

**Nocturnal Ecophysiology of the Anemonefish-Sea Anemone Mutualism:
Patterns of Dark Oxygen Consumption and Symbiont Behavior at Night**

by

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Abstract

The mutualism between anemonefishes and giant sea anemones is one of the most well known interactions on coral reefs. While the symbiotic benefits provided to each partner have been researched for over 100 years, little is known about the mutualism at night. Further, the ecophysiological mechanisms that underpin the ecological benefits of the mutualism remain greatly unexplored. Here, I conducted foundational research on the metabolic and behavioral interactions of the anemonefish-sea anemone mutualism at night. Physical contact between anemonefish and sea anemones elevates the net dark oxygen (O_2) consumption of the partners. Further, anemonefish engage in more flow-modulating activities when sea anemones are present than when anemonefish are alone. Lastly, sea anemone O_2 consumption increases with water flow. I conclude that anemonefish behavior at night modulates sea anemone O_2 consumption by forced convection of ambient water flow.

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CHAPTER I

The benefits and ecophysiology of mutualistic symbioses on coral reefs

BENEFITS OF CORAL REEF MUTUALISMS

The high biodiversity on coral reefs is unparalleled in other marine habitats, and globally is second only to that of tropical rainforests (Reaka-Kudla, 1997). Symbiotic interactions on coral reefs, such as the complex mutualistic associations between sedentary cnidarian hosts and their symbiotic guests, contribute to the biodiversity acknowledged in coral reef systems (Davies, 1992). While the ecological importance of mutualisms is recognized, the extent of the benefits and the underlying biological processes remain greatly unexplored.

Most research on mutualisms between cnidarian hosts (i.e., corals and sea anemones) and symbiotic guests on coral reefs has focused on the direct benefits provided to the participants. One of the most visible benefits is mutual protection afforded through habitat and/or physical defense. Reef cnidarians are vulnerable to predation as a result of their sedentary nature (e.g., Porat and Chadwick-Furman, 2004). However, many corals and sea anemones function as structural habitat for obligate and/or facultative fish and crustacean guests that protect their hosts from predation. Pocilloporid corals on Western Pacific coral reefs host obligate guard crabs (*Trapezia* and *Tetralia* sp.) that effectively deter predation on the coral host by the crown-of-thorns sea star (*Acanthaster planci*) (Pratchett et al., 2000). The *Trapezia* crabs drive the low frequency of pocilloporid corals in the diet of *A. planci* (Pratchett, 2001). Similarly, on Indo-Pacific coral reefs, anemonefishes (family Pomacentridae) chase away butterflyfishes (family Chaetodontidae) that prey upon host sea anemone tentacles (Fautin, 1991; Fautin and

Allen, 1997; Porat and Chadwick-Furman, 2004), and the stinging nematocysts of host sea anemones protect anemonefishes from piscivores (Mariscal, 1970b). Although not as apparent as the protection of symbiotic partners, mutualisms also contribute to the nutrient dynamics on coral reefs.

Coral reefs are considered to be productive oases amid oligotrophic marine deserts (Hoegh-Guldberg, 1999). The success of coral reefs within highly unproductive tropical waters is largely attributed to the efficient use and recycling of essential nutrients (i.e., organic and inorganic nitrogen and carbon compounds) (Davies, 1992). Corals and sea anemones metabolize organic carbon (Muscatine, 1990; Biel et al., 2007) and nitrogen (Wang and Douglas, 1998; Wang and Douglas, 1999) produced by their endosymbiotic dinoflagellate microalgae, which then utilize the inorganic carbon and nitrogen from the metabolic byproducts of the cnidarian hosts (Furla et al., 2005; Venn et al., 2008). By recycling the metabolic wastes of their symbiotic partner, the union between coral reef cnidarians and microalgae facilitates coral reef formation over a broader range of environmental conditions than possible in the absence of the mutualism (Bruno et al., 2003).

Ectosymbiotic guests of coral reef cnidarians (e.g., anemonefishes) also contribute to the tight nutrient cycling of mutualisms. Laboratory studies on anemonefish (*Amphiprion bicinctus*) and sea anemones (*Entacmaea quadricolor*) by Roopin et al. (2008) strongly indicate that sea anemones uptake ammonia excreted by anemonefish, leading to increased nutritional benefits for the host (Roopin and Chadwick, 2009). Godinot and Chadwick (2009) additionally suggested that anemonefish (*A. bicinctus*) supply phosphate to sea anemones (*E. quadricolor*), although sea anemone demand exceeds anemonefish supply. Using ¹³C- and ¹⁵N-labeled isotopes, Cleveland et al. (2010)

confirmed that sea anemones (*Heteractis crispa*) uptake nitrogen and carbon compounds from anemonefishes (*A. clarkii*, *A. perideraion*), and documented the subsequent uptake of carbon and nitrogen by microalgae within sea anemone tissue. Ammonia recycling has also been suggested between anemoneshrimps (*Periclimenes yucatanicus*) and sea anemones (*Condylactis gigantea*) (Spotte, 1996). Nutritive benefits may be universal among intimate coral reef mutualisms; however, more research on the nutrient dynamics of similar symbiotic associations on coral reefs is needed.

The defensive and nutritive benefits of mutualisms between reef cnidarians and their symbionts drive a wide range of indirect ecological benefits. For example, the temperate coral *Oculina arbuscula* is an inferior competitor to the brown seaweeds (e.g., *Sargassum*, *Padina*, and *Dictyota*) that dominate well-lit reef structure off North Carolina, USA (Stachowicz and Hay, 1999). *O. arbuscula* host facultative omnivorous crabs (*Mithrax forceps*) that consume the algal competitors of the coral host. Consequently, colonies of *O. arbuscula* that hosted *M. forceps* grow 10x faster than colonies without *M. forceps*, and *M. forceps* grow faster on live corals than dead corals (Stachowicz and Hay, 1999). Similarly, in the Red Sea, stony corals (*Stylophora pistillata*) that host obligate damselfish (*Dascyllus marginatus*) experience faster long term (>7 mo) growth and reproductive output than *S. pistillata* without damselfish guests (Lieberman et al., 1995).

The ecological benefits provided by coral reef mutualisms can extend beyond the symbiotic participants to other organisms. For example, increased nutrition to photosynthetic dinoflagellates within sea anemone tissues, such as the nitrogen provided by anemonefishes (Roopin et al., 2008; Roopin and Chadwick, 2009; Cleveland et al., 2010), increases rates of microalgal photosynthesis and mitotic division (Fit and Cook,

2001). The microalgae can then supply more organic compounds to sea anemones and increase the growth and reproduction of their host (Hoegh-Guldberg and Smith, 1989; Fit and Cook, 2001). Further, anemonefish residency enhances sea anemone asexual reproduction (Holbrook and Schmitt, 2005), growth, survivorship (Porat and Chadwick-Furman, 2004; Holbrook and Schmitt, 2005), dinoflagellate abundance, and tissue regeneration (Porat and Chadwick-Furman, 2004). The microalgae- and anemonefish-induced nutrient enhancements increase the ecological performance of sea anemones which then cover a greater net area on coral reefs (Schmitt and Holbrook, 2003). Off the coast of Moorea in French Polynesia, the increased area of sea anemone cover produced by the tripartite mutualism of sea anemones (*Heteractis magnifica*), microalgae (*Symbiodinium* sp.), and anemonefish (*A. chrysopterus*) enhances biodiversity on coral reefs, by providing more habitat for three-spot damselfish (*Dascyllus trimaculatus*), which are outcompeted by anemonefish when sea anemones cover less net area (Schmitt and Holbrook, 2003).

Mutualisms on coral reefs further benefit the reef community by connecting pelagic and littoral food webs. Planktivorous fishes, many of which are facultative or obligate guests of reef cnidarians, transfer nutrients from the open water to the benthos via nitrogen- and phosphorus-rich feces (Pinnegar and Polunin, 2005; Holbrook et al., 2008) and excretions (Porat and Chadwick-Furman, 2005; Roopin et al., 2008; Godinot and Chadwick, 2009; Roopin and Chadwick, 2009; Cleveland et al., 2010). By serving as net importers of nutrients to coral reef organisms, the ectosymbiotic guests of many marine mutualisms contribute to the productivity and biodiversity associated with coral reefs.

Mutual protection and nutrient recycling by symbiotic partners serve as essential mechanisms leading to enhanced habitat for the partners themselves, as well as other organisms on coral reefs. Currently, we have only scratched the surface of the complexity and importance of facilitative interactions, such as mutualistic symbioses (Bruno et al., 2003). Continued research on the benefits of mutualisms, as well as the underlying biological mechanisms, will lead to a more complete understanding of the ecology of tropical coral reefs.

