kg)	pCO_2 (µatm)	Ω_{Arag}
8.03 ± 0.03	26.8 ± 0.5	1760–1815	195–220	400–475	3.2–3.5
7.64 ± 0.12	26.8 ± 0.5	1995–2115	75–125	905–1660	1.2–2.0
7.31 ± 0.11	26.8 ± 0.5	2150–2205	40–60	2115–3585	0.6–1.0

Ω_{Arag}, Aragonite saturation state

The carbon parameters were calculated based on pH_{tot}, temperature, salinity of 34.0, and a total alkalinity of 2300 µmol/kg

[a] Mean ± standard deviation (SD) ($n = 7$)

maintain the good health status of the larvae. Fifty planulae (5 days old) were maintained in a 120-ml glass bottle, which was covered by a plankton net (pore size 180 µm). Five bottles were prepared in each of two aquaria for each pH treatment, and pH-adjusted seawater was supplied into each bottle in the aquaria. The survival rates of the planulae maintained at the three pH conditions were examined after 7 days of breeding by counting the surviving larvae.

Polyp and symbiosis experiment

Primary polyps were prepared by inducing the settlement of *A. digitifera* planular larvae (7 days old) with the coral metamorphosis inducer peptide Hym-248 [35]. A 20-µl aliquot of 2×10^{-4} M Hym-248 in filtered seawater (FSW: pore size 0.22 µm) was added to a well of a 6-well plastic culture plate. Four larvae in 20 µl FSW were added to one drop of peptide, and 7 drops of the peptide solution were added to each well. Larvae that settled on the seawater surface and on the side of the plastic culture plate were removed, and those that settled at the bottom of the wells were maintained at 27.0°C with a daily change of FSW for 4 days. In each treatment, eight 6-well culture plates containing settled polyps were prepared, and pH-adjusted seawater was supplied to each plate in a flow-through aquarium. The areas occupied by polyps exposed to each of the three pH conditions for 10 days were measured using a digital camera (S9; Canon, Tokyo, Japan) connected to a dissecting microscope (MZ12.5; Leica, Solms, Germany) and the ImageJ 1.38 program (National Institutes of Health, Bethesda, MD) in order to evaluate the size of polyps. We also observed the infection rate of polyps by zooxanthellae after 14 days of incubation under each pH condition, as described in the following section.

Zooxanthellae isolated from the giant clam *Tridacna crocea* and cultured at Okinawa Prefectural Sea Farming Center, Motobu, Okinawa, Japan, were used for the algal infection experiment. In this experiment, we infected *A. digitifera* primary polyps with zooxanthellae from the giant clam *T. crocea* because the primary polyps tended to acquire algae from this bivalve more efficiently than from other hosts, including *A. digitifera* [36, 37]. The number of zooxanthellae in FSW was counted using a hemocytometer, and a zooxanthella solution (1×10^6 cells/ml in FSW) was prepared. Primary polyps of *A. digitifera* were infected with zooxanthellae 14 days after being exposed to acidified seawater. A 10-ml aliquot of the zooxanthella solution was added to each flow-through aquarium. Eight 6-well plastic plates, each containing 25 primary polyps, were placed in each pH condition. The number of polyps infected with zooxanthellae in each condition was counted under a dissecting microscope (MZ12.5; Leica) on the first, second, and fourth days of the experiment. At each observation, all 6-well plastic plates with polyps in each pH condition were removed at the same time and put in FSW without zooxanthellae for counting. It took approximately 3 h to conduct all observations. A polyp that had acquired more than one zooxanthella in its tissue was counted as an infected polyp, and the rate of infected polyps was calculated.

Analyses

All statistical analyses were performed using JMP software (SAS Institute, Cary, NC). Variations in the survival rates of each *Acropora* species among pH treatments were examined using two-way factorial analysis of variance (ANOVA). Differences in planula survival rates between the two species were analyzed by the *t* test. Variations in polyp sizes among pH treatments were examined using two-way factorial ANOVA. Algal infection rates were examined using three-way factorial ANOVA to analyze the effects of pH, day, and aquarium. In each experiment, Tukey's HSD multiple comparison tests were conducted as post-hoc tests when ANOVAs detected significant differences. All ANOVA tests were conducted with appropriate transformations where necessary.

