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Feeding behavior and acquisition of zooxanthellae by planula larvae of the sea anemone *Anthopleura elegantissima*

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Abstract Symbiotic associations between cnidarians and photosynthetic dinoflagellates (i.e., zooxanthellae) are common in the marine environment. Many symbiotic cnidarians produce offspring that are initially non-symbiotic. These new hosts must acquire symbiotic algae from environmental sources. We examined zooxanthella acquisition by laboratory-reared planula larvae of the temperate sea anemone *Anthopleura elegantissima*. Larvae ingested zooxanthellae while they were feeding. However, the signal that prompted larval feeding behavior did not originate from the symbiotic algae; the addition of algal cells to larval cultures never elicited a feeding response. In contrast, the addition of macerated animal tissue from several sources invariably generated a strong feeding response, which resulted in the larvae indiscriminately ingesting any particulate matter that was present, including zooxanthellae or other unicellular algae. Ingested zooxanthellae were incorporated into endodermal cells, where they remained undigested, while all other ingested material was digested or expelled within 24 h. Our results provide evidence that one source of zooxanthellae likely to serve as a route of infection in the natural environment is zooxanthella-laden mucus egested by anemones. This egested material fulfilled both of the criteria necessary for successful infection: it prompted larvae to begin feeding and provided an abundant supply of zooxanthellae that were ingested and taken up into endodermal cells of the new host.

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Introduction

Symbiotic associations between cnidarians and photosynthetic dinoflagellates (*Symbiodinium* spp.) are common in shallow marine environments. The dinoflagellate symbionts, commonly referred to as zooxanthellae, typically reside in perialgal vacuoles within the host's endodermal cells, where they continue to photosynthesize. Nutritionally, the association between the partners is mutualistic in most cases where this has been examined (Muller-Parker and D'Elia 1997; Muller-Parker and Davy 2001). Typically, algae provide the host with photosynthate, while the host provides inorganic carbon and other nutrients to the algae (reviewed in Muscatine 1990; Muscatine and Weis 1992; Muller-Parker and D'Elia 1997).

Most cnidarians that host algae reproduce both sexually and asexually. Asexual growth is either clonal or colonial, with a new polyp produced via budding, pedal laceration, or transverse fission. The two resulting polyps contain algae from the original polyp. But the initial infection event during which a new sexually produced offspring acquires its first complement of zooxanthellae can occur in one of two ways, either vertically from the parental polyp (maternal inheritance) or horizontally from zooxanthellae present in the environment (Trench 1987; Douglas 1994).

Maternal inheritance occurs in a minority (~15%) of cnidarian species that form associations with zooxanthellae (Fadlallah 1983; Babcock and Heyward 1986; Harrison and Wallace 1990; Richmond and Hunter 1990; Richmond 1997) and tends to occur during the early stages of the host's life history, while the offspring are still associated with the parental polyp as oocytes or brooded embryos or planulae. Horizontal infection is the mode of symbiont transmission in the vast majority (~85%) of cnidarians that host zooxanthellae and occurs after offspring have left the parental polyp. This mode of transmission permits flexibility in choosing among partners that may be differentially adapted to

conditions in which the host ultimately takes up residence (Buddemeier and Fautin 1993). However, the potential also exists that the host may fail to acquire zooxanthellae altogether. In tropical systems, the failure to acquire algae would likely be lethal to the host, since the vast majority of cnidarian host species simply do not naturally occur in an aposymbiotic condition. In contrast, cnidarian hosts in temperate systems are able to survive and clonally propagate when they are aposymbiotic, but their ability to do so is presumably highly reduced, as they are restricted to microenvironments that are associated with low light availability, such as caves or areas that experience prolonged sand burial (Secord 1995).

For cnidarians that spawn gametes that undergo fertilization within the water column, infection by zooxanthellae has been shown to occur at various developmental stages depending on the host species. The scyphozoan *Linuche unguiculata* has been shown to acquire zooxanthellae as early as the 128-cell stage of embryonic development (Montgomery and Kremer 1995). The scleractinian coral *Fungia scutaria* (Krupp 1983; Schwarz et al. 1999) and the tropical sea anemone *Aiptasia tagetes* (Riggs 1988) do not acquire zooxanthellae until the planula larva stage. The scyphozoan *Cassiopeia xamachana* (Colley and Trench 1983; Fitt and Trench 1983a, b) and the gorgonians *Pseudopterogorgia bipinnata* (Kinzie 1974) and *Heteroxenia fuscescens* (Benayahu et al. 1989) do not acquire algae until after they have undergone metamorphosis and developed into the polyp stage of the life cycle.

