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Variation in *Symbiodinium* communities in juvenile *Briareum asbestinum* (Cnidaria: Octocorallia) over temporal and spatial scales

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ABSTRACT: Despite the importance of cnidarian–algal symbioses for the reef ecosystem, little is known of the pattern of symbiont acquisition in juvenile octocorals and how this varies across time and environment. To study this, aposymbiotic larvae from the common Caribbean gorgonian *Briareum asbestinum* were placed in distinct habitats in the middle Florida Keys, and the establishment of the symbiosis was monitored yearly during 1999–2002. Although *Symbiodinium* B184 (type based on length variation in domain V of chloroplast large subunit [23S]-ribosomal gene) dominated juvenile *B. asbestinum* for up to 12 mo, other symbiont types within Clades A, B, C and D co-occurred with B184 at varying frequencies across years and sites. The occurrence of some symbiont types (B184 and B224) did not differ between habitats or years monitored, while other symbiont types (A194, B220 and C180) varied significantly in prevalence depending on year and site. The diversity of symbiont types initially acquired by young juveniles was not simply a subset of the symbiont types found in nearby host cnidarians, suggesting that the source of infecting symbionts was not solely local host populations. Experimental manipulations demonstrated that symbionts continued to enter the host for several months until a single symbiont type dominated within the juvenile octocorals after 3 mo. Although some symbiont types varied significantly across habitats, the pattern of infection did not reflect a given habitat. Instead, aside from *Symbiodinium* B184 and B224, initial symbiont acquisition appeared random in *B. asbestinum* recruits.

KEY WORDS: Symbiosis · Gorgonian · Coral · Ontogeny · Zooxanthellae · Octocoral

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INTRODUCTION

Many shallow tropical marine invertebrates form obligate mutualistic endosymbioses with symbiotic dinoflagellates belonging to the genus *Symbiodinium* (Dinophyta). *Symbiodinium* is a genetically diverse genus and has been divided into 9 phylogenetic clades (A–I) that include genetically and physiologically distinct 'types' or species (Coffroth & Santos 2005, LaJeunesse et al. 2010a, Pochon & Gates 2010). These symbiotic algae supply most of the host's nutritional requirements, and stress-induced reductions in symbiont densities have severe impacts on growth and survival of the host (reviewed in Baker 2003). The diverse array of physiologies found among *Symbiodinium* types mean they are differen-

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tially affected by environmental conditions, most importantly light and temperature (e.g. Rowan et al. 1997, Savage et al. 2002, Iglesias-Prieto et al. 2004, Karako-Lampert et al. 2005) and have the potential to significantly affect host physiology (Tchernov et al. 2004, Abrego et al. 2008, Frade et al. 2008). Recent research has focused on how environmental fluctuations may lead to different host–symbiont partnerships, and whether such alterations lead to resistance or resilience to stressful conditions (Berkelmans & van Oppen 2006, Jones et al. 2008, LaJeunesse et al. 2009, 2010b, Putnam et al. 2012).

The composition of symbiotic communities within juvenile hosts also has important implications for host survival and growth (Little et al. 2004, Watanabe et al. 2007, Mieog et al. 2009, Poland 2010). For example, although adult *Acropora millepora* and *A. tenuis* harbor *Symbiodinium* ITS2-type C1 (sensu LaJeunesse 2001), juveniles establish symbioses with both Clade C and D symbionts (Little et al. 2004). Juveniles with Clade D symbionts had slower growth rates than those with *Symbiodinium* C1 (Little et al. 2004), possibly due to greater photosynthate translocation from the latter (Cantin et al. 2009). However, some symbiont types within Clade D are purported to be stress tolerant and juvenile hosts harbouring these types may benefit from enhanced survivorship when exposed to various stresses such as elevated temperatures (Baker 2001, Berkelmans & van Oppen 2006, Mieog et al. 2009).

Given the importance of these symbionts to their host, it is surprising that only a minority of cnidarians transfer *Symbiodinium* directly to their offspring (reviewed in Baird et al. 2009). Instead, aposymbiotic juveniles of most cnidarians acquire their symbionts from the water column (Coffroth et al. 2006, Manning & Gates 2008, Pochon et al. 2010), macroalgal habitats (Porto et al. 2008, Venera-Ponton et al. 2010) and the benthos (Adams et al. 2009). These environmental pools of *Symbiodinium* may consist of algae with alternating symbiotic and 'non-symbiotic' life stages, or short-term populations of symbionts, newly seeded by symbiont expulsion from neighbouring hosts (Muller-Parker 1984) or corallivorous fish (Muller-Parker 1984, Porto et al. 2008, Castro-Sanguino & Sanchez 2012). Thornhill et al. (2006) showed that the symbionts infecting *Cassiopeia xamachana* scyphistomae did not correlate with the symbiotic populations in adjacent cnidarians. This suggests that there is a more diverse environmental reservoir that includes symbionts beyond those found locally in adult symbioses, e.g. potentially free-living. This is seen in the initial *Symbiodinium* infection of juvenile cnidarians, which is diverse and often includes many heterologous types (those not typical in conspecific adults) for up to 2.5 yr (Abrego et al. 2009), yet not all available (environmental) symbiont types infect a given host (Colley & Trench 1983, Coffroth et al. 2001, Weis et al. 2001).

As the symbiosis begins, it is unclear which partner initiates/controls the establishment but, as outlined above, the initial symbiont acquisition is promiscuous, although not entirely indiscriminate (Colley & Trench 1983, Coffroth et al. 2001, 2006, Weis et al. 2001). Acquisition will be determined in part by the symbionts present in the local environmental pool (i.e. sediments and water column) and the composition of this pool can be influenced by environmental conditions, symbionts released by the local adult populations and/or symbionts that have dispersed from other locations. Within this study we examined the dynamics of the initial infection of juvenile cnidarians to assess how symbiont communities within newly settled cnidarians varied across habitats and time and to increase our understanding of factors that affect the establishment of the symbiosis. We used aposymbiotic juveniles from the octocoral *Briareum asbestinum* to 'sample' symbionts from the environment (Coffroth et al. 2001, 2006) and to test the following hypotheses: (1) the establishment of cnidarian–*Symbiodinium* associations in juvenile octocorals is characteristic of a given habitat, possibly reflecting local environmental conditions and/or host–symbiont selection; (2) the establishment of cnidarian–*Symbiodinium* associations in juvenile octocorals represents the local availability of symbionts as seeded by the local adult host populations so that newly settled recruits initially harbour those symbionts that are found in the local symbiotic cnidarian population; and finally (3) the establishment of cnidarian–*Symbiodinium* associations in juvenile octocorals is a sequential process where there is a defined pattern of infection with one specific symbiont type followed by another. To further investigate the dynamics among symbiont types over time, we examined, experimentally, whether (1) symbiont uptake/infection stops once a specific symbiont is acquired and (2) there is symbiont-specific mortality/survivorship of host juveniles.

