

Effects of anemonefish on giant sea anemones: Ammonium uptake, zooxanthella content and tissue regeneration

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Abstract

Zooxanthellae symbiotic with stony corals utilise ammonium excreted by resident fish populations. However, the contribution of ammonium produced by anemonefishes to host sea anemones has not been examined. In split-pair laboratory experiments with the giant sea anemone *Entacmaea quadricolor*, one half of each sea anemone was maintained with two individuals of the endemic anemonefish *Amphiprion bicinctus*, and the other half was maintained without anemonefish. Sea anemone fragments maintained with anemonefish regenerated significantly faster than those without anemonefish. After 8 weeks of regeneration, there were more endosymbiotic algae (zooxanthellae) in the tentacles of sea anemones with anemonefish than in those lacking fish. Adult anemonefish (8 cm total length) each excreted ammonium at a rate of about $0.97 \mu\text{M h}^{-1}$. Sea anemones that had been maintained without anemonefish for 4 weeks took up ammonia from enriched water at a faster rate than those that had been maintained with anemonefish, which absorbed very little ammonium. We conclude that anemonefishes provide ammonia for their host anemones and zooxanthellae, enhancing rates of tissue growth and regeneration.

Keywords: *Sea anemone, Actiniaria, anemone fish, symbiosis, nitrogen, nutrient, laboratory trial, Red Sea, Entacmaea quadricolor, Amphiprion bicinctus*

Introduction

One of the best known examples of symbiosis is the association between giant sea anemones and anemonefish. The only documented benefit of this association to the anemone host is protection from butterflyfish predators (Fricke 1974, 1979; Godwin & Fautin 1992; Porat & Chadwick-Furman 2004). However, other benefits have been proposed (Fautin 1991), including the fertilisation of host zooxanthella cells by ammonium excreted as a waste product from the anemonefish (Cleveland et al. 2003; Fautin 1991).

All tropical sea anemones that harbor symbiotic fish also contain zooxanthellae (endosymbiotic dinoflagellates), which provide them with photosynthetic energy, and as such they are limited to the photic zone (Fautin & Allen 1992). Zooxanthellae benefit from association with sea anemone hosts, which serve as a breeding ground and contribute inorganic nutrients (nitrogen, phosphorus, carbon dioxide) and organic molecules from host animal tissue (Lipschultz & Cook 2002; Roberts et al. 1999). The anemone host may be an important nutrient source for zooxanthellae, since this symbiotic association may occur in coral reef areas surrounded by nutrient-poor tropical waters (D'Elia & Wiebe 1990). The sea anemone receives energy in the form of photosynthetic products from the zooxanthellae, which are utilised for respiration, growth and reproduction (Achituv & Dubinsky 1990; Falkowski et al. 1984). A substantial portion of the photosynthate produced by zooxanthellae may be transferred to cnidarian hosts. In the sea anemone *Anemonia sulcata* in the Mediterranean Sea, <50% of photosynthetic products are assimilated by the algae, and the remainder is transferred to the anemone's tissues (Stambler & Dubinsky 1987).

The concentration of inorganic nitrogen in the waters surrounding coral reefs may be <1 μM (D'Elia & Wiebe 1990; Muscatine & Porter 1977), so primary producers on the reef, including symbiotic zooxanthellae, may be limited in part by lack of nitrogen (Cook et al. 1988; Falkowski et al. 1993; McGuire & Szmant 1997; Muscatine et al. 1989). Some symbiotic crustaceans transfer excreted ammonium to cnidarian hosts, which benefit via increased productivity of their zooxanthellae. The shrimp *Periclimenes yucatanicus* excretes ammonium at a rate of $0.0393 \mu\text{M g}^{-1} \text{min}^{-1}$, and provides a source of nitrogen for the zooxanthellae of its sea anemone host *Condylactis gigantea* on reefs in the Caribbean Sea (Spotte 1996). In the Red Sea, fire corals *Millepora dichotoma*, which host symbiotic barnacles (*Savignium milleporum*) absorb inorganic phosphate and carbon from the excretory products of the barnacles (Cook et al. 1991). A comparison of ammonium concentrations in the seawater surrounding barnacles and fire corals under both light and dark conditions has shown that in the light, ammonium concentration is lower, probably as a result of absorption by symbiotic zooxanthellae in the coral (Achituv & Mizrahi 1996). Also mutualistic damselfish (Lieberman et al. 1995) and boring bivalves (Mokady et al. 1998) excrete ammonium which may benefit their host corals.

