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Development and survivorship of zooxanthellate and azooxanthellate primary polyps of the soft coral *Heteroxenia fuscescens*: laboratory and field comparisons

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Abstract The zooxanthellate Red Sea soft coral *Heteroxenia fuscescens* releases aposymbiotic planulae nearly all year round, with higher numbers in the summer than in other seasons. After metamorphosis, primary polyps become infected with symbiotic dinoflagellates (zooxanthellae), derived from the ambient seawater. This study compares aspects of development and survivorship of metamorphic stages of *H. fuscescens* in relation to onset of infection by the algal symbionts in the laboratory and the field. We revealed no distinct differences in the timing and sequence of morphogenetic events during metamorphosis between zooxanthellate and azooxanthellate primary polyps in the laboratory. In the field, the polyps exhibited higher developmental synchronization during the first days after metamorphosis compared to the laboratory-reared ones, probably as result of a rapid and synchronized onset of metamorphosis in the former. Later, there were no distinct differences in these parameters between the laboratory-reared zooxanthellate polyps and those in the field. Although the symbiotic state did not appear to affect developmental sequence or timing, it did increase host survivorship. In the laboratory, the survivorship of zooxanthellate primary polyps was significantly higher compared to azooxanthellate ones. Therefore, the symbiotic state appears to be ultimately important in overall survivorship. The survivorship of zooxanthellate primary polyps developed from planulae released during the summer months was higher compared to those from other seasons, thus strengthening existing evidence that the summer is the most favorable season for the breeding and development of *H. fuscescens* sexual progeny. Comparisons between laboratory-reared zooxanthellate primary polyps and naturally settled animals in the field showed a higher survivorship in the laboratory, possibly due to unfavorable conditions commonly occurring in the field. Our findings indicate that *H. fuscescens* represents an appropriate model system for study of acquisition of algae by aposymbiotic offspring and the impact of the process on their development and survivorship.

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Introduction

Various studies have addressed the question of the developmental stage at which sexually produced offspring of symbiotic hosts acquire their dinoflagellate algal symbionts (zooxanthellae) (e.g. Muscatine 1974; Fitt 1984; Trench 1987; Douglas 1998). Trench (1987) suggested two modes of symbiont acquisition by sexual progeny: direct transmission via the eggs or brooded larvae (also known as vertical or maternal inheritance) or by post-larval stages from the ambient environment (also known also as the horizontal or open system). Direct transmission of symbionts has been documented in cnidarian species: classes Hydrozoa (Campbell 1990), Scyphozoa (Montgomery and Kremer 1995) and Anthozoa (Glynn et al. 1991; Benayahu et al. 1992; Hirose et al. 2000). Acquisition from the ambient environment is far more common than maternal inheritance, and occurs in stony corals (Harrison and Wallace 1990; Shlesinger and Loya 1991; Schwarz et al. 1999) and soft corals (Kinzie 1974; Benayahu et al. 1989a; Achituv et al. 1992; Benayahu 1997; Coffroth et al. 2001). Eggs broadcast by the vast majority of species of both coral groups lack algal symbionts upon release (azooxanthellate), whereas brooded planulae are mostly symbiotic (zooxanthellate) (Babcock and Heyward 1986; Harrison and Wallace 1990; Benayahu et al. 1992; Benayahu and

Schleyer 1998). Consequently, horizontal acquisition of symbionts is far more common than vertical acquisition.

Open and closed symbioses offer different evolutionary scenarios in regard to their influence on zooxanthella diversity (Rowan 1998). In contrast to closed symbioses, an open system should facilitate polymorphic symbioses by providing members of a host species with many opportunities to obtain different symbionts (Rowan and Knowlton 1995; Douglas 1998). Recently, it has been shown that initial zooxanthella acquisition by newly settled polyps of gorgonians was non-selective and did not reflect the adult host specificity, which appeared over time (Coffroth et al. 2001). Experimental manipulations of cnidarian sexual offspring in the field and the laboratory are still needed in order to answer open questions concerning the significance of the mode of symbiont acquisition in the processes in which the coral host and its algal partners are engaged. In choosing a system to investigate these aspects, the best strategy is undoubtedly to study an association with an open system (sensu Trench 1987), in which both azooxanthellate and zooxanthellate early ontogenetic stages exist. Therefore, experiments to date on the dynamics of initiation of algal-cnidarian symbioses have been largely confined to associations displaying an open system of symbiont transmission (see Montgomery and Kremer 1995; Schwarz et al. 1999; Weis et al. 2001).

The intriguing questions related to onset of infection of early developmental stages of a cnidarian host by zooxanthellae led us to launch a comprehensive study of the cascade of events that occur during this process. For this purpose, we chose the early developmental stages of the zooxanthellate Red Sea soft coral *Heteroxenia fuscescens*. The main nutritional source of this species is from uptake of dissolved organic material (DOM) and utilization of its symbiotic algal photosynthesis products (Schlichter 1982). *H. fuscescens* is a hermaphroditic soft coral that broods planulae (Benayahu et al. 1989a; Benayahu 1991). These planulae lack zooxanthellae, unlike brooded planulae of other soft corals, which already harbor symbiotic algae upon release (Benayahu et al. 1989b, 1992; Benayahu and Schleyer 1998). The release of *H. fuscescens* planulae can be observed nearly all year round, with highest rates occurring in summer (Ben-David-Zaslow et al. 1999). In the field, these planulae settled 5–8 h after release and then underwent metamorphosis into primary polyps (Benayahu et al. 1989b). The onset of infection by zooxanthellae is associated with the development of a mouth opening, and occurs in polyps as young as 3 days old. In the present study, we examined several aspects of development in metamorphic stages of *H. fuscescens*: (1) the sequence of morphogenetic events in zooxanthellate and azooxanthellate primary polyps reared in the laboratory and zooxanthellate primary polyps in the field, (2) the effect of seasonality on polyp survivorship, and (3) survivorship of laboratory-reared and field primary polyps. In addition, monitoring the morphogenetic events of infected and uninfected primary polyps allowed us to determine whether algal acquisition is required for normal early development of the juvenile host. Examination of these processes provides a point of departure for study of the entire complex process of symbiosis onset.