MATERIALS AND METHODS

Study organisms (host and symbionts)

As the symbiosis develops, it is unclear which partner initiates/controls the establishment. Does the

host 'take up' or 'acquire' the symbiont or does the symbiont 'infect' the host? In this study we use these terms without assigning any role to host or symbiont in the initial establishment of symbiosis. To investigate the dynamics of symbiont communities and their initial infection of juvenile cnidarians, we utilized newly settled juveniles of the encrusting form of the octocoral *Briareum asbestinum*. This species is common throughout the Caribbean and produces aposymbiotic offspring that are brooded on the surface of the maternal colony (Brazeau & Lasker 1990). Throughout its range, adult colonies of *B. asbestinum* associate primarily with the Clade B *Symbiodinium* taxon B178 but adult colonies may also harbor *Symbiodinium* B184 at some locations either alone or in combination with B178 (Santos et al. 2003, Lewis & Coffroth 2004, Hannes et al. 2009, Poland 2010) and symbiont taxa tend to be uniformly distributed throughout the adult *B. asbestinum* colonies (Hannes et al. 2009). Symbiotic algal identification is based on length variation in a hypervariable region of the chloroplast large subunit (cp23S)-rDNA domain V. Nomenclature used here (e.g. B178, B184) refers to the algal clade (i.e. B) and the fragment length of the aforementioned cp23S region (i.e. 178 or 184 bp fragment, sensu Santos et al. 2003). Symbiont types B178 and B184 are equivalent to types B19 (sensu LaJeunesse 2005) and B1 (sensu LaJeunesse 2002), respectively, as determined using internal transcribed spacer 2 (ITS-2).

Study sites

Newly settled polyps of *Briareum asbestinum* were placed at 3 environmentally distinct habitats in the middle Florida Keys: Florida Bay hard-bottom, Grassbed, and Outer Reef tract (Fig. 1). The Florida Bay site was a shallow (2 to 3 m deep) silt-covered hard-bottom habitat with sparse turtle grass *Thalassia testudinum.* The site was dominated by gorgonians (*B. asbestinum, Pseudopterogorgia americana*, *Pseudopterogorgia acerosa*, *Pterogorgia anceps* and *Pterogorgia guadalupensis*) and sediment-tolerant (Rice & Hunter 1992) scleractinians (*Porites divaricata, Manicina areolata*, *Solenastrea bournoni* and *Siderastrea siderea*) along with various anemone, zoanthid and sponge taxa (Table 1). The Grassbed site was located on the Atlantic Ocean side of Long Key, Florida, approximately 4 km from the Florida Bay site at a depth of approximately 5 m. This site was dominated by the sea grass *T. testudinum* with minimal coral cover; a preliminary survey within a

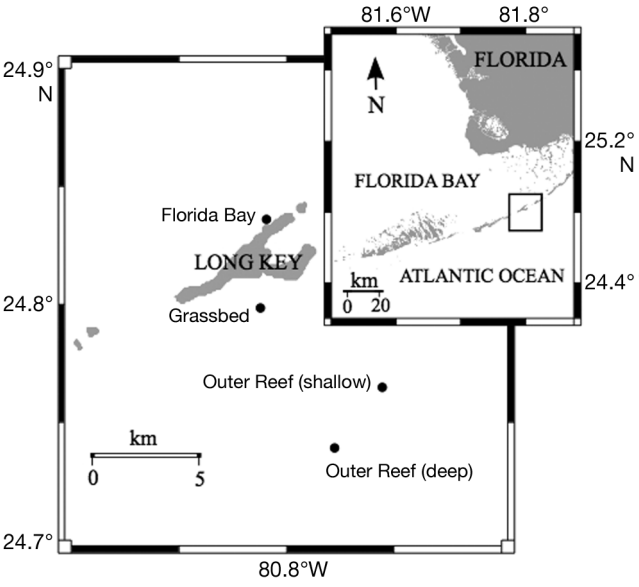

Fig. 1. Study sites (inset) in the middle Florida Keys, USA. Polyps were placed at the Florida Bay (4 m) and Grassbed (5 m) sites each year (1999–2002). At the Outer Reef tract site, polyps were placed at the shallow site in 1999 (5 m), suspended 5 m below the surface at the deep reef site (23 m) in 2000 and 2001 and placed 0.5 m above the deep reef site (23 m) in 2002

50 m radius of the transplanted juveniles encountered occasional small colonies of *Millepora alcicornis, P. divaricata, S. bournoni* and *S. siderea*, and a single colony of *B. asbestinum*. The Outer Reef tract site (5 to 23 m deep) was located above or on (respectively) an offshore reef tract (Tennessee Reef) with diverse scleractinian and octocoral fauna, including *B. asbestinum*, approximately 11 km from the Florida Bay site (Table 1). These different habitats, and associated variation in water turbidity, flow patterns, bottom substrates and cnidarian/fish fauna represent potentially different environmental pools of *Symbiodinium*.

Field *Symbiodinium* acquisition

Annual variation of symbiont infection/acquisition was measured over 4 consecutive years (1999 to 2002). During annual spawning (May to June) *Briareum asbestinum* larvae were collected directly from maternal colonies at the Florida Bay site, using 50 ml syringes. Adult *B. asbestinum* colonies at this site (as well as throughout the species range) predominantly harbor symbiont type B178. Larvae were transported to the Keys Marine Laboratory (Long Key, Florida) within 1 h of collection. Offspring from

Table 1. *Symbiodinium* types found in *Symbiodinium*-bearing hosts (adult cnidarians and sponges) encountered along 25 to 50 m belt transects centered on the juvenile rearing sites at Florida Bay, Grassbed and Outer Reef tract. **Boldface** denotes types that were also detected in juvenile *Briareum asbestinum*. The number of hosts sampled with that symbiont type is given in parentheses. Note that since some hosts harbored multiple symbiont types, the number of occurrences is often greater than the sample size. Several Clade B symbionts did not match any previously reported types and are listed as 'uncharacterized'

Site	Host genus	n	*Symbiodinium* types (no. of occurrences)
Florida Bay	Scleractinians		
	Manicina	5	**B184** (3), **B220** (1), **B170** (1), uncharacterized B (1), B190 (1)
	Porites	4	**B170** (4)
	Siderastrea	11	**B170** (10), **B220** (4), uncharacterized B (2)
	Solenastrea	9	Uncharacterized B (6), **B220** (3), B183 (2), **B170** (1)
	Octocorals		
	Briareum asbestinum	51	**B178** (49), **B184** (6)
	Pseudopterogorgia	12	**B184** (12)
	Pterogorgia	11	**B184** (11)
Grassbed	Scleractinians		
	Siderastrea	4	**B170** (3), **B184** (1)
	Porites	2	**B170** (2)
Outer Reef	Scleractinians		
	Porites	3	Uncharacterized B (3)
	Siderastrea	3	**B184** (2), **C180** (2)
	Stephanocoenia	4	**C180** (4), **B220** (1)
	Octocorals		
	Briareum asbestinum	7	**B178** (7)
	Eunicia	6	**B184** (4), uncharacterized B (3)
	Muricea	1	**B184** (1)
	Muriceopsis	2	**B184** (2)
	Plexaura	4	**B184** (4)
	Plexaurella	1	**B184** (1)
	Pseudoplexaura	4	**B184** (4)
	Pseudopterogorgia	5	**B184** (5)
	Hydrozoans		
	Millepora	6	**B224** (6)
	Sponges		
	Callyspongia	1	**D206** (1)
	Niphates	1	**D206** (1)
	Unknown spp.	1	**B184** (1)