Migratory fish such as schooling grunts (*Haemulon* spp.) also appear to contribute to stony corals on reefs via ammonium enhancement of the surrounding waters. Ammonium concentrations increased from 0.2 to 0.9 μM in the presence of these fish, causing an increase in zooxanthella abundance and nitrogen concentration in coral tissues (Meyer et al. 1983). Experimental enhancement of ammonium in the surrounding seawater has also been shown to increase the concentration of zooxanthella cells in cnidarian tissues (Dubinsky et al. 1990; Falkowski et al. 1993; Muscatine et al. 1989).

A direct contribution of nitrogen to sea anemone host tissue has been demonstrated by Lipschultz and Cook (2002), who enriched seawater with a stable isotope of nitrogen (^{15}N) and observed its absorption by tissues of the sea anemones *Aiptasia pallida* and *Bartholomea annulata*, and by their endosymbiotic zooxanthellae. Symbiotic anemones (*A. sulcata*) have also been shown to absorb ammonium from surrounding seawater via their zooxanthellae (Roberts et al. 1999). Thus, the transfer of inorganic nitrogen to host sea anemones from symbiotic anemonefish may contribute substantially to the growth of both anemone tissues and symbiotic zooxanthellae. We report here on rates of ammonium excretion by anemonefish, and on rates of ammonium uptake, tissue regeneration, and enhancement of zooxanthella abundance in sea anemone hosts in the northern Red Sea.

Methods

Six individuals of the giant sea anemone *Entacmaea quadricolor*, together with their symbiotic anemonefish *Amphiprion bicinctus*, were collected from 15 to 20 m depth on coral reefs at Eilat, northern Red Sea during March 2002 and transported to the nearby Interuniversity Institute for Marine Science (IUI).

Preliminary experiments indicated that when individuals of *E. quadricolor* were cut lengthwise into two equal halves, they completely regenerated their tentacle crowns, and regained radial symmetry in about two months (Ayalon, pers. comm.). Thus, following an acclimation period of one month in outdoor tanks supplied with flowing seawater at the IUI, each anemone was cut vertically into two equal halves through the mouth area using a sharp razor blade, and allowed to attach to a glass plate in a large ($50 \times 30 \times 20 \text{ cm}^3$) aquarium. One half of each sea anemone was maintained with its original two adult anemonefish symbionts, and the other half was maintained without fish, each in a separate aquarium for eight weeks in outdoor tanks supplied with flowing seawater ($N=6$ halves per treatment). The area of the basal disk was chosen as an indicator of the regeneration rate of sea anemone tissue. Each week for 4 weeks, the regenerating sea anemones were removed from their aquaria, placed in an upside-down position, and their basal disks were photographed through the glass plates to which they were attached. Each photograph was scanned into a computer, and the software program Image Tool was used to measure the area of the base. Light level in the outdoor tanks was about $450 \mu\text{M photons m}^{-2} \text{ s}^{-1}$ at midday during May, which simulated the natural light environment at about 5 m depth on the coral reef where members of these species were common (Chadwick-Furman unpublished data).

Zooxanthella abundance in the anemone tentacles was measured before the experiment, and afterwards every 2 weeks for 8 weeks. At each sample period, we excised the tips (2–3 cm) of 5 tentacles from each sea anemone half (Spotte 1996). This did not appear to greatly affect the anemones, as each regenerating half contained dozens of tentacles. Each tentacle tip was blotted dry and weighed to obtain its wet mass. Then each was transferred to a vial with 2 mL of seawater and homogenized. A subsample of 0.5 mL solution was pipetted from each tube and placed on a microscopic slide, and the zooxanthella cells were counted using a haemocytometer (after McGuire & Szmant 1997; Spotte 1996; Stambler & Dubinsky 1987).

