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REPORT

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Host-symbiont specificity during onset of symbiosis between the dinoflagellates *Symbiodinium* spp. and planula larvae of the scleractinian coral *Fungia scutaria*

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Abstract Many corals which engage in symbioses with dinoflagellates from the genus *Symbiodinium* (zooxanthellae) produce offspring which initially lack zooxanthellae. These species must choose their symbionts from numerous genetically distinct strains of zooxanthellae co-occurring in the environment. In most cases, symbiosis onset results in an association between a specific host coral and a specific strain of algal symbiont. This is the first study to examine host-symbiont specificity during symbiosis onset in a larval cnidarian, and the first to examine such events in a scleractinian of any life stage. We infected planula larvae of the solitary Hawaiian scleractinian *Fungia scutaria* with both homologous zooxanthellae, freshly isolated from *F. scutaria* adults, and heterologous zooxanthellae, isolated from *Montipora verrucosa, Porites compressa,* and *Pocillopora damicornis,* three species of scleractinians which co-occur with *F. scutaria*. We found that homologous zooxanthellae were better able to establish symbioses with larval hosts than were heterologous isolates, by two separate measures: percent of a larval population infected, and densities of zooxanthellae per larva. We also measured algal densities in larvae over a 4-day period until the onset of settlement and metamorphosis. We found no changes in zooxanthella population densities, regardless of zooxanthella type or the light environment in which they were incubated. Strong infection of host larvae with homologous algae compared to heterologous algae suggests that there is a specificity process which occurs sometime during the early stages of infection between the partners, and which results in the establishment of a specific symbiosis.

Keywords Cnidarian · Coral · Planula larva · *Symbiodinium* · Symbiosis · Zooxanthellae

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Introduction

Mutualistic endosymbiosis between two unrelated organisms includes a stage where the larger partner or host first acquires its smaller partner or symbiont (Trench 1993; Douglas 1994, 1998). Symbionts may be transmitted vertically where the symbiont is passed directly from host parent to offspring, or horizontally where host sexual progeny must acquire symbionts from the environment. Horizontal transmission offers the potential flexibility of choosing a partner, from a variety of possible candidates, which is best adapted to the conditions at hand. There is a risk, however, that establishment of a symbiosis could fail, leaving the non-symbiotic host with reduced growth and fitness. In contrast, vertical transmission ensures that host offspring is provided with a complement of symbionts, although these symbionts are of limited genetic diversity, a possible disadvantage should environmental conditions change.

Many members of the phylum Cnidaria engage in an endosymbiosis with photosynthetic dinoflagellates from the genus *Symbiodinium*. In most cases, the dinoflagellates, also known as zooxanthellae, reside within vacuoles in the gastrodermal cells of the host cnidarian. Zooxanthellae contribute to host nutrition by providing photosynthetically fixed carbon, whereas the host provides (to the zooxanthellae) inorganic nutrients, a high-light environment, and refuge from herbivory (reviewed in Muscatine 1990; Muscatine and Weis 1992; Muller-Parker and D'Elia 1997). Most species of cnidarians which engage in symbioses with zooxanthellae are obligately symbiotic and have severely reduced growth, survivorship, and fitness in the absence of the symbiosis (reviewed in Brown 1997a, 1997b). Despite the obligate

nature of these associations, the majority of cnidarian/algal symbioses (~85%) rely on horizontal transmission (Fadlallah 1983; Babcock and Heyward 1986; Harrison and Wallace 1990; Richmond and Hunter 1990; Richmond 1997). Many cnidarian hosts spawn azooxanthellate (i.e., lack zooxanthellae) gametes which are fertilized in the water column and in turn develop into azooxanthellate planula larvae. This new generation of hosts must, at some stage, initiate a symbiosis with a new complement of zooxanthellae from the environment.