all adults were combined and maintained in multiple 1 l containers with 0.2 µm filtered seawater (FSW) at ambient temperature (~28°C) under 40 W cool fluorescent lights (14 h:10 h light:dark photoperiod). FSW was changed daily and dead/dying larvae were removed continuously as needed. After 3 d the larvae were randomly divided into 3 replicate groups and transferred to 1 of the 3 study sites (Florida Bay, Grassbed or Outer Reef tract). At each site, larvae were placed in 2 l jugs (500–2000 larvae jug^{-1}) that contained approximately 10 dead and cleaned gorgonian branches (scrubbed in freshwater and dried in the sun for several days, hereafter referred to as branches) to provide larvae with a suitable substrate for settlement. There were between 6 and 15 jugs at each site. All jugs were suspended above the substrate with 2 windows cut out and covered with 250 µm nylon mesh. The mesh allowed water exchange and *Symbiodinium* acquisition by juveniles, but excluded predators.

At the Florida Bay and Grassbed sites, once the larvae had attached to the branches and started metamorphosis (3 to 7 d), the branches with newly settled juveniles were removed from the jugs, randomly attached to cinder blocks with monofilament line and floated 0.5 m above the substrate. Deployment techniques varied along the Outer Reef tract site. In 1999, *Briareum asbestinum* juveniles were settled onto plexi-glass plates and were floated 0.5 m above the substrate on a shallow (5 m) part of the Tennessee reef tract, while in 2000 to 2002 juveniles were settled on branches and maintained

within the jugs to avoid fish predation. In 2000 and 2001 these jugs were suspended at 5 m below the surface above the deeper (23 m) part of the reef tract and in 2002, the jugs containing juveniles were floated 0.5 to 1.0 m above the substrate at 23 m depth at this same site. Data from the outer reef tract provided a comparison of symbiont types infecting juvenile hosts in the same area over depth and time. Each cohort is referred to by the year of settlement (i.e. juveniles settled in June 1999 are referred to as 1999 offspring), although juveniles were sampled over a period of 3 to 12 mo, extending into the following year (Table 2A).

Controlled infection of *Briareum asbestinum* larvae with *Symbiodinium*

Previous studies of early ontogeny have shown that newly settled coral and octocoral juveniles are rapidly infected with a range of symbiont types that are subsequently winnowed to the single or few types that occur in the adult symbiosis (Coffroth et al. 2001, Weis et al. 2001, Little et al. 2004, Abrego et al. 2009). To investigate whether symbiont infection/acquisition occurs continuously during the early ontogeny of the juveniles and whether the type of symbiont that is initially acquired affects survivorship of juvenile hosts, larvae from *Briareum asbestinum* were collected from maternal colonies during spawning in 2001. Developing larvae were maintained in FSW as described above in 1-l containers (100 larvae per container, 15 containers per treatment) with dead and cleaned gorgonian branches (as described in previous subsection). After 7 d, the majority of the larvae had settled onto the branches and metamorphosed into polyps. At this time, branches with juvenile *B. asbestinum* were randomly divided into 3 treatments and inoculated with either (1) *Symbiodinium* A194 (A1 sensu LaJeunesse 2002) (500 cells ml^{-1}), (2) *Symbiodinium* B184 (B1 sensu LaJeunesse 2002) (500 cells ml^{-1}), or (3) no *Symbiodinium* (Control). The cultures were originally isolated from newly settled *B. asbestinum* polyps and had been maintained in our laboratory (BURR Culture Collection) for approximately 2 yr. These cultures were monoclonal, but not axenic. Water was changed in the containers every 3 d and then reinoculated with the appropriate symbiont type to ensure that all polyps became infected. Infection was first observed at 8 to 16 d after settlement and a subset of the juveniles (n = 15 to 19; Table 2B) was sampled to verify initial symbiont type. After 8 wk, branches with attached juveniles

Table 2. Number of *Briareum asbestinum* juveniles sampled (by removing individual polyps from settlement branch) for characterization of the symbiont type, using cp23S-rDNA in (A) the 1999–2002 field symbiont acquisition studies and (B) the controlled infection study performed in 2001. For (B), juveniles were maintained in the laboratory for ~8 wk before being placed in the field. –: juveniles were not sampled

(A) 1999–2002 field studies

Year	Site	Month collected											
		Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
1999	Florida Bay	17	69	63	61	–	30	13	–	–	–	–	–
	Outer Reef	29	14	23	–	–	–	–	–	–	–	–	–
2000	Florida Bay	40	36	–	28	25	30	10	17	–	13	–	42
	Grassbed	50	40	70	31	17	20	19	14	–	23	–	23
	Outer Reef	22	–	44	–	–	–	–	–	–	–	–	–
2001	Florida Bay	60	24	27	19	24	23	26	29	23	–	28	–
	Grassbed	26	24	24	32	31	30	16	22	–	23	–	–
	Outer Reef	22	18	24	21	–	–	–	–	–	–	–	–
2002	Florida Bay	38	28	–	–	44	–	–	58	–	–	15	–
	Grassbed	45	93	–	–	23	–	–	45	–	–	23	–
	Outer Reef	36	25	–	–	29	–	–	–	–	–	–	–

(B) 2001 control study

Treatment	Week collected							
	5	7	12	16	20	24	27	35
A194	18	19	17	11	16	14	14	26
B184	18	18	23	20	20	6	15	25
Control	19	15	9	12	14	16	–	17

were placed at the Florida Bay site where *B. asbestinum* is common. Juvenile survivorship was monitored by counting all colonies attached to the branches at 2 to 4 wk intervals, for 27 wk. Juveniles were subsampled at regular intervals for molecular characterization of symbionts (see 'Molecular identification', n = 6 to 25, mean = 16.6; Table 2B). These data were used to determine if uptake continues in the juveniles previously infected in the laboratory and whether initial symbiont type had an effect on survivorship of the offspring.

Surveys of symbionts within adjacent cnidarian hosts

To investigate the potential contribution of local, *Symbiodinium*-bearing hosts, to the environmental pool of *Symbiodinium*, tissue samples were taken from symbiotic anthozoans and sponges encountered along 25 to 50 m belt transects centered on the juvenile rearing sites at the deep Outer Reef tract (approximately 23 m), Florida Bay, and Grassbed sites (Fig. 1). Not all hosts recorded in earlier surveys (see 'Study sites') were encountered on these transects.