The rate of ammonium excretion by anemonefish was determined by placing each of five adult anemonefish (of 5–10 cm total length and 3.4–15.0 g wet mass each) in a separate aquarium containing 7 L of aerated seawater. Water samples of 1 mL were taken from each aquarium every 20 min for 2 h ($N=6$ samples total per fish, modified after Spotte 1996). Each fish was then removed from the aquarium, gently blotted and weighed to obtain wet mass, and returned to its anemone host on the coral reef adjacent to the IUI. The fish appeared calm and swam normally during the 2 h of excretion rate monitoring, and did not appear to be stressed or breathing rapidly (according to gill cover opening rates). Ammonium levels in all water samples were determined fluorometrically using the method of Holmes et al. (1999). Excretion was calculated as μM of ammonium in each 7-L tank at each sample period, and then converted to μM of ammonium excreted per gram fish.

We compared the rate of ammonium uptake by whole, undamaged *E. quadricolor* anemones after culture with anemonefish for 4 weeks in the laboratory, and then after 4 weeks without anemonefish (after Spotte 1996). Culture conditions and light level in this experiment were as described above. Prior to each uptake measurement, the six

anemones were each transferred to a separate 5.5-L tank with flowing seawater and allowed to acclimate for 24 h. Then the water flow was stopped, air stones were added for aeration, and the ammonium level in each tank was raised by about $8 \mu\text{M}$ (the approximate amount of ammonium that 2 adult anemonefish emit over 4 h, see Results). A water sample of 1 mL was taken from each tank prior to enrichment and then for every 30 min thereafter for 3 h. Ammonium levels were calculated as μM in each 5.5-L tank at each sample period. All statistical analyses were conducted using the SPSS software program. Differences among treatments were compared using paired t -tests (after McGuire & Szmant 1997; Spotte 1996).

Results

After we cut them in half, the anemones contracted their tissues around the damaged areas. Then they slowly re-expanded and attached their basal disks to the glass plates provided. At 1 week after tissue damage, there was no significant difference in anemone size between the treatments, but by 2 weeks, anemones with anemonefish had regenerated significantly more tissue than had those without anemonefish (Paired t -test, $P < 0.01$; Figure 1). After 2 weeks following tissue damage, some of the anemone halves in each treatment died, apparently due to an inability to heal following the cutting process, so statistical tests were not applied after this point. In the surviving anemones, a trend continued of faster

