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Metamorphosis of an Octocoral Primary Polyp and Its Infection by Algal Symbiosis

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Abstract

This study describes the post larval development of the octocoral alcyonacean *Heterozenia fuscescens*. Planulae of this species exhibit rapid settlement, within 7–8 hr after their release from parent corals, the vast majority of larvae settle and attach to reef substrata covered with crustose coralline algae.

Metamorphosis of the primary polyps is accompanied by contraction of the planular body and subsequent development of tentacles. The aposymbiotic polyps acquire, *in situ*, their micro algal symbionts (zooxanthellae) during the third day of metamorphosis. Initiation of symbiont infection is associated with the development of a mouth opening. Symbionts first reside in the tentacles and in the tentacle pinnules. A fully metamorphosed 30-day-old polyp has dense algal cells in the endodermal tissues. Oogenesis and embryogenesis of *H. fuscescens* occur in the algal rich tissues of the parent coral. Nevertheless, algal infection takes place only after adequate development of the primary polyp at a stage that can facilitate symbiont uptake.

Keywords: Octocorallia, *Heterozenia fuscescens*, metamorphosis, algal symbionts, polyp

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1. Introduction

Settlement of cnidarian planulae involves substantial constructive and destructive morphological changes (Martin et al., 1983). Planular settlement and post larval metamorphosis constitute major developmental stages in cnidarian life history (Babcock, 1985; Chia and Bickell, 1987). Various aspects of structural and morphological changes during transformation of octocoral planulae into primary polyps have been described (Matthews, 1917; Gohar, 1940; Chia and Crawford, 1977; Weinberg and Weinberg, 1979; Benayahu and Loya, 1984a; Sebens, 1983; Farrant, 1986). Many reef-dwelling octocorals harbour symbiotic dinoflagellate algae (zooxanthellae) within their endodermal tissues (Kinzie, 1974; Berner et al., 1987). This symbiosis is newly established each generation by the sexually produced offspring (Trench, 1987; Benayahu et al., 1988). The algal symbionts may be derived by parental inheritance or from the external environment (Trench, 1987). Some octocorals produce aposymbiotic planulae which gain their symbionts in the course of their post larval metamorphosis (Gohar, 1940; Kinzie, 1974; Benayahu et al., 1988).

Heteroxenia fuscescens is a common Indo-Pacific soft coral (Octocorallia: Alcyonacea) associated with symbiotic algae (Schlichter, 1982). The algal cells play a major role in metabolism of this species, supplying assimilates to host tissues (Schlichter et al., 1983). Mature colonies of *H. fuscescens* are dimorphic with autozooids and siphonozooids (Gohar, 1940). Colonies are hermaphroditic with highly specialized brood care involving retention of embryos in internal polyp cavities and inter-siphonozooid spaces (Benayahu et al., 1989). Expelled planulae of *H. fuscescens* lack algal symbionts (Benayahu et al., 1989).

This study investigates post larval development of *H. fuscescens* and examines metamorphosis of a settled planula into a primary polyp. A major question addressed in the study is: At what developmental stage do the sexually produced progeny become infected by the symbiotic algae? This is the first study dating *in situ* algal acquisition by an aposymbiotic alcyonacean offspring in the course of its post larval development.

2. Materials and Methods

The study was conducted during April/May 1987 at the Interuniversity Institute of Marine Biology in Eilat (Red Sea), Israel. Planulae of *Heteroxenia fuscescens* were obtained from freshly collected colonies kept in aerated aquaria. Released planulae were pipetted out of these aquaria

and transferred to experimental transparent plastic jars which later were covered with plankton net (200 μm mesh). Branches of dead colonies of the hermatypic coral *Stylophora pistillata*, covered by crustose coralline algae, collected shortly prior to the experiment were used as substrata for planular settlement. There were six settling jars each supplied with approximately 250 planulae.