Within an individual species, the initial infection event occurs during discrete developmental stages, and some species have a longer window during which the host can acquire its initial complement of zooxanthellae. For instance, in all gorgonian corals and most scyphozoans studied to date (described above), the host is not capable of acquiring algae until after larvae have undergone metamorphosis and developed into the polyp stage (an exception is *L. unguiculata*, which can become infected with zooxanthellae during early embryonic development). In contrast, the mushroom coral *F. scutaria* has a fairly long window during which it can acquire its first complement of zooxanthellae; planula larvae can take up algae throughout most of the larval stage as well as after metamorphosis into the polyp stage (Schwarz et al. 1999).

The polyp stage in the cnidarian life cycle is sedentary, and it has been documented in several host species that motile zooxanthellae swim toward and congregate near the mouths of host polyps, where they are engulfed by the mouth and are then presumably taken up into endodermal cells by phagocytosis (Kinzie 1974; Fitt 1984; T. Yacobovitch, personal communication). It has also been shown that zooxanthellae ingested by live brine shrimp can establish an association with the polyp stage (scyphistomae) of the scyphozoan *C. xamachana* after the host ingests and digests the brine shrimp (Fitt 1984). But for species that acquire algae during the planula larval stage, the mechanisms by which algae enter the host's gastric cavity have not been investigated in any detail.

We examined the process of symbiont acquisition in the temperate sea anemone, *Anthopleura elegantissima*. This species is an abundant inhabitant of the rocky intertidal from Mexico through to Alaska and forms associations with two different species of the dinoflagellate *Symbiodinium* (LaJeunesse and Trench 2000) as well as a taxonomically undescribed green alga. During the summer months, both male and female anemones spawn gametes that undergo fertilization in the water column (Ford 1964; Siebert 1974). The resulting planula larvae lack symbiotic algae entirely and develop the ability to feed when they are 4 days old (Siebert 1974). The feeding behavior in this species has not been studied in detail, although Smith (1986) found that the addition of dried fish flakes caused larvae to extend their mouths and attach to the nearest substratum and remain attached for 1–3 h. Since spawning events in this species occur in an unpredictable pattern and experimental efforts to induce spawning have historically proved difficult (Ford 1964; Siebert 1974; Jennison 1979; Smith 1986; Schwarz 1996), information regarding the larval life history stage of this species is sparse. In particular, there have been no published reports detailing the processes by which sexually produced offspring first acquire zooxanthellae.

In this article we focus on general questions regarding when and how laboratory-reared sexually produced offspring of *A. elegantissima* acquire their first complement of zooxanthellae. In particular we investigated the mechanisms by which *Symbiodinium* spp. is brought into contact with the host's gastrodermal cells and the role that host feeding behavior plays in this process.

Materials and methods

Gamete collection and larval cultures

All experiments were performed at the University of California Santa Cruz Long Marine Laboratory. Approximately 50 adult anemones representing several clones (collected from Tatoosh Island and Friday Harbor, both in Washington, and Soquel Point, Calif.) were maintained in outdoor tanks and fed previously frozen brine shrimp or minced mussels one to three times a week for several years. Throughout the summer months, individual anemones were selected and placed in standing nonaerated seawater in the afternoon sun for 4 h and then placed in glass bowls in an indoor flow-through seawater table overnight. The following morning, gametes were collected with a turkey baster and placed into 0.2-μm filtered seawater (FSW), and the eggs were fertilized with a dilute suspension of sperm collected from all the males that had spawned. Two hours later, the fertilized eggs were collected on a 50-μm filter, rinsed with FSW, and placed into bowls containing FSW. Larval cultures were maintained in a 13°C cold room on a 12:12 light cycle. Water was changed daily for the first 4 days, and then every 2 or 3 days thereafter.

Preparation of algal cells, animal tissue, and *Artemia*

Cultured algal cells

Three species of axenically cultured, motile unicellular algae were used in feeding experiments. These were (1) *Symbiodinium califor-*

nium, the species of dinoflagellate that forms associations with *Anthopleura elegantissima* living in central and southern California (cultures were obtained from Bob Trench's lab and represent *S. californium* as cited in LaJeunesse and Trench 2000); (2) *Amphidinium carterae*, a free-living dinoflagellate (i.e., does not form symbioses with cnidarians); and (3) *Rhodomonas* sp., a free-living red alga. Algae were cleaned of culture media by rinsing several times with FSW and concentration by light centrifugation. Algae were resuspended in FSW prior to use in feeding experiments.

