Proceedings of 10th International Coral Reef Symposium, 38-42 (2006)

Temperature tolerance of symbiotic and non-symbiotic coral larvae

Andrew H BAIRD[1, 2*], James P GILMOUR[3,4], Takayuki M KAMIKI[1], Masanori NONAKA[5], Morgan S PRATCHETT[2], Hiromi H Yamamoto[5] and Hideo YAMASAKI[1]

[1]Center of Molecular Biosciences (COMB), University of the Ryukyus, Nishihara, Okinawa, 903-0213 Japan
[2]Centre for Coral Reef Biodiversity, School of Marine Biology and Aquaculture, James Cook University, Townsville, Queensland 4811 Australia
[3] School of Animal Biology M092, University of Western Australia, 35 Stirling Hwy, Crawley, Western Australia, 6009
[4]The Australian Institute of Marine Science, PO Box 83, Fremantle, Western Australia, 6959
[5]Okinawa Churaumi Aquarium, Motobu-cho, Okinawa, Japan 905-0206
*Corresponding author: Andrew H Baird, e-mail: ahbaird@sigmaxi.org

Abstract Coral larvae represent the ideal model with which to test many aspects of the relationship between symbiotic zooxanthellae and the coral host because this is the only stage in the life history of the organism where it is easy to produce both symbiotic and non-symbiotic individuals for comparison. We used this larval model to investigate the effect of the presence of the symbiont on the thermal tolerance of coral larvae. We exposed symbiotic and non-symbiotic larvae of *Acropora muricata* to 3 temperatures under a mildly stressful level of light (125-150 μE m^{-2} s^{-1}) for a 7-day period. Many larvae survived well at 28 and 32° C. In contrast, all larvae died within 40 h at 36° C. Interestingly, there was no major difference in survivorship between symbiotic and non-symbiotic larvae at any temperature indicating that the symbionts had little influence on larval susceptibility to heat and light. However, the density of symbionts in the larvae when the experiments were initiated was low and therefore, unlikely to affect the physiology of the host.

Key words: adaptation, bleaching, climate change, coral reef, symbiosis

Introduction

Reef corals form obligate symbiotic associations with dinoflagellates commonly known as zooxanthellae. Under stress, such as high sea water temperature, this association can break down with consequent loss of the pigmented algal symbionts. This process is known as coral bleaching. Zooxanthellae provide 30 % of the total nitrogen and 91 % of carbon needs of the coral host (Bythell 1988). Consequently, when the density of zooxanthellae is reduced following bleaching the coral loses an important source of energy (Porter et al. 1989) with a subsequent reduction in colony growth and/or reproduction (Baird and Marshall 2002). If the loss of symbionts is very high, or prolonged, the colony will die (Glynn and D'Croz 1990).

Many aspects of the bleaching phenomenon remain puzzling from a physiological perspective (Brown and Ogden 1993). In many coral species, the relationship breaks down at as little as 1° C above long term summer average temperatures (Goreau and Hayes 1994). The absolute temperatures that induce the breakdown in symbiosis are not that high, with bleaching typically occurring between 28 and 32° C, depending on the thermal history of the location (Coles and Brown 2003). Few other animals are affected by these thermal anomalies (at least in the initial stages of the event) and nor would they be predicted to be affected because cellular processes, such as the denaturing of proteins, are affected below 37° C. Similarly, few free-living algae bleach during these events. Furthermore, zooxanthellae isolated from coral hosts can be maintained in cultures for long periods at 37° C (Peter Ralph pers comm.), and many of the zooxanthellae recovered from corals following bleaching are healthy (Ralph et al. 2001). Most theories assume, therefore, that it is the communication between the host and the symbiont that is being disrupted. However, it is also possible that the coral host is unusually susceptible to temperature stress.

Coral larvae are the ideal model with which to test many aspects of the relationship between symbiont and host. In many broadcast spawning coral species, gametes do not contain zooxanthellae (Harrison and Wallace 1990), however, larvae can readily be infected with different strains of zooxanthellae under experimental conditions (Schwarz et al. 1999; van Oppen 2001). Consequently, it is possible to compare symbiotic and non-symbiotic individuals of the same species, and furthermore, readily control the identity of both the host and the symbiont. This control over the make up of the association is not possible in adults, because even completely bleached adults still contain residual densities of symbionts (Hoegh-Guldberg and Smith 1989), and it is not known whether they can take up symbionts from the environment (Hoegh-Guldberg et al. 2002). Furthermore, coral larvae are non-feeding (Fadlallah 1983) with an extraordinary capacity to delay

metamorphosis (Harii et al. 2002) and can therefore be maintained for long periods in sterile conditions (Morse et al. 1996). In addition, larvae are small and abundant making them easy to manipulate and allowing for many individuals to be compared for statistical power. The aim of this research was to use this larval model to compare the thermal tolerance of symbiotic and non-symbiotic individuals.