On the night of planulation the plastic containers were fastened on the reef adjacent to the Interuniversity Institute at a depth of 2–3 m. During the following 2-week period several randomly-selected branches of dead colonies with 15–20 settled polyps were removed daily from the containers. During the next 3 weeks similar sampling was conducted twice a week. Because larval settlement was very rapid the plankton net coverings were removed 12 hr after the containers were fastened to the reef. Settled polyps were examined in the laboratory under a stereoscopic microscope immersed in finger bowls filled with sea-water. Polyps for light microscopy were fixed in Bouin's solution and then decalcified in a mixture of equal volumes of formic acid (50%) and sodium citrate (15%). Serial paraffin section (8–10 μm) of 6–8 polyps from each sample were lightly stained by hematoxylin-eosin. Sections were examined under an epifluorescence microscope (excitation at 390, emission at 450). Material used for scanning electron microscopy (SEM) was fixed in 2% glutaraldehyde, followed by GTGO procedure (Gamliel, 1963), and examined in JEOL JSM 840 scanning electron microscope.

3. Results

Polyp metamorphosis

Planulae of *Heteroxenia fuscescens* were released at dusk from the intersiphonozooid spaces of the parent colony (Fig. 1) and settled rapidly. Within 5–8 hr after planulation more than 95% of the larvae had attached to the *Stylophora* fragments. Metamorphosis of the larvae began immediately after settling and was highly synchronized. On the first day settled planulae contract and, apparently, become shorter with a remarkable, flat oral end (Fig. 2A). On the second day the young polyp exhibits 8 tentacular grooves of unequal size (Fig. 2B). On the third day each polyp has 8 primary tentacles still lacking pinnules (Fig. 2C). During the following days tentacles elongate and gradually the pinnules increase in number along each of them. A 6-day-old polyp has 1–2 pairs of pinnules per tentacle (Fig. 2D), while on the ninth day four pairs occur along each tentacle (Fig. 2E). A 30-day-old polyp bears 11–13 pairs of pinnules on a tentacle (Fig. 2F).

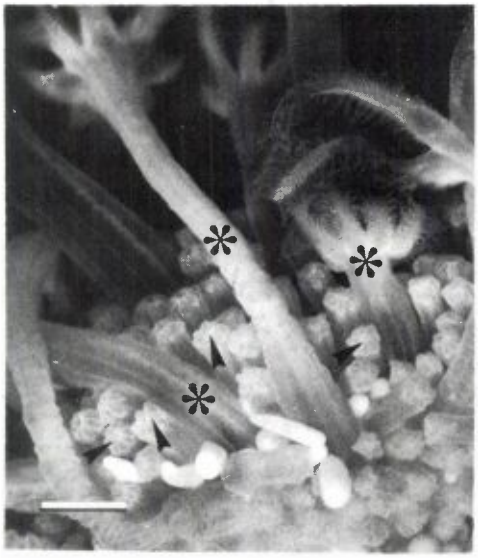

Figure 1. Dimorphic colony of *Heterozenia fuscescens* in the process of expelling planulae. Siphonozooids are indicated by arrowheads and autozooids by asterisks. Scale, 3 mm.

Algal infection

Within the first 2 days of metamorphosis no algal symbionts were found in any of the sectioned primary polyps. Examination of numerous histological sections of 12 polyps indicated that infection is only on the third day of metamorphosis. Such a polyp (Fig. 3A) has a clearly recognizable mouth opening (Fig. 3B). The tentacular endoderm contains only very few algal cells. In the course of the first 8 days of metamorphosis algal symbionts are sparsely scattered only in tentacular endoderm (Fig. 3C and D), predominantly in the pinnules (Fig. 3E). Subsequent metamorphosis is associated with infection of the basal polyp gastrovascular-endoderm (Fig. 3F). Note that at such an early developmental stage the symbionts appear as patches of algal cells within the host tissues (Figs. 3D–F).

Thirty-day-old polyps (Fig. 4A and B) are densely infected by symbionts in all their endodermal tissues (Fig. 4C). The basal gastrovascular endoderm of such a fully metamorphosed primary polyp has a high density of symbionts (Fig. 4D). In addition, pinnules of the living polyps are dark brown due to the infection by numerous algal cells (Fig. 4E).