Despite their relatively uniform morphology, zooxanthellae are taxonomically highly diverse, as evidenced by varying physiological (e.g., Iglesias-Prieto et al. 1992; Iglesias-Prieto and Trench 1997), biochemical (e.g., Schoenberg and Trench 1980a; Blank and Trench 1985; Iglesias-Prieto et al. 1991), and molecular phylogenetic characteristics (e.g., Rowan and Powers 1991a, 1991b; McNally et al. 1994; Baker and Rowan 1997; Wilcox 1998). Recently, zooxanthellae have been divided into four distinct taxa or clades, namely A, B, C, and D, based on ribosomal RNA gene sequences and RFLP patterns (Rowan and Powers 1991a, 1991b; Wilcox 1998; Baker 1999; LaJeunesse and Trench 2000). It is difficult to generalize about the specificity of algal clades and host taxa, in part because the study of zooxanthellae diversity is still in its infancy. There are numerous examples of host species containing a single algal clade (e.g., Rowan and Powers 1991a, 1991b; Rowan 1998; Wilcox 1998), as well as those harboring more than one clade (Rowan and Knowlton 1995; Darius et al. 1998; LaJeunesse and Trench 2000). The side-by-side occurrence on reefs of numerous corals and other cnidarian species harboring different algal clades suggests that there is a specificity process which occurs at some point during the initiation of the symbiosis, where host and symbiont select some partners and exclude others (Douglas 1994). Indeed, this process of specificity likely extends beyond the very broad level of the clade to genetically distinct populations within single clades. Ongoing work by several investigators is beginning to describe within-clade genetic diversity (Goulet and Coffroth 1997; Hunter et al. 1997; Snell and Coffroth 1999; Wilcox et al. 1999), similar to that detailed in earlier classical investigations (e.g., Schoenberg and Trench 1980a, 1980b; Trench and Blank 1987; Banaszak et al. 1993). This diversity provides the opportunity for recognition and specificity during the initiation of the partnership.

The role of host-zooxanthella specificity during the onset and establishment of the symbioses has been examined in the laboratory in a variety of adult (Kinzie and Chee 1979; Schoenberg and Trench 1980c; Fitt 1984; Davy et al. 1997) and juvenile (Kinzie 1974; Colley and Trench 1983; Fitt and Trench 1983a, 1983b; Colley and Trench 1985; Coffroth et al. 2001) cnidarian hosts. In addition, one study has examined specificity during symbiosis onset in juveniles in the field (Coffroth et al. 2001). Zooxanthellae establish residence in (infect) these polyp stages of the host by being taken into the animal during the feeding process and being phagocytosed by gastrodermal cells (Colley and Trench 1983; Fitt and Trench 1983a, 1983b). In these studies of infection, azooxanthellate hosts were challenged with both homologous zooxanthellae, i.e., algae obtained from the same host species, and heterologous zooxanthellae, i.e., algae isolated from a different species of host. In most cases, some strains of heterologous zooxanthellae were capable of infecting hosts but, by various measures, were less effective than homologous zooxanthellae in establishing a stable association with the host. Other heterologous algal strains completely failed to successfully infect hosts.

Less attention has been devoted to studying symbiosis onset in the planula larva stage of cnidarians, despite the likelihood that in nature it is this life-history stage where symbiosis is most often initiated. Montgomery and Kremer (1995) found that planulae of the scyphozoan *Linuche unguiculata* became infected by experimentally added homologous zooxanthellae. Schwarz and coworkers (Schwarz 1996; Schwarz et al. 2001) found that planulae of the temperate sea anemone *Anthopleura elegantissima* acquired zooxanthellae after feeding on host tissue which contained zooxanthellae recently isolated from an adult. We have described larval development and symbiosis onset with homologous zooxanthellae in the solitary scleractinian *Fungia scutaria* (Krupp 1983; Schwarz et al. 1999). We determined that larval *F. scutaria* were competent to acquire zooxanthellae after development of a mouth, that the zooxanthellae were ingested during the feeding process and rapidly phagocytosed by gastrodermal cells, and that the zooxanthellae persisted in larvae through metamorphosis to juvenile polyps. Infection with zooxanthellae was not required for metamorphosis and further, azooxanthellate juvenile polyps could successfully acquire zooxanthellae.