Materials and methods

Collection of planulae

Mature colonies of *Heteroxenia fuscescens* (>36 mm in diameter, see Achituv and Benayahu 1990) were sampled randomly from the coral reef (3–8 m deep) across from the Interuniversity Institute of Eilat (IUI) over a 22-month period (November 1998–August 2000). In the laboratory, these colonies were placed in containers with running seawater, at a flow rate of 2 l min^{-1}. Prior to sunset, each colony was transferred to a separate aerated aquarium, and examined the following morning for the presence of planulae (see also Ben-David-Zaslow and Benayahu 1996). For each colony, released planulae were counted and placed into 250-ml PVC containers filled with Millipore-filtered (0.22 μm) seawater (FSW), to avoid bacterial contamination. Planulation was monitored for three to five successive nights using 10–15 colonies per sampling month. The average number of planulae ($\pm$SD) released per colony per night in the different months was calculated. The calculation of each monthly average was based on colonies that produced planulae during at least one of the successive monitoring nights, including zero results from these colonies when applicable (i.e. n-value for each month = number of colonies that produced planulae at least one night each month×number of monitoring nights). The planulae and their respective parental colonies were transported to Tel Aviv for further experiments. Availability of planulae and the resulting primary polyps throughout the study period dictated the number of replications of each experiment, in both the laboratory and the field (see below).

Development of primary polyps in the laboratory and in the field

We conducted a series of experiments to examine several aspects of development in metamorphic stages of *H. fuscescens*. For this purpose, we reared zooxanthellate and azooxanthellate primary polyps in the laboratory and zooxanthellate primary polyps in the field. A scoring system was developed, corresponding to the different morphogenetic stages of the primary polyps, which were also visualized by scanning electron microscope (see below). The polyps were monitored under a dissecting microscope, and the timing and sequence of morphogenetic events of the primary polyps were routinely recorded and scored accordingly. This enabled us to compare the morphogenetic events of the three types of polyps at different ages. The number of surviving primary polyps at different ages was quantified as the ratio between the number of counted primary polyps and the initial number of planulae for each of the experiments detailed below.

Laboratory experiments

Preliminary experiments indicated that freshly released planulae of *H. fuscescens* maintained in 0.45 μm FSW from Eilat initiated normal metamorphosis 10–14 days after release, and subsequently successfully developed into azooxanthellate primary polyps (see also Ben-David-Zaslow and Benayahu 1996). We refer to this specific timing as commencement of primary polyp stage, and age of the primary polyps was determined after onset of metamorphosis, excluding the 14 days elapsed since release of planulae. This procedure, which was adopted throughout these experiments, avoided the use of artificial inducers (Henning et al. 1996, 1998). In Tel Aviv, planulae were routinely placed in 24-well tissue-culture

plates (Corning, COSTAR), at three planulae per well (72 planulae per plate), which was filled with 3 ml of 0.45 μm FSW. Half of the 0.45 μm FSW volume in each well was changed every other day. Each plate with 72 planulae was considered a single replicate. The plates were placed in incubators (Yihdern, LE-509) with a 12 h light:12 h dark lighting regime (30 μmol quanta m^{-2} s^{-1}), at a temperature corresponding to ambient Red Sea seawater temperature when the parental colonies were collected (21–28°C). We obtained temperature data from IUI routine measurements.

To facilitate development of zooxanthellate primary polyps, planulae were treated as above and were infected with freshly isolated algae obtained from adult polyps removed from their respective parental colonies by homogenization and centrifugation, following the methodology described by Davy et al. (1997). The symbionts were introduced to each well at the initiation of metamorphosis, yielding a final concentration of $5–7\times10^{-4}$ algal cells ml^{-1}. Half of the 0.45 μm FSW volume in each well was changed every other day throughout the experiments. The above procedure has been proven successful, and algal infection coincided with commencement of metamorphosis (Yacobovitch 2001).

In order to examine survivorship of laboratory-reared primary polyps as a function of seasonality, we monitored the zooxanthellate primary polyps weekly in a series of experiments during different months throughout the study period (November 1998–August 2000). Survivorship of the primary polyps from initiation of metamorphosis up to 35 days of age was calculated separately for each plate (replicate) as the ratio between the number of counted primary polyps and the initial number of planulae (i.e. 72) introduced to each plate. The seasons were defined in this study according to the stratification of the water column in the Gulf of Eilat (Lindell and Post 1995): "winter" (December–March), "spring" (April and May), "summer" (June–September) and "autumn" (October and November). The months refer to the time of release of planulae, with the following initial number of replicates for each examined month: November ($n=3$ plates) and December 1998 ($n=3$); March ($n=5$), May ($n=5$), August ($n=8$) and November 1999 ($n=9$); and February ($n=8$), April ($n=6$), June ($n=4$) and July 2000 ($n=7$). For each date, we monitored survivorship of the resulting infected primary polyps for a period of 35 days, with the exception of April 2000, when it was monitored for 28 days.