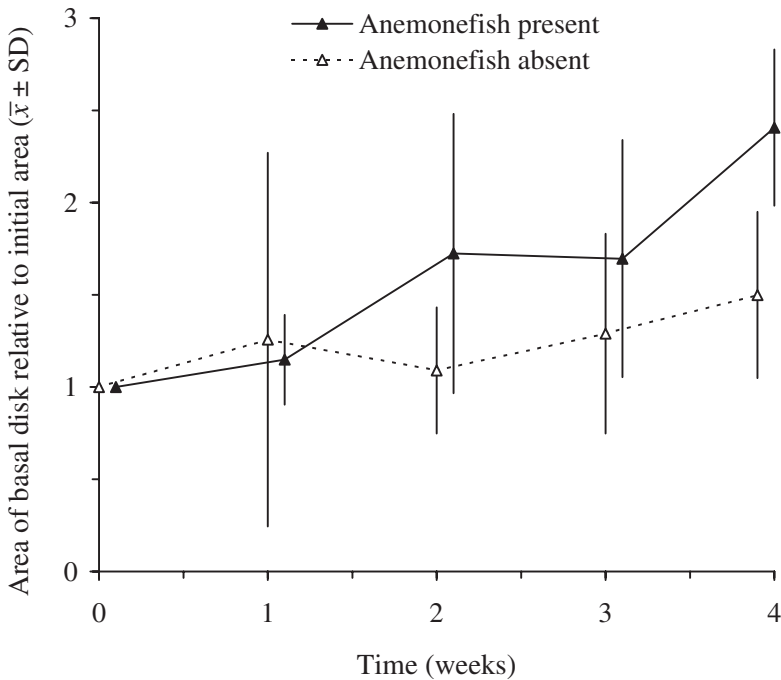


Figure 1. Variation in the regeneration rate of giant sea anemones (*E. quadricolor*) with the presence of anemonefish (*A. bicinctus*) over 4 weeks under laboratory conditions at Eilat, northern Red Sea. Paired observations on $N=6$ regenerating halves of anemones up to 2 weeks, and $N=4$ afterwards, due to the death of some anemones.

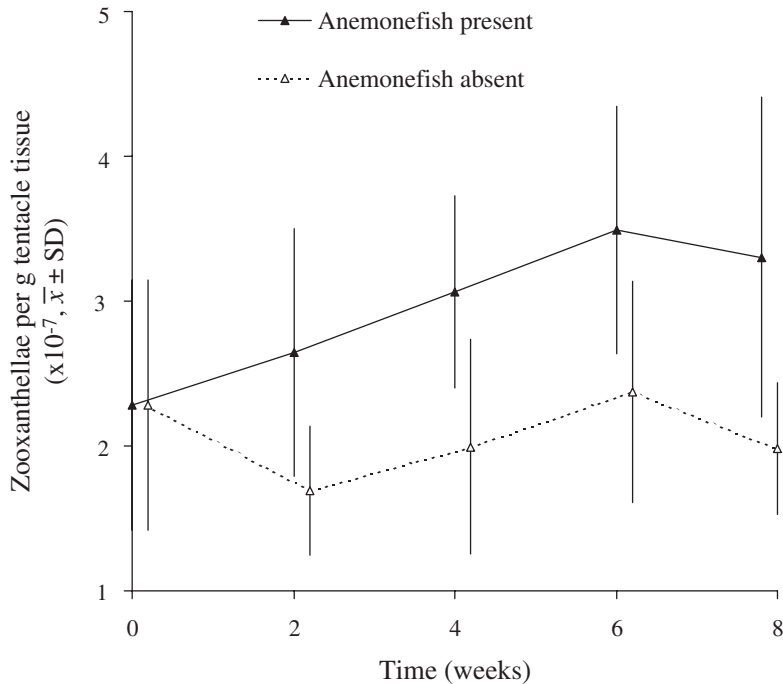


Figure 2. Variation in zooxanthella abundance in the tentacle tissues of regenerating anemones (*E. quadricolor*) with the presence of anemonefish (*A. bicinctus*), as measured at biweekly intervals under laboratory conditions at Eilat, northern Red Sea. Paired observations on $N=6$ regenerating halves of anemones up to 2 weeks, and $N=4$ afterwards, due to the death of some anemones. Zooxanthella abundance reported from each regenerating anemone was calculated as a mean from 5 tentacles sampled each 2 weeks on each anemone.

regeneration in halves with anemonefish than in those without, up to the final measurement of this trait at 4 weeks (Figure 1).

In the tentacles of anemones with resident anemonefish, the abundance of zooxanthellae increased during regeneration, and after 8 weeks was significantly higher than in anemones lacking anemonefish (Paired t -test, $P < 0.04$; Figure 2).

The rate of ammonium excretion by each adult anemonefish averaged about $0.175 \mu\text{M g}^{-1}$ in 2 h (Figure 3), or $0.0015 \mu\text{M g}^{-1} \text{min}^{-1}$. Since the anemonefish weighed about 10.8 g each on an average ($x + \text{SD} = 10.8 + 4.5 \text{ g}$, range = 3.4–15.0 g), each adult anemonefish excreted on an average about $0.972 \mu\text{M ammonium h}^{-1}$ ($0.0015 \mu\text{M g}^{-1} \text{min}^{-1} \times 10.8 \text{ g fish}^{-1} \times 60 \text{ min h}^{-1}$).