In this report we extend our studies of symbiosis onset in larval *F. scutaria* to examine host-symbiont specificity and the kinetics of zooxanthellae infection. We introduced homologous and heterologous zooxanthellae, all members of zooxanthella clade C, to larval *F. scutaria* and used the proportion of larvae infected in a population and the density of zooxanthellae within the host larvae as measures of infection success. We examined algal density in larvae through time, up to metamorphosis, to determine if algal population size changed over the duration of the larval phase of the host lifecycle. We also studied changes in algal density between populations of larvae incubated in high-light and low-light environments to determine if algal population changes were affected by irradiance level.

Materials and methods

Gamete collection and larval cultures

All experiments were performed at the Hawaii Institute of Marine Biology on Coconut Island, Kaneohe Bay, Oahu, Hawaii. Approximately 50 previously tagged specimens of *F. scutaria*, around 25 cm in diameter, were collected from the reef and placed in

seawater tables. Spawning and gamete collection has been described in detail previously (Krupp 1983; Schwarz et al. 1999). Briefly, *F. scutaria* generally spawns between 1,700 and 1,900 h, 2–4 days after the full moon from June through September. We performed our experiments on larvae resulting from spawns in August and September of 1998. Approximately 1 h before a predicted spawning event, the corals were placed in standing, 0.45-μm filtered seawater (FSW) in individual glass or plastic bowls. During spawning, gametes were released directly into the isolated bowls containing single adults. After spawning, females were removed from their bowls, leaving a layer of eggs on the bottom of the dish. Within 30 min after spawning, water collected from the bowls of all spawning males was combined and a small volume was added to each bowl of eggs. The bowls were left in seawater tables overnight for fertilization and early larval development. Larvae from all parental crosses were combined and maintained in bowls in FSW which was changed every other day. After the summer spawning events, the tagged adults were returned to the reef for use in future spawns.

Preparation of zooxanthella isolates

Zooxanthellae were isolated from freshly collected fist-sized chunks of adult specimens of *F. scutaria* (defined as homologous zooxanthellae), *Montipora verrucosa, Pocillopora damicornis,* and *Porites compressa* (termed heterologous zooxanthellae) by using the spray from a Water Pik filled with FSW to remove and homogenize coral tissue. The resulting homogenate was spun in a tabletop centrifuge at 2,000 *g* for 2 min to separate the algae from the animal tissue. The zooxanthella pellet was twice resuspended in FSW and repelleted by centrifugation to partially clean the algae of contaminating animal tissue. Following this, zooxanthellae were resuspended into a very dense suspension in FSW before being added to the larval cultures. Zooxanthella isolates were used within 1 h of preparation. Zooxanthellae from all four coral species, from specimens collected in Kaneohe Bay, have been described as members of clade C (Rowan and Powers 1991a, 1991b; Baker and Rowan 1997). We did not independently confirm the algal clades for our corals for this study.

In preliminary experiments, we attempted to quantify the number of algae which we added to a population of larvae. After the two rinses in FSW (described above) the algae from all coral species were highly clumped and contaminated with animal debris, making quantification impossible. In an attempt to obtain more uniform suspensions of zooxanthella isolates and to remove animal debris, we performed additional and more vigorous washes. In doing so, we considerably reduced the ability of the algae to infect the host, which resulted in inconsistent and greatly reduced percentages of larvae infected (data not shown). We therefore decided to provide non-quantified but saturating concentrations of algae to the larvae (see below), which resulted in consistent and repeatable infection results.

Percent of *F. scutaria* larvae infected with homologous and heterologous zooxanthellae

In order to compare the abilities of different zooxanthella types to take up residence in *F. scutaria* larvae, we measured the proportion of larvae infected with different algal types one day after an experimental infection event. Approximately 10,000 larvae from a single mixed population were divided into 12 separate finger bowls. Zooxanthella isolates were prepared from three different individuals of each of four species of corals, homologous *F. scutaria,* and heterologous *P. compressa, P. damicornis,* and *M. verrucosa*.