Results

Larval survival rate

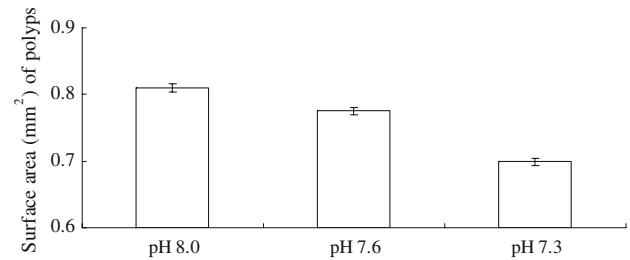The *A. digitifera* larval survival rate did not differ significantly among pH treatments (two-way ANOVA,
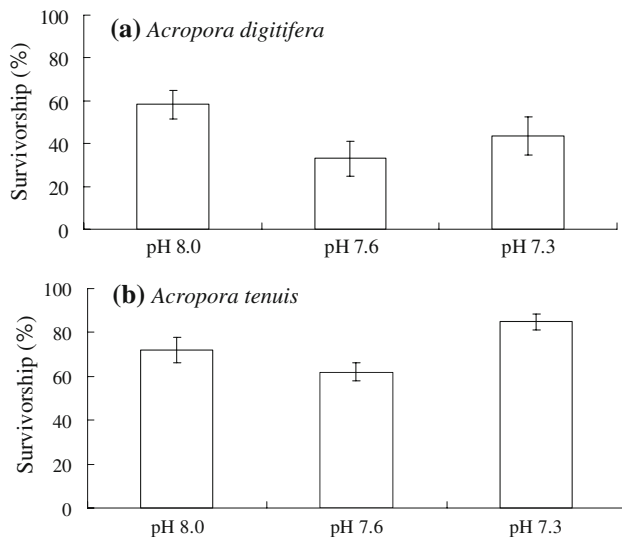
Fig. 2 Mean survival rates of *Acropora* corals after 7 days of breeding in three pH conditions: **a** *Acropora digitifera*; **b** *A. tenuis*. *Bars* indicate means and standard deviations ($n = 10$)
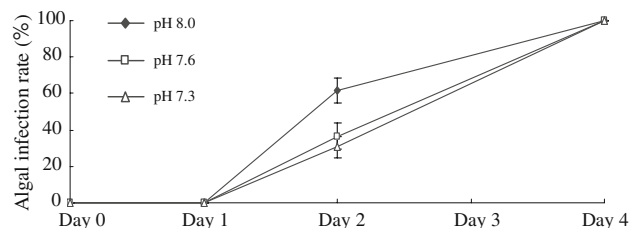

Fig. 3 Mean areas of occupation by primary polyps of *Acropora digitifera* after 10 days of incubation under three pH conditions. *Bars* indicate means and standard deviations ($n = 332$ for pH 7.3, $n = 279$ for pH 7.6, $n = 362$ for pH 8.0)

Fig. 4 Mean algal infection rates of *Acropora digitifera* primary polyps exposed to zooxanthellae from giant clams under three pH conditions. Aposymbiotic polyps were pre-incubated under the three pH conditions for 14 days after settlement. *Bars* indicate means and standard deviations ($n = 8$)

$F_{2,29} = 2.403$, $P = 0.112$, Fig. 2a). The highest survival rate was observed under control conditions [58.2% $\pm$ 6.7, mean $\pm$ standard error (SE)] and the lowest survival was at pH 7.6 (33.0% $\pm$ 8.0). However, the survivorship of *A. tenuis* was significantly different among pH conditions (two-way ANOVA, $F_{2,29} = 5.756$, $P = 0.010$, Fig. 2b), and there was a significant difference in survivorship of this between pH 7.6 (62.0% $\pm$ 4.2; the lowest) and pH 7.3 (84.8% $\pm$ 3.8; the highest) (Tukey's HSD post-hoc test, $P < 0.05$). Survivorship also differed between species (t test, $P < 0.0001$).

