

Environmental controls on the establishment and development of algal symbiosis in corals

A. H. Baird¹, R. Bhagooli^{2, 3}, M. Nonaka⁴, I. Yakovleva^{2, 5}, H. H. Yamamoto⁴, M. Hidaka², H. Yamasaki²

1) ARC Centre of Excellence for Coral Reef Studies, James Cook University, Townsville, Qld, 4811, Australia

2) Department of Chemistry, Biology and Marine Science, University of the Ryukyus, Nishihara, Okinawa, 903-0213, Japan

3) Department of Biosciences, Faculty of Science, Reunion, Mauritius

4) Churaumi Aquarium, Motobu-cho, Okinawa, 905-0206, Japan

5) A. V Zhirmunsky Institute of Marine Biology, Far Eastern Branch of the Russian Academy of Sciences, Palchevskogo 17, Vladivostok, 690041, Russia

Abstract: Coral reefs are under severe threat from changing climate, yet little is known about how environmental variables affect the establishment and development of coral symbiosis. Here, we use a coral larval model to test whether temperature affects the establishment and development of symbiosis using three different strains of algal symbionts (zooxanthellae) with contrasting thermal tolerance. We also compare the growth of these clades within the larval host, *Acropora intermedia*, and follow the survival of three different larval holobionts under three different temperatures (26, 30 and 34°C). Initial rates of infection were influenced by temperature in two of the three clades: in clade C9b infection rates peaked at 30°C, while in clade A6 infection rates declined with increasing temperature. Infection by clade C1 zooxanthellae was uniformly low. The thermal tolerance of the zooxanthellae did not appear to influence survival of the holobiont, however, at high temperature larvae with high densities of zooxanthellae had lower survival, suggesting zooxanthellae may be an added burden to larvae when under stress.

Keywords: coral reefs, climate change, larval ecology, reproductive biology, symbiosis

Introduction

Coral reefs are under severe threat from many sources, including rising sea-surface temperature caused by climate change (Hughes et al. 2003). A major concern is that the accelerating rate of environmental change could exceed the capacity of coral species to acclimate or adapt (Hoegh-Guldberg et al. 2007). Corals are a symbiosis between the animal host and micro-algae from the genus *Symbiodinium*. While the association is in most cases highly specific, with a given coral species associating predominantly with a single sub-clade (Knowlton and Rohwer 2003), some species associate with more than one strain and multiple strains can occur within a single colony (Rowan and Knowlton 1995, van Oppen et al. 2001). Under stress, such as high sea water temperature, the association can break down with a consequent loss of the algal symbionts. This process is known as coral bleaching, and can lead to the death of the coral host (Baird and Marshall 2002). Because symbiotic strains differ considerably in physiological characteristics, such as tolerance to high temperatures (Bhagooli and Hidaka 2003,

Rowan 2004), a switch from a thermally susceptible to a tolerant strain is one mechanism by which corals may respond to rising seawater temperature associated with climate change (Buddemeier and Fautin 1993). This mechanism has received considerable attention in the recent literature (Baker et al. 2004), however, experimental support for it is inconclusive. For example, while there is some evidence that the identity of the symbiont can affect the physiology of the host (Little et al. 2004), it has only recently been demonstrated that heat tolerant symbionts can increase the tolerance of the host (Berkelmans and van Oppen 2006). Similarly, while a shift in the dominant clade of zooxanthellae within coral colonies has been documented following stress, such as disease (Toller et al. 2001), and transplantation (Baker 2001) there is no direct evidence to show that this change had been induced by a change in temperature nor that these shifts increase the fitness of the host (Hoegh-Guldberg et al. 2002, Douglas 2003). Furthermore, there is no evidence to show that scleractinian corals can acquire novel, and superior, symbiont clades from the

environment (Sotka and Thacker 2005). The appeal of the adaptive bleaching hypothesis is that it provides a mechanism by which corals can adjust to stress as soon as change is induced rather than over generations, as would be required via adaptation. However, changes in the composition of a species complement of *Symbiodinium* are most likely to occur between generations at least in those species which do not inherit zooxanthellae from the parents (LaJeunesse et al. 2004). For most coral species, initial infection occurs as larvae or early juveniles, which appear, at least initially, to be much less specific in their choice of algal partner (Baird et al. 2007). Consequently, each larval recruitment event provides the opportunity for novel symbioses to be established.

The absence of zooxanthellae in oocytes of most broadcast spawning corals providing an opportunity to manipulate symbioses to explore the effects of different symbionts on the performance of the holobiont. Larvae which lack symbionts can be readily infected with many different species of symbionts under experimental conditions (van Oppen 2001, Weis et al. 2001). Consequently, it is possible to compare symbiotic and non-symbiotic individuals of the same species and to readily control the identity of both the host and the symbiont. Control over the make-up of the association is not possible in adults, because even completely bleached adults still contain residual densities of symbionts (Jones 2008) and it is yet to be established whether adults can acquire novel strains from the external environment (Hoegh-Guldberg et al. 2002).