ECOPHYSIOLOGICAL ADAPTATIONS TO ENVIRONMENTAL STRESS

The sedentary nature of the organisms involved in many marine symbioses (e.g., mutualisms between cnidarian hosts and obligate guests) may make them more susceptible than free-ranging organisms to environmental stressors on coral reefs. For example, the oxygen concentration ($[O_2]$) surrounding coral reefs is highly variable, especially during low tide and at night. The $[O_2]$ of the ambient water around coral reefs off Heron Island on the Great Barrier Reef decreases substantially when the lagoons are cut off from the ocean by the reef crest during low tide (Kinsey and Kinsey, 1967). Additionally, phase shifts from coral- to algal-dominated reefs reduce the ambient $[O_2]$ above the reef structure, most likely by increasing labile dissolved organic matter and subsequent microbial metabolism (Niggli et al., 2010; Wild et al., 2010). On a microhabitat scale, the $[O_2]$ of the water among coral branches becomes severely hypoxic at night, as documented on reefs off Lizard Island at the Great Barrier Reef (Nilsson et al., 2004) and in the northern Red Sea at Eilat (Goldshmid et al., 2004). Lastly, reef cnidarians experience diel cycles of O_2 availability within their tissues, which become hyperoxic during the daytime due to microalgal photosynthesis, and hypoxic at night due

to the respiration of both microalgae and host (Shashar et al., 1993; Richier et al., 2003). Even when the $[O_2]$ is normoxic (70-100% saturation), local flow variability can restrict the availability of O_2 and other substances to many sedentary coral reef organisms.

Water flow is one of the most important abiotic factors affecting the growth and survivorship of sessile marine invertebrates (Sebens et al., 2003), which generally lack the ability to self-regulate the mass transfer of dissolved particles across their tissues (Shick, 1990). The flow-induced reduction or elimination of the diffusive boundary layer surrounding sedentary organisms notably enhances gas exchange (Patterson and Sebens, 1989; Patterson et al., 1991; Bruno and Edmunds, 1998; Sebens et al., 2003; Finelli et al., 2006; Schutter et al., 2010), nutrient uptake (Stambler et al., 1991; Atkinson and Bilger, 1992; Lesser et al., 1994; Thomas and Atkinson, 1997), prey capture (Helmuth and Sebens, 1993; Sebens, 1997; Sebens et al., 1998), and debris removal (Nugues and Roberts, 2003; Box and Mumby, 2007). The waters above coral reefs are generally associated with wave-induced oscillatory flow (Helmuth and Sebens, 1993), which reduces the thickness of the diffusive boundary layer that limits gas and nutrient transfer in sedentary invertebrates (Reidenbach et al., 2006). However, coral reefs are topographically complex and water flow at the surface of the reef structure is likely restricted. For example reef crests and flats experience high flow regimes from waves and tides, while protected lagoons, fore reefs, and reefs at greater depths are sheltered from wave action and experience weaker water flow regimes (Sebens, 1997; Sebens et al., 1997). Moreover, within a microhabitat, water flow can be reduced or diverted by dense colonies of structurally complex organisms (e.g., reef-building corals) (Sebens, 1997). Similarly, the flow regime around crevice dwellers (e.g., sea anemones) may be obstructed by protrusions of the reef structure.

The variable [O₂] and water flow encountered by sedentary invertebrates on coral reefs threatens the residency of their symbiotic guests. However, symbiotic guests employ unique ecophysiological adaptations to maintain the benefits of the mutualisms. At Lizard Island on the Great Barrier Reef, coral-dwelling fishes (Gobiidae, Scorpaenidae) that take refuge among coral branches at night exhibit substantial hypoxia tolerance and air breathing abilities (Nilsson et al., 2004; Nilsson and Ostlund-Nilsson, 2004; Nilsson et al., 2007b). Further, the relative expression of these adaptations is correlated to species-specific habitat preferences, such that fishes among the branches of coral colonies near the water surface (i.e., more likely to become air-exposed) possess greater air breathing abilities than fishes among coral colonies at greater depths (Nilsson et al., 2007b). Similarly, low rates of water flow on coral reefs at Eilat in the northern Red Sea contribute to hypoxic conditions among coral branches at night (Goldshmid et al., 2004). Three species of damselfishes that reside among the corals actively aerate their hosts by beating their fins at stroke frequencies 2x faster than during diurnal swimming. This modulation of the hydrodynamic conditions among coral branches effectively restores [O₂] to that of the ambient water (Goldshmid et al., 2004).

Cnidarian hosts and their endosymbiotic dinoflagellates have evolved physiological adaptations to oxidative stress within host tissue. The hyperoxic environment within cnidarian tissues during the day can increase the abundance of harmful O₂ derivatives (i.e., reactive oxygen species, ROS) that cause severe cellular damage (Li and Jackson, 2002; Lushchak and Bagnyukova, 2006). To protect against oxidative stress, corals and sea anemones possess antioxidant systems, such as the superoxide dismutase enzyme (SOD), which reduces ROS to less harmful derivatives (Li and Jackson, 2002). Richier et al. (2005) reported that symbiotic cells of the sea anemone

Anemonia viridis have higher SOD activity and diversity than aposymbiotic cells. Furthermore, within symbiotic sea anemone tissue (*Anthopleura elegantissima*), SOD activity positively correlates with chlorophyll concentrations (proxy for endosymbiotic microalgae) (Dyken and Shick, 1984).

Coral reef mutualisms employ a myriad of ecophysiological adaptations to buffer the symbiotic participants against environmental stressors, such as O₂ variability and water flow patterns. The evolution of essential physiological and behavioral connections between mutualistic partners underpins the nutritive and ecological advantages associated with these facilitative interactions. Further investigation of the ecophysiology and behavior of mutualistic interactions will clarify how coral reef organisms adapt to changes in their naturally variable environment, as well as to the human- and climate-induced changes currently affecting the world's coral reefs.

The goal of this thesis is to explore potential nocturnal benefits and underlying ecophysiological components of the anemonefish-sea anemone mutualism. As a model, I used the two-band anemonefish (*Amphiprion bicinctus*) and its preferred host in the Red Sea, the bulb-tentacle sea anemone (*Entacmaea quadricolor*) (Chadwick and Arvedlund, 2005). While previous investigations demonstrate that nocturnal physiology and associated behaviors are essential components to the maintenance of similar symbiotic associations (Goldshmid et al., 2004; Nilsson et al., 2004), little is known about the nocturnal interactions between anemonefishes and sea anemones. Further, the Red Sea anemonefish-sea anemone mutualism is a practical model to address the metabolic and behavioral interactions of mutualisms on coral reefs at night because of the intimate nature of the partners at night, in which anemonefish spend the entire night nestled among the sea anemone tentacles (Allen, 1974; Fautin and Allen, 1997).

CHAPTER II

Anemonefish behavior at night modulates sea anemone oxygen consumption

SUMMARY

Mutualisms between sessile cnidarian hosts (i.e., corals and sea anemones) and their fish guests involve complex metabolic and behavioral components, especially at night when the fishes rest in association with the host. While some aspects of the mutualism between anemonefishes and giant sea anemones have been well examined, the benefits derived by the partners at night, and the underlying biological processes involved, remain greatly unexplored. The present study investigated the metabolic and behavioral components of the anemonefish-sea anemone mutualism at night, using two-band anemonefish (*Amphiprion bicinctus*) and bulb-tentacle sea anemones (*Entacmaea quadricolor*). The net dark oxygen consumption (VO_2 , $\mu\text{mol O}_2 \text{ hr}^{-1}$) of pairs (fish+anemone) were measured separately, together as a unit, and together but separated by a mesh screen that prevented physical contact. The net VO_2 of the symbionts when incubated together was 1.4x higher than that of the partners in isolation of each other, or separated by a mesh barrier. The symbiotic association between anemonefish and sea anemones elevates the VO_2 of at least one partner, and physical contact between partners is needed to induce the metabolic elevation. The VO_2 of isolated sea anemones increased with water flow until 2 cm s^{-1} , after which VO_2 remained constant up to 8 cm s^{-1} . Using infrared video, I observed the nocturnal behavior of anemonefish in the absence and

presence of sea anemone hosts to categorize the behavioral repertoire of anemonefish at night and to discern the effect of sea anemone residency on anemonefish behavior. The percent time and bout frequency of several types of anemonefish behavior (i.e., fanning, wedging, switching) increased significantly when the host sea anemone was present. Based on the enhancement of flow-modulating behaviors by anemonefish when they occurred with sea anemones, and the increase of sea anemone VO_2 with flow, I conclude that anemonefish behavior at night likely oxygenates host anemones and augments metabolism in both partners.