Each of the zooxanthella isolates was added to one of the 12 bowls of larvae (i.e., three replicates of each species). An even layer of freshly isolated algae was added to the bottom of the bowls containing 4-day-old larvae, which are competent to feed and acquire symbionts (Schwarz et al. 1999). Several drops of homogenized *Artemia* were added to the bowls to stimulate a feeding response as described in Schwarz et al. (1999). The *Artemia* slurry prompts the swimming larvae to drop to the bottom of the dish and begin ingestion of any material they encounter (Schwarz et al. 1999).

After 4 h, zooxanthellae were still present in large quantities on the bottom of the dish. The larvae were removed from the zooxanthella isolates, by concentration onto a 60-μm mesh filter and subsequently the larvae were placed into a clean bowl of FSW. Non-quantitative inspections of larvae under the compound microscope immediately after the 4-h incubation revealed that virtually all larvae, regardless of which algal strain they were challenged with, had gastric cavities full of algae as described previously (see Schwarz et al. 1999). The fate of ingested zooxanthellae was not followed at the microscopic level in the study. In previous studies on infection with homologous zooxanthellae, however, we found that as soon as 1 h post-feeding, algae were present in vacuoles in the endoderm with very few algae remaining in the gastric cavity (see Schwarz et al. 1999).

The following day, three separate counts of approximately 100 larvae each were made from each of the 12 bowls. Approximately 1 ml of larvae in solution was dispensed into a 1.5-ml microfuge tube and spun at 500 *g* for 1–2 min to gently pellet the larvae. The larval pellet was immediately removed with a fine-bore pipette and placed on a glass microscope slide. A cover slip was placed on the drop and pressed onto the slide with a pencil eraser. This pressure was sufficient to squash the larvae, without completely dispersing their contents. Depending on the density of the larval culture, each glass slide contained from 50 to 200 larvae. Larvae were counted and scored as either symbiotic (regardless of the number of algae/larva) or aposymbiotic, using a compound microscope.

Density of zooxanthellae in larvae over time and in different light regimes

In a separate experiment, to compare the density of homologous vs heterologous zooxanthellae within larvae and to determine changes in numbers of zooxanthellae per host larva through time, we quantified algae in larvae which had been infected with homologous and heterologous zooxanthellae. We were also interested in determining what effect light level had on algal density within larvae, hypothesizing that zooxanthellae in larvae kept in a high-light environment would increase in density at a faster rate than those in larvae incubated in low light. Approximately 3,000 larvae from a single mixed population were divided into nine separate finger bowls. Zooxanthella isolates were prepared from three different individuals of each of three species of corals, homologous *F. scutaria*, and heterologous *P. compressa* and *P. damicornis*. Each of these isolates was added to one of the nine bowls of larvae as described above.

Following rinsing and placement into fresh FSW, each of the nine bowls of infected larvae was further subdivided into two bowls, one destined for a high-light treatment and the other for a low-light treatment. The final experimental design therefore involved 18 bowls: zooxanthella isolates from three coral species × algae from three individuals per coral species × two light treatments.

High-light treatment bowls were placed in a seawater table which was exposed in the mornings to full ambient light, but which was in the shade of an overhanging eave in the afternoons. Low-light treatment bowls were in the same seawater table but were covered by dark plastic tubs which allowed in virtually no light.

The number of zooxanthellae per larva was quantified for 25 symbiotic larvae per bowl on each of 4 days following infection, and an average algal density per larva was calculated per bowl per day.

Statistical analyses

Analysis of variance (ANOVA), nested ANOVA, and two-tailed unpaired student t-tests were performed using SAS 6.12 software. Data expressed as percentages were arcsin transformed before t-tests were performed. The kinetics data were log transformed to meet the assumptions of homogeneity of variance, and a nested ANOVA was performed with the following effects: zooxanthella isolate type, bowls of larvae nested within isolate type, light

treatment, and time. Student t-tests were corrected for multiple comparisons using the Bonferroni procedure.