Materials and Methods

Inoculation of larvae with homologous symbionts

The first priority was to develop techniques to infect a high proportion of the larvae offered symbionts, to ensure the comparisons between the treatments groups were meaningful. Previous research has demonstrated that rates of infection are influenced by a number of variables including the density of the inoculum (Kinzie et al. 2001), the light environment (van Oppen 2001), the strain of zooxanthellae (Weis et al. 2001) and the presence of food (Schwarz et al. 1999). Temperature is also likely to be important. The conditions we settled on for the initial inoculation of larvae were: a temperature of 26-28° C i.e. similar to the sea surface temperature in the adult habitat in June in Okinawa; a low, non-stressful light level (50-80 μE m^{-2} s^{-1}); and very high densities of homologous zooxanthellae (approximately 1.0 x 10^6 per ml). The addition of artemia to induce zooxanthellae uptake was considered necessary, because coral larvae are generally considered to be non-feeding and the behaviour previously described as feeding in *Fungia scutaria* larvae (Schwarz et al. 1999) is more likely to be typical settlement behaviour. Larvae of the common scleractinian *Acropora muricata* were cultured following the methods of Babcock et al. (2003). Approximately 72 h after spawning, when larvae were motile with an oral pore and had begun typical settlement behaviour including "testing" the substratum (Harrison and Wallace 1990) they were placed in 1 l glass jars in 0.2 μm filtered seawater (FSW) at initial densities of approximately 1000 larvae per l under cool fluorescent lights (50-80 μE m^{-2} s^{-1}) with a 12:12 h light: dark photo-period. Freshly isolated zooxanthellae were used for infection of larvae. *Acropora muricata* nubbins were rinsed gently with 0.2 μm FSW and then coral tissue was removed from the coral skeleton by Water-Pik with 0.45 μm FSW (Johannes and Wiebe 1970). To remove the mucus produced during the isolation procedure, coral blastate was first filtered through a 350-μm-nylon mesh. The blastate was then homogenized with a potter homogenizer. Following centrifugation at 115 g for 5 min, filtration through 40-μm mesh was performed. The algal pellet was washed three times by centrifugation (1500 g for 5 min, 2000 g for 10 min and 3000 g for 15 min). This method of isolating and cleaning zooxanthellae yields intact algal cells. This was checked microscopically under 400 × magnification using a Nikon OPTIPHOT-2 microscope. The isolated zooxanthellae were then introduced into the larval culture. Under these condition 100 % of larvae became infected within 72 h of the addition of the zooxanthellae. In control culture, where no zooxanthellae were added, none of the larvae became infected. The cultures of infected larvae were followed for another 32 days to estimate the population growth rate of zooxanthellae within the larval hosts. Ten larvae were removed from the cultures, squashed and mounted on a slide, and viewed under blue-violet light using a fluorescent microscope at 7, 12, 15, 18, 24 and 32 days after spawning. The blue-violet light causes the chlorophyll with the zooxanthellae to fluoresce bright red allowing even a single symbiont to be seen in a larva.

Larval survivorship under different temperatures

Following the successful inoculation of *Acropora muricata* larvae with homologous zooxanthellae, 3 replicate batches of symbiotic and non-symbiotic larvae were introduced into 25 ml glass jars containing 0.2 μm FSW in water baths maintained at 28, 32 and 36° C with RACOMACE, Model HT-10D heaters under a mildly stressful level of light (125-150 μE m^{-2} s^{-1}) provided by cool fluorescent globes. Some light stress is required, in addition to temperature, to induce a stress response in zooxanthellae. Water was changed approximately every 72 h. Twenty larvae were placed in each jar, providing a total of 60 larvae per combination of temperature and treatment (i.e. zooxanthellate vs. non-zooxanthellate). The number of larvae alive in each jar was counted directly after 6, 18, 24, 40, 96 and 198 h to estimate survivorship through time. Dead larvae quickly dissolve and any larva visible was considered to be alive.