INTRODUCTION

The symbiosis between anemonefishes and giant sea anemones on Indo-Pacific coral reefs is one of the most conspicuous and endeared mutualisms in marine environments, and the benefits provided to the participants have been heavily researched. A fundamental benefit to this symbiotic association is mutual protection against predation; anemonefishes chase away butterflyfishes (Chaetodontidae) that prey on sea anemone tentacles (Fautin, 1991; Fautin and Allen, 1997; Porat and Chadwick-Furman, 2004), and the nematocysts within sea anemone tissue ward off piscivorous predators of anemonefishes (Mariscal, 1970b). Moreover, the stinging tentacles of sea anemones provide a protective veil behind which anemonefishes lay their benthic egg clutches (Moyer, 1976; Moyer and Steene, 1979; Fautin, 1991; Arvedlund et al., 2000).

In addition to protection from predators, anemonefish residence provides nutritional benefits that enhance host sea anemone growth, reproduction, and survivorship (Porat and Chadwick-Furman, 2004; Holbrook and Schmitt, 2005; Porat and

Chadwick-Furman, 2005). Through excretion, anemonefishes provide inorganic nitrogen, phosphate, and carbon to the endosymbiotic dinoflagellates within sea anemone tissue (Porat and Chadwick-Furman, 2005; Roopin et al., 2008; Godinot and Chadwick, 2009; Roopin and Chadwick, 2009; Cleveland et al., 2010). Nutrient fertilization of sea anemones by resident anemonefishes and their microalgae contributes to increased rates of sea anemone regeneration and proliferation, which in turn enhances the net area of sea anemone habitat for anemonefishes and other facultative fish residents that are outcompeted by anemonefishes when sea anemones are smaller and less abundant (Schmitt and Holbrook, 2003).

Despite over 100 yr of research on the symbiotic interactions between sea anemones and their anemonefishes, the net benefits and underlying biological processes of the mutualism at night remain greatly unexplored. This is particularly surprising given the intimate nature of the mutualism at night. During the day, anemonefishes swim meters above host sea anemones to forage for zooplankton in the water column, but spend the entire night among sea anemone tentacles for rest and protection (Allen, 1974; Fautin and Allen, 1997).

Further, intimate symbioses between coral reef cnidarians (i.e., corals and sea anemones) and fishes are common in marine systems, and the unique metabolic and behavioral adaptations of these symbionts at night are essential to maintain symbiotic benefits during variable nocturnal conditions. At Lizard Island on the Great Barrier Reef, the oxygen availability among living coral branches can drop severely at night (Nilsson et al., 2004), and coral-dwelling fishes that take refuge among coral branches exhibit substantial hypoxia tolerance and air breathing abilities (Nilsson et al., 2004; Nilsson and

Ostlund-Nilsson, 2004; Nilsson et al., 2007b). Moreover, in the northern Red Sea, hypoxic conditions at night are compounded by reduced water flow over reef structure (Goldshmid et al., 2004), and damselfishes that reside among coral branches engage in sleep-swimming behaviors to increase oxygen availability among coral branches (Goldshmid et al., 2004).

Sea anemones, like many sessile marine invertebrates on coral reefs, are largely unable to self-modulate the bulk flow of sea water across their tissues, and thus rely on ambient water flow for the mass transfer of essential gases and nutrients (Sebens, 1987). Currently, the potential for anemonefishes to modulate the flow regime surrounding host sea anemones has only been hypothesized (Mariscal, 1970b; Allen, 1974; Fautin, 1991; Porat and Chadwick-Furman, 2004, 2005). Further, qualitative observations of *in situ* nocturnal behavior suggest that anemonefishes are generally inactive at night (Allen, 1974). Regardless, the variable oxygen concentrations (Kinsey and Kinsey, 1967; Niggel et al., 2010; Wild et al., 2010) and flow rates (Sebens and Done, 1992; Helmuth and Sebens, 1993; Johnson and Sebens, 1993) of coral reefs demonstrate the potential importance of nocturnal interactions between sea anemones and anemonefishes. Like similar symbiotic networks on coral reefs, the metabolic and behavioral association of sea anemones and anemonefish at night may be an essential aspect of this mutualism.

In the present study, I explored the nocturnal interactions between the bulb-tentacle sea anemone and its obligate fish guest in the northern Red Sea, the two-band anemonefish. I measured the effects of the symbiotic association on the metabolism (oxygen consumption) of the symbionts at night, and the effect of water motion on the gas exchange of sea anemones. Further, I conducted nocturnal surveillance on

anemonefish to characterize fish activity at night and determine the effect of fish behaviors on the nocturnal metabolism of the mutualism.

MATERIALS AND METHODS

Animal collection and maintenance

At the Marine Science Station (MSS) at Aqaba, Jordan, two-band anemonefish (*Amphiprion bicinctus*, Rüppell 1828), 7-11 cm fork length [FL], and bulb-tentacle sea anemones (*Entacmaea quadricolor*, Rüppell and Leuckart 1828), 11-16 cm tentacle crown diameter [TCD]), were obtained in June 2010 from shallow coral reefs adjacent to the MSS (29°27.250' N, 34°58.359' E). All animals were distributed haphazardly among flow-through aquaria (80 L) circulating seawater pumped from the Red Sea. Aquaria received a 12:12 light:dark photoperiod using halogen lighting (Aqua-Medic, Fort Collins, CO, USA). Fish were fed Formula One Marine Flake (Ocean Nutrition, San Diego, CA, USA) daily, and anemones were hand fed frozen fish (*Atherinomorus* spp.) weekly.

At Auburn University (AU) in Alabama, USA, *A. bicinctus* (10-12 cm FL) were obtained from Oceans, Reefs & Aquariums (Florida, USA) in 2006, and *E. quadricolor* (10-18 cm TCD) were obtained from SunPet, Inc. (GA, USA) and from the New England Aquarium (MA, USA) during August 2010-August 2011. At AU, animals were distributed haphazardly among 150 L glass aquaria (two fish, 1 anemone tank⁻¹) circulating artificial seawater and receiving a 12:12 light:dark photoperiod using high-output fluorescent lighting (Sunlight Supply, Inc., Pompano, FL, USA). Fish were fed a mixed diet of Formula One Marine Pellet (Ocean Nutrition, San Diego, CA, USA) and

frozen foods (Mysid Shrimp and Emerald Entrée; San Francisco Bay Brand, Inc., Newark, CA, USA) daily, and anemones were hand-fed raw shrimp weekly. All animals appeared to be in good physiological condition prior to and during experimental use.

Nocturnal oxygen consumption patterns

The metabolic effects of the symbiotic association between fish and anemones were assessed at both the MSS and AU using flow-through respirometry (Fig. 1). Animals were placed in custom-made acrylic chambers connected to a recirculating seawater reservoir (150 L, flow= $1.0 \pm 0.1 \text{ cm s}^{-1}$). Two Clark-type oxygen (O_2) electrodes (Strathkelvin Instruments, Ltd., North Lanarkshire, Motherwell, Scotland) were attached to the inflow and outflow ports of the chamber. To ensure a uniform O_2 concentration ($[\text{O}_2]$) within the chamber, a stir bar covered by a small mesh cage in the bottom of the chamber gently mixed the seawater. To attain a standard metabolic state (post-absorptive and quiescent), animals were starved for ≥ 24 hr prior to experimental use, and all experiments were conducted under dark conditions by draping darkroom curtains over the chamber. Dark conditions also simulated nighttime, when anemonefishes reside among sea anemone tentacles for rest and protection (Allen, 1974). Animals were allowed to acclimate within the chamber until standard metabolism was reached (3-6 hr), at which point 20 min of dark O_2 consumption ($\text{VO}_2, \mu\text{mol O}_2 \text{ hr}^{-1}$) was measured (1 measurement 6 s^{-1}) using a two-channel O_2 meter (Strathkelvin Instruments, Ltd.). The time at which animals were reduced to standard metabolism was determined visually, based on stable VO_2 on the O_2 meter display (Fig. 2).

Dark VO_2 of symbiotic pairs (1 fish+1 anemone) was measured in three experimental treatments in random order (Table 1). The control treatment measured the dark VO_2 of the fish and anemone separately, and the two rates were added together for a single net dark VO_2 . The unit treatment measured the net dark VO_2 of the fish and anemone within the chamber as a pair. Lastly, the mesh treatment measured the net dark VO_2 of the fish and anemone within the same chamber, but physically separated by a flow-permeable screen (1 mm mesh) that permitted visual and chemical interaction but prevented any physical contact between partners. A subsample of pairs ($N=6$) was subjected to the mesh treatment twice; once with the anemone in the bottom of the chamber such that incoming seawater passed by the anemone before reaching the fish (mesh₁), and again with the fish in the bottom of the chamber such that incoming seawater first passed by the fish before reaching the anemone (mesh₂). The mean VO_2 difference between mesh₁ and mesh₂ was compared to assess the possibility that (a) basal nitrogen excretion by fish (Roopin et al., 2008) effects anemone VO_2 (mesh₂>mesh₁), or (b) fish detection of anemone scent effects fish VO_2 (mesh₁>mesh₂).