In order to examine primary polyp survivorship and development as a function of the symbiotic state, we conducted two experiments comparing zooxanthellate and azooxanthellate polyps, each monitored weekly, for 70 days following onset of metamorphosis. The first was conducted in December 1998 and consisted of three plates of each polyp type. The second was conducted in July 1999 and consisted of two plates with zooxanthellate and one plate with azooxanthellate primary polyps. Infected primary polyps were obtained as described above.

Field experiments

In order to monitor the development and survivorship of zooxanthellate primary polyps naturally settled in the field, batches of freshly released planulae, ranging between 50 and 400, were transferred to rectangular 1-l transparent PVC containers, fastened to the IUI reef at a depth of 3–4 m. Each container held granite gravel collected from the reef as substratum for settlement. Two sides of the containers were removed and replaced with 375 μm nylon mesh to allow water flow. Containers with planulae were placed on the reef, in June–August 1999 and again in June–August 2000. Metamorphosis was initiated on the day following introduction to the natural substrata (see also Benayahu et al. 1989b), and polyp age was determined accordingly. In these experiments, different containers were removed on days 3, 7, 14, 21, 28 and 56. On each sampling day, we counted the primary polyps under a dissecting microscope, and survivorship was calculated in relation to the number of planulae initially introduced to each container. We removed the primary polyps and fixed them for electron microscopy (see below).

Electron microscopy

For each batch of planulae obtained throughout the study, the remaining planulae (<72), an insufficient number for replication in the laboratory experiments (see above), were reared to primary polyps as described above. Selected zooxanthellate and azooxanthellate developmental stages from these animals (each 5–15 primary polyps) were periodically fixed in 2.5% glutaraldehyde in seawater. In addition, samples of field-reared primary polyps obtained from the periodically removed containers (see above) were similarly treated. This enabled us to visualize the developmental sequence of animals subjected to different treatments and to develop a scoring system corresponding to the different developmental stages. The samples were decalcified in a mixture of equal volumes of formic acid (50%) and sodium citrate (15%) for 30 min (see also Benayahu et al. 1992) and then placed in 2.5% glutaraldehhyde. Samples underwent dehydration through a graded series of ethanol, and were critically point dried with liquid CO_2, coated with gold and examined under a JEOL JSM 840 scanning electron microscope.

Statistical analyses

Analysis of variance (ANOVA) was performed after data expressed as percentages were arcsine transformed. The tests were corrected for multiple comparisons using the Bonferroni procedure.

Results

Release of planulae

Release of planulae occurred throughout most of the study period (November 1998–August 2000). Table 1 shows the proportion of colonies for each month that produced planulae during at least one night, out of the total number of monitored colonies. Notably, only a few colonies released planulae each month. The table also presents the average number of planulae released per colony per night in every month; monthly averages ($\pm$ SD) are based only on colonies that produced planulae at least one night in a given month (n = number of planulating colonies×number of monitoring nights, see also "Materials and methods"). For each month, the range of numbers of planulae released per colony is also provided: a zero value indicates that a colony failed to produce planulae during any of the monitoring nights of a given month, but did planulate on another night. A remarkably high number of planulae were produced during summer months, i.e. July and August 1999 and July 2000 (Table 1).

Figure 1 presents the frequency distribution of the number of planulae released per colony during the study period ($n=58$ nights). Planulation occurred on 38 nights (66% of total nights) and 17,297 planulae were released. On any of these nights, only one to three colonies planulated, and most colonies released >101 planulae each.

Development and survivorship of primary polyps in the laboratory

In all laboratory experiments, the vast majority ($>90\%$) of planulae initiated metamorphosis within 12–14 days

Table 1 *Heteroxenia fuscescens*. Proportion of colonies for each month that produced planulae during at least one night out of the total number of minitored colonies. And average (±SD) number of planulae released per colony per night (min.–max.), out of the total number of colonies collected in each examined month (*n* number of colonies that produced planulae at least one night each month×number of monitoring nights; *N.S.* no sampling)

Month	Proportion of planulating colonies of total number of colonies	Average (±SD) number of planulae released per colony per night (min.–max.)
Nov 1998	4/44	105.6 ± 103.3 (22–303), $n = 12$
Dec 1998	5/24	126.2 ± 125.3 (0–302), $n = 15$
Jan 1999	N.S.	
Feb 1999	1/28	72 ± 101.8 (0–216), $n = 3$
Mar 1999	2/15	203.3 ± 114.2 (43–300), $n = 6$
Apr 1999	N.S.	
May 1999	4/40	225 ± 83.6 (148–350), $n = 12$
Jun 1999	N.S.	
Jul 1999	2/15	1,100 ± 400 (700–1,500), $n = 6$
Aug 1999	3/20	451.25 ± 171.9 (210–580), n = 15
Sep 1999	N.S.	
Oct 1999	0/40	
Nov 1999	6/20	182.9 ± 288.7 (0–948), $n = 30$
Dec 1999	1/16	113 ± 67 (46–180), $n = 3$
Jan 2000	N.S.	
Feb 2000	2/30	201 ± 59.2 (150–300), $n = 6$
Mar 2000	0/25	
Apr 2000	1/65	112.5 ± 194.9 (0–450), $n = 4$
May 2000	1/72	50 ± 86.6 (0–200), $n = 4$
Jun 2000	1/22	125 ± 125 (0–250), $n = 3$
Jul 2000	3/30	904 ± 254.7 (520–1200), $n = 12$
Aug 2000	2/10	86.7 ± 102.1 (0–230), $n = 6$