Results

Percent of *Fungia scutaria* larvae infected with homologous and heterologous zooxanthellae

To compare infection success in *F. scutaria* larvae infected with homologous vs heterologous algae, we sampled subsets of larval populations 1 day after exposure to different zooxanthella isolates, and determined the percentage of larvae which contained symbionts. These treatments were highly significantly different from one another (ANOVA, $p < 0.001$). Nearly all larvae incubated with *F. scutaria* algae contained zooxanthellae, with an average of $96 \pm 1.5\%$ (mean $\pm$ SD; $n =$ three bowls of larvae) of larvae being symbiotic (Fig. 1). High percentages of larvae incubated with *P. compressa* and *P. damicornis* algae were also symbiotic (84 ± 5.2 and $83 \pm 4.4\%$, respectively). However, these values were significantly lower (t-test, $p < 0.05$) than those from larvae with *F. scutaria* algae. In contrast, larvae incubated with *M. verrucosa* zooxanthellae were nearly all aposymbiotic, with only $3 \pm 3.7\%$ of larvae being infected (different than *F. scutaria* by $p < 0.001$).

Density of homologous and heterologous zooxanthellae in larvae

To compare the density of different algal types within *F. scutaria* larval hosts, we quantified the number of algae within larvae infected with homologous vs heterologous zooxanthella isolates. *F. scutaria* larvae containing homologous *F. scutaria* algae had much higher algal densities than those larvae with heterologous *P. compressa* or *P. damicornis* zooxanthellae (Fig. 2), despite very high variability in the homologous algal densities on all days and in both high-light and low-light treatments. The average number of *F. scutaria* zooxanthellae resident within larvae ($n = 24$ total bowls of larvae counted, across all three replicates, four times and both light treatments) was 22.2 ± 5.5 (mean $\pm$ SD), almost 3 times higher than the 8.5 ± 1.7 for *P. compressa* algae, and 5.5 times higher than the 3.9 ± 1.2 for *P. damicornis* algae. A nested ANOVA of algal density data comparing zooxanthella isolate type vs time vs light treatment showed a highly significant zooxanthella isolate type effect ($p < 0.001$). Further, t-tests showed a significant difference between the density in larvae of *F. scutaria* algae vs *P. compressa* algae and vs *P. damicornis* algae (both $p < 0.001$).

Changes in algal density with time and between light treatments

We were interested in determining whether algal population densities within larvae changed over the duration of the larval life-history stage, and whether altering light regime would affect algal densities. As shown in Fig. 2, surprisingly, algal densities remained very stable over the 4-day sampling period, regardless of zooxanthella isolate or treatment type (nested ANOVA time effect,

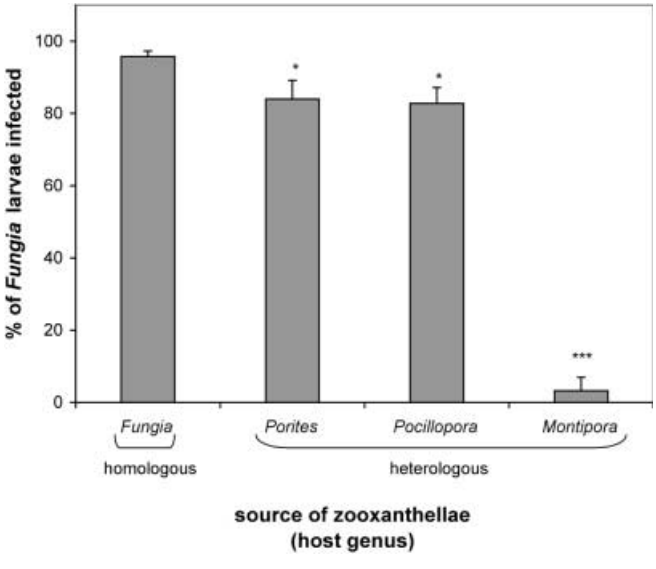

Fig. 1 Percentage of *Fungia scutaria* larvae symbiotic with homologous *F. scutaria* algae and heterologous *Porites compressa, Pocillopora damicornis,* and *Montipora verrucosa* zooxanthellae 1 day after experimental infection. *Bars* represent means $\pm$ SD, $n =$ three bowls of larvae with 100 larvae counted per bowl. * and *** indicate values different from homologous *F. scutaria* percentage, $p < 0.05$ and $p < 0.001$, respectively