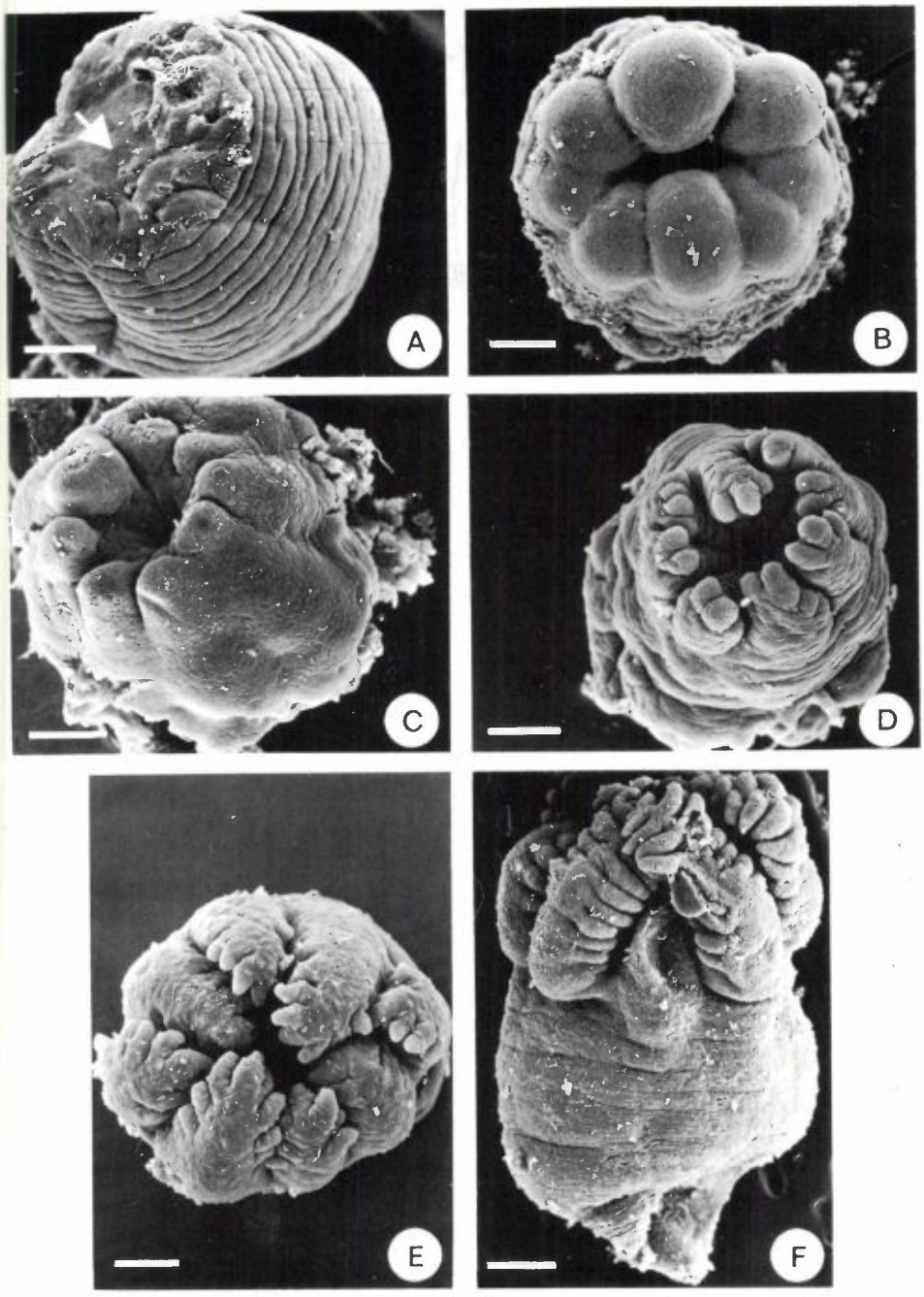

Figure 2. Scanning electron micrographs (SEM) of metamorphosed primary polyps. Scale, 100 μm. (A) 1-day-old polyp with a flat oral side (arrow). (B) 2-day-old polyp with 8 tentacular grooves. (C) 3-day-old polyp with 8 tentacles. (D) 6-day-old polyp with first indication for pinnules. (E) 9-day-old polyp. (F) 30-day-old primary polyp.