Here, we use the coral larval model to test whether temperature effects the establishment of zooxanthella strains of contrasting thermal tolerance in larvae of the coral *Acropora intermedia*. Next we explore how the growth rate of zooxanthellae within the host is affected by temperature and finally we explore survival of three holobionts under different temperatures.

Material and Methods

We first tested the photo-physiological response of four strains of *Symbiodinium* to temperature (Fig. 1). *Symbiodinium* were isolated from four different cnidarian hosts following Yakovleva and Hidaka (2004) and identified following using internal transcribed spacer 2 (ITS2) region amplified for DGGE following Lajeunesse (2002). The host and the corresponding strains were *Seriatopora caliendrum* (C1) *Acropora intermedia* (C3), *Platygyra ryukyuensis* (C9b) and *Tridacna crocera* (A6). Isolated zooxanthellae were placed on Millipore filters and then exposed to four temperature levels, 26, 29, 32 and 34°C, under a moderate light

level of 110 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. A pulse amplitude modulated chlorophyll fluorometer (PAM) was used to assess the maximum quantum yield (20 min dark-adapted F_v/F_m) of zooxanthellae following Bhagooli and Hidaka (2003). Measurements were taken prior to thermal treatment (0h) and then after 6 and 12h.

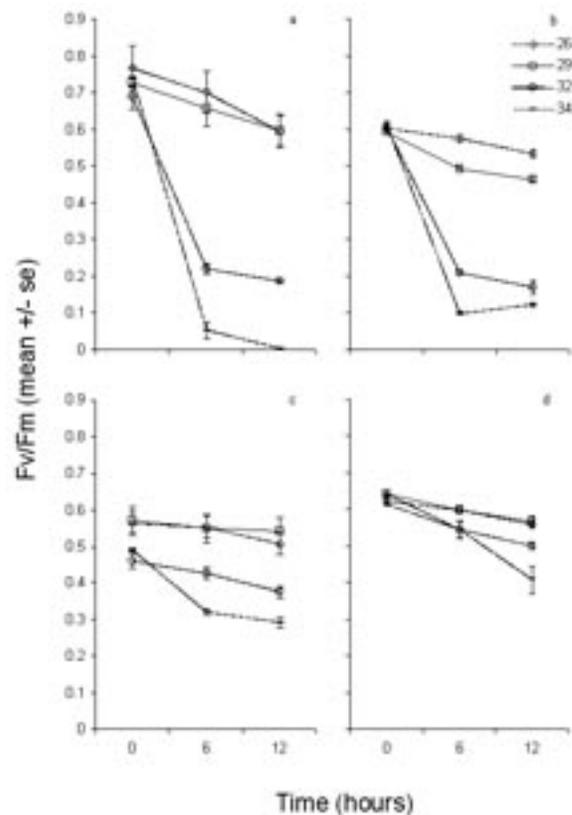


Figure 1. Thermal tolerance in four strains of zooxanthellae (a = C1, b = C3, c = A6, d = C9b).

Next we exposed larvae of *Acropora intermedia* (a species which does not contain zooxanthellae in the oocytes) collected from colonies maintained at the Churaumi Aquaria, Okinawa Japan to freshly isolated batches of three strains of zooxanthellae (all except strain C3) at densities of 4.3×10^6 cells per mL^{-1} at three different temperatures 26, 30 and 34°C. Larvae were cultured and maintained in 0.2 FSW for three days post-spawning (Baird et al. 2006). Temperatures were maintained using temperature control units (EYELA, Thermister Tempet T-80) and larvae were kept under 110 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ of PAR with a 12:12 h light: dark photoperiod. A sub-sample of 6-8 larvae was removed from each of three replicate jars after 24h and squashed under a coverslip and observed under a fluorescent microscope and the number of zooxanthellae per larva counted. In a second experiment, larvae were infected with zooxanthellae of strains C3, C9b and A6, and 50

larvae placed in 200 ml glass jars at three temperatures, 26, 30 and 32°C and larval survival was followed for 8 d. The density of zooxanthellae at the end of this time was counted to compare the growth rate of strains at each temperature.