Dark VO_2 of animals at the MSS was measured within 5-10 d of collection. Due to time and collection limitations, VO_2 of MSS animals was measured between only the control and unit treatments, and a single anemone was used with all fish examined ($N=6$). Dark VO_2 of animals at AU was measured after ≥ 2 yr (fish) or 4-5 wk (anemones) of maintenance in laboratory aquaria. Dark VO_2 of AU animals was measured across all three treatments, and each pair ($N=12$) consisted of a unique fish and anemone.

Effects of water motion on sea anemone respiration at night

The effects of water motion on dark VO_2 of anemones ($N=8$) were assessed at AU using flow-through respirometry (Fig 1). Anemones were transferred to the respirometry chamber and acclimated for 3-6 hr, as described above. In contrast to the low uniform water flow rate ($1.0\pm 0.1 \text{ cm s}^{-1}$) used in the above experiments, this experiment exposed anemones to increasing levels of water motion by varying the speed of the caged stir bar within the chamber. Water flow levels generated by the stir bar were estimated with a Flo-Mate 2000 portable flow meter (Marsh-McBirney, Inc., Frederick, MD, USA). Anemones were exposed to flow speeds ($N=9$, $0.5\text{--}8.0 \text{ cm s}^{-1}$) commonly encountered by *A. bicinctus* and *E. quadricolor* at coral reef field sites in the northern Red Sea (Goldshmid et al., 2004). At each flow speed, 10 min of VO_2 was measured (1 measurement 6 s^{-1}). Maximum O_2 consumption ($\text{VO}_{2 \text{ max}}$) and maximum change in O_2 consumption across flow regime ($\text{VO}_{2 \text{ diff}}$) were calculated.

Anemonefish nocturnal behavior

Observations in experimental aquaria

Potential effects of fish behavior on net dark VO_2 of the symbiotic partners were investigated at AU by observing the nocturnal activity of the fish. Six symbiotic pairs (fish and anemone) were selected randomly from laboratory stock ($N=13$). In random order, each fish was observed under two treatments: anemone absent and present. For each treatment, the individual(s) was transferred to experimental aquaria (150L) and allowed to acclimate for 24 hr. Eight 20-min video segments (1 hr apart) were recorded throughout the night (20:00-6:00) per fish per treatment using a Sony DCR-SR68 IR

video camera (Sony, San Diego, CA, USA) and IRLamp6 LED infrared lamps (Wildlife Engineering, Carlisle, PA, USA). From each 20 min segment, a random 5 min subsegment (8 subsegments x 5 min each = 40 min total per fish per treatment) was observed to (a) categorize the nocturnal behavioral repertoire of the fish, and (b) determine effects of anemone presence on fish behavior at night.

The percent time and bout frequency of five distinct fish behaviors were measured: fanning, wedging, switching, swimming, and no motion. When fanning, fish are motionless, aside from continuous pectoral fin strokes. When wedging (1-2 s), fish use using both caudal and pectoral fins to forcefully wiggle deeper into the anemone tentacle crown (or into the bottom substrate, in the absence of an anemone). When switching (1-2 s), fish change orientation (usually 180°) while wedging. During swimming events (2-8 s), fish hover in one spot or slowly move around the aquarium, usually to forage. Lastly, during periods of no motion, fish lay completely still on the substrate or among anemone tentacles.

Fish fin stroke frequencies (pectoral and caudal, reported as strokes per 5 s) were compared between the two treatments using five random 5 s segments (25 s per fish per treatment) selected from 24:00-3:00. Nocturnal fin stroke frequencies were compared to diurnal fin stroke frequencies, using five random 5 s video segments collected for the same fish from 12:00-15:00.

Observations in respirometry chambers

To assess variation in fish behavior when in experimental aquaria (above) versus in the smaller respirometry chambers, IR video of a random subsample of the fish and

anemones ($N=6$) during the four respirometry treatments (control, unit, mesh₁, mesh₂) was recorded during the 20 min of VO_2 used for respirometry analysis. From each 20 min segment, one random 5 min subsegment was observed (fish⁻¹ treatment⁻¹) for behavioral analysis. Similar to the behavioral analysis in the experimental aquaria, the percent time and bout frequency of the five distinct fish behaviors were analyzed.

Data analysis

Statistical analyses were conducted using SAS 9.2 (Cary, NC, USA). Differences in fish and anemone VO_2 among treatments, and the effects of water motion (semi-ln transformed) on anemone VO_2 , were examined using one-way repeated-measures analysis of variance (rmANOVA). The VO_2 of mesh₁ and mesh₂ were compared using a paired t-test. Effects of treatment (i.e., anemone absent, present) and time of night on fish behavior within the experimental aquaria (percent time and bout frequency) and the effect of anemone presence on fish fin stroke frequency (pectoral and caudal) were analyzed with one- or two-way rmANOVA. The effects of treatment on fish behavior within the respirometry chamber were analyzed with the nonparametric Friedman's Chi Square test. When appropriate, post hoc multiple pairwise comparisons were analyzed using Tukey's studentized range (HSD) tests. For rmANOVA models, where the assumption of sphericity was not met, Greenhouse-Geisser approximations were used. The significance level for all analyses was set at $P<0.05$. All reported values are means \pm 1 s.e.m.

RESULTS

Nocturnal oxygen consumption patterns

The net dark oxygen consumption (VO_2) of fish and anemones at the MSS was significantly higher (1.4x) when measured together within the same chamber (unit) than the summed VO_2 of both partners in isolation (control) (Fig. 3A, Table 2A). Similarly, at AU, the net VO_2 of anemonefish and anemone partners together (unit) was significantly higher (1.4x) than both the summed VO_2 of the partners in isolation (control) and the VO_2 of the partners separated by a mesh barrier (mesh) (Fig. 3B, Table 2B). The positions of the anemonefish and anemone in the chamber during the mesh treatments (mesh₁, mesh₂) had no significant effect on the net VO_2 of the symbionts at night (Table 2C). Mean $VO_{2\text{ diff}}$ between the unit treatment and control or mesh treatments was 70.96 ± 10.03 or $83.34 \pm 14.81 \mu\text{mol O}_2 \text{ hr}^{-1}$, respectively.

Effects of water motion and sea anemone oxygen consumption at night

Anemone VO_2 increased significantly when exposed to water flow rates between $0.5\text{-}2.0 \text{ cm s}^{-1}$, then reached an asymptote at 2.0 cm s^{-1} (rmANOVA, $F=41.32$, $P<0.0001$) (Fig. 4). $VO_{2\text{ max}}$ for anemones was $111.53 \pm 13.32 \mu\text{mol O}_2 \text{ hr}^{-1}$. Maximum $VO_{2\text{ diff}}$ across flow regimes (mean difference between VO_2 at 0.5 and 3.0 cm s^{-1}) was $23.69 \pm 2.64 \mu\text{mol O}_2 \text{ hr}^{-1}$.

Anemonefish nocturnal behavior

In the experimental aquaria, regardless of treatment (i.e., anemone absent or present), fish spent $\geq 98\%$ of the night in a single location. When the anemone was

present, it always served as the singular location. In the absence of the anemone, the fish rested against a rock or the aquarium wall. In the respirometry chambers, fish spent $\geq 80\%$ of the night in a single location. When the anemone was accessible (unit), it always served as the singular location.

Percent time spent performing each behavior

Within the experimental aquarium, anemone presence had no significant effect on the percent time fish spent fanning, swimming, and not moving. However, time spent wedging and switching increased 11x and 47x, respectively, when the anemone was present (Fig. 5A, Table 3A). Similarly, within the respirometry chambers, treatment (control, unit, mesh₁, mesh₂) had no significant effect on the percent time fish spent fanning, swimming, or not moving. When the anemone was accessible (unit treatment), fish spent a significantly higher percent time wedging and switching (20x and 2.5x, respectively) than during treatments when the anemone was absent or inaccessible (control, mesh₁, mesh₂) (Fig. 5B, Table 4A).

Frequencies of behaviors

In the experimental aquarium, bouts of fanning, wedging, and switching were more frequent when the anemone was present (1.6x, 20x, and 36x, respectively), while the frequency of swimming and periods of no motion were unaffected (Fig. 6A, Table 3B). In the respirometry chambers, fish fanned, wedged, and switched more frequently (3x, 6x, and 30x) when the anemone was accessible (unit) than when inaccessible (control, mesh₁, mesh₂) (Fig. 6B, Table 4B). Further, during the mesh₁ treatment (fish

downstream of anemone), fish engaged in switching more frequently than during the control and mesh₂ treatments.

Within the experimental aquarium, anemone presence did not affect the pectoral fin stroke frequency of fish (rmANOVA, $F=0.32$, $P=0.32$) (Fig. 7). Further, both nocturnal fin stroke frequencies (i.e., anemone absent, present) were significantly lower than diurnal pectoral stroke frequencies (rmANOVA, $F=3.32$, $P=0.12$). Caudal fin stroke frequency, however, was significantly higher when the anemone was present (rmANOVA, $F=14.29$, $P=0.013$) and was comparable to the diurnal caudal fin stroke frequency (rmANOVA, $F=0.01$, $P=0.95$).