Molecular identification of *Symbiodinium* type

A scalpel or razor blade was used to remove individual polyps from the experimental branches or small sections (1 cm^2) of host tissue (respectively). Polyps were randomly sampled from the branches, but only one polyp per colony was sampled each time. Samples were preserved in 1.5 ml 95% ethanol or 20% salt-saturated dimethyl sulfoxide–ethylenediaminetetraacetic acid buffer (Seutin et al. 1991). Total genomic nucleic acids were extracted using a modification of the 2× hexadecyltrimethylammonium bromide (CTAB) extraction protocol (Coffroth et al. 1992). For characterization of the symbiont taxa, cp23S-rDNA was amplified using Santos et al. (2003)'s PCR protocol. Amplified DNA fragments were separated according to molecular weight on a 6.5% non-denaturing polyacrylamide gel (LI-COR 4200 NEN® Global IR2 DNA sequencing system; LI-COR Biosciences) and individual DNA fragments scored using size standards of a mixture of known symbiont types. This method allows detection of individual symbiont types present at 10 to 1000 cells among a mixture of other types, or a relative proportion of 1% of the sample (Santos et al. 2003, D. M. Poland unpubl. data).

Statistical analyses

Inter-annual and habitat variation in *Symbiodinium* acquisition/infection

Juveniles generally associated with multiple symbiont types simultaneously. To examine overall symbiont diversity over spatial and temporal scales, the number of juveniles harboring each symbiont type was recorded. Multiple symbiont types within a single juvenile were counted independently. Given that a single juvenile polyp harbored up to 6 different symbiont types (see 'Results'), the number of occurrences of symbionts was greater than the number of juveniles sampled per site or year. To test for variation in symbiont types between sites within a given year and between years at a given site, the frequency of a given symbiont type was compared using the χ^2 test of independence with Bonferroni corrections for significance thresholds. Between-polyp symbiont acquisition was highly variable during early ontogeny (the first 3 mo; see 'Results') resulting in many empty cells when the data were analyzed month by month. To achieve sample sizes needed for appropriate statistical analyses, data for the first 3 mo were pooled within a site+year. The low frequencies of some symbiont types (i.e. found in 0 to 2 juveniles per 3 mo sampling period) precluded statistical analysis of these data.

Controlled infection experiments

Per capita mortality rates of experimentally infected juveniles were calculated by dividing the number of polyps on a branch in one time period by counts at the previous time period. These values were ln-transformed and analyzed using repeated-measures ANOVA with time as the within-subjects factor and treatment (A194, B184 or Control) as the between-subjects factor using SPSS v.12 (SPSS). The use of treatment does not reflect the precise symbiont composition within juveniles of each treatment, as multiple symbionts were detected once the treatments were placed in the field, but represents the category of symbionts that were initially offered. Thus, the use of treatments as the between-subjects factor is in accordance with the question of what effects initial uptake/exposure had on survival.

RESULTS

Field *Symbiodinium* acquisition

Field-reared larvae settled upon the dead gorgonian skeletal branches in 3 to 7 d and were all visibly infected within 2 wk of being placed in the field. *Symbiodinium* B184 was found in 69 to 100% of juveniles during the first 3 mo (June to August; average approximately 96% across all populations and years; Fig. 2, Table S1 in the supplement at www.int-res.com/articles/suppl/m476p023_supp.pdf). In addition, at least 10 other symbiont types were found in the newly settled recruits (ITS-types given in parentheses when known): Clade A symbionts A188 (A2), A194 (A1) and A198 (A3), Clade B symbionts B178 (B19, homologous to parental population at Florida Bay), B170, B211, B220 and B224 (B2), Clade C symbiont C180 and Clade D symbiont type D206 (D1) (Fig. 2, Table S1). Most often these symbionts co-occurred with B184 but after 3 mo the diversity of symbiont types decreased, leaving B184 as the most frequent symbiont inhabiting the juveniles.

