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Benefits to host sea anemones from ammonia contributions of resident anemonefish

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ABSTRACT

Large ectosymbionts (especially fishes and crustaceans) may have major impacts on the physiology of host cnidarians (sea anemones and corals), but these effects have not been well quantified. Here we describe impacts on giant sea anemone hosts (*Entacmaea quadricolor*) and their endosymbiotic zooxanthellae (*Symbiodinium* spp.) from the excretion products of anemonefish guests (*Amphiprion bicinctus*) under laboratory conditions. Starved host anemones were maintained with anemonefish, ammonia supplements (= NH_3 gas and NH_4^+ ion), or neither for 2 mo. In the presence of external ammonia supplements or resident anemonefish, the zooxanthellae within host anemones increased in abundance (173% and 139% respectively), and provided the hosts with energy that minimized host body size loss. In contrast, anemones cultured with neither ammonia nor anemonefish harbored significantly lower abundances of zooxanthellae (84% of initial abundance) and decreased >60% in body size. Although they maintained higher zooxanthella abundances, anemones cultured with either ammonia supplements or resident anemonefish exhibited significantly lower ammonia uptake rates (0.065±0.005 μmol g^{-1} h^{-1}, and 0.052±0.018 μmol g^{-1} h^{-1} respectively) than did control anemones (0.119±0.009 μmol g^{-1} h^{-1}), indicating that their zooxanthellae were more nitrogen sufficient. We conclude that, in this multi-level mutualism, ammonia supplements provide essentially the same level of physiological contribution to host anemones and zooxanthellae as do live resident fish. This nutrient supplement reduces the dependence of the zooxanthellae on host feeding, and allows them to provide abundant photosynthetically-produced energy to the host.

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1. Introduction

Mutualisms are ubiquitous in nature, yet the mechanisms underlying most mutualistic interactions, especially in terms of the potential benefits to partner species, remain poorly understood and inadequately quantified (Bronstein, 1994; Bruno et al., 2003). In the association between anemonefishes (28 species of damselfishes in the genera *Amphiprion* and *Premnas*) and host sea anemones (10 species in the order Actiniaria, see Fautin and Allen, 1997), the obligate fishes cannot survive in nature without the safe haven they find among the anemones' tentacles (Mariscal, 1970; Allen, 1972; Fautin and Allen, 1997). The sea anemone hosts, on the other hand, are not obligate partners, and some species appear able to survive without anemonefishes (Godwin and Fautin, 1992; Fautin and Allen, 1997). Both partners can survive separately in laboratory aquaria (Fautin and Allen, 1997), but the anemones and also possibly the fishes grow faster when cultured together (Porat and Chadwick-Furman, 2005; N.E. Chadwick, unpublished data). In the wild, individuals of some host anemone species also survive and grow better when they harbor anemonefishes (Porat and Chadwick-Furman, 2004; Holbrook and Schmitt, 2005), but the underlying mechanisms through which anemonefishes benefit their hosts are not fully understood. Anemonefishes can defend some host anemones from cnidarian predators (Mariscal, 1970; Fricke, 1975; Fautin, 1991; Godwin and Fautin, 1992; Fautin and Allen, 1997; Porat and Chadwick-Furman, 2004). Physiological benefits also potentially accrue to host anemones through the utilization of resident anemonefish waste products (Fautin, 1991; Porat and Chadwick-Furman, 2005; Cleveland et al., 2008). We have demonstrated that anemonefish generate ammonia more rapidly than their host anemones can absorb it, thus providing a major source of external nitrogen for uptake by the endosymbiotic zooxanthellae within the hosts (Roopin et al., 2008). However, impacts of ammonia excretion by anemonefishes on the physiology of host anemones have not been well quantified.