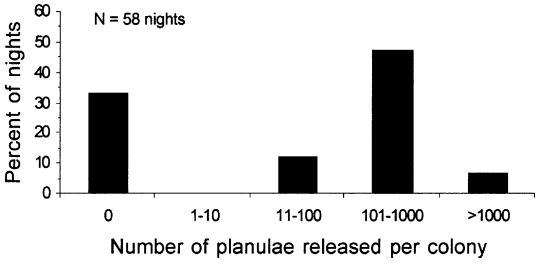

Fig. 1 *Heteroxenia fuscescens*. Frequency distribution of number of planulae released per colony per night throughout the entire study period (November 1998–August 2000)

after release (see also "Materials and methods"). At this particular stage, the indication for onset of metamorphosis was contraction of planula, followed by the immediate appearance of a mouth opening (Fig. 2a). Thereafter, the morphogenetic events were relatively synchronized among primary polyps of a given age, as demonstrated by the SEM images. A 2-day-old primary polyp had eight tentacular grooves (Fig. 2b), and at 3 days old distinct tentacular buds appeared (Fig. 2c). During the following 7 days the tentacles elongated (Fig. 2d) and, starting with 14-day-old primary polyps, pinnules appeared along the tentacles (Fig. 2e), gradually increasing in number (Fig. 2f), until 28-day-old primary polyps had 11–13 pairs along each tentacle.

In order to compare the developmental sequence and timing of animals subjected to different treatments, a scoring system was developed to correspond to the different developmental stages, and visualized by SEMs (Fig. 2). Animals were routinely scored under a dissecting microscope and categorized as: (1) attached and contracted planulae; (2) polyps with the presence of a mouth opening (Fig. 2a); (3) polyps with the presence of tentacular grooves (Fig. 2b); (4) polyps with tentacles (Fig. 2c, d); (5) polyps with the presence of the first pair of pinnules on the tentacles (Fig. 2e); and (6) advanced primary polyps, with more than six pairs of pinnules (Fig. 2f).

There were no distinct differences in the timing or sequence of morphogenetic events in zooxanthellate compared to azooxanthellate primary polyps reared in the laboratory (Fig. 3). In both polyp types, variation occurred in the timing of morphogenetic events during the first 21 days following onset of metamorphosis. This variation then decreased, and development of both types of animals became synchronized on day 28 (Fig. 3).

Figure 4 presents the average survivorship values of laboratory zooxanthellate primary polyps released at different months during the study period. Highest survivorship was recorded for primary polyps developed from planulae released in the summer months. We found no significant difference between survivorship in summer and spring (Fig. 4, two-way ANOVA with Bonferroni correction $\alpha/10$, $P > 0.005$), when seawater temperatures were 26–28°C and 23–24°C, respectively. For example, 28-day-old polyps had a survivorship of 52 ± 15.4% ($n = 9$ plates) and 36.9 ± 15.1% ($n = 6$) for summer and spring, respectively (Fig. 4e). Similarly, we found no significant difference between winter and autumn (Fig. 4, two-way ANOVA with Bonferroni correction $\alpha/10$, $P > 0.005$), when seawater temperature was 21–22°C and 22–23°C, respectively. Survivorship of polyps of the same age in winter and autumn was 12.4 ± 6.8% ($n = 6$) and 20.6 ± 9.1% ($n = 3$), respectively (Fig. 4e). However, significant differences in survivorship were found between summer and autumn (starting on day 28), summer and winter (starting on day 21) and spring and winter (on all days) (Fig. 4, two-way ANOVA with Bonferroni correction $\alpha/10$, $P < 0.005$). Autumn 1999 was an exception (24°C), with no significant difference being found in polyp survivorship between this month (average survivorship of a 28-day-old primary polyp = 57.6 ± 16.0%, $n = 4$) and the summer months (Fig. 4, two-way ANOVA with Bonferroni correction $\alpha/10$, $P > 0.005$).

Figure 5 presents the survivorship of zooxanthellate and azooxanthellate primary polyps in the laboratory during a 70-day period. Day 0 represents onset of metamorphosis for both types of polyp and initiation of symbiont infection for the zooxanthellate ones (see "Materials and methods"). Average survivorship (±SD) of zooxanthellate primary polyps compared to azooxanthellate ones became significantly higher on