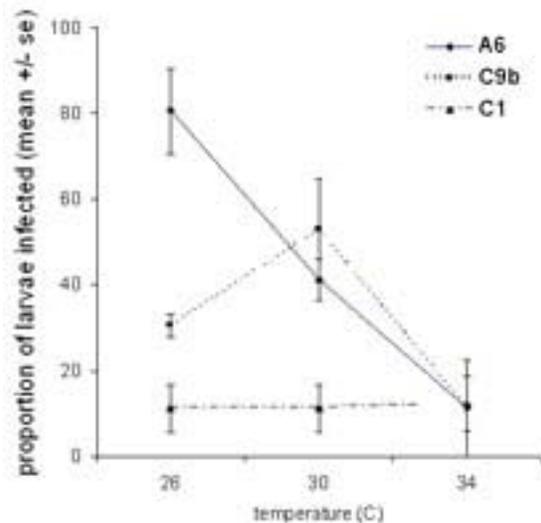


Figure 2. Percentage of *Acropora intermedia* larvae infected by zooxanthellae

Results and Discussion

The four ITS2 types of zooxanthellae clearly exhibited different functional responses to temperature (Fig. 1). Maximum quantum yield (F_v/F_m) was not affected under either 26 or 29°C treatments in any strain (Fig. 1). In strains C3 and C1, F_v/F_m dropped sharply after 6h exposure at 32°C. In contrast a significant decline in F_v/F_m did not occur until 12h at 34°C in strains C9b and A6. The zooxanthellae were consequently ranked in terms of their thermal tolerance as follows: C1 = C3 < A6 = C9b. Also of interest is the wide variation in thermal tolerance among strains of clade C, strengthening the argument that the clade of zooxanthellae is not necessarily an accurate indication of its thermal sensitivity (Toller et al. 2001). Finally, clade A zooxanthellae were both highly infective at ambient temperature, and grew rapidly within the host at temperatures up to 32°C (Fig. 3), supporting the idea that symbionts from this clade are opportunists with life history features that enable them to take advantage of ecological opportunity provided by the loss of other strains of zooxanthellae from the host following stress (Knowlton and Rohwer 2003).

Initial rates of infection were affected by temperature in two of the three symbionts (Fig. 2). In strain A6, the proportion of larvae infected decreased with temperature from 80% infection at 26°C to 10%

at 34°C (Fig. 2). In strain C9b the proportion of larvae infected at 30°C was twice as high as at 26°C (Fig. 2). The proportion of larvae infected by strain C1 was uniformly low, perhaps as a result of the fact that this strain may not have a free living form and therefore be unsuitable for culture (see below).

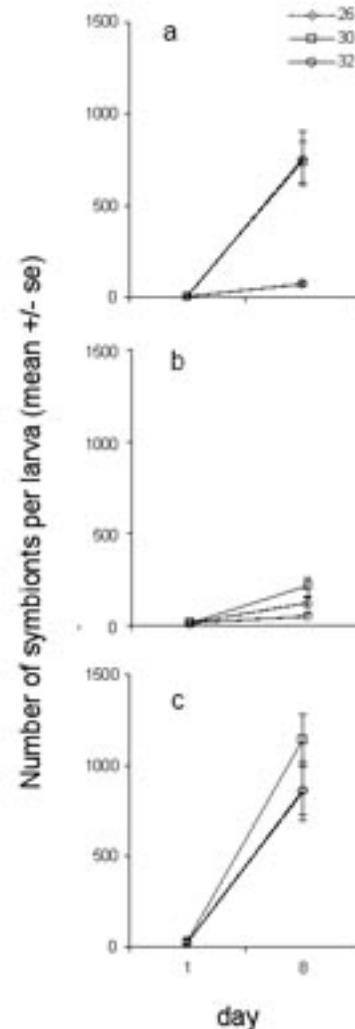


Figure 3. Change in the density of zooxanthellae strains (a = C3, b = C9b; c = A6) in *A. intermedia* larvae (n = 6).

Similarly, at 34°C the proportion of larvae infected was uniformly low (Fig. 2), suggesting none of the strains are capable of dealing with temperature 6°C above ambient. The different optimum temperature of infection in strains A6 (26°C) and C9b (30°C) suggests that certain strains may have a competitive advantage under different environments (Baird et al. 2007). However, within the range of temperatures examined these patterns of infection were not directly related to thermal tolerance of the zooxanthella strains.