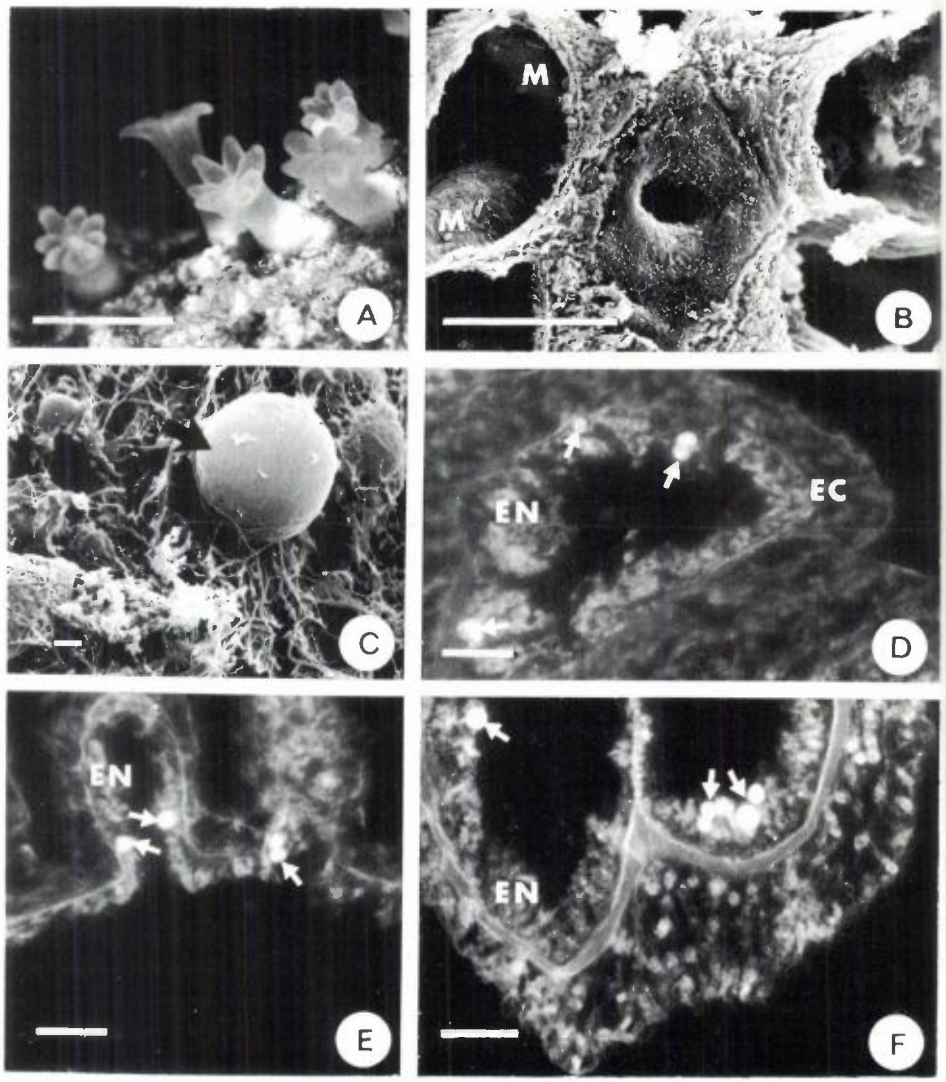

Figure 3. The initial stages of algal infection. Scale, 3 mm (A), 100 μm (B), 1 μm (C), 50 μm (D–F). (A) 3-day-old living polyp. (B) Mouth of a 3-day-old polyp. M, mesentary. (C) Algal cell resides the polyp endoderm (arrow). (D) Algal cells (arrows) in tentacular endoderm (EN). EC: Ectoderm. (E) Algal cells (arrows) in endodermal lining (EN) of pinnules. (F) Basal polyp endoderm (EN) with algal cells (arrows).