Results and Discussion

Many larvae survived well at 28 and 32° C with approximately 30 % of the cohort remaining alive after 198 h (Fig. 1). While there was no major difference in survivorship between zooxanthellate and non-zooxanthellate larvae at these temperatures, at 28° C survivorship of zooxanthellate larvae was consistently higher than the non-zooxanthellate larvae, and it is possible this trend could have resulted in significant difference in survivorship if the experiments had been continued (Fig. 1). Higher survivorship of zooxanthellate larvae at non-stressful temperatures is to be expected because the acquisition of symbionts give the larvae the potential to be autotrophic and supplement the initial maternal energy reserves with nutrition from the zooxanthellae (Richmond 1981).

In contrast, all zooxanthellate larvae died within 18 h, and all non-zooxanthellate larvae within 40 h, at 36° C (Fig. 1). While this small difference in survivorship between the groups is unlikely to be ecological meaningful, this is the result we would have predicted. Current theories to explain coral bleaching suggest that heat makes light toxic. At high temperatures even normal light levels overload photosystem II of the zooxanthellae (Jones et al. 1998; Takahashi et al. 2004) resulting in the production of reactive oxygen species (ROS) by photosynthetic electron

transport. At least some of these ROS are permeable through the cell wall (Nakamura and van Woesik 2001) and may damage tissue beyond the zooxanthellae. Consequently, we would have predicted that at high temperature larvae containing zooxanthellae would be affected by these ROS and mortality rates would be higher than larvae that lacked zooxanthellae. However, it is also clear that the upper thermal limit of *Acropora muricata* larvae lies somewhere between 32 and 36° C suggesting that the coral host, or at least these early life history stages, are susceptible to temperatures that do not typically challenge many other marine animals.

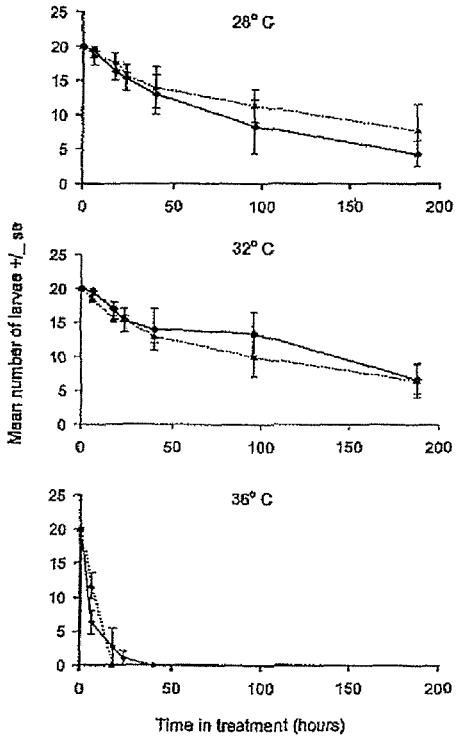

Fig. 1. Survivorship of *Acropora muricata* larvae through time at 3 different temperatures. Dotted lines represent symbiotic larvae and the solid lines are non-symbiotic larvae.

The failure to detect any major difference between zooxanthellate and non-zooxanthellate larvae may have been due to the low the density of symbionts when the experiments were initiated. The mean density of zooxanthellae in larvae at start of the experiments was 7.1 ± 2.45 per larva and possibly too low to affect the physiology of the host. By way of comparison, values for the mean number of zooxanthellae per egg in broadcast spawning species with vertical transmission include 3000 in *Porites cylindrica* and 1300 in *Montipora digitata* (Hirose et al. 2001). In the original larvae cultures maintained at 26° C under low light the mean symbiont density continued to grow, reaching a mean of 360 ± 60 per larva after 30 d, with no sign of stabilizing (Fig. 2). Clearly, these larvae can accommodate much higher densities of zooxanthellae than those in the larvae at the time the temperature manipulations commenced. Future studies, using the larval model, should be conducted after these densities have stabilized.

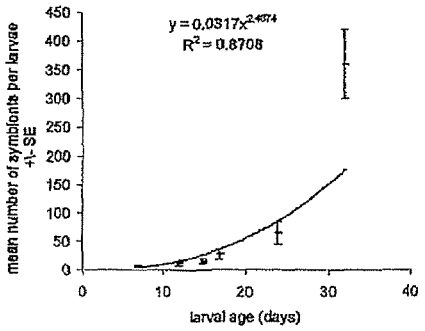

Fig. 2. Mean density of zooxanthellae (± 1 SE) in larvae of *Acropora muricata* through time.