Effect of time of night on anemonefish behavior

Time of night had no significant effect on the percent time fish engaged in any of the five behaviors exhibited in the experimental aquarium, regardless of treatment (i.e., anemone absent, present) (Table 5A). However, fish engaged in wedging at a substantially higher frequency during the first time segment (20:00–20:20) than during the remaining seven time segments (20:20–6:00) (rmANOVA, $F=4.12$, $P=0.002$) (Table 5B).

DISCUSSION

The symbiotic association between anemonefish (*A. bicinctus*) and sea anemones (*E. quadricolor*) increases the net dark O₂ consumption (VO₂) of one or both partners at night. Additionally, physical contact between the partners is needed to produce metabolic elevation. The relative importance of each partner's contribution to this metabolic

elevation could not be discerned because of the technical constraints of measuring the VO_2 of multiple species incubated together. However, it is likely that fish behavior elevates anemone VO_2 when the partners are together, because (a) artificially increasing water motion elevates anemone VO_2 , and (b) anemone presence increases the expression of flow-modulating behaviors by the fish. It is unlikely that anemones are wholly responsible for the elevated VO_2 when the partners are together. The mean $\text{VO}_{2\text{diff}}$ between control and unit treatments ($70.96 \pm 10.03 \mu\text{mol O}_2 \text{ hr}^{-1}$), when added to the mean VO_2 of isolated sea anemones (control treatment, $108.28 \pm 9.44 \mu\text{mol O}_2 \text{ hr}^{-1}$), is substantially higher than the mean $\text{VO}_{2\text{max}}$ of anemones during the flow experiments ($114.21 \pm 13.89 \mu\text{mol O}_2 \text{ hr}^{-1}$). Thus, the metabolic elevation observed during the unit treatment is too large to be achieved by the anemone alone, and increased fish metabolism is also required to explain the total increase in VO_2 when the partners are together.

Contrary to previous studies that report that anemonefishes, like most pomacentrids, remain generally inactive at night (Allen, 1974), we demonstrate that anemonefish spend the majority of the night in some form of localized motion. Further, some anemonefish behaviors appear to be tailored specifically toward interactions with sea anemone hosts (i.e., wedging and switching). Within both the experimental aquaria and the respirometry chamber, anemonefish spent significantly more time wedging and switching when resting among sea anemone tentacles than when alone. Increased instances of wedging and switching by anemonefish potentially (a) elevated the energy expenditure of the anemonefish through increased activity and (b) increased sea anemone gas exchange through enhanced ambient water flow. Together, these effects provide a

likely explanation for the increased net dark VO_2 of the anemonefish and sea anemone partners during the unit treatment.

Bouts of wedging and switching, though brief in duration, involve rapid caudal and pectoral fin movement, and forcefully propel the fish deeper into the anemone tentacle crown. These vigorous behaviors rely heavily upon the caudal fin and posterior musculature, and likely require more energy than the other localized movements. For example, bouts of fanning involve no fish movement aside from alternating pectoral fin strokes, and swimming primarily involves simultaneous pectoral rowing. Further, fish change behaviors at a substantially higher rate when anemones are present than when not. High levels of activity at night have been documented for other marine fishes that rely on symbiosis with sedentary invertebrates on coral reefs. Sleep-swimming behaviors have been documented in three species of damselfishes (*Dascyllus marginatus*, *D. aruanus*, *Chromis viridis*) that shelter among the branches of stony corals at night (Goldshmid et al., 2004). During sleep swimming, the damselfishes move among coral branches at fin strokes frequencies 2x higher than during diurnal activities.

Enhanced activity by anemonefish among sea anemone tentacles also affects the cnidarian host. The wedging and switching behaviors of anemonefish clearly enhance the hydrodynamic conditions surrounding their host, as evidenced by the neutrally buoyant tentacle crown of sea anemones, which moves passively with ambient water flow. The rapid and forceful bouts of wedging and switching by the anemonefish likely disrupt the diffusive boundary layer surrounding sea anemone tissue, and enhance gas exchange with ambient water. Ultimately, anemonefish facilitate regular bouts of tentacle movement

over most of the sea anemone tentacle crown that is noticeably greater than tentacle movement induced by ambient water flow alone.

Anemonefish-induced water flow among sea anemone tentacles could have pronounced effects on the physiology and biology of the host. For example, in the present study, sea anemone dark VO_2 increased with water flow, suggesting sea anemones are potentially flow-limited. Similarly, Patterson and Sebens (1989) demonstrated that reduced diffusive boundary layer thickness increased gas exchange in the temperate sea anemone *Metridium senile*. Oxygenation of host sea anemone through anemonefish behavior could be especially important in the light of recent research that implicates O_2 limitation as a major abiotic selection pressure on coral reefs (Goldshmid et al., 2004; Nilsson et al., 2007a; Niggel et al., 2010; Wild et al., 2010).

Similar to the benefits provided to sea anemones by increased water flow, anemonefish residency catalyzes sea anemone growth, reproduction, and survivorship (Porat and Chadwick-Furman, 2004; Holbrook and Schmitt, 2005; Porat and Chadwick-Furman, 2005). Some benefits are directly attributed to the nutrient contributions from anemonefishes to their host sea anemones and endosymbiotic dinoflagellates, in the form of phosphorous (Godinot and Chadwick, 2009), nitrogen (Porat and Chadwick-Furman, 2005; Roopin et al., 2008; Roopin and Chadwick, 2009; Cleveland et al., 2010), and carbon (Cleveland et al., 2010). The results of the present study suggest that anemonefishes likely aid in the efficient uptake of nutrients and dissolved gases, via forced convection of ambient seawater.

Beyond the nocturnal patterns presented here, anemonefish-induced flow modulation also could have important diurnal effects. While anemonefishes generally

spend most of the daylight hours in the water column above their host sea anemones (Allen, 1974; Fautin and Allen, 1997), periodic “bathing” forays by anemonefishes back to their sea anemones (Allen, 1974) can flush the diffusive boundary layer surrounding the host and increase primary production within sea anemone tissue. Positive effects of enhanced water flow on intracellular primary production have been documented for other reef cnidarians, including sea anemones (Patterson and Sebens, 1989) and reef-building corals (Patterson and Sebens, 1989; Patterson et al., 1991; Bruno and Edmunds, 1998; Sebens et al., 2003; Finelli et al., 2006). Moreover, anemonefish-induced flow modulation may clear sediments and algae from sea anemone tentacles (Nugues and Roberts, 2003; Box and Mumby, 2007), and flush metabolic wastes, such as harmful O_2 species, that can accrue within sea anemone tissues (Lushchak and Bagnyukova, 2006). The enhanced removal of free O_2 radicals by anemonefish could buffer sea anemones against bleaching, or expedite recovery after a bleaching event (Nakamura and van Woesik, 2001; Nakamura et al., 2003).

Physical interaction between anemonefishes and sea anemones appears to be required for increased sea anemone gas exchange; however, chemical compounds released by sea anemones may play a role in initiating the anemonefish behaviors responsible for the elevation in net VO_2 of the partners when together. For example, anemonefish engaged in switching behaviors significantly more frequently when positioned downstream of sea anemones (mesh₁ treatment) than upstream (mesh₂ treatment). Further, an indication of the same pattern was observed for the percent time anemonefish spent fanning, but the difference was not significant. Sea anemone chemical compounds directly influence the recruitment and recognition behaviors of

anemonefishes toward host sea anemones (Murata et al., 1986; Arvedlund et al., 1999); however, the extent to which sea anemone chemical cues influence anemonefish behavior at night has yet to be discerned.

While it is clear that anemonefish behavior at night modulates the hydrodynamic conditions surrounding host sea anemones, it is unclear if flow modulation is the intended purpose of these behaviors. In both French Polynesia and the Red Sea, the number and size of resident anemonefishes correlate positively with sea anemone body size (Holbrook and Schmitt, 2005; Porat and Chadwick-Furman, 2005). It is possible that anemonefish wedging and switching stimulate sea anemones to alter their morphology (e.g., expand). If anemonefish behavior promotes sea anemone expansion, this process may contribute to increased sea anemone VO_2 by exposing more surface area for gas exchange. Alternatively, anemonefishes kept in captivity without sea anemone hosts occasionally “bathe” in airstream bubbles and stringy algal tufts (Mariscal, 1970a). As such, the tactile stimulation that anemonefishes receive from sea anemone tentacles may be beneficial to anemonefish well being (Mariscal, 1970a). More research is needed to clarify the factors that enhance the expression of certain behaviors (i.e., wedging and switching) when anemonefishes reside among sea anemone tentacles.

