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Microscopic observation of symbiotic and aposymbiotic juvenile corals in nutrient-enriched seawater

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ABSTRACT

Symbiotic and aposymbiotic juvenile corals, which were grown in the laboratory from the gametes of the scleractinian coral *Acropora digitifera* and had settled down onto plastic culture plates, were observed with a microscope under different nutrient conditions. The symbiotic corals successfully removed the surrounding benthic microalgae (BMA), whereas the aposymbiotic corals were in close physical contact with BMA. The areal growth rate of the symbiotic corals was significantly higher than that of the aposymbiotic corals. The addition of nutrients to the culture seawater increased the chlorophyll *a* content in the symbiotic coral polyps and enhanced the growth of some of the symbiotic corals, however the average growth rate was not significantly affected, most likely because of the competition with BMA. The comparison between the symbiotic and aposymbiotic juvenile corals showed that the establishment of a symbiotic association could be imperative for post-settlement juvenile corals to survive in high-nutrient seawater.

1. Introduction

Over the past several decades, coral reef ecosystems have globally degraded due to various environmental changes, such as global warming and decline of the water quality (Hoegh-Guldberg et al., 2007; Burke et al., 2011). Reef degradation is caused by the loss of adult corals, such as with bleaching, and also by decreases in coral recruitment. In fact, low levels of sexual recruitment throughout the Florida Keys and the Caribbean have been reported in recruitment surveys (Hughes and Tanner, 2000) and settlement plate studies (Shearer and Coffroth, 2006). Because coral recruitment processes will strongly influence the recovery and distribution of adult corals in the near future, many laboratory experiments have been performed to examine the effects of environmental changes on juvenile corals.

Global warming is well known to increase the bleaching frequency of adult corals (Hoegh-Guldberg et al., 2007) and is also a serious concern for juvenile corals (Edmunds, 2008; Howells et al., 2012; Schnitzler et al., 2012). Schnitzler et al. (2012) observed that, when the seawater temperature was elevated from 27 °C to 29–31 °C, the ability of *Fungia scutaria* larvae to establish symbiosis with *Symbiodinium* sp. (clade C1) was impaired and the larval survivorship was reduced. Inoue et al. (2012) reported that the threshold temperature affecting bleaching and growth rate reduction for *Acropora digitifera* juvenile coral was between 29 °C and 31 °C.

Ocean acidification caused by elevated pCO_2 levels has also received attention in recent years because the decreased aragonite saturation state in seawater might negatively impact coral calcification (Kleypas et al., 2006; McCulloch et al., 2012). Juvenile corals were also reported to decrease their skeletal growth rate due to ocean acidification (Albright et al., 2008, 2010; Suwa et al., 2010). Albright et al. (2010) showed that all of the processes of fertilization, settlement, and growth of *Acropora palmata* juvenile coral were negatively impacted by increasing pCO_2.

In addition to global environmental changes, local water quality decline has also received attention as an accelerator of coral reef degradation (Wooldridge, 2009; Lapointe et al., 2010; Reopanichkul et al., 2010). Human activity has increased the production of organic and inorganic nitrogen (N) by such processes as the use of chemical fertilizer and the combustion of fossil fuel (Galloway et al., 2004), and the N loading in the terrestrial biosphere has contaminated rivers and groundwater (Umezawa et al., 2002). The concentration of dissolved inorganic N (DIN: nitrate + nitrite + ammonium) is normally maintained at <1 μmol l^{-1} in oligotrophic coral reefs, but the concentration is sometimes elevated to 1–5 μmol l^{-1} at sites

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that receive anthropogenic nutrient input (Lapointe et al., 2010; Reopanichkul et al., 2010) and infrequently increases up to 5–10 μmol l^{-1} (Costa et al., 2000). Despite the problem of coastal eutrophication, the effect on post-settlement juvenile corals has rarely been studied (Wittenberg and Hunte, 1992).

When the concentration of nutrients increases in seawater, the endosymbiotic algae (zooxanthellae) of adult corals incorporate more nutrients and proliferate in the host coral tissue (Hoegh-Guldberg and Smith, 1989; Dubinsky and Berman-Frank, 2001). Increases in the zooxanthellate density and the algal photosynthetic rate per unit coral surface area are also stimulated with nutrients (Chauvin et al., 2011; Sawall et al., 2011). A recent study showed that the imbalanced enrichment of DIN resulted in phosphate (PO_4^{3-}) starvation of zooxanthellae and increased the susceptibility of corals to bleaching (Wiedenmann et al., 2012). The observed effects of nutrients on coral growth (calcification) rates are not consistent, with both positive (Chauvin et al., 2011; Sawall et al., 2011) and negative (Marubini and Davies, 1996; Ferrier-Pagès et al., 2000; Renegar and Riegl, 2005) effects being reported.

From an ecological aspect, the competition between adult corals and benthic algae under nutrient enrichment has been surveyed in field manipulative experiments (Burkepile and Hay, 2006; Furman and Heck, 2008; Sotka and Hay, 2009; Smith et al., 2010; Vermeij et al., 2010). Factors of reduced grazing pressure on benthic algae and elevated eutrophication were found to increase the abundance of benthic algae, resulting in decreases in coral coverage. However, it is presently not well understood which condition (grazing pressure on algae or eutrophication) regulates the competitive interaction between corals and benthic algae. Vermeij et al. (2010) observed that herbivores had no effect on the rate at which turf algae overgrew corals, but other researchers concluded that nutrients did not negatively affect coral growth and that the grazing pressure on benthic algae influenced the coral-algal interaction more strongly than nutrient loading (Burkepile and Hay, 2006; Furman and Heck, 2008; Sotka and Hay, 2009; Smith et al., 2010).