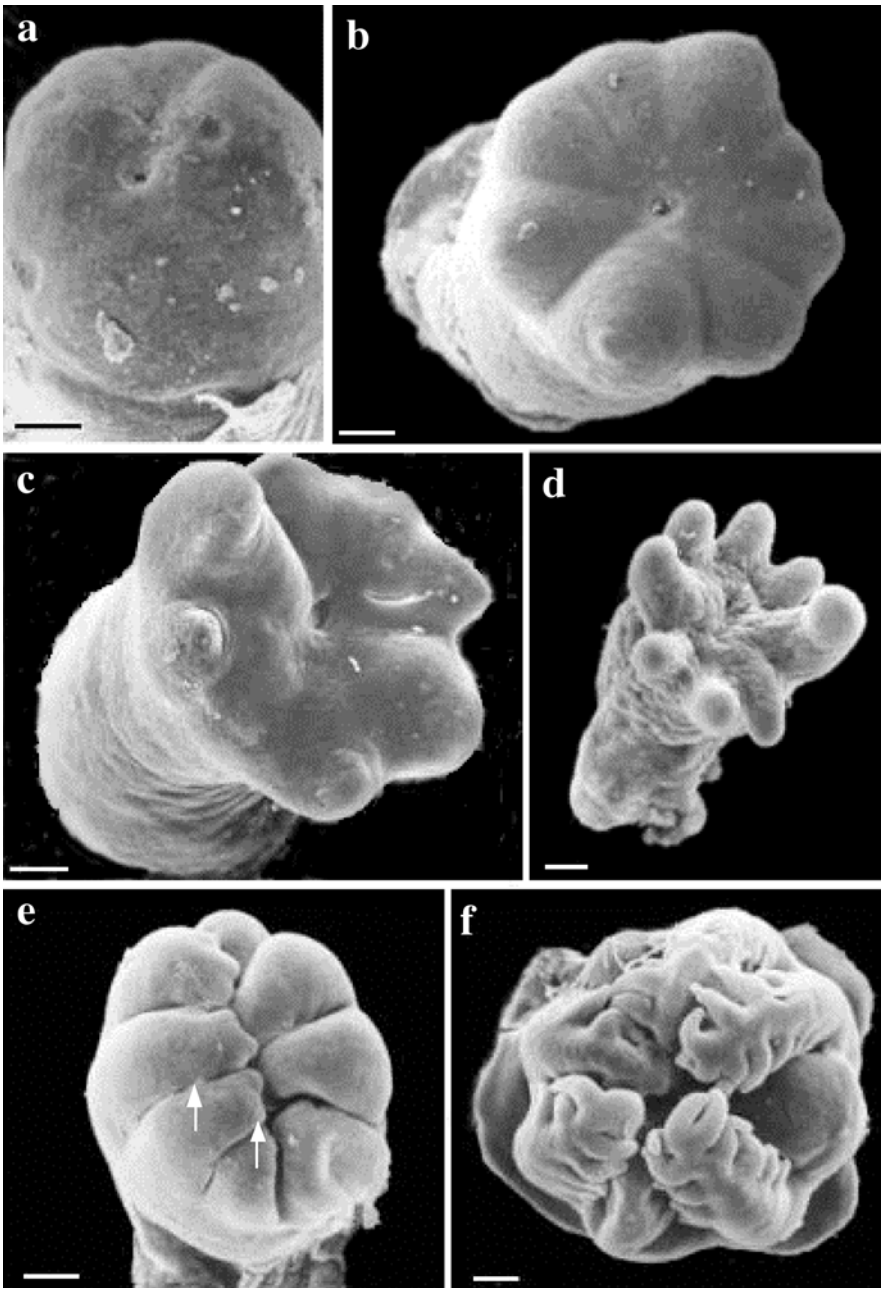

Fig. 2a–f *Heteroxenia fuscescens*. Scanning electron micrographs of metamorphosed primary polyps: **a** initiation of metamorphosis, polyp with mouth opening; **b** 2-day-old polyp with eight tentacular grooves; **c** 3-day-old polyp with eight tentacular buds; **d** 7-day-old polyp with elongated tentacles; **e** 10-day-old polyp with first indication of pinnules (*arrows* point to two of them); and **f** 21-day-old polyp with six pairs of pinnules along each tentacle. *Scale bars*: 100 μm

day 21 and remained so until day 70 in both experiments (Fig. 5, two-way ANOVA, $P < 0.01$).

Development and survivorship of primary polyps in the field

Within 5–12 h after introducing freshly released planulae into the 1-l experimental containers in the field, $>90\%$ of the planulae had settled on the granite gravel and metamorphosis had commenced. All primary polyps became naturally infected by zooxanthellae upon appearance of their mouth opening (Yacobovitch 2001). From day 21 on, polyp development tended to become synchronized among individuals, achieving complete synchronization on day 28 (Fig. 3). There were no distinct differences in timing and sequence of morphogenetic events between the laboratory-reared zooxanthellate primary polyps and those in the field (Fig. 3).

Survivorship of field primary polyps in the two experiments decreased with time, and we noted no distinct differences in values obtained for each age between the 2 years (Fig. 6). There was a significant difference in survivorship between laboratory-reared and field zooxanthellate polyps with the former showing higher survivorship (Fig. 7, two-way ANOVA, $P < 0.01$), as revealed by comparisons between primary polyps from both groups that developed in the summer months (1999, 2000).