My findings demonstrate that the association between anemonefishes and sea anemones can elevate the VO_2 of the symbionts at night. Also, I observed that anemonefish activity is affected by sea anemone presence, and certain behaviors (i.e., wedging and switching) modulate water flow among sea anemone tentacles and appear to increase sea anemone gas exchange. It is important to note that wild individuals of *A. bicinctus* and *E. quadricolor* in the Red Sea can be much larger than the specimens used

in the present study (Chadwick and Arvedlund, 2005). Further, in the Red Sea, *E. quadricolor* usually host multiple anemonefish, including mated pairs of *A. bicinctus* plus 0-3 juveniles (Chadwick and Arvedlund, 2005). The effects of anemonefish size, quantity, and social hierarchy on their nocturnal behavior in the wild are currently not well understood. Regardless, these results provide foundational evidence of anemonefish-induced flow modulation of sea anemone hosts, a previously debated benefit of this mutualism. Further, this study documents the metabolic consequences to both partners of anemonefish behavior at night, and thus joins a growing body of knowledge indicating the importance of ecophysiological underpinnings to the ecological advantages associated with symbiotic associations on coral reefs.

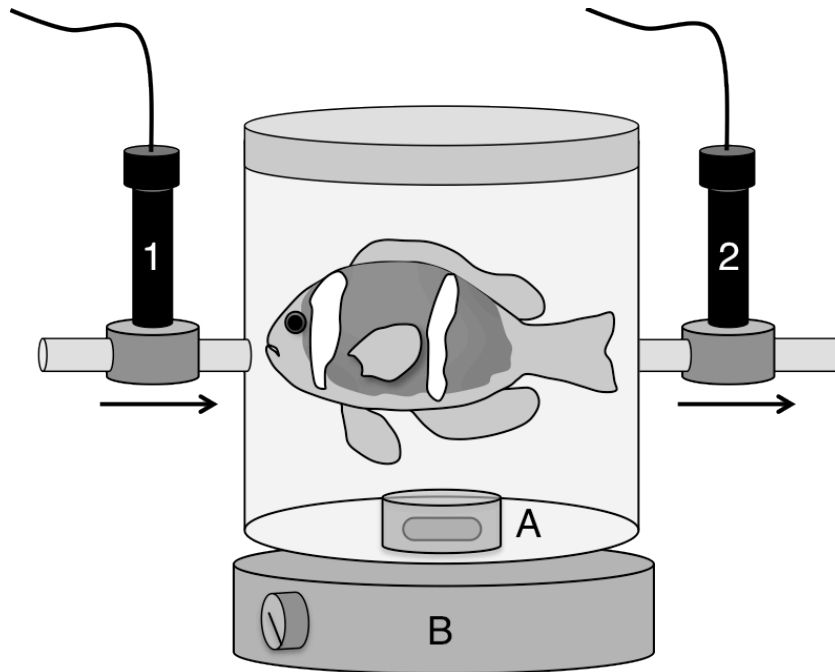
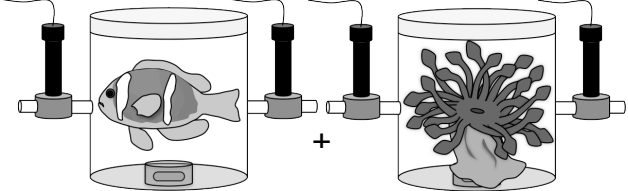
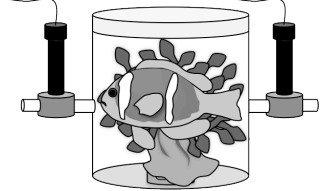
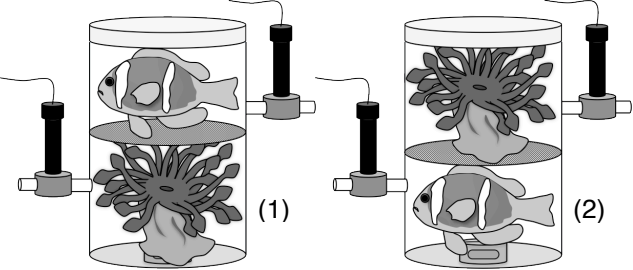


Fig. 1. Flow-through respirometry setup used to measure dark oxygen consumption ($\mu\text{mol O}_2 \text{ hr}^{-1}$) of anemonefish (*Amphiprion bicinctus*) and sea anemones (*Entacmaea quadricolor*). Arrows indicate water flow direction ($1.0 \pm 0.1 \text{ cm s}^{-1}$). Numbers 1 and 2 depict inflow and outflow oxygen electrodes, respectively. Caged-enclosed stir bar (A) and magnetic stir plate (B) are displayed.

Table 1. Flow-through respirometry treatments used to assess the effects of symbiotic interactions on dark oxygen consumption (VO_2) of anemonefish (*Amphiprion bicinctus*) and sea anemones (*Entacmaea quadricolor*). See Fig. 1 for details of chamber setup. (1) and (2) depict the position of the anemonefish and sea anemone during mesh₁ and mesh₂ treatments, respectively.

Treatment	Description	Chamber setup
Control	Anemonefish and sea anemone VO_2 measured separately, then summed for a single VO_2	
Unit	Anemonefish and sea anemone VO_2 measured together	
Mesh	Anemonefish and sea anemone VO_2 measured together, but separated by a mesh barrier that prevented physical contact	

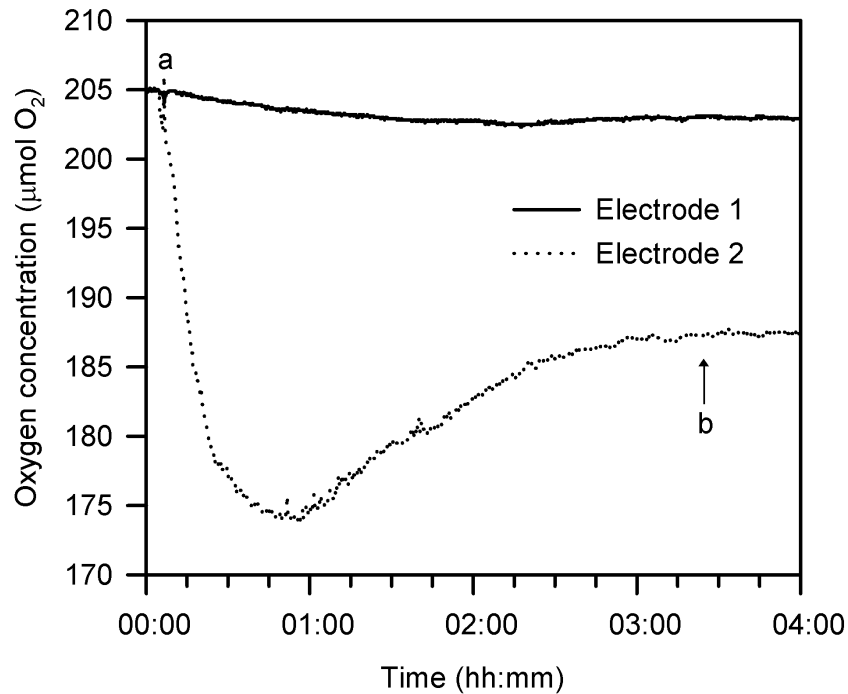


Fig. 2. Representative plot of an oxygen meter reading during a flow-through respirometry experiment (unit treatment) on an anemonefish (*Amphiprion bicinctus*) and sea anemone (*Entacmaea quadricolor*) at Auburn University. Plot depicts the oxygen concentrations of seawater passing electrode 1 (immediately before entering the respirometry chamber) and electrode 2 (immediately after exiting the chamber). Letters refer to the time at which experimental animals were added to the chamber (a), and the time at which standard metabolic rate was achieved (b). Oxygen consumption rate of the experimental animals was derived from the mean difference between the two electrode readings for 20 min after (b).