Homogenized anemone tissue and zooxanthella isolates

To prepare anemone tissue for feeding experiments, whole anemones were homogenized using a Sorvall homogenizer. To prepare "freshly isolated zooxanthellae," we further isolated zooxanthellae from this anemone homogenate using three cycles of centrifugation and resuspension in FSW. Despite our attempts to remove zooxanthellae from host tissue, it was not possible to obtain clean resuspensions of algae, as there was always significant contamination by anemone tissue, which caused the algae to form clumps.

To prepare zooxanthella isolates from naturally occurring sources, we collected two different types of zooxanthella-containing egesta from anemones that were maintained in an illuminated flow-through seawater tank. Black egesta, material that is released from anemones' mouths, is composed primarily of zooxanthellae but also contains live ciliates, undigested food, degraded material, and possibly endodermal cells from the host (Gates et al. 1992). Brown egesta, material that is sloughed from the body of anemones and accumulates in a ring around the pedal disk, is much more highly degraded than black egesta and contains only a small amount of zooxanthellae. Each type was collected separately and was resuspended as much as possible, by rapidly pipetting the chunks in FSW.

Artemia

Previously frozen *Artemia* (brine shrimp) was macerated in a ground glass homogenizer and filtered through a 50-μm mesh to remove large particulate matter.

Feeding experiments

To assess the feeding preferences of larvae, we prepared various "food" items of either algal or animal origin and presented them separately to groups of several hundred larvae in clean FSW in six-well dishes. For "algal-only" food, aliquots from cultures of three species of unicellular algae were prepared as described above and added to groups of larvae for a final concentration of approximately 10,000 algal cells per milliliter. For "animal-only" food, two types of animal tissues were used: filtered homogenized *Artemia* and dry fish flakes (Tetramin brand). For "animal + algae" food, we used homogenized anemone tissue, which contains numerous zooxanthellae, or a mixture of one of the three species of unicellular algae with homogenized *Artemia*. For these treatments, algae were added to larvae first, and then several drops of filtered, homogenized *Artemia* were swirled into the dishes containing larvae and algae.

Upon addition of the food item, larvae were immediately observed under a dissecting microscope for changes in their behavior that indicated that they were feeding and ingesting material. After several minutes, the number of feeding versus nonfeeding larvae was determined. After a 3–4 h feeding period, larvae were placed into clean FSW and were observed 24 h later using both light and epifluorescence microscopy to determine whether any material, specifically algal cells (which appear red due to the fluorescence of chlorophyll under blue light), remained within their bodies.

Transmission electron microscopy

Larvae that had acquired freshly isolated zooxanthellae during feeding experiments were preserved 4 days later in 2% glutaraldehyde in phosphate-buffered saline (PBS, 0.1 *M* sodium phosphate, 0.45 *M* sodium chloride, pH 7.2) for 1 h, rinsed 3×10 min in PBS, post-fixed for 1 h in 1% osmium tetroxide in PBS, rinsed twice, and dehydrated in an ethanol series (10%, 25%, 50%, 75%, 90%, and 100%). Samples were infiltrated with Epon resin in a series of acetone dilutions (3:1, 1:1, 1:3 acetone:Epon) and then placed in 100% Epon and allowed to polymerize. Thin sections were stained in 0.2% lead citrate and 0.5% uranyl acetate and observed on a Philips CM12 transmission electron microscope.

Effect of zooxanthella source on infection

We conducted infection experiments using four different zooxanthella isolates that ranged from being completely free from any host tissue to zooxanthellae that were still associated with host tissue: (1) cultured specimens of *S. californium*, which are free from host tissue; (2) zooxanthellae freshly isolated from an anemone host, which are contaminated with host tissue and may remain within vacuolar membranes of host origin or within entire intact host cells (Gates et al. 1992; Nii 1997; Colley and Trench 1983); (3) zooxanthellae from black egesta; (4) zooxanthellae from brown egesta.