After they were maintained with resident anemonefish for 4 weeks, host anemones showed very low rates of uptake of ammonium from enriched seawater (Figure 4). In contrast, after the same anemones were isolated from anemonefish for an additional 4 weeks, they took up ammonium from the surrounding seawater at a much higher rate (Paired t -test of ammonium concentration at 3 h, $P < 0.01$; Figure 4).

Discussion

We show here that under laboratory conditions, giant sea anemones regenerate faster and contain higher concentrations of zooxanthellae in the presence of anemonefish than

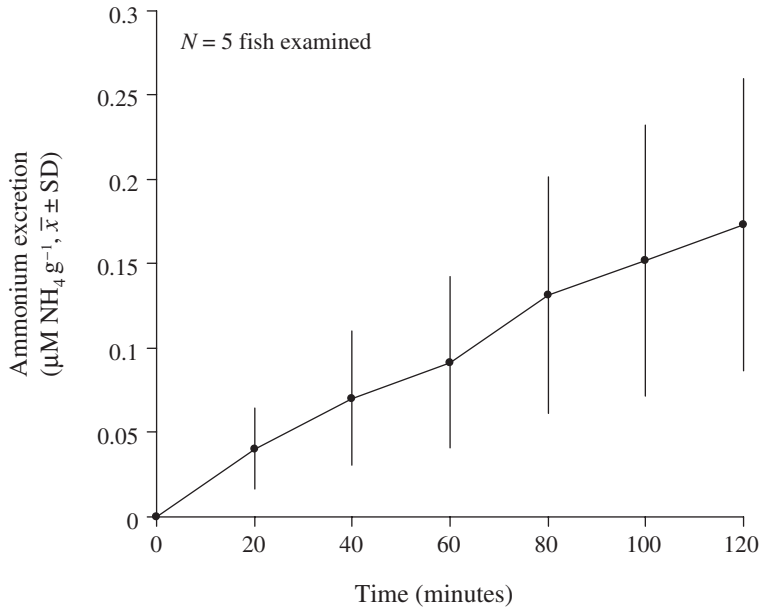


Figure 3. Excretion of ammonium by adult anemonefish (*A. bicinctus*) under laboratory conditions at Eilat, northern Red Sea. Ammonium is reported as μM excreted per gram fish into 7-L seawater tanks.

in their absence. We also demonstrate that adult anemonefish excrete substantial amounts of ammonium, and that anemone hosts which have been isolated from anemonefish appear starved for ammonium, in that they absorb ammonium from surrounding seawater at high rates. We conclude that anemonefish contribute ammonium to their hosts, causing enhanced growth of zooxanthellae and host tissue. The amount of ammonium excreted by adult anemonefish ($0.972 \mu\text{M h}^{-1}$ on average, see Results) is a substantial contribution to the host anemone's environment, which is poor in nutrients (ammonium concentration in waters surrounding the coral reef at Eilat = $0.1 \mu\text{M}$; Muscatine et al. 1989).

As expected, the contribution of anemonefish to ammonium enrichment of their hosts is much higher than that of anemoneshrimps, in part because each anemonefish is about 77 times the mass of each anemoneshrimp ($x + SD = 10.8 + 4.5 \text{ g}$ per fish compared to $0.14 + 0.02 \text{ g}$ per shrimp; Spotte 1996). However, anemonefish excrete ammonium at a lower rate per gram body mass ($0.0015 \mu\text{M g}^{-1} \text{ min}^{-1}$; see Results) than shrimps do ($0.0393 \mu\text{M g}^{-1} \text{ min}^{-1}$; Spotte 1996). Symbiotic fish and shrimps also differ in their behaviour. Anemoneshrimps remain close to the anemone, and may consume anemone tentacles as food (Fautin et al. 1995). In contrast, anemonefish consume zooplankton, and during feeding they may range several meters from their host (Fricke 1974, 1979; Meroz & Fishelson 1997; Porat & Chadwick-Furman 2004). Thus, anemonefish act as importers of nutrients from the plankton to the reef, and enrich the environment of the anemone host with excreted nutrients obtained from a source external to the reef benthos.