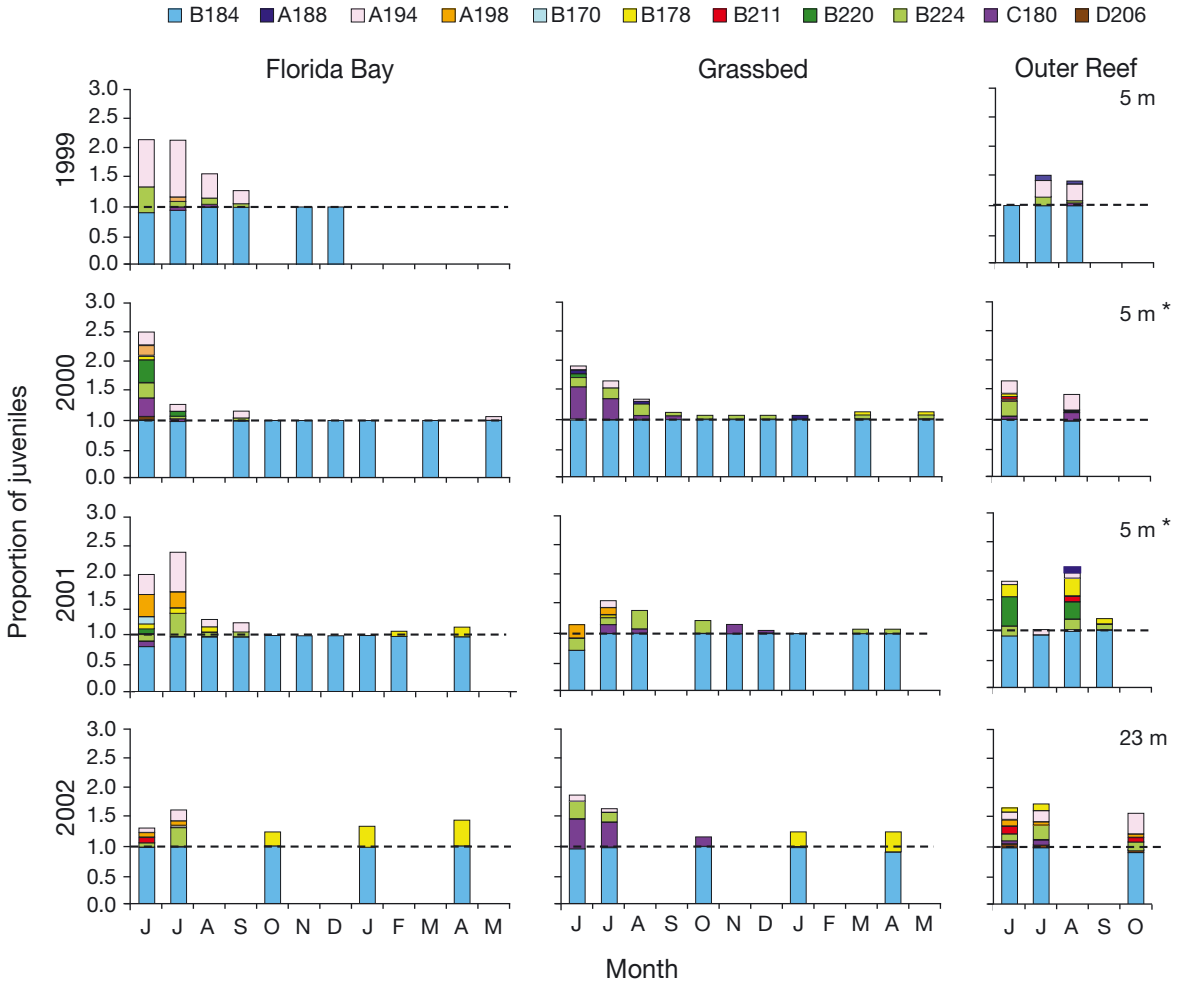

Fig. 2. *Symbiodinium* spp. Proportion of *Briareum asbestinum* juveniles at the different sites (columns) with a given *Symbiodinium* type over time (months) during the different years (rows). Each cohort was monitored starting in June (J) and the last samples were taken in May (M) of the following year for some cohorts at the Florida Bay and Grassbed sites. Since symbiont types often co-occur in juvenile *B. asbestinum,* the number of symbiont types often exceeds the number of juveniles sampled, resulting in total values in excess of 1.0 (100%). See 'Materials and methods' for description of the site depth variability at Outer Reef between years. Panels for the Outer Reef tract are noted with the treatment depth (m); asterisk (*) indicates treatments that were suspended at a depth of 5 m below the surface/18 m above the underlying reef (see 'Materials and methods: Field *Symbiodinium* acquisition'). For specific symbiont occurrences data refer to Table S1 in the supplement at www.int-res.com/articles/suppl/m476p023_supp.pdf

Variation among *Symbiodinium* within Clade B

Statistical testing of differences in prevalence of B178 and B184 symbionts was hampered due to their rarity and dominance, respectively, during the early ontogenetic stage (up to 3 mo). However, the frequency of B178 within juveniles varied significantly depending on habitat during June to August 2001, where it was found in a higher proportion of recruits at the Florida Bay and Outer Reef tract sites than at Grassbed (χ^2 = 13.2, df = 2, p < 0.01; Fig. 2). The occurrence of *Symbiodinium* B224 did not differ among sites within a year (p > 0.0125 for all years, Bonferroni-corrected α; Table 3A) or between years at a given site (p > 0.017, Bonferroni-corrected α; Table 3B). *Symbiodinium* B220, on the other hand, was significantly more abundant in juveniles raised at Florida Bay in 2000 (p < 0.001; Table 2A), and at the Outer Reef tract in 2001 (p < 0.001). The numbers of juveniles with this symbiont (B220) were too low in the other years for statistical comparisons. *Symbiodinium* B170 was only detected in approximately 8% of juveniles at the Florida Bay site in 2001 (Fig. 2). *Symbiodinium* B211 was never detected in juveniles at the Grassbed site (total n = 764) but was present at the Florida Bay and Outer Reef tract sites in 2000–2002 (Fig. 2, Table 3) and was absent from all juveniles sampled from all sites in 1999.

Table 3. Chi-square test of independence of symbiont types in *Briareum asbestinum* juveniles in the first 3 mo (June to August). (A) Between-site differences among symbiont types, and (B) between-year differences at each of the sites where juveniles were reared. Corrected p: threshold p-value determined by Bonferroni corrections on α = 0.05, based on number of comparisons made. Cases where the observed counts were different than expected are given. ns: no significant differences. na: insufficient sample sizes for statistical comparisons. FL Bay: Florida Bay site (natal site); Reef: Outer Reef tract site. Total numbers of juveniles analyzed are listed in Table 2A

Symbiont type	Year/ site	Corrected p	Significance	Obs ≠ Exp
(A) Between sites				
B224	1999	0.0125	ns	
	2000	0.0125	ns	
	2001	0.0125	ns	
	2002	0.0125	ns	
A194	1999	0.0125	<0.001	FL Bay, Reef
	2000	0.0125	<0.001	FL Bay
	2001	0.0125	<0.001	FL Bay, Reef
	2002	0.0125	ns	
A198	1999		na	
	2000		na	
	2001	0.05	<0.05	FL Bay
	2002		na	
C180	1999		na	
	2000	0.025	ns	p = 0.028
	2001		na	
	2002	0.025	<0.001	Grassbed
B220	1999		na	
	2000	0.025	<0.001	FL Bay
	2001	0.025	<0.001	Reef
	2002		na	
(B) Between years				
A194	FL Bay	0.017	<0.001	2001
	Grassbed	0.017	ns	
	Reef	0.017	ns	
B224	FL Bay	0.017	ns	
	Grassbed	0.017	ns	
	Reef	0.017	ns	

Variation among *Symbiodinium* within Clade A

Several symbionts within Clade A (A188, A194 and A198; Fig. 2) were also detected in juvenile *Briareum asbestinum*. The frequency of *Symbiodinium* A198 varied and was generally too low to compare between years. However, significantly more Florida Bay juveniles harbored this symbiont than those at the Grassbed site in 2001 (p < 0.05, within-year comparisons; Table 3A), while 2002 was the only year this symbiont type was observed at the Outer Reef tract site, appearing in 7.5% of the juveniles in the first 3 mo (Fig. 2). *Symbiodinium* A194 often infected juvenile *B. asbestinum* but its frequency varied significantly between sites for each year 1999–2001 (p < 0.017, Bonferroni-corrected α) but not in 2002 (p = 0.029, Bonferroni-corrected α = 0.017). The frequency of juveniles with A194 symbionts for the first 3 mo at Florida Bay also varied significantly between years, with the highest occurrence in 1999 (χ^2 = 80.7, df = 3, p < 0.017; Table 3B).

Symbiodinium A188 was not found in any juveniles sampled at the Florida Bay site (total n = 960) and only appeared in juveniles at the Grassbed site after the first 5 mo (January 2000, n = 2; and November 2001, n = 3). At the Outer Reef tract, *Symbiodinium* A188 appeared in 1 and 2

samples, out of 23 and 24 juveniles sampled during 1999 and 2001, respectively (Fig. 2).

Variation among other symbiont types

Among non-Clade A or B symbionts, *Symbiodinium* C180 was found most frequently in juveniles at the Grassbed site (Fig. 2). *Symbiodinium* D206 was detected at low levels in juveniles at the Outer Reef tract site during 2002 (3%) and found in Florida Bay and Grassbed juveniles in 2000 (~8 and 2%, respectively; Fig. 2).

Symbiont richness

While total symbiont richness (the number of symbiont types encountered) at any site/year ranged between 5 and 9 types, 73 to 97% of juveniles harbored only 1 or 2 types of symbionts simultaneously, depending on site or year (Fig. 3). Rarely (≤ 0.2%) did juveniles harbor 5 or 6 symbiont types simultaneously, even when the symbiont richness at a particular site and/or year was high (Fig. 3). For example, in 2000 at the Florida Bay site, 9 symbiont types were found among all the juveniles sampled, but only 2 of these juveniles (2.7%) harbored 5 or more symbiont types simultaneously (Fig. 3).