Polyp size and algal infection rate

Polyps were significantly smaller under lower pH conditions (two-way ANOVA, $F_{2,967} = 92.142$, $P < 0.0001$; Fig. 3). Significant decreases were detected with each decrease in pH (Tukey's HSD post-hoc test, $P < 0.05$). The largest area was observed under pH 8.0 (0.81 mm^2 $\pm$ 0.006, mean $\pm$ SE) and the smallest under pH 7.3 (0.70 mm^2 $\pm$ 0.006). Algal infection rates differed among pH treatments over observation days (three-way ANOVA, $F_{6,72} = 13.157$, $P < 0.0001$; Fig. 4). No polyps contained zooxanthellae 1 day after the addition of algae to the seawater, but from the second day of incubation onwards, polyps started to acquire zooxanthellae. The number of polyps acquiring zooxanthellae tended to decrease with increasing acidity (three-way ANOVA, $F_{2,18} = 14.043$, $P = 0.0002$; Fig. 4). Significant differences between the control (pH 8.0) and other pH conditions (pH 7.6 and 7.3) were detected (Tukey's HSD post-hoc test, $P < 0.05$, respectively), but all polyps in all treatments acquired zooxanthellae 4 days after the algal treatment (Fig. 4).

Discussion

We examined the effects of reduced seawater pH [pH 7.3, 7.6, and control (pH 8.0)] on early life stages of *Acropora* spp. using a pH-stat system and found that under lower pH conditions, *A. digitifera* coral polyps were smaller, and their establishment of symbiosis with zooxanthellae was delayed. However, the survivorship of coral larvae was not obviously affected by acidified seawater, as no consistent patterns in survivorship were observed with reduced pH conditions.

Our data indicate the possibility that the survival of coral larvae may not be strongly affected by pH change. In other words, coral larvae may be able to tolerate ambient pH decreases of at least 0.7 pH units. This does not correspond with results from other studies on the effects of acidification on larval stages of marine benthos ([12, 18], but see [38]). High mortality (100%) of *Ophiothrix fragilis* larvae was reported within 8 days under higher pH conditions than those used in this study as the low pH conditions [18]. Kurihara and others reported abnormalities in larval development and skeleton and shell synthesis in echinoderms and mollusks (reviewed in [12]). A reason for the discrepancies between our results and those of previous studies may be that coral larvae do not have any calcareous structure. An earlier study showed that the soft bodies

(polyps) of scleractinian coral species can survive for 12 months at pH 7.3–7.6, as evidence by maintaining algal symbionts and developing gametes [4]. The soft bodies of corals may regulate the acid–base balance in their tissue, although it is known that increasing external CO_2 causes the acidification of body fluids and changes in ion balances within the bodies of marine organisms [11]. During larval dispersal, coral larvae of the genus *Acropora* are likely to be exposed to large diurnal changes in pH in near-surface water caused by high uptakes of CO_2 for benthic photosynthesis and slow mixing with offshore waters (e.g., ranging from a nighttime pH of 7.8 to a daytime pH of 8.8 on the reef flats and crests of Shiraho Reef [39]) and, therefore, larvae may be able to tolerate short exposures to the low pH levels used in our study. Alternatively, the inferred tolerance of coral to low pH could result from a reduction in metabolic rate. Some marine invertebrates demonstrate metabolic depression under low ambient pH conditions [40, 41], and this is thought to be a strategy for enhancing survival under stressful conditions [42]. Further investigations are necessary to understand the response mechanisms of coral larvae to acidified seawater, such as regulation of the acid–base balance and/or reductions in metabolic rate.

The findings of our study also imply that differences in the stress tolerance of coral larvae to ocean acidification during the dispersal phase may be species-specific because survivorship after 1 week of exposure to pH-controlled seawater was higher in *A. tenuis* than in *A. digitifera* at all pH levels (Fig. 2). *A. tenuis* planulae have previously been shown to have a higher survival rate 10 days after spawning than those of *A. digitifera* in ambient seawater [43, 44]. Differences in calcification and net production in response to acidification between species (genera) have been previously reported for adult corals [9]. Further studies are required to examine the potential stress responses of the larvae of various coral species as well as the physiological mechanisms that govern those differences.