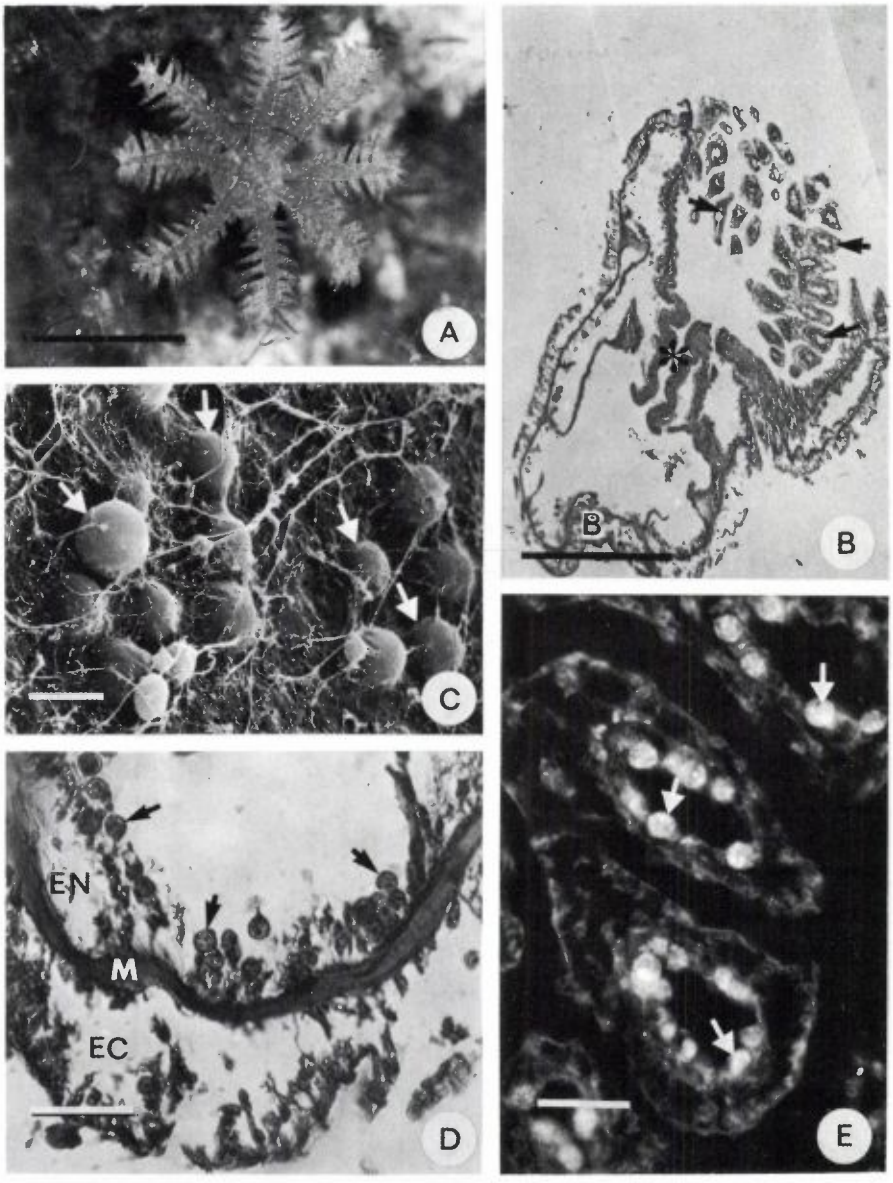

Figure 4. Further stages of algal infection of a 30-day-old polyp. Scale, 3 mm (A), 1 mm (B), 10 μm (C), 50 μm (D–E). (A) 30-day-old living polyp. (B) Long section of a 30-day-old polyp. Pinnules are indicated by arrows, pharynx by an asterisk and the basal polyp tissues by (B). (C) Algal cells (arrows) inhabiting the endoderm. (D) Basal polyp tissues. EN: Endoderm with algal cells (arrows). M: Mesaglea. EC: Ectoderm. E: Algal cells (arrows) in the endoderm of pinnules.