The infection experiments consisted of three replicate dishes of larvae (all larvae were from the same spawning date). The treatments consisted of one of the four sources of zooxanthellae in either the presence or absence of a feeding elicitor (homogenzied *Artemia*). For each replicate, approximately 100 larvae were placed into 5 ml FSW in one well of a six-well dish. Resuspended zooxanthellae (and associated material) were layered along the bottom of the well to cover the entire surface. If food was to be added, an aliquot of filtered, homogenized *Artemia* was swirled into each dish. Larvae were allowed to feed for 3 h and then were placed into clean FSW. The following day, approximately 20–25 larvae per replicate were selected and observed using epifluorescence microscopy. The autofluorescence of chlorophyll under blue light made it possible to determine the number of larvae that contained zooxanthellae.

Effect of feeding and availability of zooxanthellae

To determine whether the number of larvae infected by zooxanthellae was a function of the feeding behavior of the larvae or of the relative numbers of zooxanthellae present, we conducted a 2×2 factorial experiment. Three replicate wells of eight treatments were prepared. The eight treatments consisted of four different "doses" of zooxanthellae in combination with two feeding treatments (no food or plus food). Each zooxanthella dose was prepared from a stock preparation of freshly isolated zooxanthellae and consisted of different volumes (100 μl, 300 μl, 600 μl, 1,000 μl) of the stock added into six-well dishes containing several hundred larvae in 5 ml FSW. Efforts to quantify the numbers of zooxanthellae in each treatment proved impossible, due to clumping of the zooxanthella isolates. However, the highest dose was sufficient to create a fairly even layer of zooxanthellae along the bottom of each well. If food was to be added to the treatment, a 100-μl aliquot was taken from a stock solution of filtered, homogenized *Artemia* and swirled into the dishes containing larvae and zooxanthellae.

Larvae were allowed to feed for 3 h and then were placed into clean FSW. The following day, approximately 20–25 larvae per replicate were selected and observed using fluorescence microscopy. The autofluorescence of chlorophyll under blue light made it possible to determine the number of larvae containing zooxanthellae.

Results

Larval development

Between 10 and 70% of anemones released gametes during the night following exposure to sunlight. Eggs

were brown, approximately 150 μm in diameter, and had numerous spines (approximately 10 μm in length). Larvae developed as described in Siebert (1974). Specifically, within 2 days the fertilized eggs developed into actively swimming planula larvae that were approximately 150 μm in length. The development of a mouth and gastric cavity occurred approximately 4 days following fertilization. While maintained in the laboratory, larvae typically exhibited continuous active swimming behavior, occasionally sweeping their ciliary tufts across surfaces that they encountered. Larvae never appeared to exhibit behavior that suggested they were ready to undergo settlement or metamorphosis and their lifespan in culture was 3–8 weeks, depending on the conditions under which they were maintained.

Feeding behavior and infection

To determine whether infection of larvae might occur as a consequence of feeding on motile, free-living unicellular algae, we observed larval feeding response to three species of motile algae. Larvae did not initiate feeding behavior when any of the algal types were presented to them and failed to take any algae, including *Symbiodinium californium*, into their gastric cavities (Fig. 1). From our observations, it appeared that algal cells were not able to gain entry to the gastric cavity because currents created by the ciliary motion of swimming larvae swept algae away from the mouths of the larvae. We observed the larvae 0.5–3 h later using a compound microscope to confirm that larvae had not ingested any algal cells.

To determine which cues would prompt larvae to begin feeding, we placed animal-derived tissues into larval cultures. Regardless of the source, within seconds, almost all of the larvae immediately began to feed, as illustrated in Figs. 1 and 2 (a representative, nonfeeding larva is shown in Fig. 2A). They ceased active swimming, descended to the substratum, and expanded their mouths to ingest any particulate matter that they encountered. After some period of time (within seconds to hours), larvae resumed swimming, often with mucus trailing from their oral end (Fig. 2B). These mucus threads trapped particulate matter that was drawn into the mouth as the mucus was ingested.

To determine whether larvae would ingest algae while they were feeding, we presented them with either (1) *Artemia* plus one of the three types of unicellular algae that we used previously, or (2) freshly isolated zooxanthellae with no additional feeding elicitor. The addition of *Artemia* prompted larvae to secrete mucus and to open their mouths, and as shown in Fig. 2C, all three types of algae became mired in these threads. As larvae ingested the mucus, algae entered the gastric cavity. In the treatment that consisted of freshly isolated zooxanthellae alone with no *Artemia*, larvae exhibited a strong feeding response that was most likely triggered by the presence of residual host tissue. This feeding behavior resulted in ingestion of zooxanthellae. The following day, the only algae remaining within larvae from any treatment were zooxanthellae that had been contained within homogenized anemone tissue (i.e., freshly isolated zooxanthellae; Fig. 2D). All others, including cultured *S. californium*, had been eliminated. Transmission electron micrographs of larvae 4 days after acquiring freshly isolated zooxanthellae revealed that the zooxanthellae that remained within larvae had become incorporated into the gastrodermal cells, as shown in Fig. 2E.