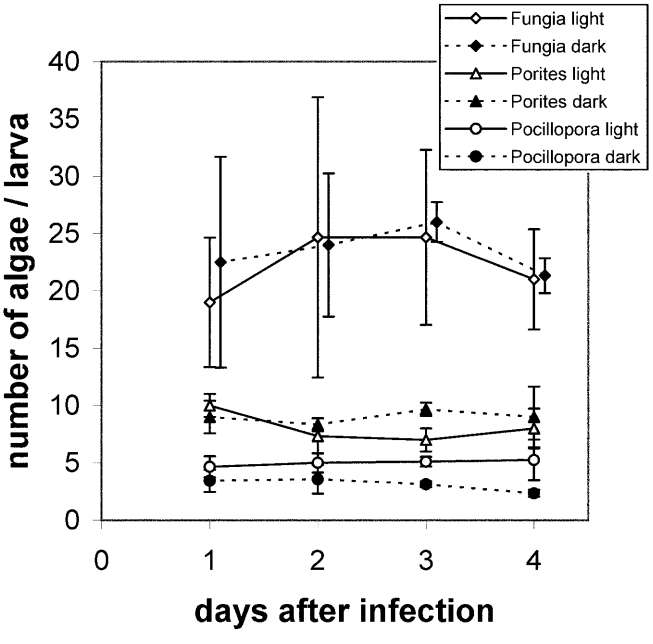

Fig. 2 Densities of homologous and heterologous zooxanthellae in *Fungia scutaria* larvae over time and in different light regimes. *Data points* represent means $\pm$ SD, $n =$ three bowls of larvae with 25 larvae counted per bowl. Values for the *Fungia* zooxanthella type dark treatment are slightly offset for clarity

$p > 0.1$). Similarly, there were no differences in zooxanthellae densities between larvae incubated in high-light vs low-light treatments within any species or on any day (nested ANOVA light treatment effect, $p > 0.1$).

Discussion

This study is the first to examine specificity events during the early stages of symbiosis between scleractinian larvae and symbiotic dinoflagellates. By two separate measures, percent of larvae infected and density of zooxanthellae per larva, we found that homologous algae were better able to colonize larvae than were heterologous algae. In addition, we found no changes in algal densities per larva over 4 days, regardless of the type of algae added or light regime.

Recognition and specificity

This investigation of symbiosis onset in *F. scutaria* larvae provides evidence of recognition and specificity between host and symbiont, two processes which are central to the biology of cooperative associations (Trench 1993; Douglas 1994). Under saturating conditions, that is, with abundant zooxanthella isolates available for infection, *F. scutaria* larvae became infected in higher proportions (Fig. 1) as well as with greater numbers of zooxanthellae (Fig. 2) when challenged with homologous compared to heterologous zooxanthellae. Whereas homologous *F. scutaria* algae infected nearly 100% of larvae, heterologous zooxanthellae from *M. verrucosa* established populations in only 3% of larvae. In addition, algae from *P. compressa* and *P. damicornis* were, respectively, 2.7 and 5.5 times less numerous within larvae than were homologous algae from *F. scutaria*. This differential ability to establish a partnership suggests that there is a recognition process between the partners which plays a role in the ultimate establishment of a specific combination of host and symbiont (Trench 1987, 1993; Douglas 1994).