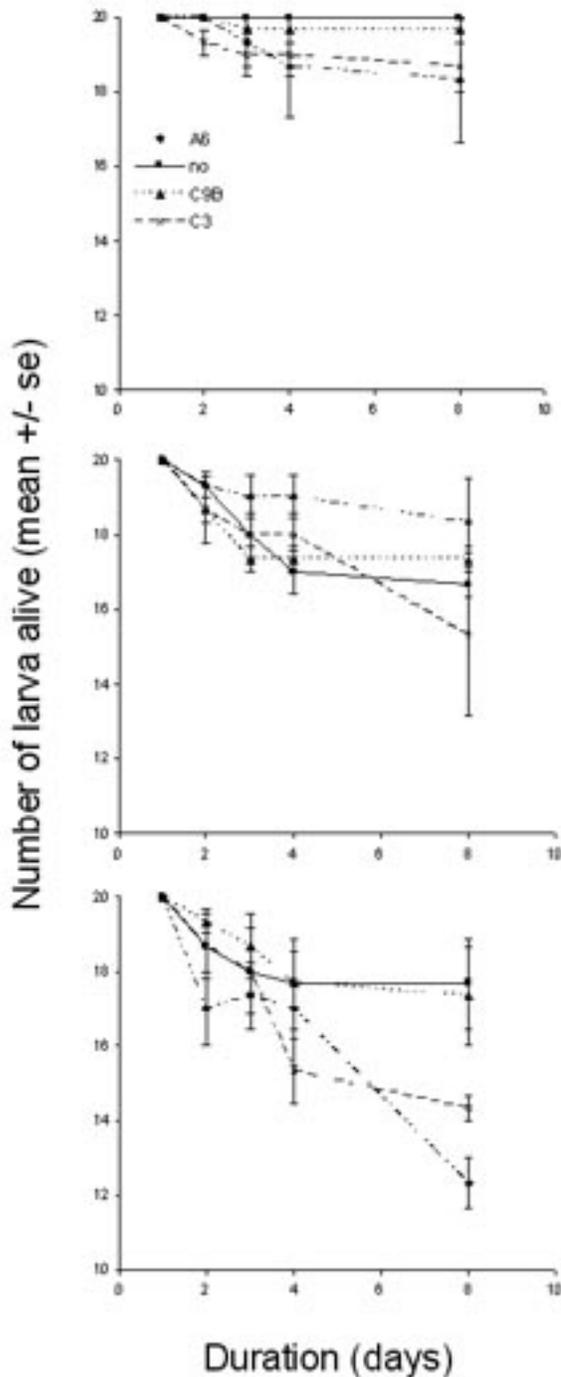


Figure 4. Survivorship of *A. intermedia* larvae infected with three strains of zooxanthellae plus non-zooxanthellate larvae at three temperatures (a = C3, b = C9b, c = A6; n = 6).

The growth rate of the zooxanthellae strains within larvae varied dramatically among strains and with temperature (Fig. 3). Strain C3 increased more quickly at higher temperature (i.e. $30 + 32 > 26$) (Fig. 3). Strain C9b increase slowly at all temperatures with little difference in density among temperature

treatments after 8 days (Fig. 4). Strain A6 increased rapidly at all temperatures (Fig. 3).

These data suggest there may be some trade-off in terms of thermal tolerance vs. growth rate, with strain C9b being the most thermally tolerant and also having the slowest rate of population increase in the host. Similar trade-offs between growth and tolerance have been suggested for clade D vs. clade C zooxanthellae in coral on the GBR (Berkelmans and van Oppen 2006).

There was little difference in the performance of the different holobionts at 26 or 30°C, however, at 32°C both A6 and C3 holobionts had lower survival (Fig. 4). This suggests that zooxanthellae may be an added burden to larvae when under stress (Yakovleva et al. 2009).

A number of methodological issues may have affected the results. More recent research indicates that the maximum rates of infection are not achieved until 5-7 d post spawning (Yasuda et al. 2007). Our infection experiments commenced 3 d following spawning. While a high proportion of larvae were infected in some groups, for example, 80% of larvae were infected by strain A6 at 26°C, the results may have been different if larvae were at a stage more susceptible for infection. Furthermore, the high temperature treatment was probably too extreme. Very few larvae were infected by any strains at 34°C which is nearly 6°C above ambient at this time of year in Okinawa. Future work should examine infection using more treatments over a narrower range to more completely describe the functional response of larval infection to temperature. Also, other differences between some of the zooxanthellar strains could potentially affect rates of infection. In particular, strain A6 is not an intra-cellular symbiont, rather it is found in the haemal sinuses of the clam mantle (Farmer et al. 2001). Also, strain C1, isolated from *Seriatopora caliendrum*, is transmitted vertically i.e. directly into the oocytes of offspring and therefore may not exist outside the host. In contrast, strains C3 and C9b are both inherited horizontally i.e. they must be acquired anew in each generation, and therefore must have a free living stage in their life history. Finally, to effectively explore the effect of functionally different strains on the host performance it would have been ideal to wait until zooxanthella densities had stabilized within the host. This may take 4-6 weeks in coral larvae (Baird et al. 2006) which have the potential to host 1000s, to tens of thousands of zooxanthellae per larvae (Edmunds et al. 2005).

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