Surveys of symbiont composition within juveniles continued for up to 1 yr at the Grassbed and Florida Bay sites (Fig. 3). Following the initial 3 mo, overall symbiont type richness decreased and all juveniles harbored *Symbiodinium* B184 either alone or together with other symbiont types. The occurrence of B178, the dominant symbiont in the parental population in Florida Bay and throughout the Florida Keys (Poland 2010), was variable in new recruits and generally appeared later in ontogeny (e.g. at 7 to 12 mo; Fig. 2). This symbiont type was generally observed in 5% or less of the colonies sampled during the first 6 mo. Exceptions include the Outer Reef tract and Florida Bay sites in 2001 where B178 was found in 4 to 23% of the samples (Fig. 2) and the Grassbed and Florida Bay sites in 2002 where 14 to 46% of juveniles sampled harbored B178 after 5 or 8 mo (October and January, respectively; Fig. 2). However, this symbiont almost always occurred in mixed communities (generally with B184) and rarely alone (n = 2, Florida Bay, 2002; n = 1, Outer Reef tract, 2002).

Controlled infection of *Briareum asbestinum* larvae with *Symbiodinium*

Analyses of *Briareum asbestinum* juveniles during the laboratory infection period detected A194 in 67% of the juveniles that were exposed to A194 at Week 5 and 47% at Week 7; *Symbiodinium* B184 was detected in 89% (Week 5) and 84% (Week 7) of the juveniles that had been exposed to B184 symbionts (Fig. 4). In the control treatment no *Symbiodinium* cells were offered to the juveniles, yet 4 of the 34 juveniles that were screened during the laboratory stage harbored Clade B symbionts (Fig. 4). In addition, of the polyps sampled from the A194 treatment, 6 juveniles harbored symbionts other than A194 (5 polyps contained B184 and 1 polyp contained A198). One of the polyps sampled from the B184 treatment harbored A194 (Fig. 4). This is likely attributed to cross-infection from other experiments, despite the use of dedicated equipment (rinsed in freshwater between uses) for each strain, or transferred in with the initial collections. Although finding cross-infection presents a challenge, it does not prevent us from addressing our question: Does symbiont

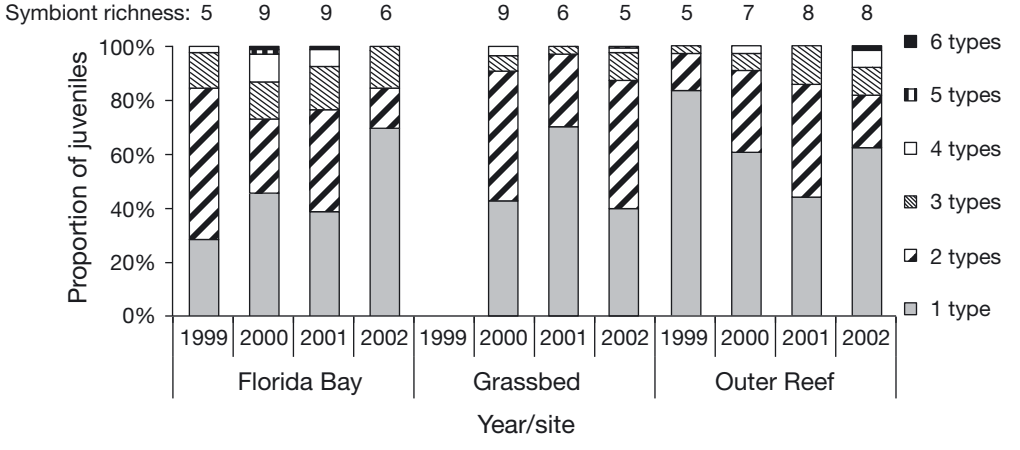

Fig. 3. *Symbiodinium* spp. Proportion of *Briareum asbestinum* juveniles with single (1 type) or multiple (2 to 6 types) simultaneous *Symbiodinium* populations. Symbiont richness indicates total number of symbiont types within *B. asbestinum* juveniles for each site during this study. Total numbers of juveniles analyzed are listed in Table 2A

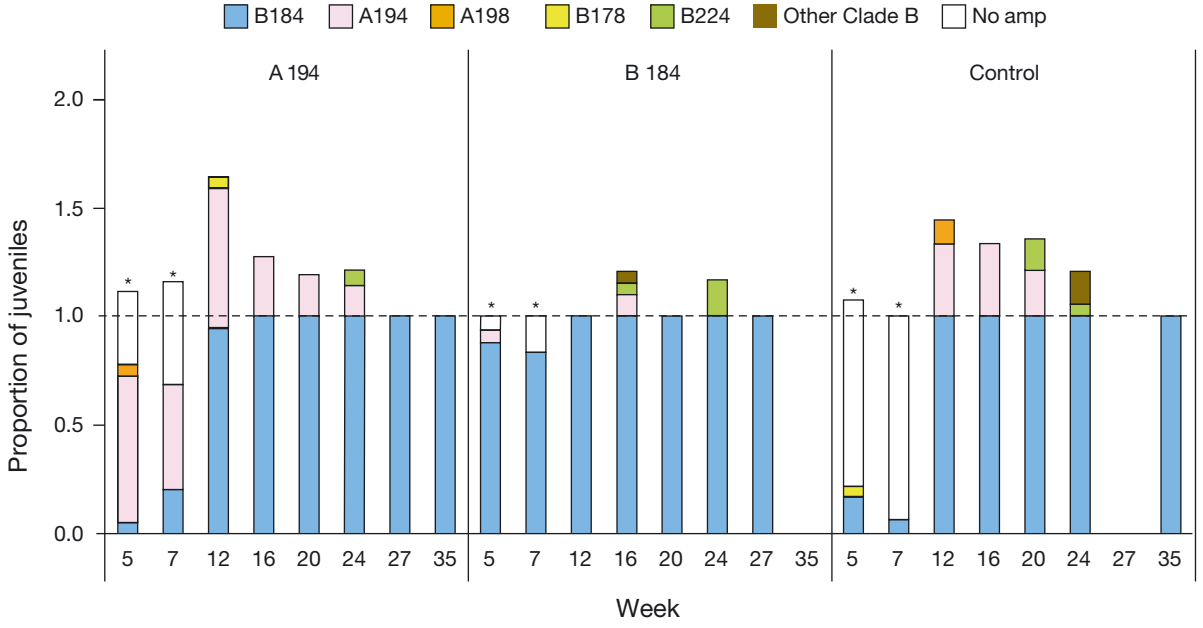

Fig. 4. *Symbiodinium* spp. Proportion of *Briareum asbestinum* juveniles with a given *Symbiodinium* type in the controlled infection experiments. Since different types of symbionts often co-occur in juvenile *B. asbestinum,* the number of symbiont types often exceeds the number of juveniles, resulting in total proportions in excess of 1.0 (100%). Asterisk (*) indicates the laboratory infection period after which juveniles were relocated to the field. *Symbiodinium* types in the 'other Clade B' category refer to symbionts where cladal identity is confirmed (Clade B), but cp23S-type was not established. In several cases, no *Symbiodinium* types were detected and are shown as 'No amp'

uptake/infection stop once a specific symbiont is acquired? After approximately 1 mo in the field, at Week 12, 94 to 100% of all juveniles sampled harbored B184 *Symbiodinium*, regardless of the initial treatments or cross-infection observed (Fig. 4). Of the juveniles sampled, all that were initially offered *Symbiodinium* A194 symbionts had acquired additional symbionts (98.9% with B184, 1.1% with B178) and by Week 16 (8 wk in the field) all polyps from the A-treatment had acquired B184 (Fig. 4). Juveniles initially offered *Symbiodinium* A194 symbionts maintained A194 symbionts for up to 24 wk post-inoculation (14%; Fig. 4). Among the sampled polyps from the B184 treatment, only 3 polyps (6.9%) harbored symbiont types other than B184 (A194, B224 and B170) (Fig. 4) after 8 wk in the field (Week 16). Overall, those polyps initially infected with B184 were significantly less likely to acquire other symbionts (e.g. types other than B184) after placement in the field compared to those polyps initially inoculated with A194, which instead readily acquired *Symbiodinium* B184 (χ^2 = 1769.8, df = 1, p < 0.001).