In contrast to findings reported here for anemonefish (Figure 4), Spotte (1996) found that host anemones cultured with shrimp absorbed ammonium at higher rates than those that had lacked shrimp. This was explained by a higher density of zooxanthellae in anemones

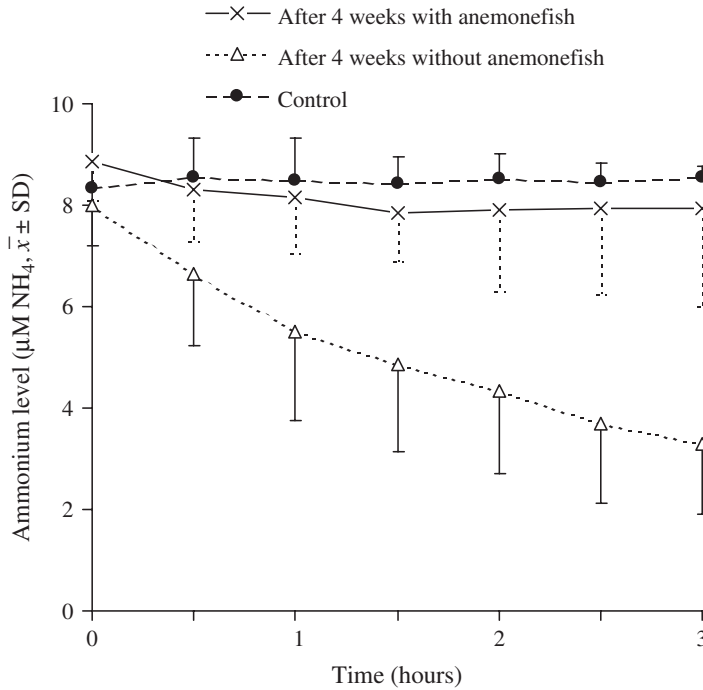


Figure 4. Variation in ammonium absorption from enriched seawater by sea anemones (*E. quadricolor*) under laboratory culture conditions at Eilat, northern Red Sea. Anemones were maintained for 4 weeks with anemonefish (*A. bicinctus*) and tested in enriched seawater, then the same anemones were cultured in isolation from anemonefish for an additional 4 weeks and retested ($N=6$ anemones). Control = enriched seawater with no anemones present ($N=2$ tanks). Ammonium levels are reported as μM in each 5.5-L tank of seawater.

cultured with shrimps. Another contributing factor may be inherent differences between fish and shrimps, as described above. Therefore the contribution of anemonefish is much higher than that of anemone shrimps, and could affect the relationship between an excess versus a deficiency of nutrients in the system. The amount of time spent by anemone hosts without symbionts prior to testing nutrient uptake could also have an effect on the system; this parameter was not reported in the shrimp experiment (Spotte 1996).

Past work which demonstrated an increase in zooxanthella density in cnidarians as a result of water enrichment was based on ammonium concentrations ($2\text{--}20\ \mu\text{M}$) that were held constant over time (Dubinsky et al. 1990; Falkowski et al. 1993; Muscatine et al. 1989). We observed here that zooxanthella abundance also increases when ammonium enrichment is from a natural source (anemonefish, Figure 2) which varies greatly due to differences in behaviour and size among fish. Zooxanthella abundances in the anemone tentacles that we observed here ($\approx 2\text{--}4 \times 10^7\ \text{cells g}^{-1}$; Figure 2) were lower than those found in tentacles of the anemone *C. gigantea* in the Caribbean Sea ($13\text{--}16 \times 10^7\ \text{cells g}^{-1}$; Spotte 1996) and in entire individuals of *A. sulcata* in the Mediterranean Sea ($4.5\text{--}6 \times 10^7\ \text{cells g}^{-1}$; Stambler & Dubinsky 1987), and may reflect the extremely high levels of irradiance occurring in the Red Sea area.