Larvae were able to acquire algae via the feeding method starting when they were 4 days old (the age at which the gastric cavity had fully developed) and until they were at least 2 weeks old (data not shown). We did not attempt to infect larvae older than 2 weeks, but it seems likely that larvae are capable of acquiring algae throughout the entire larval period since older larvae continued to feed in a manner identical to that of younger larvae and their morphology did not change significantly.

Larval acquisition of zooxanthellae derived from different sources

To explore more fully the degree to which zooxanthella acquisition is a function of the source of zooxanthellae and of larval feeding response, we determined the infection success of four different zooxanthella isolates – cultured zooxanthellae, zooxanthellae freshly isolated from an anemone host, zooxanthellae contained within black egesta, and zooxanthellae contained within brown egesta. Of these, the two sources that produced a significant rate of infection were zooxanthellae that had been freshly isolated from an adult host and zooxan-

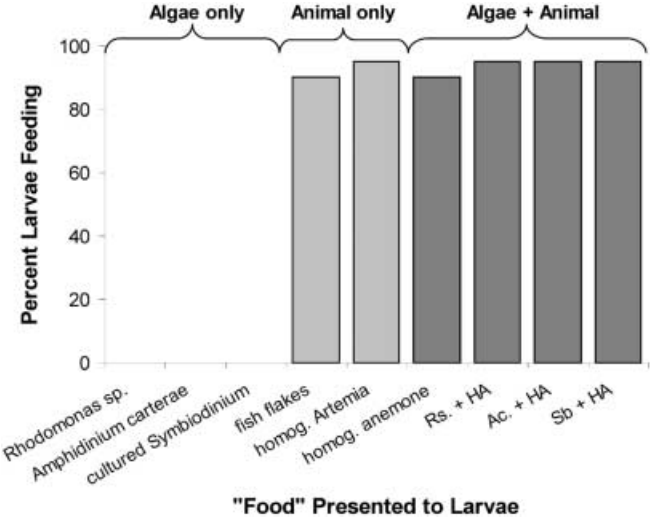

Fig. 1 Percentage of larvae exhibiting feeding behavior after addition of *algal-only*, *animal-only*, or *algal + animal* food items. *HA* homogenzied *Artemia*, *Ac Amphidinium carterae*, *Rs Rhodomonas* sp., *Sb Symbiodinium californium*. $N = 1$ group of several hundred larvae for each treatment