Over the entire 27 wk study period there were no significant differences in survivorship between juveniles that were initially infected with *Symbiodinium* types A194 or B184 or those in the control treatment ($F_{2,6}$ = 0.32, p > 0.05) (Fig. 5). Survivorship among juveniles that did not receive symbionts in the laboratory phase (control) appeared lower initially (August to October; Fig. 5) but was not significantly different from juveniles that were offered symbionts ($F_{2.33,40}$ = 2.59, p = 0.09; Fig. 5).

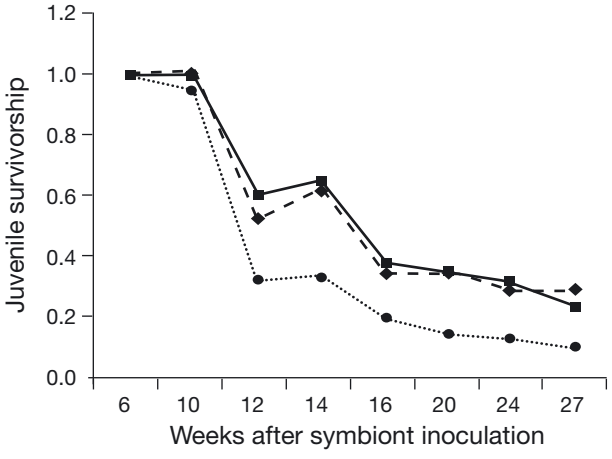

Fig. 5. *Briareum asbestinum*. Survivorship of juvenile *B. asbestinum* placed in the field after approximately 1 mo inoculation with *Symbiodinium* type A194 (dashed line) or B184 (solid line) *Symbiodinium*, and control (no *Symbiodinium* added, dotted line)

Symbiodinium types within adjacent cnidarians

An analysis of samples taken from other symbiont-harboring cnidarians (and potential sources of *Symbiodinium*) along belt transects at each site identified 2 (Grassbed), 6 (Florida Bay) and 8 (Outer Reef tract) different symbiont types at the resolution level of the chloroplast 23S rDNA marker (Table 1). Of the symbionts found in juvenile *Briareum asbestinum*, A188, A198 and B211 symbionts were not found in any of the cnidarian or sponge hosts sampled within the detection threshold of the chloroplast 23S rDNA marker. The reef tract was the only site where hosts harbored *Symbiodinium* B224 (in *Millepora* sp. and *Montastraea* sp.), *Symbiodinium* C180 (in *Agaricia* sp., *Diploria* sp., *Montastraea* sp., *Mycetophyllia* sp., *Siderastrea* sp. and *Stephancoenia* sp.) and *Symbiodinium* D206 (in *Diploria* sp., *Montastraea* sp., *Mycetophyllia* sp. and 2 sponges). Many of the symbiont types detected in juvenile *B. asbestinum* placed at the Florida Bay and Grassbed sites (i.e. the Clade A symbionts A188 and A198 Clade B symbiont B224, Clade C symbiont C180 and Clade D symbiont D206) were not found in local hosts (Table 1).

DISCUSSION

The present study describes the infection dynamics of symbionts that are acquired by juvenile octocorals over temporal and spatial scales. Initial infection of juvenile *Briareum asbestinum* by *Symbiodinium* was dynamic with juveniles acquiring an assortment of symbiont types, similar to findings in other juvenile cnidarians (Kinzie 1974, Colley & Trench 1983, Coffroth et al. 2001, Little et al. 2004, Abrego et al. 2009). The abundance and distribution of most symbiont types that initially infected juveniles varied significantly over time and space but did not follow a consistent pattern or sequence across sites and years. In contrast, the occurrence of symbiont types B184 and B224 in juvenile *B. asbestinum* did not change across site, depth, or years. Moreover, *Symbiodinium* B184 dominated the symbiont community and appeared in an average of 96% of *B. asbestinum* juveniles sampled during the first 3 mo (Fig. 2). These findings suggest that the sequence in which symbionts enter *B. asbestinum* juveniles is not important in determining the final outcome, namely the dominance of *Symbiodinium* B184 in the 3 to 6 mo old recruits.

Symbiont sources: local environmental pools

Although *Symbiodinium* B184 quickly dominated in juveniles, followed by a change to *Symbiodinium* B178 in the adult host, the fact that juvenile *Briareum asbestinum* are able to acquire a range of symbionts raises the possibility that other symbionts might carry an advantage under different environmental conditions. It is thus worthwhile to consider the source of this diversity. *B. asbestinum* recruits acquire symbionts from the environment so that the diversity observed in the early stages could reflect the diversity in local environmental pools. Although we did not directly sample environmental *Symbiodinium*, the diversity within the newly settled polyps may provide a conservative picture of the temporal and spatial variation in the environmental pool of symbionts. Symbiont richness varied across sites with a given site having up to 9 different types distributed among the sampled polyps. Over the course of this study, 11 different symbiont types belonging to the 4 common *Symbiodinium* Clades A to D were found associated with *B. asbestinum* recruits either singly or co-occurring in multiple simultaneous assemblages with the dominant *Symbiodinium* B184 (Fig. 2). The variation in initial acquisition seen across sites and years (Table 3) may be attributed to stochastic symbiont acquisition with diversity in local pools controlled by one or more of the following: (1) changing environmental conditions such as temperature and/or light that could favor particular symbiont types, (2) variation in symbionts in local host populations which 'seed' the pool, or (3) variation of symbionts due to variation in dispersal/immigration (which again may reflect changing environmental conditions such as currents).

Although the variation in symbiont types that infect *Briareum asbestinum* recruits may reflect local environmental conditions, this study did not find a relationship between habitat and symbiont type. For example, at the Outer Reef tract (Tennessee Reef) polyps were placed at 3 different depths over the course of the study (at 5 m depth in 1999, 5 m below the surface [18 m above the reef] in 2000 and 2001 and on the reef at 23 m in 2002). If a given site, environment, or year affect the symbiont type that a juvenile host initially acquires, one would predict differences in symbiont type between depths and/or years due to the variation in experimental design (differences in environmental conditions associated with the different depths at which the juveniles developed and/or years). However, regardless of depth or year, the occurrence of specific symbiont types did not

differ. B184 dominated the symbiosis during all years, and the occurrence of B224 and A194 did not vary significantly between years (or depths) at the Outer Reef tract site (Table 2B). In contrast, the occurrence of *Symbiodinium* A194 varied annually in Florida Bay, where juveniles developed at the same depth each year. While depth (i.e. light) does not seem to be a consistent controlling factor in initial symbiont uptake/infection of these particular symbiont types, it is possible that the distribution of other symbiont types is affected by light conditions as found in some adult corals (Rowan & Knowlton 1995, Diekmann et al. 2003, Ulstrup & van Oppen 2003). Although we found no evidence that initial infection reflected a given environment, we did not measure environmental parameters and cannot dismiss the possibility that initial symbiont infection may reflect some local environmental conditions (e.g. alkalinity, ultraviolet radiation), affecting free-living symbiont abundances and/or the infection/acquisition process itself for some symbiont types.