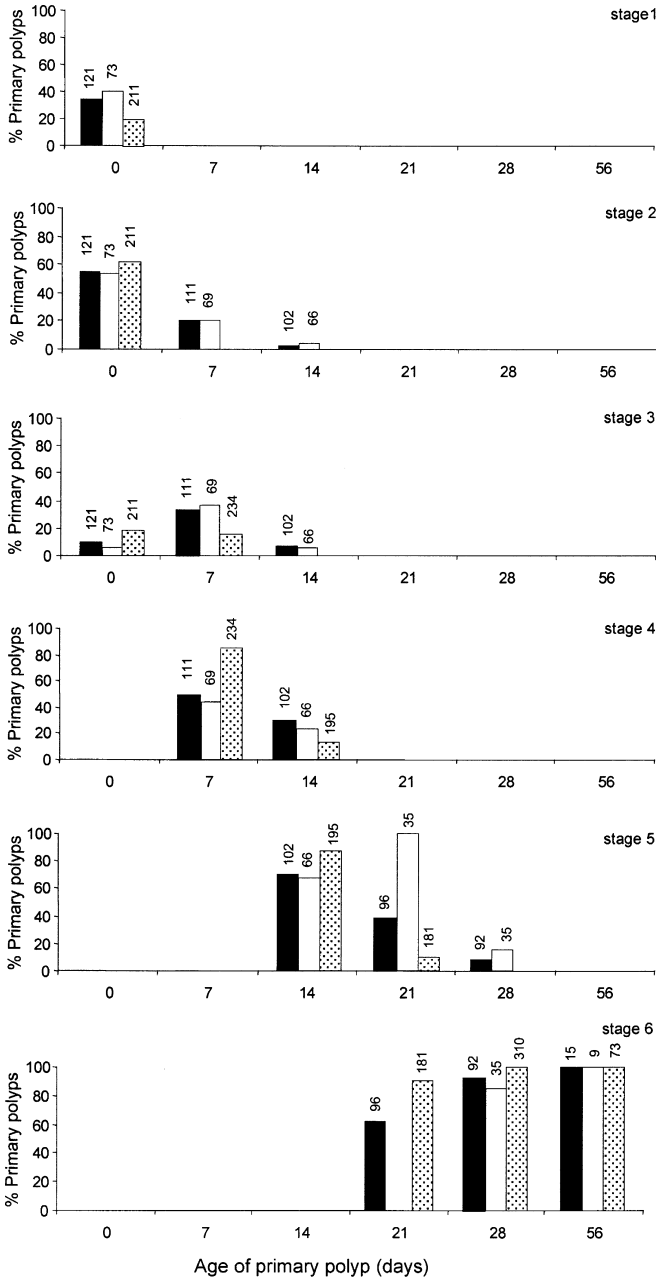

Fig. 3 *Heteroxenia fuscescens*. Morphogenetic events of laboratory reared zooxanthellate (*closed bars*) and azooxanthellate (*open bars*) primary polyps and naturally settled ones in the field (*dotted bars*) at different ages (days), scored into six stages as follows: (1) attached and contracted planulae; (2) appearance of mouth opening; (3) appearance of tentacular groves; (4) appearance of tentacles; (5) appearance of pinnules; and (6) advanced primary polyp, with more than six pairs of pinnules (*number above bars* number of primary polyps for each group at a given age)

Discussion

The findings presented in the current study support previous findings on annual breeding activity of the soft coral *Heteroxenia fuscescens* (Benayahu 1991; Ben-David-Zaslow et al. 1999), indicating year-round planulation, with the highest rate occurring during the summer months. The present results further reveal that during summer, the highest planulation was reached in July and August (Table 1). Although a rather low percentage of colonies planulated on each date, a relatively high planula yield was obtained (Table 1; Fig. 1), indicating high fecundity of the reproducing colonies. Thus, the successful sexual reproduction of this soft coral appears to be due to the constant breeding activity of a low percentage of colonies at any time.

Planulae of *H. fuscescens* are able to successfully undergo metamorphosis in 0.45 μm filtered (Millipore) seawater, starting 10–14 days after their release. This further strengthens previous results, indicating that in the laboratory no artificial induction is needed, and metamorphosis of these planulae can be endogenously controlled (Ben-David-Zaslow and Benayahu 1996). Metamorphosis of larvae in FSW (0.22 μm or 0.45 μm) without artificial inducers has been reported for the giant clam *Tridacna squamosa* (Fitt and Trench 1981) and the stony coral *Fungia scutaria* (Schwarz et al. 1999). Our results suggest that the use of 0.45 μm FSW will effectively yield synchronously metamorphosed aposymbiotic primary polyps. Such a procedure, which avoids contamination of azooxanthellate primary polyps by unicellular algae or other microorganisms that may be found in unfiltered seawater, is therefore recommended for controlled experiments that examine acquisition of zooxanthellae in cnidarians (Yacobovitch 2001).

The survivorship of primary polyps developed from planulae released during the summer months was higher compared to the other seasons (Fig. 4). The only exception was November 1999, when survivorship was similar to that in summer. This might be explained by the exceptionally high seawater temperature measured at the time when planulae were released (24°C), compared to the usual autumn temperature (22–23°C). Previous studies report that summer planulae of *H. fuscescens* are longer, weigh more (Ben-David-Zaslow and Benayahu 1999; Ben-David-Zaslow et al. 1999), and have a higher rate of metamorphosis than those in other seasons (Ben-David-Zaslow and Benayahu 1996). In addition, mature colonies of *H. fuscescens* have higher energetic content in summer (Ben-David-Zaslow and Benayahu 1999). All existing evidence suggests that summer is the most favorable season for breeding and development of the sexual progeny in *H. fuscescens*.

This study reveals a similarity between the morphogenetic events during metamorphosis of zooxanthellate and azooxanthellate primary polyps of *H. fuscescens* in the laboratory (Fig. 3). Our findings are in agreement with some other studies that have examined the effects of symbiotic algae on host juvenile stages. Kinzie (1974) observed no difference in development of zooxanthellate and azooxanthellate primary polyps of the gorgonian *Pseudopterogorgia bipinnata*. Fitt and Trench (1981) showed no differences in metamorphosis initiation of veligers of the giant clam *Tridacna squamosa*. In