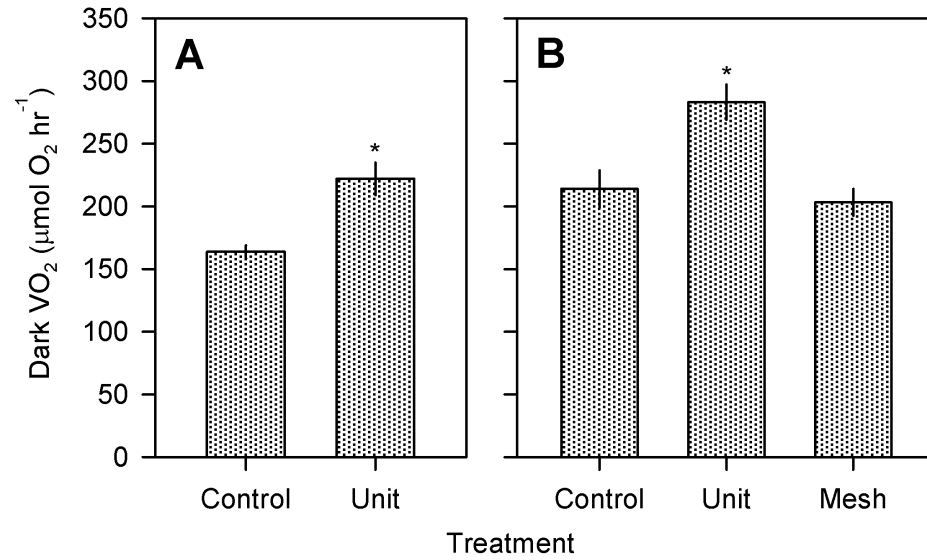


Fig. 3. Dark oxygen consumption (mean±1 s.e.m.) of anemonefish (*Amphiprion bicinctus*) and sea anemones (*Entacmaea quadricolor*) across respirometry treatments (Table 1) at the Marine Science Station in Aqaba, Jordan (A) and at Auburn University in Alabama, USA (B). Asterisks depict significant difference.

Table 2. Statistical summary (repeated-measures ANOVA) of effects of respirometry treatment on oxygen consumption (mean $VO_2 \pm 1$ s.e.m.) of anemonefish (*Amphiprion bicinctus*) and sea anemones (*Entacmaea quadricolor*) at the Marine Science Station in Aqaba, Jordan (A) and at Auburn University in Alabama, USA, (B,C). Significant results are in bold type.

	Treatment	VO_2	df	MS	F	P
A	Control	163.62 ± 4.78	1	10168.578	16.26	0.01
	Unit	221.27 ± 12.24				
B	Control	213.84 ± 14.92	2	24266.53	19.22	0.0001
	Unit	283.10 ± 14.08				
	Mesh*	203.14 ± 10.71				
C	Mesh ₁	213.84 ± 14.92	1	351.84	0.29	0.6
	Mesh ₂	283.10 ± 14.08				

* No difference in VO_2 across mesh treatments (C), so only mesh₁ is presented.

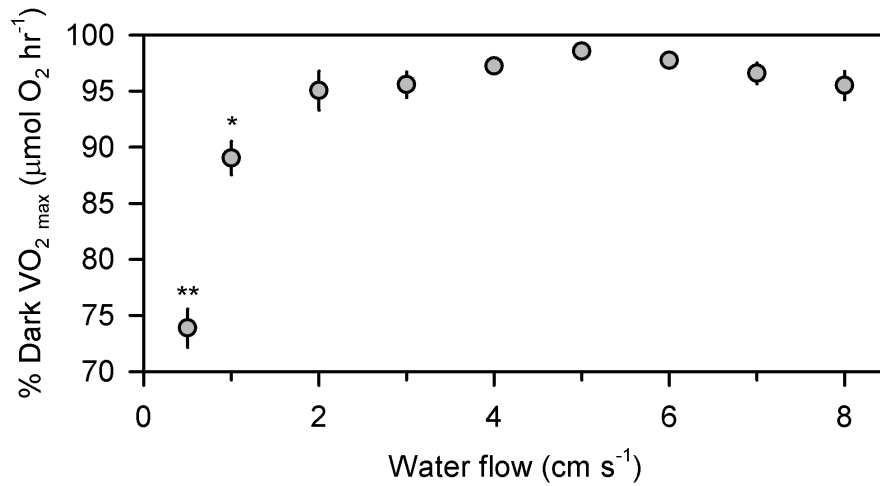


Fig. 4. Effects of rate of water flow on the dark oxygen consumption (mean±1 s.e.m.) of the sea anemone *Entacmaea quadricolor* in flow-through respirometry. Mean VO_{2 max} = 114.40±14.47 μmol O₂ hr⁻¹. Asterisks depict significant difference.

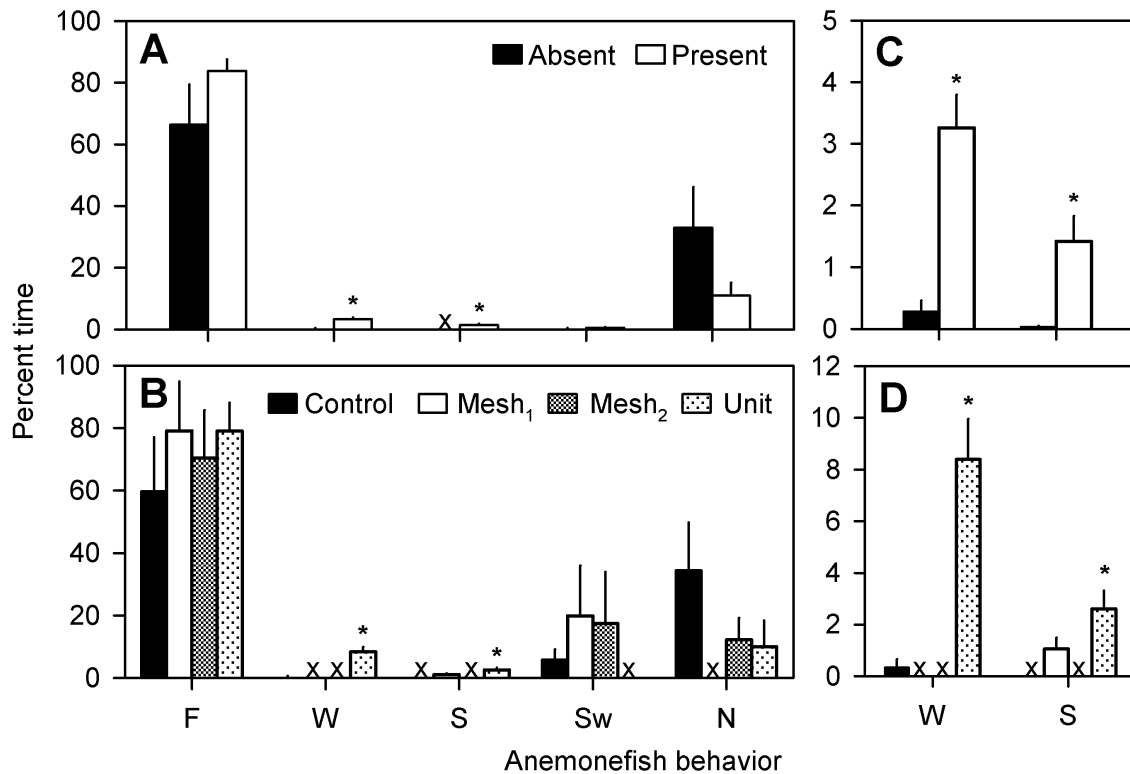


Fig. 5. Percent time (mean \pm 1 s.e.m.) that anemonefish (*Amphiprion bicinctus*) engaged in five types of nocturnal behavior in experimental aquaria (A) and in respirometry chambers (B). F=fanning, W=wedging, S=switching, Sw=swimming, and N=no motion. C and D depict magnified views of the percent time anemonefish engaged in wedging and switching behaviors, which were significantly different across treatments in experimental aquaria and respirometry chambers. In experimental aquaria, anemonefish were observed alone (Fish) and with host sea anemone (*Entacmaea quadricolor*, Fish+Anem). In the respirometry chambers, anemonefish were observed in each of four experimental treatments (Table 1). Asterisks depict significant difference within each behavior type.

Table 3. Statistical summary (repeated-measures ANOVA) of percent time (*A*) and bout frequency (bouts 5 min⁻¹, *B*) for five types of nocturnal behavior by anemonefish (*Amphiprion bicinctus*) when alone (Fish) and when with host sea anemone (*Entacmaea quadricolor*, Fish+Anem). Significant results are in bold type.

	Behavior	Treatment		Repeated-measures ANOVA			
		Fish	Fish+Anem	df	MS	<i>F</i>	<i>P</i>
<i>A</i>	Fanning	66.38 ± 13.08	83.79 ± 3.85	1	0.808	1.13	0.337
	Wedging	0.28 ± 0.18	3.26 ± 0.54	1	0.399	54.41	0.001
	Switching	0.03 ± 0.02	1.42 ± 0.41	1	0.154	13.57	0.014
	Swimming	0.29 ± 0.16	0.49 ± 0.28	1	0.003	1.02	0.359
	No Motion	33.01 ± 13.15	11.03 ± 4.15	1	1.669	2.33	0.188
<i>B</i>	Fanning	7.67 ± 0.75	12.80 ± 1.70	1	640.667	24.09	0.004
	Wedging	0.44 ± 0.12	9.02 ± 1.88	1	1768.167	66.62	0.001
	Switching	0.06 ± 0.04	2.19 ± 0.79	1	108.375	20.84	0.006
	Swimming	0.17 ± 0.09	0.31 ± 0.09	1	0.510	1.32	0.302
	No Motion	6.79 ± 0.78	5.38 ± 0.55	1	48.167	0.94	0.376

Table 4. Statistical summary (Friedman's Chi Square Test) of effects of respirometry treatments (Table 1) on the percent time (A) and bout frequency (bouts 5 min⁻¹, B) for five types of nocturnal behavior by anemonefish (*Amphiprion bicinctus*). Significant results are in bold type.