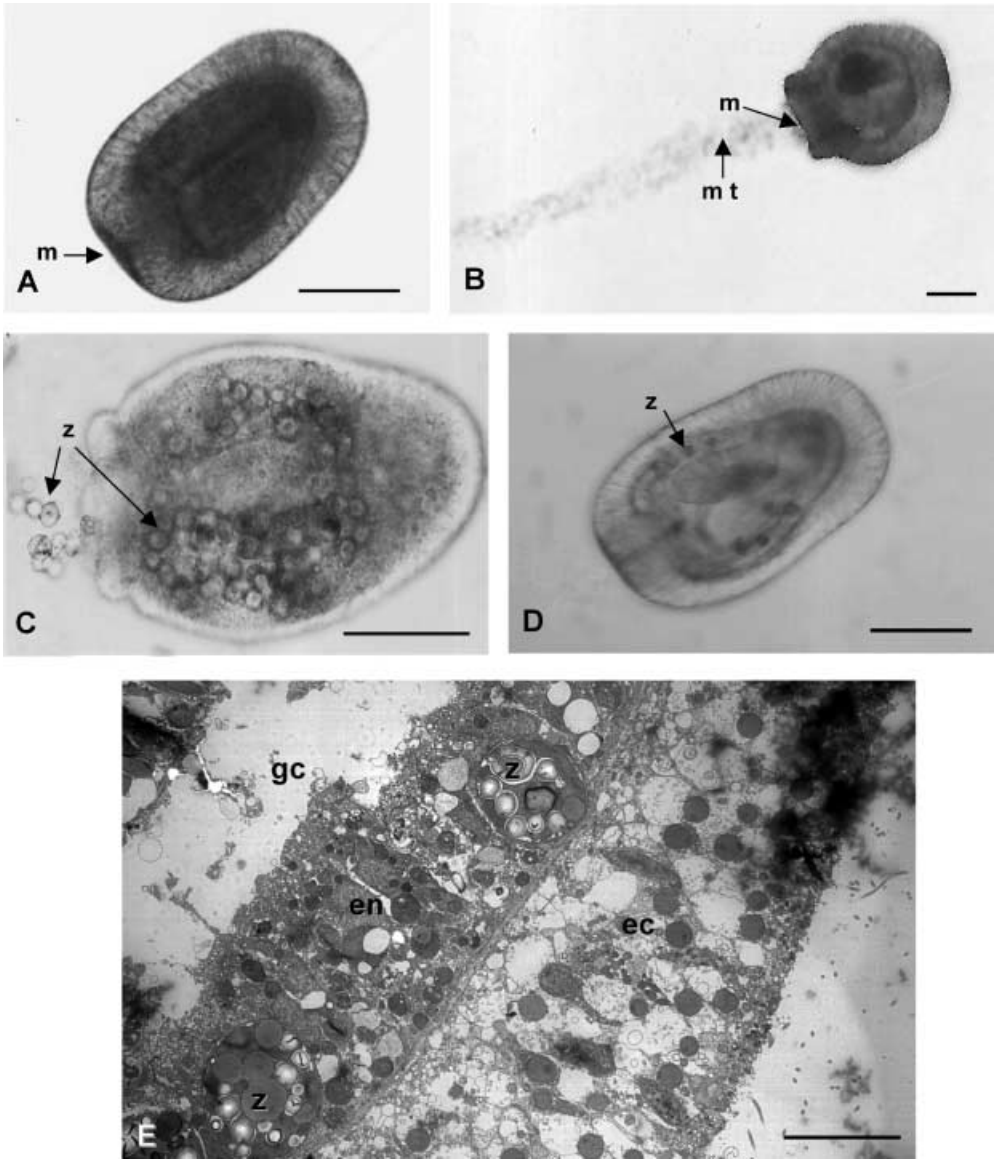

Fig. 2A–E Light and transmission electron micrographs illustrating the process of zooxanthella acquisition by feeding *Anthopleura elegantissima* planulae. **A** Three-day-old, nonsymbiotic planula larva. **B** Feeding larva, with mouth open and mucus "thread" trailing out of the oral end (*m* mouth, *mt* mucus thread). **C** Feeding larva with gastric cavity filled with zooxanthellae (*z* zooxanthellae). **D** Larva infected with zooxanthellae that it had ingested 24 h previously. **E** Transmission electron micrograph showing zooxanthellae contained within endodermal cells of the host larva (*gc* gastric cavity, *en* endoderm, *ec* ectoderm, *z* zooxanthella). *Scale bars* 50 μm for light micrographs (**A–D**) and 10 μm for transmission electron micrograph (**E**)

thellae contained within black egesta. These represent the conditions in which zooxanthellae are most closely associated with residual host tissue. Cultured zooxanthellae (*S. californium*) and zooxanthellae contained within brown egesta rarely infected larvae, even when larvae had been prompted to ingest significant quantities of zooxanthellae (Fig. 2C shows a larva whose gastric cavity is entirely filled with cultured zooxanthellae).

To determine whether larvae would acquire zooxanthellae at a higher rate when a very strong feeding response was stimulated experimentally, we examined the rate of infection both with and without the addition of homogenized *Artemia*. We performed *t*-tests on the plus-food and no-food treatments for each zooxanthella source, using arcsine square root transformed data, $\alpha = 0.05$. As Fig. 3 shows, stimulation of a strong feeding response resulted in a statistically significant higher infection rate only by freshly isolated zooxanthellae ($P < 0.001$).

During the process of feeding, the gastric cavities of larvae became filled with zooxanthellae, regardless of the source (Fig. 2C). However, when observed 24 h later, the average number of zooxanthellae per larva was less than five and few larvae contained ten or more zooxanthellae (data not shown). In only one treatment (freshly isolated zooxanthellae plus *Artemia* slurry) did larvae retain large numbers of zooxanthellae 24 h after infection (data not shown).