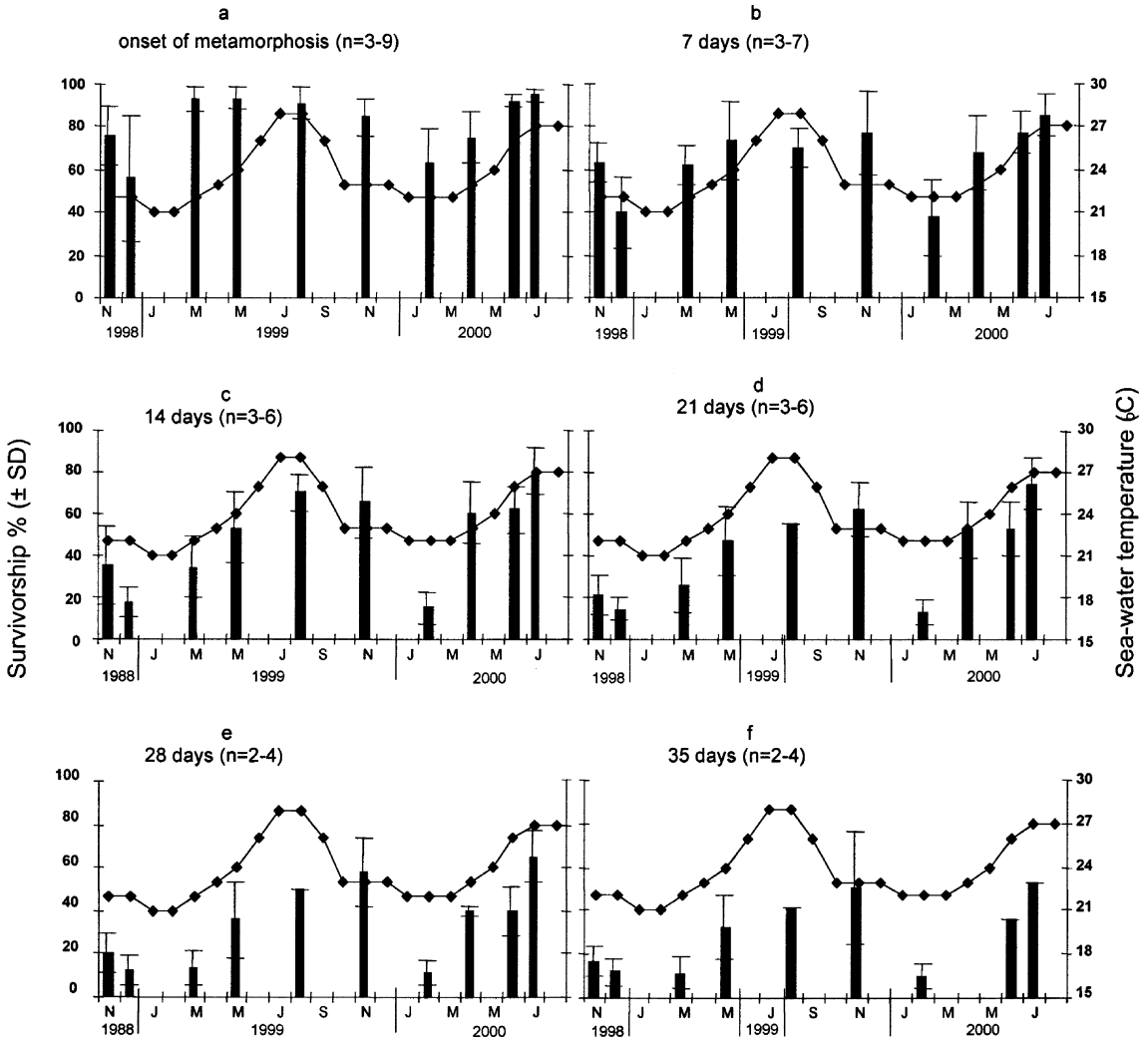

Fig. 4a–f *Heteroxenia fuscescens*. Average survivorship of primary polyps (±SD) derived from planulae released in different months during the study period (November 1998–August 2000). Results are presented for different polyp ages from initiation of metamorphosis up to 35 days (**a–f**). *Line* represents the water temperature curve. Ranges for number of plates (*n*) are given for each age

contrast, other studies have shown that the symbiotic state does affect juvenile host development. For example, strobilation of the scyphystomae of *Cassiopeia xamachana* requires infection with symbiotic algae (Trench 1987). Schwarz et al. (1999) showed that zooxanthellate planulae of the stony coral *Fungia scutaria* settled and metamorphosed earlier than azooxanthellate ones.

Although the symbiotic state did not appear to affect developmental sequence or timing (Fig. 3), it did significantly increase host survivorship (Fig. 5). This suggests that the presence of zooxanthellae benefits the juvenile host's physiology. A study on the energetics of symbiotic *Pocillopora damicornis* planulae suggests that the zooxanthellae contribute 13–27% of their total fixed carbon to their larval host (Richmond 1987). It is possible that zooxanthellate *H. fuscescens* primary polyps receive enough carbon from their symbionts, even at this early stage, to increase their survivorship. Therefore, although the symbiotic state does not appear to directly affect development, it does seem to have a positive impact on the overall survivorship of the juvenile soft corals.

Comparisons between laboratory-reared zooxanthellate primary polyps and naturally settled ones showed a similarity in timing and sequence of morphogenetic events (Fig. 3). In the field, however, the polyps revealed a higher developmental synchronization, probably resulting from a rapid and synchronized onset of metamorphosis (5–12 h following upon initiation of the experiment), compared to a delayed and longer period in the laboratory (day 10–14 after planulae release). The higher survivorship in the laboratory (Fig. 7) might be due to the lack of predation, smothering by sediment, turf algae and other unfavorable conditions commonly occurring in the field (e.g. Benayahu and Loya 1987).