The decrease in *A. digitifera* polyp size is thought to be due to the decrease in skeletal growth in acidified seawater. Skeletal calcification of primary polyps is also thought to be affected by a reduced aragonite saturation state in lower pH conditions, which has been well reported in adult corals (e.g., [4, 5, 12, 24]). Our results showing decreased polyp size in lowered pH conditions are in accordance with a previous report that showed decreased *Porites astreoides* primary polyp size in HCl-acidified seawater [25]. This smaller size of polyps may imply lower survivorship of juveniles [25]. The reduced infected polyp ratio at pH 7.3 and 7.6 on the second day of the experiment in our study also implies reduced survivorship because reduced zooxanthellae availability under natural conditions may make it more difficult for juvenile corals to establish symbiosis.

A possible factor delaying zooxanthellae infection under low pH conditions is the decreased feeding ability related to smaller polyps in acidified seawater. Ciliated gastrodermal cells in the primary polyps of *Acropora* spp. are known to produce water currents for capturing material in their coelenterons [37]. A larger polyp may have a greater ability to capture zooxanthellae because of its higher number of ciliated gastrodermal cells. Therefore, reduced polyp size may weaken the ability to capture food and zooxanthellae. Further studies are needed to investigate the effects of ocean acidification on symbiosis establishment.

The results of our study provide new insights into the effects of ocean acidification on the planular larval and post-settlement stages of corals. They imply that ocean acidification may have the potential to negatively affect the maintenance of coral communities through reductions in juvenile polyp size and delays in the acquisition of symbiotic algae. Increased risks of mortality have been demonstrated for smaller corals [45, 46]. For evaluating the near future effects of ocean acidification on corals, the effects of several levels of pH between the ambient and pH 7.6 must be examined. Moreover, stress exposure experiments using longer experimental time scales and lower levels of CO_2 concentrations than those used in this study are necessary for establishing the threshold of CO_2 emissions required to sustain coral reef ecosystems [38, 47].

References

1. Hoegh-Guldberg O, Mumby PJ, Hooten AJ, Steneck RS, Greenfield P, Gomez E, Harvell CD, Sale PF, Edwards AJ, Caldeira K, Knowlton N, Eakin CM, Iglesias-Prieto R, Muthiga N, Bradbury RH, Dubi A, Hatziolos ME (2007) Coral reefs under rapid climate change and ocean acidification. Science 318:1737–1742
2. Caldeira K, Wickett ME (2003) Anthropogenic carbon and ocean pH. Nature 425:365
3. Orr JC, Fabry VJ, Aumont O, Bopp L, Doney SC, Feely RA, Gnanadesikan A, Gruber N, Ishida A, Joos F, Key RM, Lindsay K, Maier-Reimer E, Matear RJ, Monfray P, Mouchet A, Najjar R, Plattner GK, Rodgers KB, Sabine CL, Sarmiento JL, Schlitzer R, Slater RD, Totterdell IJ, Weirig MF, Yamanaka Y, Yool A (2005) Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. Nature 437:681–686
4. Fine M, Tchernov D (2007) Scleractinian coral species survive and recover from decalcification. Science 315:1811
5. Marubini F, Christine AE, Ferrier-Page's AE, Furla P, Allemand D (2008) Coral calcification responds to seawater acidification: a working hypothesis towards a physiological mechanism. Coral Reefs 27:491–499
6. Bijma J, Honisch B, Zeebe RE (2002) Impact of the ocean carbonate chemistry on living foraminiferal shell weight: comment

on "Carbonate ion concentration in glacial-age deep waters of the Caribbean Sea" by Broecker WS and Clark E. Geochem Geophys Geosyst 3:1064. doi:10.1029/2002GC000388

7. Erez J (2003) The sources of ions for biomineralization in foraminifera and their implications for paleoceanographic proxies. In: Dove PM, De Yoreo JJ, Weiner S (eds) Reviews in minerology and geochemistry, vol 54: biomineralization. Minerological Society of America, Washington D.C., pp 115–149