Effect of feeding behavior and availability of zooxanthellae

Since it appeared that the addition of an exogenous feeding elicitor (homogenized *Artemia*) might enhance larval susceptibility to infection by freshly isolated zooxanthellae, we examined rates of infection after experimentally manipulating both larval feeding response

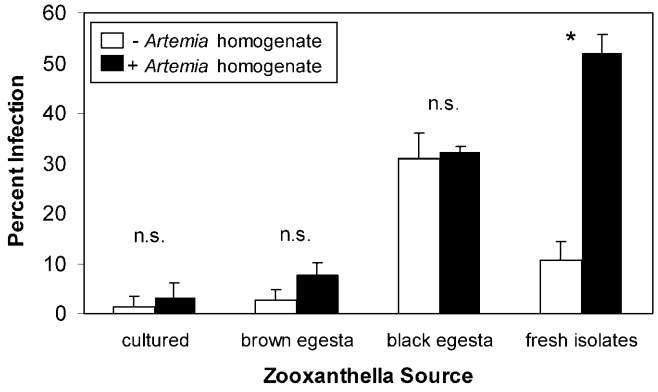

Fig. 3 Percentage of larvae (mean ± SD) infected by zooxanthellae from four sources that differ in the degree to which zooxanthellae are associated with host tissue, either with or without the addition of homogenized *Artemia* as a feeding elicitor. *Fresh isolates* are most intimately associated with host cells, whereas *cultured* zooxanthellae represent a "host-free" condition. *Black egesta* and *brown egesta* represent intermediate conditions (see text for details). An *asterisk* denotes statistical significance ($P < 0.001$) between the feeding treatments for each zooxanthella source; *n.s.* nonsignificant ($P > 0.05$). $N = 3$ replicate groups of approximately 20–25 larvae per group

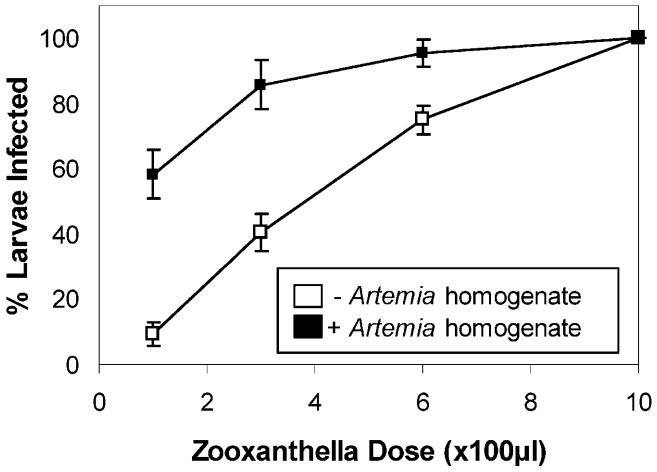

Fig. 4 Percentage of larvae infected (mean ± SD) by increasing doses of freshly isolated zooxanthellae either with or without addition of homogenized *Artemia* as a feeding elicitor. $N = 3$ replicate groups of approximately 20–25 larvae per group

and availability of zooxanthellae (differing concentrations of zooxanthellae). As shown in Fig. 4, both the addition of a feeding elicitor (two-way ANOVA, $P < 0.001$) and increasing availability of zooxanthellae (two-way ANOVA, $P < 0.001$) showed significant effects. The interaction term was also significant ($P < 0.001$).

Discussion

Most cnidarians that form associations with the dinoflagellate *Symbiodinium* do not directly transmit algae to their sexually produced offspring and instead produce offspring that must independently acquire algae from environmental sources. From the few studies that have examined horizontal transmission of zooxanthellae, it appears that there are two main "modes" of infection. First, motile zooxanthellae may congregate around and enter the host's mouth (this has been called "swarming"). Subsequently, they enter the gastric cavity and are taken up into gastrodermal cells (Kinzie 1974; Fitt 1984; T. Yacobovitch personal communication). Second, the host may acquire zooxanthellae that they ingest while they are feeding (Fitt 1984; Riggs 1988; Schwarz et al. 1999). This study suggests that the "swarming" mode would not be a common mode of infection in planulae, as larval ciliary motion generated by their swimming behavior tends to sweep particulate matter away from their mouths. This constrains larvae to acquiring zooxanthellae that they actively ingest.