The purpose of the present study was to observe the growth of symbiotic and aposymbiotic juvenile corals under nutrient enrichment on a microscale level and to evaluate the importance of symbiotic algal acquisition for post-settlement juvenile corals. The use of juvenile corals enabled us to measure the coral growth rate in a relatively short time period and to directly observe the competition against the surrounding benthic microalgae (BMA) under a microscope. Moreover, the comparison between symbiotic and aposymbiotic corals was effective to investigate the function of zooxanthellae for juvenile corals. This new experimental approach was expected to lead to a better understanding of coral growth from both ecological and physiological aspects.

2. Materials and methods

2.1. Preparation of post-settlement juvenile corals

The scleractinian coral *A. digitifera*, which is one of the most common species in the Ryukyu Islands, Japan, were collected from a fringing reef of Sesoko Island (26°37–39′ N, 127° 51–52′ E) on 6 Jun 2011. The specimens were maintained in outdoor continuous-flow aquaria at Sesoko Station, University of the Ryukyus. Gametes, which were spawned from ten colonies of the collected *A. digitifera* on 13 Jun 2011, were collected and thoroughly mixed in buckets. The gametes were then transferred to the indoor laboratory and cultured in plastic containers filled with sufficient reef seawater. The seawater was filtered through a cartridge-type filter (pore size, 1 μm) in advance and was freshened every day until the ensuing settlement treatment. The fertilized eggs developed to planula larvae approximately 4 d after the spawning. Light was provided by fluorescent lamps at an intensity of 40 μmol m^{-2} s^{-1} for 12 h per day. The seawater temperature in the containers was controlled by maintaining the room temperature at 27 °C, which is the seawater temperature during the coral spawning season in the Ryukyu Islands (Negri et al., 2007).

After 8 d of the larval period, the larvae settled onto 6-well plastic culture plates (volume, 10 ml in each well) with the coral metamorphosis inducer peptide Hym-248 (Hirose et al., 2008; Suwa et al., 2010). It was observed that approximately 50 larvae settled onto each plate within 6 h after the addition of the peptide. The culture plates were supplied with fresh filtered seawater every day. Two days after the settlement, a solution containing zooxanthellae (*Symbiodinium*, clade A), obtained from the giant clam *Tridacna crocea* because this type of zooxanthellae easily infects young *A. digitifera* polyps (Hirose et al., 2008), was added to each culture well (final concentration, 1.4×10^4 cells ml^{-1}) for polyp infection. One day after the infection treatment, the culture plates with corals were submerged in aquaria (inner volume, 11 l) filled with fresh filtered seawater. The aquaria were illuminated with metal-halide lamps for 12 h a day (0700–1900 h), and the average irradiance was 110 μmol m^{-2} s^{-1} at the depth of the corals. The seawater temperature was maintained at 27 °C with a thermostat and a heater. The seawater was freshened every 2–3 d, and the juvenile corals were incubated for 4 d until the start of the experiment. Coral infection with the symbiotic algae was confirmed with a microscope approximately 3 d after the infection treatment. To evaluate the growth difference between the symbiotic and aposymbiotic corals, juvenile corals without algal infection were also prepared simultaneously.

2.2. Culture experiment in flow-through aquaria

Filtered seawater (pore size, 1 μm) was continuously supplied to six aquaria (inner volume, 11 l each) under laboratory conditions at the rate of 300 ml min^{-1} each. Three culture plates with symbiotic juvenile corals were placed in three of the aquaria (one plate in each aquarium), and three plates with aposymbiotic corals were placed in the other three aquaria. The seawater temperature was maintained at 27 °C with a thermostat and a heater, and the seawater was circulated (approximately 5 cm s^{-1}) with a water-jet pump. The aquaria were illuminated with metal-halide lamps for 12 h a day (0700–1900 h), and the average irradiance was 110 μmol m^{-2} s^{-1} at the depth of corals. The nutrient concentrations in the running seawater of the aquaria were 0.2 ± 0.0, 0.1 ± 0.0, 0.2 ± 0.0, and 0.2 ± 0.1 μmol l^{-1} for NO_3^-, NO_2^-, NH_4^+, and PO_4^{3-}, respectively (mean ± SD, $n = 4$). The concentrations were measured every 2 or 3 d for the symbiotic and aposymbiotic control aquaria (hereafter referred to as SC and AC, respectively) during the 10-d culture experiment. A mixed solution of KNO_3 and NaH_2PO_4 was supplied to the two aquaria at the rate of 5.5 ml h^{-1} for the symbiotic and aposymbiotic low-nutrient additions (SL and AL, respectively) and to the other two at the rate of 13 ml h^{-1} for the symbiotic and aposymbiotic high-nutrient additions (SH and AH, respectively). The final concentrations of NO_3^- and PO_4^{3-} in the nutrient-enriched aquaria (measured every 2 or 3 d) were 3.9 ± 0.1 and 0.4 ± 0.1 μmol l^{-1} in SL, 3.7 ± 0.1 and 0.4 ± 0.1 μmol l^{-1} in AL, 8.8 ± 0.2 and 0.8 ± 0.2 μmol l^{-1} in SH, and 8.8 ± 0.2 and 0.6 ± 0.1 μmol l^{-1} in AH, respectively (mean ± SD, $n = 4$ or 3). To measure the growth rate of each juvenile coral, 15 corals were randomly chosen from each aquarium and photographed under a microscope on days 0, 6, and 10 of the culture experiment. After the 10-d incubation, the organic soft tissue of the 15 corals was removed by a water-pick method, and the carbonate skeletons were dried to measure the skeletal dry weight, as described below. To measure