8. Gao K, Aruga Y, Asada K, Ishihara T, Akano T, Kiyohara M (1993) Calcification in the articulated coralline alga *Corallina pilulifera*, with special reference to the effect of elevated CO_2 concentration. Mar Biol 117:129–132

9. Anthony KRN, Kline DI, Diaz-Pulido G, Dove S, Hoegh-Guldberg O (2008) Ocean acidification causes bleaching and productivity loss in coral reef builders. Proc Natl Acad Sci USA 105:17442–17446

10. Kuffner IB, Andersson AJ, Jokiel PL, Rodgers KS, Mackenzie FT (2008) Decreased abundance of crustose coralline algae due to ocean acidification. Nat Geosci 1:114–117

11. Raven J, Caldeira K, Elderfield H, Hoegh-Guldberg O, Liss P, Riebesell U, Shepherd J, Turley C, Watson A (2005) Ocean acidification due to increasing atmospheric carbon dioxide. Policy document 12/05. Royal Society, London

12. Kurihara H (2008) Effects of CO_2-driven ocean acidification on the early developmental stages of invertebrates. Mar Ecol Prog Ser 373:275–284

13. Pörtner HO (2008) Ecosystem effects of ocean acidification in times of ocean warming: a physiologist's view. Mar Ecol Prog Ser 373:203–217. doi:10.3354/meps07768

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

15. Havenhand JN, Buttler FR, Thorndyke MC, Williamson JE (2008) Near-future levels of ocean acidification reduce fertilization success in a sea urchin. Curr Biol 18:R651–R652

16. Morita M, Suwa R, Iguchi A, Nakamura M, Shimada K, Sakai K, Suzuki A (2009) Ocean acidification reduces sperm flagellar motility in broadcast spawning reef invertebrates. Zygote. doi:10.1017/S0967199409990177

17. Kurihara H, Shirayama Y (2004) Effects of increased atmospheric CO_2 on sea urchin early development. Mar Ecol Prog Ser 274:161–169

18. Dopont S, Harenhand J, Thorndyke W, Peck L, Thorndyke M (2008) Near-future level of CO_2-driven ocean acidification radically affects larval survival and development in the brittlestar *Ophiothrix fragilis*. Mar Ecol Prog Ser 373:285–294. doi:10.3354/meps07800

19. Kurihara H, Ishimatsu A (2008) Effects of high-CO_2 seawater on the copepod *Acartia tsuensi* through all life stages and subsequent generations. Mar Pollut Bull 56:1086–1090

20. Kurihara H, Kato S, Ishimatsu A (2007) Effects of increased seawater pCO_2 on early development of the oyster *Crassostrea gigas*. Aquatic Biol 1:91–98

21. Kurihara H, Asai T, Kato S, Ishimatsu A (2008) Effects of elevated pCO_2 on early development in the mussel *Mytilus galloprovincialis*. Aquat Biol 4:225–233

22. Ellis R, Bersey J, Rundle SD, Hall-Spencer JM, Spicer JI (2009) Subtle but significant effects of CO_2 acidified seawater on embryos of the intertidal snail, *Littorina obtusata*. Aquat Biol 5:41–48. doi:10.3354/ab00118

23. Fabry VJ, Seibel BA, Feely RA, Orr JC (2008) Impacts of ocean acidification on marine fauna and ecosystem processes. J Mar Sci 65:414–432

24. Kleypas JA, Feely RA, Fabry VJ, Langdon C, Sabine CL, Robbins LL (2006) Impacts of ocean acidification on coral reefs and other marine calcifiers: a guide for future research. Report of a

workshop. NSF, NOAA, and the US Geological Survey, St Petersburg, FL

25. Albright R, Mason B, Langdon L (2008) Effect of aragonite saturation state on settlement and post-settlement growth of *Porites astreoides* larvae. Coral Reefs 27:485–490

26. Veron JEN (2000) Corals of the world. Australian Institute of Marine Science, Townsville

27. Wallace CC (1999) Staghorn corals of the world: a revision of the genus *Acropora*. CSIRO Publ, Collingwood

28. Schneider K, Erez J (2006) The effect of carbonate chemistry on calcification and photosynthesis in the hermatypic coral *Acropora eurystoma*. Limnol Oceanogr 51:1284–1293