This is the first study which documents in detail the specificity of symbiosis onset in the planula larva stage of cnidarians. We reported previously on the ability of *F. scutaria* larvae to initiate a symbiosis with zooxanthellae from *Aiptasia pallida* (*Symbiodinium pulchrorum*, clade B) and *Cassiopeia xamachana* (*S. microadriaticum,* clade A), zooxanthellae which are distantly related to those from *F. scutaria* (clade C, Rowan and Powers 1991a, 1991b; Baker and Rowan 1997). Based on these findings, we suggested that *F. scutaria* exhibited a low degree of specificity (Schwarz et al. 1999). We did not, however, quantify the infections or compare them with those achieved with homologous zooxanthella isolates. In contrast, this study quantifies symbiosis onset when using four zooxanthella isolates grouped in clade C and obtained from four species of scleractinians which co-occur on reefs in Hawaii. Despite their occurrence in the same clade, there were clear differences in their abilities to establish a symbiosis in *F. scutaria* larvae. These within-clade differences in specificity contribute to overwhelming biochemical, physiological, and molecular evidence (reviewed in Trench 1993, 1997; Rowan 1998) of the taxonomic diversity of zooxanthellae resident within cnidarians. Further, this specificity helps to explain the observed pattern of specific hosts harboring specific symbionts in the field (Rowan and Powers 1991a, 1991b) despite the likely availability of multiple symbionts in the surrounding environment.

Our findings of host-symbiont specificity in *F. scutaria* larvae agree with numerous previous reports of specificity with algal infection in the polyp stage of the host lifecycle. These studies examined both repopulation of adult polyps which had been rendered aposymbiotic, specifically several species of the anemone *Aiptasia* (Kinzie and Chee 1979; Schoenberg and Trench 1980c; Fitt 1984) and the temperate anemone *Cereus pedunculatus* (Davy et al. 1997), and symbiosis onset in juvenile polyps, specifically scyphistomae of *C. xamachana* (Colley and Trench 1983; Fitt and Trench 1983b; Fitt 1984; Colley and Trench 1985) and newly settled primary polyps of the gorgonians *Pseudopterogorgia bipinnata* (Kinzie 1974), *P. porosa,* and *Plexaura kuna* (Coffroth et al. 2001). It is difficult to generalize about these studies, as widely varying measures of infection success were used, ranging from densities of algae per unit animal (similar to this study) to growth rates of, or developmental changes in the host. In addition, infections were monitored over periods ranging from a few days (similar to this study) to 36 weeks. In all cases, however, homologous zooxanthellae appeared to establish stronger symbioses with hosts than did heterologous ones. For example, in repopulation studies of *Aiptasia tagetes*, algae from 14 species of hosts were introduced (Schoenberg and Trench 1980c). Algae from three species failed to infect anemones, results similar to our infections with *M. verrucosa* algae in *F. scutaria*. The remaining heterologous strains infected, but at lower rates and lower densities than the homologous strain, which is again similar to our infections with *P. compressa* and *P. damicornis* zooxanthellae. In studies of symbiosis onset in scyphistomae of *C. xamachana,* four heterologous strains of algae were initially able to infect hosts, but two of these subsequently failed to establish stable symbioses, and the other two infected but at a slower rate than did homologous *S. microadriaticum* (Colley and Trench 1983). In studies of symbiosis onset in *Plexaura kuna* and *Pseudoptergorgia porosa*, Coffroth and coworkers (2001) found that juveniles of both gorgonian species could be infected with *Symbiodinium* spp. from clades A, B, and C. After being placed for 3 months in the field, however, the vast majority of juvenile polyps contained only clade B zooxanthellae, the clade which is found in field-collected juveniles and adults. The similarity in specificity phenomena between larval and adult stages of cnidarians suggests that these events are not particular to a host developmental stage,

rather that the mechanisms driving initial symbiosis onset in planula larvae are the same as those which occur in juvenile and adult polyps.

The very early cellular events in host-symbiont recognition and specificity were not examined in this study. Future work on these initial cellular events between the partners will be required before we can gain a complete understanding of these early events in this symbiosis. It is worthwhile here to briefly discuss some of the experimental constraints which were encountered in the execution of this study, how they limit our interpretation of the data, and how they relate to other studies on the cellular nature of the zooxanthella–cnidarian recognition process.