Fig. 3 outlines a generalized stress response in coral larvae. The term "stress" was first proposed by Hans Selye (Selye 1936). Stress was originally defined a nonspecific response independent of the type of stress (Selye 1946). For example, corals bleach in response to high temperature, high light or high salinity stress, and therefore bleaching can be considered a stress response. It is important to note that this stress theory is relevant for all organisms; invertebrates, humans, plants, fungus, and bacteria. In most stress responses, the production of ROS in cells is a common early event. Superoxide ($O_2^{\cdot-}$) is the most commonly produced ROS. Hydrogen peroxide (H_2O_2), which is the only diffusive ROS molecule, is produced secondarily. Highly toxic hydroxyl radical ($^{\cdot}OH$) or singlet oxygen ($^1O_2$) can also form under severe stress conditions.

Potentially toxic ROS formed in the cells are removed by antioxidant systems. As long as these scavenging mechanisms are functional, ROS will not accumulate. Under severe stress, however, these antioxidant systems may not be able to destroy all ROS produced, in which case oxidative damage will occur. Oxidative destruction or modification of biomolecules (protein, lipid, DNA) causes enzyme inactivation, lipid peroxidation and DNA damage that leads to metabolic dysfunction, cell destruction, mutation or sterilization. In addition to antioxidant systems, there are repair systems to remove damaged molecules and to replace them with new ones. The photosynthetic reaction center protein D1 requires a protein repair system to maintain photosynthetic activity under light (Takahashi et al. 2004). In this context, antioxidant

systems function as the primary line of defense and the repair systems act as a secondary line of defense against oxidative stress (Fig. 3). If these mechanisms cannot limit or suppress stress damage, living organisms will eventually die.

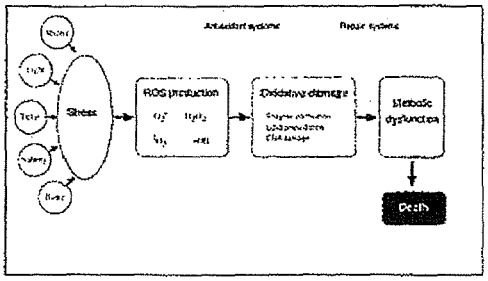

Fig. 3 A sequential diagram for stress-induced events

Except in the eyes, animals are not susceptible to visible light stress because there are few pigments which absorb visible light energy. Thus, animals normally are tolerant of visible light stress. This is not the case for plants and algae because of the presence of photosynthetic pigments such as chlorophylls; even visible light is a potential cause of stress for zooxanthellae. This may account for the difference in survivorship between zooxanthellate and non-zooxanthellate larvae at 36° C. Thus, harboring symbiotic zooxanthellae involves a risk for coral larvae in that the zooxanthellae are an additional source of ROS that may cause oxidative damage. However, this risk may be outweighed by advantages in terms of the acquisition of stress tolerance. One of the obvious benefits of harbouring symbionts is a continuous supply of energy via photosynthetic activity of zooxanthellate.

Marine invertebrates are known to be susceptible to ammonium (NH_4^+) or nitrite ion (NO_2^-), i.e. nitrogen toxicity. Recent biochemical investigations have suggested that such inorganic nitrogen can be converted to toxic molecules whose characteristics are very similar to those of ROS; these are referred to as reactive nitrogen species (RNS). Photosynthetic organisms, including zooxanthellae, can remove potential source of RNS (NH_4^+ or NO_2^-) by driving nitrogen assimilation metabolism. It is important to note that nitrogen assimilation requires the reducing power provided by photosynthesis. The involvement of RNS in stress damage has been proposed in land plants (Yamasaki 2000). For evaluating the contribution of photosynthetic symbionts in stress tolerance, we need to pay much attention to nitrogen assimilation metabolism as well as carbon assimilation process.

Acknowledgements

The Japanese Society for the Promotion of Science supported this research with a Grant in Aid of Research to AHB and HY. We thank the many members in HY's lab for assistance in the field, in particular, Dr. Takashi Nakamura and Dr. Senzo Uchida from the Okinawa Churaumi Aquarium for his support. This is RPP No. 2.