The findings presented here indicate that primary polyps of *H. fuscescens* can be successfully maintained in the laboratory for use in experimental manipulations that address the onset of symbiosis and the competence of

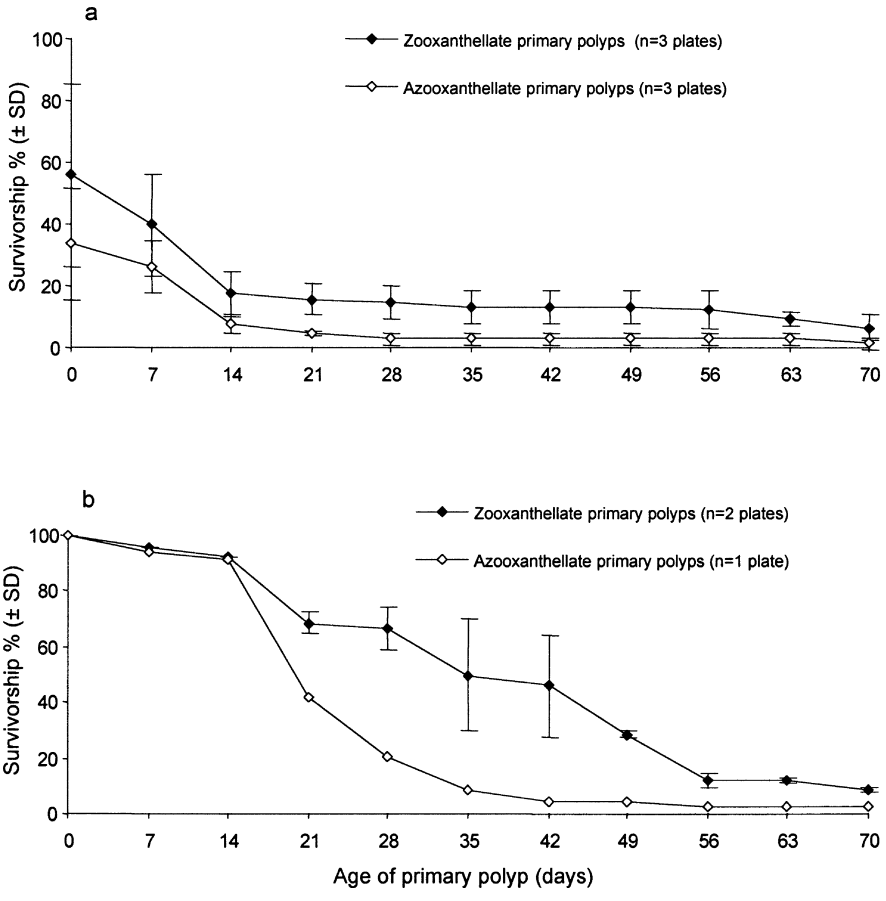

Fig. 5a, b *Heteroxenia fuscescens*. Average survivorship of zooxanthellate and azooxanthellate laboratory-reared primary polyps (±SD): **a** experiment conducted in December 1998, **b** experiment conducted in July 1999. Each replication initially contained 72 planulae

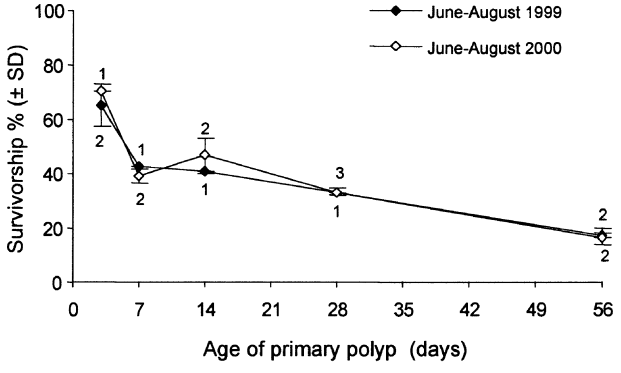

Fig. 6 *Heteroxenia fuscescens*. Survivorship of naturally settled primary polyps in the field at different ages (days) in June–August 1999 and June–August 2000. *Each point* represents primary polyp survivorship in a given 1-l PVC container that was fastened to the IUI reef (*number above data points* number of sampled 1-l PVC containers initially with batches of planulae ranging between 50 and 400)

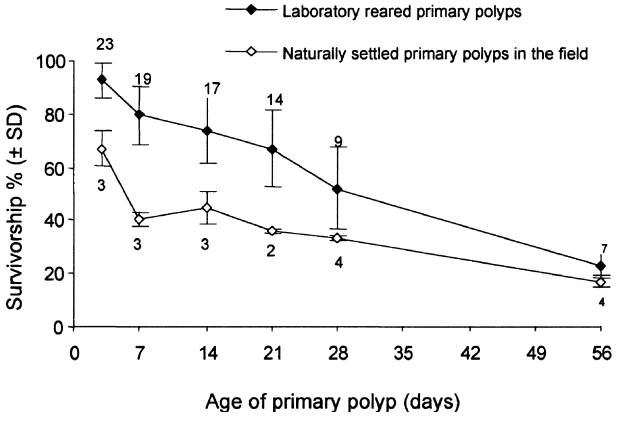

Fig. 7 *Heteroxenia fuscescens*. Average survivorship of laboratory-reared primary polyps and naturally settled ones in the field (±SD), derived from June–August 1999 and June–August 2000 experiments (*number above data points* sample size: in the laboratory, number of 24-well tissue-culture plates and, in the field, number of sampled 1-l PVC containers)

aposymbiotic cnidarian sexual progeny to algal acquisition (Yacobovitch 2001). Our study is the first to thoroughly compare the development and survivorship of zooxanthellate and azooxanthellate anthozoan primary polyps in the laboratory, and to examine this in relation to the field scenario. Thus, *H. fuscescens* represents an appropriate model system for study of the acquisition of algae by an aposymbiotic offspring and the impact of this process on their development and survivorship.

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