The anemonefish presence may affect the regeneration of damaged sea anemone tissue in several ways. This association may be important in times of stress and injury to anemone tissues, since the anemone directly absorbs ammonium excreted by the fish,

thus accumulating materials to rebuild proteins. Enrichment of seawater with ammonium is known to cause increased protein content in the tissue of other sea anemones and corals (Muscatine et al. 1989). The presence of the anemonefish also causes an increase in the abundance of the symbiotic zooxanthellae (Figure 2), which are a major source of energy for the anemone. In addition to contributing nutrients, anemonefish may stimulate their hosts mechanically and increase water movement among the tentacles, thus increasing rates of gas exchange in host tissues (Fautin 1991; Porat & Chadwick-Furman 2004); however, this effect has never been tested experimentally.

Experimental damage and subsequent tissue rehabilitation of sea anemones in the laboratory may simulate natural processes on the coral reef, such as partial predation and asexual reproduction. When sea anemone hosts are damaged by the attacks of predators, the presence of anemonefish may strongly affect their tissue regeneration, both in terms of defense from further attack (Fricke 1979; Godwin & Fautin 1992; Porat & Chadwick-Furman 2004), and contribution of nutrients for tissue growth (Figure 1). The results presented here are the first indication of how inorganic nutrient excretion by anemonefish may contribute to host anemone tissue growth and symbiotic algal populations. Direct evidence of transfer of labelled nitrogen (^{15}N) from anemonefish to hosts further demonstrates that nutrients are contributed to the host in this association (Cleveland et al. 2003). These studies add to the growing body of knowledge (Godwin & Fautin 1992; Porat & Chadwick-Furman 2004) on how anemonefish affect the biology of their host sea anemones.

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References

- Achituv Y, Dubinsky Z. 1990. Carbon budgets in marine, mutualistic associations between microalgae and cnidarians. *Comp. Physiol.* 5:36–48.
- Achituv Y, Mizrahi L. 1996. Recycling of ammonium within a hydrocoral (*Millepora dichotoma*)-zooxanthellae-cirripede (*Savignium milleporum*) symbiotic association. *Bull. Mar. Sci.* 58:856–860.
- Cleveland A, Verde EA, Lee R. 2003. Investigating the physiological basis for the clownfish/host anemone symbiosis: Do resident fish provide their host with nitrogen? Abstract, 4th Internat. Symbiosis Soc. Conf. Halifax, Nova Scotia, Canada.
- Cook CB, D'Elia CF, Muller-Parker G. 1988. Host feeding and nutrient sufficiency for zooxanthellae in the sea anemone *Aiptasia pallida*. *Mar. Biol.* 98:253–262.
- Cook PA, Stewart BA, Achituv Y. 1991. The symbiotic relationship between the hydrocoral *Millepora dichotoma* and the barnacle *Savignium milleporum*. *Hydrobiologia* 216/7:285–290.
- D'Elia CF, Wiebe WJ. 1990. Biogeochemical cycle in coral-reef ecosystems. In: Dubinsky Z. editor, *Ecosystems of the world. Coral reefs*. Amsterdam: Elsevier. pp. 49–74.
- Dubinsky Z, Stambler N, Ben-Zion M, McCloskey LR, Muscatine L, Falkowski PG. 1990. The effect of external nutrient resources on the optical properties and photosynthetic efficiency of *Stylophora pistillata*. *Proc. Royal Soc. Lond. B* 239:231–246.
- Falkowski PG, Dubinsky Z, Muscatine L, Porter JW. 1984. Light and the bioenergetics of a symbiotic coral. *Bioscience* 34:705–709.
- Falkowski PG, Dubinsky Z, Muscatine L, McCloskey L. 1993. Population control in symbiotic corals: Ammonium ions and organic materials maintain the density of zooxanthellae. *Bioscience* 43:606–611.