In this study, all zooxanthella isolates which were used to infect larvae were highly contaminated with animal debris. Indeed, when we attempted to produce cleaner, less contaminated isolates, as described above in the Materials and methods, we found that the infection success decreased dramatically, even in homologous algal infections. There are three possible explanations for these observations of decreased infection with cleaner zooxanthella isolates. The first possibility is that the algae are somehow compromised by vigorous cleaning, and therefore exhibit reduced infection success because fewer algae are viable. The second possibility is that recognition events occur not between host and symbiont but between host and contaminating host source debris, for example, an animal vacuole membrane surrounding the algae. The third possibility is that algae are ingested non-specifically during feeding, and that host debris acts as a non-specific feeding cue which increases infection success of highly contaminated algal isolates by increasing the number of algae which enter the host.

Evidence from other studies on host feeding during symbiosis onset suggests that our data are explained, at least in part, by the third possibility, i.e., zooxanthellae are taken up non-specifically during feeding and host debris acts as a feeding cue. In studies of early events in the symbiosis between zooxanthellae and *C. xamachana*, algal infections of host scyphistomae were less successful, regardless of host source, when using naked, motile cultured algae, or when using freshly isolated algae which had been treated with a detergent to remove host membranes, compared to untreated, freshly isolated algae (Colley and Trench 1983). These results led the authors to contend (1) that the contaminating animal debris was important in a non-specific feeding response which resulted in algal phagocytosis, and (2) that specificity events between the partners are likely to happen after non-specific phagocytosis of potential algal symbionts. Similarly, Schwarz and coworkers (Schwarz 1996; Schwarz et al. 2001) describe non-specific feeding by planula larvae of the anemone *Anthopleura elegantissima* on zooxanthellae and non-symbiotic species of algae. Larvae were shown to ingest all algal types in the presence of *Artemia* slurry used as a feeding cue. They ultimately retained only freshly isolated zooxanthellae from *A. elegantissima* adult hosts (*Symbiodinium californium*; Banaszak et al. 1993; LaJeunesse and Trench 2000) and rejected all other algal species as well as cultured *S. californium*.

Our insight into recognition and specificity events in algal–cnidarian symbioses is further limited by our lack of information on the nature of infective zooxanthellae in the field. For example, we do not know if source zooxanthellae are naked motile swarmers or, alternatively, freshly expelled coccoid symbionts surrounded by host tissue. Despite these limitations in our understanding of the mechanisms of recognition and specificity, our data suggest these events occur in *F. scutaria* and result in discrimination between algal types.

Kinetics of algal infection

Densities of zooxanthellae in larval *F. scutaria* did not change over the 4-day sampling period, regardless of the algal type infecting the larval populations, or of the light level at which the larvae were incubated (Fig. 2). This was surprising, as we hypothesized that algae resident in larvae incubated in high light would be more productive, and therefore grow faster than those in low-light conditions. Other studies measuring algal population changes in hosts over time have reported the occurrence of initial lag periods, lasting several days after inoculation, before algal populations started to increase (Colley and Trench 1983; Berner et al. 1993; Davy et al. 1997). It is possible that our short sampling period of 4 days, the duration of the larval stage before the initiation of settlement, falls within such a lag phase of population growth in the algae. The duration of the planula larva stage of *F. scutaria* in the field is unknown. If it is similar in length to that of larvae in the laboratory, our data indicate that significant changes in symbiont–host biomass ratios do not occur in *F. scutaria* planulae. Static zooxanthellae densities in larvae could also be explained by the existence of a maximum symbiont–host biomass ratio, a carrying capacity for algae within the host. If this were the case, zooxanthellae densities could only increase with the growth of the juvenile polyp after metamorphosis. Our data do not necessarily support this hypothesis, as variability in homologous algal densities remained high throughout the 4-day sampling period. If densities reached a carrying capacity, one would predict that the variability would decrease over the 4-day period. Further investigations which monitor mitotic index of algae in larvae would give more information on algal growth and host regulation.

This study enhances our understanding of the specificity events occurring during the initial establishment of the symbiosis between a larval scleractinian and *Symbiodinium* spp. Complementary field studies, which monitor long-term dynamics between developing corals and zooxanthella strains, such as those recently undertaken on gorgonians (Coffroth et al. 2001), would provide additional information to help explain the complex patterns of specificity observed in nature.

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