All tropical sea anemones that host anemonefishes also harbor zooxanthellae (Dunn, 1981; Fautin, 1991), which supply the anemones with energy-rich photosynthetic compounds for respiration, growth, and reproduction (e.g., Steen, 1988; Achituv and Dubinsky, 1990; Whitehead and Douglas, 2003). In algal-cnidarian symbioses, zooxanthellae obtain inorganic nutrients from host catabolism (Szmant-Froelich and Pilson, 1984), host holozoic feeding (Steen, 1986), and the surrounding sea water (Muscatine, 1980; Wilkerson and Trench, 1986). The net excretory ammonia of host cnidarians often is insufficient to sustain zooxanthellae (Szmant-Froelich and Pilson, 1984; McAuley and Cook, 1994). For example, in the Red Sea coral *Stylophora pistillata*, excretory ammonia at steady state can potentially

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support only about 1/3 of the growth rate of its endosymbiotic zooxanthellae (Rahav et al., 1989). Cook and D'Elia (1987) first proposed that zooxanthellae may be nutrient-limited in hospite. Regulation of intracellular ammonium levels in the host potentially allows the host to control the biomass of resident zooxanthellae, and to maintain a steady-state ratio of symbiont to host in which neither outgrows the other (D'Elia and Cook, 1988; Falkowski et al., 1993). Several lines of evidence support this nitrogen-limitation theory, including the enhancement of dark carbon fixation in isolated zooanthellae following addition of ammonium chloride (Cook et al., 1994), and an increase in cnidarian zooxanthella densities with the addition of inorganic nitrogen (e.g., Hoegh-Guldberg and Smith, 1989; Muscatine et al., 1989; Hoegh-Guldberg, 1994). Thus, the contribution of ammonia waste products by anemonefishes (Roopin et al., 2008) can augment the capacity of sea anemone hosts to support and potentially increase their zooxanthella densities (e.g., Meyer and Schultz, 1985a,b; Porat and Chadwick-Furman, 2005), which in turn may provide additional photosynthetic energy to the host as needed when heterotrophic food sources are limited (Roberts et al., 1999).

The direct transfer of nitrogen from anemonefish to sea anemone host tissues has been demonstrated by Cleveland et al. (2008) using stable isotope markers. However, the physiological impacts of this nutritional benefit, and the extent to which nitrogen versus other fish-related factors contribute to host body size, remain unknown. Here we compare the impacts of symbiotic anemonefish presence versus ammonia supplements on the body size and zooxanthella population dynamics of starved host sea anemones under laboratory conditions.

2. Methods

2.1. Study organisms and maintenance

Giant sea anemones *Entacmaea quadricolor* (Rüppell and Leuckart, 1828) were transported to Auburn University in 2006 from Waikiki Aquarium (Hawaii, USA), where they had been propagated via clonal replication from individuals collected in Palau in 1985. All of the zooxanthellae contained within the endodermal tissues of these host sea anemones belonged to *Symbiodinium* clade C1 (Roopin, 2007). Anemonefish *Amphiprion bicinctus* Rüppell, 1828 also were transported to Auburn in 2006 from Oceans Reefs and Aquariums (ORA), an aquaculture facility of Harbor Branch Oceanographic Institution at Fort Pierce, Florida, USA, where they had been propagated from brood stock collected in Saudi Arabia in 2000. One anemone was placed on each side of a rigid plastic screen (2 cm mesh) in each of 12 identical closed-system aquaria (n=24 anemones total), together with 1 - 2 anemonefish per anemone (n=30 anemonefish total). All fish and anemones were acclimated to these culture conditions for at least 4 mo prior to experiments, and grew actively during this period. Each closed-system aquarium circulated 160 L of artificial seawater (Reef Crystals, Aquarium System, Inc., Ohio, USA) between an upper tank (77 cm×32 cm×33 cm) containing the animals, and a lower sump (77 cm×32 cm×33 cm) with filters. Water flowed into the upper tank from the sump alternately through 2 pipe outlets using a SCWD–wave maker (3iQ Ventures LLC, Manhattan Beach, California, USA). Each sump contained a protein skimmer, live rock, and macroalgal cultures as filters, and both upper tanks and sumps contained a layer of fine sand. Anemones were allowed to attach to pieces of flat coral rock that were placed in each upper tank. All systems were maintained at 34 - 35 ppt salinity, temperature of 26 °C, and a 12 h light : 12 h dark photoperiod. Concentrations of dissolved ammonia in the tanks were consistently low, <0.5 μmol L^{-1}. Over each aquarium was suspended a 6-bulb TEK-LIGHT™T5 high output fluorescent light, with a combination of 3 39W T5 Midday 6000K, and 3 39W T5 Pure actinic Giesemann PowerChrome fluorescent bulbs, which provided photosynthetically-active radiation (PAR) of about 200 μmol quanta m^{-2} s^{-1} at the bottom of the aquarium to 800 μmol quanta m^{-2} s^{-1} at the water surface (QSL-2101 Scalar PAR Sensor, Biospherical Instruments, San Diego, California, USA), equivalent to PAR at 7-20 m depth on coral reefs in the Red Sea (Stambler and Dubinsky, 2005) where these organisms occur (Fautin and Allen, 1997; Chadwick and Arvedlund, 2005).