Local source host populations that 'seed' environmental pools could also affect symbiont availability. Although it is possible that some symbionts came from local adult hosts, the majority of symbiont types initially infecting juveniles were not found in local symbiotic host taxa, challenging the idea that the expulsion of symbionts by local hosts is a major source for environmental pools of symbionts. Lack of correspondence with symbionts within local cnidarian hosts was also observed in symbiont infection studies of the scyphistomae of the upside-down jellyfish *Cassiopea xamachama* (Thornhill et al. 2006). These data suggest that many of the symbionts infecting juvenile *B. asbestinum* were either free-living in the water column and/or the benthos, or were found in low (undetectable by cp23S-rDNA screening) frequencies in local host taxa. This raises the possibility that some *Symbiodinium* may be transient within the host and have alternate niches that differ from the typical cnidarian–algal symbiosis. Finally, dispersal abilities (or conditions that affect dispersal) may contribute to the variable abundances of the different symbiont types at a given site, leading to variation in the source pool.

Symbiodinium community dynamics during host development

As opposed to other symbiont types detected in juvenile *Briareum asbestinum*, the occurrence of *Symbiodinium* B184 and B224 did not vary across site, depth, or years. These symbiont types may (1) be more resilient to annual, or site-related, environmental variations, (2) be locally more abundant (3) have higher infection rates, (4) represent generalist or opportunistic types (LaJeunesse 2002, LaJeunesse et al. 2009), and/or (5) be preferred by *B. asbestinum* juveniles. *Symbiodinium* B184, in particular, occurred in the majority of the polyps during the first 3 mo and was the dominant sole symbiont type by 6 mo. This could indicate that this species is opportunistic and able to rapidly infect newly settled corals. For example, LaJeunesse et al. (2009) observed that *Symbiodinium* B1 (equivalent to B184; Coffroth & Santos 2005) colonized *Pocillopora damicornis* after a bleaching event, but was eventually replaced by the symbiont that *P. damicornis* had harbored prior to the bleaching event.

While the benefits of harbouring one symbiont type vs. another were not examined in this study, data from the laboratory infection study clearly show that the early ontogeny is a dynamic period where uptake/infection continues for many months. The outcome of this experiment demonstrates that survivorship of the individual polyps did not vary significantly regardless of whether the polyps were initially infected with *Symbiodinium* A194 or B184 (Fig. 5). The similar survival of juveniles from the different treatments may indicate that symbiont types A194 and B184 both meet the host's needs during the time frame they were followed. Alternatively, and more likely, the similar survivorship may be attributed to the fact that all juveniles quickly acquired *Symbiodinium* B184, regardless of what they initially harbored (Fig. 4). Once transferred to the field, those juveniles that initially harbored *Symbiodinium* A194 did not die, but continuously acquired additional symbiont types (almost exclusively B184 symbionts). This demonstrates that previously acquired symbionts do not necessarily prevent further uptake. In contrast, in those polyps that were initially infected with *Symbiodinium* B184, uptake of (or infection by) other symbiont types appeared to be limited, or retarded, at the level of detection of the cp23S rDNA marker. However, other symbionts belonging to Clade A and B entered a few 12 to 16 wk old juveniles even if these hosts were originally dominated by B184 symbionts (Fig. 4), suggesting that the incorporation of the potentially beneficial or opportunistic *Symbiodinium* B184 does not preclude others from, at least temporarily, entering into an association.

Diverse and sequential symbioses appear to increase fitness of hosts in ant–plant symbioses, even if not all symbionts are by themselves beneficial

(Palmer et al. 2010). In cnidarians, these questions require further testing but the early and rapid dominance of *Symbiodinium* B184 in early *Briareum asbestinum* ontogeny suggests a benefit to the juvenile host not discerned here, such as satisfying different metabolic needs. *Symbiodinium* type B224 also occurred in a predictable fashion in early ontogeny, but failed to establish a long-term symbiosis in field-settled juveniles (Fig. 4). Acquisition of other, co-dominant symbiont types appeared random which suggests that the other symbiont types (e.g. A194, A198, C180, D206) may be non-essential incidental occurrences or become established as background symbionts that fall outside the detection limit of our molecular screening methods.

Together, the findings presented here show that *Symbiodinium* type B184 becomes the dominant symbiont early in ontogeny regardless of year or habitat, which suggests that the formation of juvenile symbioses in *Briareum asbestinum* is not strongly influenced by environmental factors. However, since up to 9 other symbiont types were found in the juvenile hosts, albeit occurring randomly between habitats and years, it appears that the juvenile host may not actively control initial acquisition. The mechanism by which *Symbiodinium* B184 then becomes dominant in juvenile *B. asbestinum* is unknown; is the *in hospite* symbiont sorting during ontogeny due to higher symbiont growth rates, competitive interactions between symbiont types, host control or a function of the host's metabolic needs? These are not mutually exclusive processes and should be considered in future field or laboratory studies focusing on the early ontogeny of cnidarian symbioses. Our experimental design does not distinguish between these alternatives, but does establish for the first time that the initial infection/acquisition does not follow a set 'turnover' sequence.

Although not addressed in this study, future research should also be directed towards understanding the subsequent change from B184 in the juveniles to the dominance of B178 in the adult host. A change in the dominant symbiont type over ontogeny occurs in other cnidarians (Weis et al. 2001, Little et al. 2004, Abrego et al. 2009) and the physiology of juvenile corals can vary with symbiont composition, suggesting different metabolic needs over the course of ontogeny (Little et al. 2004, Watanabe et al. 2007, Cantin et al. 2009, Mieog et al. 2009). Yet, the mechanisms of this sorting to the final symbiont type have not been clearly identified and are a topic for continued research (e.g. Yuyama et al. 2005, Weis et al. 2008, Davy et al. 2012).

Finally, the establishment of the symbiosis and host–symbiont pairing is suggested to be genetically controlled (Coffroth et al. 2001, Weis et al. 2001, Rodriguez-Lanetty et al. 2006, Voolstra et al. 2009). This also appears to be the case for *Briareum asbestinum*; the apparent selectivity for a single symbiont type in juvenile *B. asbestinum* leaves the ability of this host to respond to the current threats of climate change, by switching or shuffling symbionts, in question. Continued explorations of the early establishment of these ecologically important symbioses remains important because they will help to identify the possible processes that may, or may not, mitigate environmental changes associated with anthropogenic activities.

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