Regardless of the mechanism by which algae are brought into contact with the host (swarming or feeding), the incorporation of algae into host cells usually occurs via phagocytic uptake of zooxanthellae present in the gastric cavity of the host (Colley and Trench 1983, 1985; Fitt and Trench 1983a, b; Schwarz et al. 1999). In the life history of the host, therefore, the development of a mouth is the critical event marking the onset of competence for infection. The timing of the development of the mouth and gastric cavity differs widely in cnidarians. Some species produce planula larvae that develop a mouth within a day or two while others produce offspring that do not develop a mouth until the metamorphic event that produces the polyp stage, which may not occur for weeks. Thus the age at which a host becomes competent to acquire algae probably varies widely among host species.

Not all sources of zooxanthellae are equally "infective." Differential abilities to form an association with a new host may result from recognition and specificity events that occur during the phagocytic process, or after the symbiont has been taken into the host cell. One confounding factor is the presence of remnant host tissue, which appears to play a role in this recognition process. Colley and Trench (1983) measured a significantly higher rate of phagocytosis of zooxanthellae that were associated with a host membrane, and it has been hypothesized that the presence of the host membrane surrounding zooxanthellae plays a role in the recognition process.

This study did not directly address the question of whether the presence of a host membrane around zooxanthellae influences the overall infection success. However, there was a trend suggesting that contamination with host tissue was correlated with infection success. The most successful infections occurred as a result of using zooxanthellae freshly isolated from an anemone host and zooxanthellae contained within black egesta, both of which are contaminated with host tissue and may still be contained within host membranes (Colley and Trench 1983; Gates et al. 1992; Nii 1997). Zooxanthellae from brown egesta, which were likely free from host tissues, and cultured *S. californium* were much

less successful in forming an association with planulae. This suggests that the presence of host tissue, and possibly the presence of a host membrane around zooxanthellae, plays a role. Alternatively, these results could reflect differences in viability of zooxanthellae rather than presence of host tissue. Cultured *S. californium*, although reported to be the species that associates with *A. elegantissima* along the central California coast (LaJeunesse and Trench 2000), was perhaps incompatible due to the conditions under which planulae were maintained in the lab. To date there have been no reports of using these cultures successfully to infect either adult or larval *A. elegantissima*, so it is unknown whether these cultures can be used successfully to infect a suitable host.

The role of feeding is, similarly, not clear. Feeding does cause larvae to bring material into their digestive cavities that they may have otherwise ignored, but the degree to which it can enhance the rate of infection seems to differ depending on the source of zooxanthellae. Feeding played a dramatic role in increasing the infection rate by freshly isolated zooxanthellae (Figs. 3, 4) but did not significantly influence the ability of larvae to acquire zooxanthellae derived from any other source. The reasons for this remain to be explored.

This study provides insights into possible sources of zooxanthellae that may be important in the initial infection process as it occurs in the natural environment. It has been observed that hosts routinely, and under stressful conditions, expel mucus and/or intact gastrodermal cells that contain zooxanthellae (Steele 1975, 1977; Gates et al. 1992; Montgomery and Kremer 1995; McCloskey et al. 1996; Schwarz et al 1999). This study has demonstrated that this egested material fills both of the requirements for infection of motile, feeding larvae (eliciting a feeding response and providing viable zooxanthellae). Other potential sources of zooxanthellae that were not examined as a part of this study (but have been shown to infect aposymbiotic host polyps) include zooxanthella-laden fecal pellets from species that prey on anemones (Muller-Parker 1984), and ingestion of a prey item that contains zooxanthellae (Fitt 1984).

From patterns that are emerging from this and other studies, we can make predictions regarding when initially aposymbiotic hosts might first acquire algae. We would predict that in host species that produce a nonfeeding (lecithotrophic) larva, the host would be constrained to acquiring algae after metamorphosis, and that this infection event could occur either due to the "swarming" of symbionts toward the host, or upon ingestion of algae during feeding. This has been found to be the case in several species of octocorals and jellyfish, which all produce nonfeeding larvae that, after metamorphosis, become infected by motile zooxanthellae that swarm to the mouth of the host (Kinzie 1974; Fitt 1984; T. Yacobovitch, personal communication). However, for species that produce a feeding planula larva, it appears that the host may not have to wait until after metamorphosis; infection could occur during the larval stage. It also seems to be the case that infection via motile zooxanthellae might not be possible. Larvae might be constrained to becoming infected only as a result of their feeding behavior. It will be interesting to see whether future studies support these predictions.

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