- Fautin DG. 1991. The anemonefish symbiosis: What is known and what is not. *Symbiosis* 10:23–46.
- Fautin DG, Allen GR. 1992. Field guide to anemonefishes and their host sea anemones. Perth: Western Australian Museum. p. 60.
- Fautin DG, Guo C-C, Hwang J-S. 1995. Costs and benefits of the symbiosis between the anemoneshrimp *Periclimenes brevicarpalis* and its host *Entacmaea quadricolor*. *Mar. Ecol. Prog. Ser.* 129:77–84.
- Fricke VHW. 1974. Öko-ethologie des monogamen anemonefisches *Amphiprion bicinctus* (Freiwasseruntersuchung aus dem Roten Meer). *Zeits. Tierpsychol.* 36:492–512.
- Fricke HW. 1979. Mating system, resource defense and sex change in the anemonefish *Amphiprion akallopisos*. *Zeits. Tierpsychol.* 50:313–326.
- Godwin J, Fautin DG. 1992. Defense of host actinians by anemonefishes. *Copeia* 3:902–908.
- Holmes RM, Aminot A, Kerouel R, Hooker BA, Peterson BJ. 1999. A simple precise method for measuring ammonium in marine and freshwater ecosystems. *Can. J. Fish. Aquat. Sci.* 56:1801–1808.
- Lieberman T, Genin A, Loya Y. 1995. Effects on growth and reproduction of the coral *Stylophora pistillata* by the mutualistic damselfish *Dascyllus marginatus*. *Mar. Biol.* 121:741–746.
- Lipschultz F, Cook CB. 2002. Uptake and assimilation of ¹⁵N-ammonium by the symbiotic sea anemones *Bartholomea annulata* and *Aiptasia pallida*: Conservation versus recycling of nitrogen. *Mar. Biol.* 140:489–502.
- McGuire MP, Szmant AM. 1997. Time course of physiological responses to NH₄ enrichment by a coral-zooxanthellae symbiosis. *Proc. Eighth Internat. Coral Reef Symp.* 1:909–914.
- Meroz A, Fishelson L. 1997. Juvenile production of *Amphiprion bicinctus* (Pomacentridae, Teleostei) and rehabilitation of impoverished habitats. *Mar. Ecol. Prog. Ser.* 151:295–297.
- Meyer JL, Schultz ET, Helfman GS. 1983. Fish schools: An asset to corals. *Science* 220:1047–1049.
- Mokady O, Loya Y, Lazar B. 1998. Ammonium contribution from boring bivalves to their coral host – a mutualistic symbiosis? *Mar. Ecol. Prog. Ser.* 169:295–301.
- Muscatine L, Falkowski PG, Dubinsky Z, Cook PA, McCloskey LR. 1989. The effect of external nutrient resources on the population dynamics of zooxanthellae in a reef coral. *Proc. Royal Soc. Lond. B* 236:311–324.
- Muscatine L, Porter JW. 1977. Reef corals: Mutualistic symbioses adapted to nutrient-poor environments. *Bioscience* 27:454–460.
- Porat D, Chadwick-Furman NE. 2004. Effects of anemonefish on giant sea anemones: Expansion behavior, growth and survival. *Hydrobiologia* 530/531:513–520.
- Roberts JM, Davies PS, Fixter LM, Preston T. 1999. Primary site and initial products of ammonium assimilation in the symbiotic sea anemone *Anemonia viridis*. *Mar. Biol.* 135:223–236.
- Spotte S. 1996. Supply of regenerated nitrogen to sea anemones by their symbiotic shrimp. *J. Exp. Mar. Biol. Ecol.* 198:27–36.
- Stambler N, Dubinsky Z. 1987. Energy relationships between *Anemonia sulcata* and its endosymbiotic zooxanthellae. *Symbiosis* 3:233–248.

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