4. Discussion

Planulae of several octocoral species spend a short period of time in the water column and, therefore, may have low dispersal (Benayahu and Loya, 1983; 1984b). Similarly, though more dramatically, the present study demonstrates that planulae of *Heteroxenia fuscescens* exhibit rapid settlement on leaving the parent colony. Among invertebrates it is not yet clear whether negative phototaxis or increased sinking rate are mechanisms by which ready-to-settle larvae seek the sea floor (Cameron, 1986). Planulae of *H. fuscescens* exhibit a directional response towards available substrata (in prep.), most probably cued by gravity. Attachment of these planulae is accompanied by an abrupt metamorphosis. Gohar (1940) described an 11-day pelagic phase for planulae of *H. fuscescens*, coupled with initiation of metamorphosis in the laboratory prior to settlement. This agrees with our observations that in the absence of an appropriate substrata settlement is delayed. These results suggest that natural reef substratum is a major cue for normal and complete post larval development of *H. fuscescens*.

Many marine invertebrate larvae settle on surfaces covered by microorganisms and their extra-cellular products (Cameron, 1986). In addition, algal coating is a major cue determining the final site of planular attachment (Chia and Bickell, 1987). Larvae of agariciid hermatypic corals were found to be induced to metamorphose by substrata covered with crustose coralline algae (Moorse et al., 1988). Settlement and metamorphosis of alcyonacean planulae of *Alcyonium siderium* (Sebens, 1983), *Xenia macrospiculata* and *Parerythropodium fulvum fulvum* (Benayahu and Loya, 1984b) are similarly induced by crustose coralline algae. On the reef, primary polyps of *H. fuscescens* are almost always found directly attached to such substrata. *Heteroxenia* planulae are highly attracted by these appropriate surfaces whose abundance may strongly determine the dispersal of the developing recruits (in prep.). In addition, the immediate and rapid metamorphosis may cause the patchy distribution of *H. fuscescens* (Benayahu, 1982), and largely determines its occurrence in Eilat reefs.

The intimate association between symbiotic algal cells (zooxanthellae) and *Heteroxenia fuscescens* is critically important for the coral's nutritional physiology (Schlichter, 1982; Schlichter et al., 1983). However, neither the eggs nor mature planulae contain algae (Benayahu et al., 1989). The results of the present study indicate that the symbiosis between *H. fuscescens* and the algae is re-established in each new generation of host colonies. Many of the invertebrate hosts which do not pass their symbionts directly to the

offspring, require that the larval or juvenile stages acquire their complement of algae from the environment (Trench, 1979; Rahat and Adar, 1980; Fitt, 1984). Among invertebrate hosts, such acquisition of symbionts can be achieved through feeding (Trench, 1979; 1987; Kempf, 1984). In octocorals, aposymbiotic planulae have been observed in the gorgonians *Eunicella stricta aphyta*, *Briarium asbestinium*, *Pseudopterogorgia elizabethae* and *P. bipinnata* (Kinzie, 1974). Recently settled polyps of *Pseudopterogorgia bipinnata* open their mouth and trap algae with their tentacles (Kinzie, 1974). These symbionts pass into the gastrovascular cavity, and are subsequently ingested by gravitational cells and infection is initiated. Our similar results may indicate that symbiont acquisition by *H. fuscescens* coincides with development of a mouth opening in the metamorphosed polyp (Figs. 3A and B). In spite of the fact that oogenesis and embryogenesis of *H. fuscescens* occur in algae-rich tissues (Benayahu et al., 1989), no algal incorporation takes place at these stages. Algal intake is further delayed to a 3-day-old metamorphosed polyp which settles in an environment teaming with algal swarmers (Krupp, 1983). The patches of algal cells in this young polyp (Fig. 3D–F) indicate that they have proliferated by cell division while residing in the host endoderm. Kinzie (1974) suggested that aposymbiotic offspring would be advantageous in order to acquire those algae most suited to the site where the polyp settles. We further propose that benthic planulae with a diminished planktonic phase are most probably dependent on their algal symbionts for successful and complete metamorphosis. Therefore, the rapid post larval development largely assists algal infection of the young recruits of *H. fuscescens*. Further experimental studies are required in order to determine the mechanism and processes which cue the timing of infection in the course of octocoral offspring development.

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