Behavior	Treatment				Friedman's Chi Square Test		
	Control	Unit	Mesh ₁	Mesh ₂	df	Q	P
A Fanning	59.56 ± 17.55	79.00 ± 9.10	79.06 ± 15.87	70.33 ± 15.40	3	2.39	0.4955
Wedging	0.33 ± 0.33	8.39 ± 1.57	0.00 ± 0.00	0.00 ± 0.00	3	18.85	0.0008*
Switching	0.00 ± 0.00	2.61 ± 0.71	1.06 ± 0.44	0.00 ± 0.00	3	14.36	0.0025*
Swimming	5.78 ± 3.45	0.00 ± 0.00	19.89 ± 16.10	17.44 ± 16.53	3	5.03	0.1697
No Motion	34.33 ± 15.44	10.00 ± 8.63	0.00 ± 0.00	12.22 ± 7.01	3	4.36	0.2254
B Fanning	4.67 ± 1.65	17.67 ± 3.85	4.50 ± 1.31	3.33 ± 1.15	3	10.16	0.0173*
Wedging	0.50 ± 0.50	15.17 ± 6.86	0.00 ± 0.00	0.00 ± 0.00	3	12.75	0.0052*
Switching	0.00 ± 0.00	6.00 ± 1.39	3.00 ± 1.27	0.00 ± 0.00	3	14.36	0.0025**
Swimming	1.00 ± 0.45	0.00 ± 0.00	1.00 ± 0.37	0.33 ± 0.21	3	7.69	0.0529
No Motion	5.00 ± 2.42	3.67 ± 2.89	0.00 ± 0.00	2.50 ± 1.15	3	4.36	0.2254

* Unit treatment was significantly higher than control, mesh₁, and mesh₂ treatments.

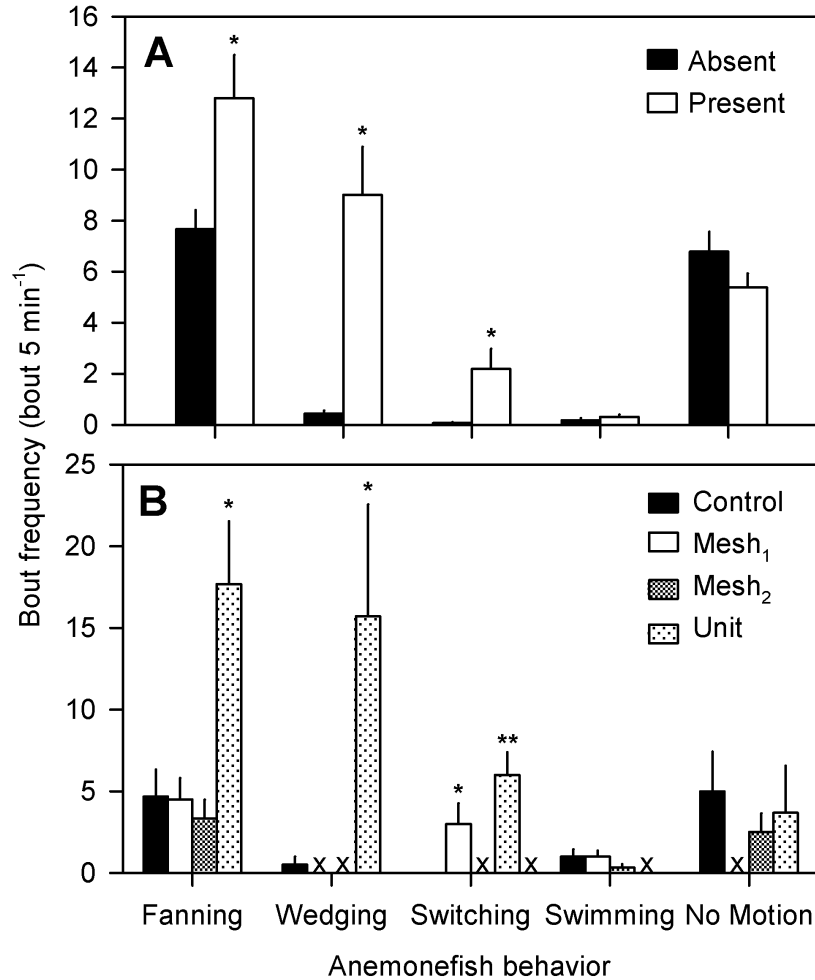


Fig. 6. Bout frequencies (mean \pm 1 s.e.m.) for five types of nocturnal behavior by anemonefish (*Amphiprion bicinctus*) in experimental aquaria (A) and in respirometry chambers (B). F=fanning, W=wedging, S=switching, Sw=swimming, and N=no motion. In experimental aquaria, anemonefish were observed alone (Fish) and with host sea anemone (*Entacmaea quadricolor*, Fish+Anem). In the respirometry chambers, anemonefish were observed in each of four experimental treatments (Table 1). Asterisks depict significant difference within each behavior type.

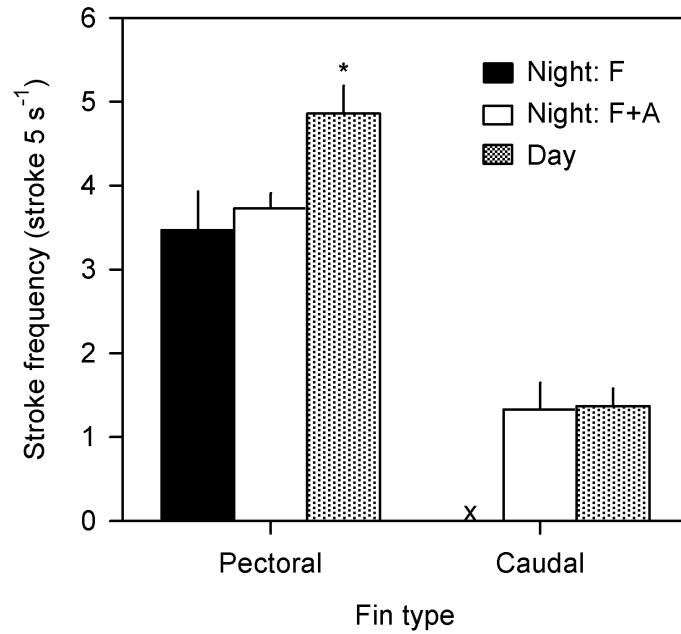


Fig. 7. Nocturnal and diurnal stroke frequencies (mean \pm 1 s.e.m.) of dorsal and caudal fins of anemonefish (*Amphiprion bicinctus*). During the night, fin stroke frequencies were measured when each anemonefish was alone (F) and with host sea anemone (*Entacmaea quadricolor*, F+A). Asterisks depict significant difference within fin type.

Table 5. Statistical summary (repeated-measures ANOVA) of effects of time (20:00-6:00) on percent time (A) and bout frequency (bouts 5 min⁻¹, B) for five types of nocturnal behavior by anemonefish (*Amphiprion bicinctus*) in the presence of host sea anemone (*Entacmaea quadricolor*). The assumption of sphericity was not met and Greenhouse and Geisser (G-G) approximations were used. Significant results are in bold type.

	Behavior	Source	G-G epsilon	G-G df1	G-G df2	G-G adjusted P	
A	Fanning	time	0.354	2.475	12.376	0.589	
		trt X time	0.400	2.799	13.997	0.447	
	Wedging	time	0.473	3.309	16.545	0.199	
		trt X time	0.336	2.351	11.753	0.192	
	Switching	time	0.403	2.823	14.116	0.087	
		trt X time	0.411	2.879	14.396	0.052	
	Swimming	time	0.300	2.099	10.497	0.459	
		trt X time	0.296	2.073	10.367	0.176	
	No Motion	time	0.351	2.458	12.289	0.505	
		trt X time	0.370	2.588	12.940	0.628	
	B	Fanning	time	0.416	2.909	14.546	0.061
			trt X time	0.321	2.249	11.246	0.118
		Wedging	time	0.287	2.012	10.059	0.0338*
			trt X time	0.269	1.882	9.408	0.046
Switching		time	0.253	1.768	8.838	0.076	
		trt X time	0.265	1.854	9.268	0.070	
Swimming		time	0.313	2.193	10.966	0.470	
		trt X time	0.338	2.367	11.837	0.212	
No Motion		time	0.429	3.005	15.026	0.350	
		trt X time	0.406	2.843	14.217	0.504	

* Wedging occurred at a significantly higher frequency during the first time segment (20:00-20:20) than the seven remaining segments.

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