References

Babcock RC, Baird AH, Piromvaragorn S, Thomson DP, Willis BL (2003) Identification of scleractinian coral recruits from Indo-Pacific reefs. Zool. Stud. 42: 211-226

Baird AH, Marshall PA (2002) Mortality, growth and reproduction in scleractinian corals following bleaching on the Great Barrier Reef. Mar. Ecol. Prog. Ser 237: 133-141

Brown BE, Ogden JC (1993) Coral Bleaching. Sci.Am. 268: 64-70

Bythell JC (1988) A total nitrogen and carbon budget for the elkhorn coral Acropora palmata (Lamarck). Proc 6th Int Coral Reef Symp 10: 535-540

Coles SL, Brown BE (2003) Coral bleaching - Capacity for acclimatization and adaptation. Adv. Mar. Biol. 46: 183-223

Fadlallah YH (1983) Sexual reproduction, development and larval biology in scleractinian corals: A review. Coral Reefs. 2: 129-150

Glynn P, D'Croz L (1990) Experimental evidence for high temperature stress as the cause of El Nino-coincident coral mortality. Coral Reefs. 8: 181-191

Goreau TJ, Hayes RL (1994) Coral Bleaching and Ocean Hot-Spots. Ambio 23: 176-180

Harii S, Kayanne H, Takigawa H, Hayashibara T, Yamamoto M (2002) Larval survivorship, competency periods and settlement of two brooding corals, Heliopora coerulea and Pocillopora damicornis. Marine Biology 141: 39-46

Harrison PL, Wallace CC (1990) Reproduction, dispersal and recruitment of scleractinian corals. In: Dubinsky Z (ed) Coral Reefs. Elsevier, Amsterdam, pp 133-207

Hirose M, Kinzie RA, Hidaka M (2001) Timing and process of entry of zooxanthellae into oocytes of hermatypic corals. Coral Reefs 20: 273-280

Hoegh-Guldberg O, Jones RJ, Ward S, Loh WK (2002) Is coral bleaching really adaptive? Nature 415: 601-602

Hoegh-Guldberg O, Smith GJ (1989) The effect of sudden changes in temperature, light and salinity on the population density and export of zooxanthellae from the reef corals Stylophora pistillata Esper and Seriatopora hystrix Dana. Joural of Experimental Marine Biology and Ecology 129: 279-303

Johannes RE, Wiebe WJ (1970) Method for determination of coral tissue biomass and composition. Limnol. Oceanogr. 15: 822-824

Jones RJ, Hoegh-Guldberg O, Larkum AWL, Schreiber U (1998) Temperature induced bleaching of corals begins with impairment of dark metabolism in zooxanthellae. Plant, Cell Environ. 21: 1219-1230

Kinzie RA, Takayama M, Santos SR, Coffroth MA (2001) The adaptive bleaching hypothesis: Experimental tests of critical assumptions. Biol. Bull. 200: 51-58

Morse ANC, Iwao K, Baba M, Shimoike K, Hayashibara T, Omori M (1996) An ancient chemosensory mechanism brings new life to coral reefs. Biol. Bull. 191: 149-154

Nakamura T, van Woesik R (2001) Water-flow rates and passive diffusion partially explain differential survival of corals during the 1998 bleaching event. Mar. Ecol. Prog. Ser 212: 301-304

Porter JW, Fitt WK, Spero HJ, Rogers CS, White MW (1989) Bleaching in reef corals: Physiological and stable isotopic responses. Proc. Natl. Acad. Sci. USA. 86: 9342-9346

Ralph PJ, Gademann R, Larkum AWD (2001) Zooxanthellae expelled from bleached corals at 33 degrees C are photosynthetically competent. Mar. Ecol.-Prog. Ser. 220: 163-168

Richmond RH (1981) Energetic considerations in the dispersal of Pocillopora damicornis (L.) planulae. Proc 4th Int Coral Reef Symp 2: 153-156

Schwarz JA, Krupp DA, Weis VM (1999) Late larval development and onset of symbiosis in the scleractinian coral Fungia scutaria. Biol. Bull. 196: 70-79

Selye H (1936) A syndrome produced by diverse nocuous agents. Nature 138: 32

Selye H (1946) The general adaptation syndrome and the diseases of adaptation. J. Clin. Endocrinol. 6: 117-230

Takahashi S, Nakamura T, Sakamizu M, van Woesik R, Yamasaki H (2004) Repair machinery of symbiotic photosynthesis as the primary target of heat stress for reef-building corals. Plant Cell Physiol. 45: 251-255

van Oppen M (2001) In vitro establishment of symbiosis in Acropora millepora planulae. Coral Reefs 20: 200-200

Weis VM, Reynolds WS, deBoer MD, Krupp DA (2001) Host-symbiont specificity during onset of symbiosis between the dinoflagellates Symbodinium spp and planula larvae of the scleractinian coral Fungia scutaria. Coral Reefs 20: 301-308

Yamasaki H (2000) Nitric oxide produced by nitrate reductase: implications for involvement of active nitrogen species in photoinhibition in vivo. Proc. R. Soc. Lond. Ser. B-Biol. Sci. 355: 1477-1488