29. Morita M, Nishikawa A, Nakajima A, Iguchi A, Sakai K, Takemura A, Okuno M (2006) Eggs regulate sperm flagellar motility initiation, chemotaxis, and inhibition in the coral, *Acropora digitifera*, *A. gemmifera*, and *A. tenuis*. J Exp Biol 209:4574–4579

30. Leclercq N, Gattuso JP, Jaubert J (2002) Primary production, respiration, and calcification of a coral reef mesocosm under increased CO_2 partial pressure. Limnol Oceanogr 47:558–564

31. Suwa R, Hirose M, Hidaka M (2008) Seasonal fluctuation in zooxanthella composition and photophysiology in the corals *Pavona divaricata* and *P. decussata* in Okinawa. Mar Ecol Prog Ser 361:129–137

32. Dickson AG, Sabine CL, Christian JR (eds) (2007) Guide to best practices for ocean CO_2 measurements. PICES Special Publication 3. North Pacific Marine Science Organization (PICES), Ottawa

33. Fujimura H, Oomori T, Maehira T, Miyahira K (2001) Change of coral carbon metabolism influenced by coral bleaching. Galaxea 3:41–50

34. Lewis E, Wallace DWR (1998) Program developed for CO_2 system calculations, ORNL/ CDIAC-105. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, Oak Ridge

35. Iwao K, Fujisawa T, Hatta M (2002) A cnidarian neuropeptide of the GLWamide family induces metamorphosis of reef-building corals in the genus *Acropora*. Coral Reefs 21:127–129

36. Yuyama I, Hayakawa H, Endo H, Iwao K, Takeyama H, Maruyama T, Watanabe T (2005) Identification of symbiotically expressed coral mRNAs using a model infection system. Biochem Biophys Res Commun 336:793–798

37. Hirose M, Yamamoto H, Nonaka M (2008) Metamorphosis and acquisition of symbiotic algae in planula larvae and primary polyps of *Acropora* spp. Coral Reefs 27:247–254

38. Clark D, Lamare M, Barker M (2009) Response of sea urchin pluteus larvae (Echinodermata: Echinoidea) to reduced seawater pH: a comparison among a tropical, temperate, and a polar species. Mar Biol 156:1125–1137. doi:10.1007/s00227-009-1155-8

39. Suzuki A, Nakamori T, Kayanne H (1995) The mechanism of production enhancement in coral reef carbonate systems: model and empirical results. Sediment Geol 99:259–280

40. Reipschläger A, Pörtner HO (1996) Metabolic depression during environmental stress: the role of extra- versus intracellular pH in *Sipunculus nudus*. J Exp Biol 199:1801–1807

41. Michaelidis B, Ouzounis C, Paleras A, Pörtner HO (2005) Effects of long-term moderate hypercapnia on acid–base balance and growth rate in marine mussels *Mytilus galloprovinciallis*. Mar Ecol Prog Ser 293:109–118

42. Guppy M, Withers PC (1999) Metabolic depression in animals: physiological perspectives and biochemical generalizations. Biol Rev 74:1–40

43. Nishikawa A, Katoh M, Sakai K (2003) Larval settlement rates and gene flow of broadcast-spawning (*Acropora tenuis*) and planula-brooding (*Stylophora pistillata*) corals. Mar Ecol Prog Ser 256:87–97

44. Nishikawa A, Sakai K (2005) Settlement-competency period of planulae and genetic differentiation of the scleractinian coral *Acropora digitifera*. Zool Sci 22:391–399

45. Babcock RC (1991) Comparative demography of three species of scleractinian corals using age- and size-dependent classifications. Ecol Monogr 61:225–244

46. Babcock R, Mundy C (1996) Coral recruitment: consequences of settlement choice for early growth and survivorship in two scleractinians. J Exp Mar Biol Ecol 206:179–201

47. Shirayama T, Thornton H (2005) Effects of increased atmospheric CO_2 on shallow water marine benthos. J Geophys Res 110:1–7