2.2. Experimental design

All anemones were fed weekly to satiation with small pieces of fish or shrimp. Anemonefish were fed each morning to satiation with a combination of dry pellets (Formula one, AquaPet Americans, Utah, USA) and frozen foods (copepods, brine shrimps, mysids).

To assess effects of ammonia enrichment on starved sea anemones, each individual anemone was assigned haphazardly to 1 of 3 treatments for a period of 2 mo: (1) without anemonefish in nutrient-poor seawater, referred to as the control treatment (n=8 anemones), (2) with 1 - 2 anemonefish in nutrient-poor seawater, referred to as the anemonefish treatment (n=8 anemones), and (3) without anemonefish in ammonia-enriched seawater (daily ammonia treatment of ~10 μmol L^{-1} ammonia for 1 - 1.5 h), referred to as the ammonia treatment (n=8 anemones). Identical treatments were assigned to both sides of each aquarium system, to avoid treatment effects from one side of the mesh barrier to the other. Each aquarium system was assigned randomly to a treatment using a random number generator. During the experiment, food was withheld from the anemones, but the anemonefish were fed daily with dry pellets only (Formula one, AquaPet Americans, Utah, USA). Pellets were introduced to the fish one by one and their feeding behavior was monitored carefully. Uningested pellets were removed from the tanks, and the anemones did not ingest any of the food intended for the fish. After 2 mo, nutrient supply treatments were reversed as follows: (1) anemonefishes were removed from the anemonefish treatment, (2) daily ammonia supplements were stopped for the ammonia treatment, and (3) 1 - 2 anemonefish per anemone were added to the control treatment. These reversed treatments were maintained for an additional 3 wk, then all animals were returned to their original culture conditions.

2.3. Size changes in unfed sea anemones

To assess patterns of change in body size during starvation, each sea anemone was photographed at the start of the experiment and after 2 mo, prior to treatment reversal. Photographs were taken only when anemones were fully expanded. Three individuals, 1 in each treatment, were excluded from the size analysis because they consistently remained contracted, and could not be measured accurately. Photographs were scanned into a computer, and the software program Image Tool (Ver.3.00, UTHSCSA) was used to determine the long and short axial lengths of the sea anemones (tentacle tip to tentacle tip, an approximation of tentacle crown diameter). The area covered by the tentacles of each anemone (tentacle crown surface area) was regarded as an oval and was estimated as (long axial length) × (short axial length) × π/4 (after Hirose, 1985). Changes in sea anemone body sizes were estimated by calculating the final tentacle crown surface area of each as a percentage of initial area.

2.4. Zooxanthella parameters

To assess the condition of the zooxanthellae within host sea anemones in each treatment during starvation, we determined the abundance, cell division rate (mitotic index), and chlorophyll *a* content of the zooxanthellae every 2 - 3 wk during the study. During each sample period, 3 tentacle tips (2 - 3 cm long each) were removed from each anemone and immediately analyzed. Sampling did not appear to adversely affect the anemones, as each possessed many tentacles and rapidly regenerated lost tentacle tips (Porat and Chadwick-Furman, 2005). Each tentacle tip was blotted dry and