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Sexual reproduction and settlement of the coral reef sponge *Chalinula* sp. from the Red Sea

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Abstract

Characteristics of the sexual reproduction and larval settlement of the haplosclerid sponge *Chalinula* sp., which inhabits the shallow waters (1 to 6 m) of Eilat, Red Sea, were investigated from September 1985 through to November 1987. This species was found to be a simultaneous hermaphroditic brooder, hence gonochorism is not the rule in the order Haplosclerida. Brooding always takes place in special brooding chambers. While the oocytes in the brooding chambers are among the largest known in sponges ($355 \pm 37 \mu m$), the spermatic cysts distributed in the choanosome are among the smallest known for this phylum (average $26 \pm 7 \mu m$). *Chalinula* sp. breeds throughout the year and in experiments most larvae (74%) settled within 1 to 8 h post-release, generally within 4.5 h. Metamorphosis from larval shape to a sessile sponge lasts 1 to 6 h. Thus, larvae had a short swimming period, settled fast, and metamorphosed rapidly (within 1 to 6 h). The large size of the larvae may contribute to their ability to rapidly reorganize their body shape into that of a sessile sponge. In addition, the existence of already differentiated choanocyte chambers in the larvae, facilitates fast construction of the water filtration system in the newly settled sponges. The reproductive and larval characteristics of *Chalinula* sp. enable the larvae to settle on any vacant space in the reef, which may explain its abundance in the Red Sea.

Introduction

During the last decade there has been a major increase in knowledge of the reproduction of benthic coral reef organisms, mainly stony corals (e.g. Fadlallah 1983, Harrison et al. 1984, Shlesinger and Loya 1985, Babcock et al. 1986, Szmant 1986) and soft corals (Yamazato et al. 1981, Benayahu and Loya 1983, 1984, 1986, Dinesen 1985, Babcock et al. 1986, Benayahu 1989).

Although sponges are an important component in marine tropical ecosystems, little attention has been paid to their reproduction (Reiswig 1973, Bergquist 1978, Wilkinson 1987); the publications on sponge reproduction from the coral reefs of the Caribbean remain as the only available literature (Reiswig 1973, Hoppe and Reichert 1987, Hoppe 1988). Recently we have described the reproduction and settlement of a coral reef sponge *Niphates* sp. (Haplosclerida) from the Red Sea (Ilan and Loya 1990). This hermaphroditic species reproduces mostly during summer, the embryos are brooded in special chambers, and the free-swimming larval stage lasts from 6 h to 17 d after larval release from the parental sponge.

The present study compares the reproduction and settlement of the haplosclerid sponge *Chalinula* sp. with that of the sympatric sponge *Niphates* sp. from the same order. Both sponges are very similar in size (height 2 ± 0.5 cm; length 10 ± 5 cm), shape (encrusting), and color (gray), and are distributed at the same habitat, microhabitat (sides of rocks with low coral coverage), and depth (0.5 to 6 m), where they are the most abundant sponge species (Fig. 4a). An additional aim of this study was to obtain *Chalinula* sp. larvae for experimental studies of the process of their settlement, in comparison with that of *Niphates* sp., the only other coral reef sponge in which this process has been studied so far.

Materials and methods

The field study was conducted in an area ca 300×30 m, situated on the coral reef in front of the Marine Biological Laboratory at Eilat, Red Sea. Most of the *Chalinula* sp. individuals in the study area were marked, enabling sampling of five different known individuals each month.

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For light microscopy, samples were fixed in 4% formaldehyde in sea water for 24 h. Following rinsing in 70% ethanol, siliceous spicules were desilicified using 4% hydrofluoric acid in 70% ethanol for 3 h. Further processing of sponges for histological analysis was carried out using standart dehydration series, followed by embedding in paraffin, sectioning to 10 μm and staining with haematoxylin and eosin.

For electron microscopy (EM), the fixative used was 2.5% glutaraldehyde buffered in seawater. Before scanning, samples were washed, dehydrated, critical-point-dried using liquid CO_2, coated with gold-palladium and finally viewed in a JEOL T-35 and JEOL JSM-840A SEM. Following fixation, samples used for TEM were postfixed with 1% OsO_4, dehydrated, embedded in Epon 812, sectioned, stained with uranyl acetate and lead citrate and then viewed in a JEOL 1200-EX TEM.

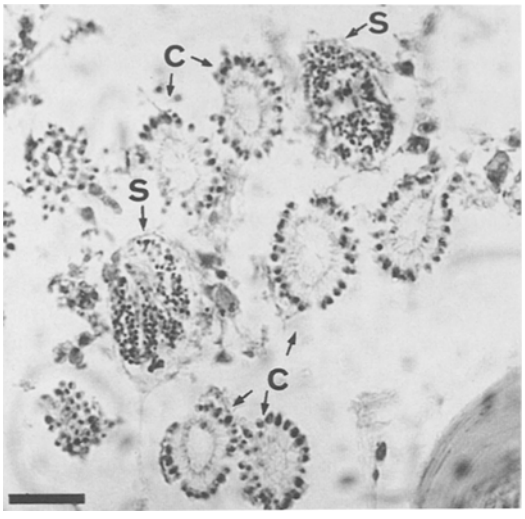


Fig. 1. *Chalinula* sp. Spermatic cysts (s) and choanocyte chamber (c); scale bar = 12.5 μm

For each individual sponge the histological preparations were examined for presence of reproductive elements to determine its sex, duration of gamete development, size and position within the choanosome. In addition, we wanted to establish (1) whether this species brooded its larvae or released gametes for external fertilization; (2) the seasonality of larval release; and (3) the type of larva developed. By slicing live sponges, free-swimming larvae were obtained for settlement experiments. These were placed in petri dishes, the bottom of which were covered with a thin layer of celluloid (rougher than the dish) and then filled with unfiltered seawater, which was replaced daily. Settled larvae were checked at 1 h intervals from their release until 10 h after settlement. The time intervals for settlement checking were then gradually increased to once daily.

Results

Many individual *Chalinula* sp. were observed to simultaneously contain both spermatozoa and oocytes. Thus it was established that *Chalinula* sp. is a simultaneous hermaphroditic species. Small spermatic cysts full of spermatids (Fig. 1) were observed distributed in the choanosome. They ranged in size from 12.5 to 45 μm with an average of 26 ± 7 μm. Oocytes could be detected at 15 μm, but ripe oocytes reached a diameter of 420 μm (average 355 ± 37 μm) (Fig. 2a). The mode of oocyte growth was by accumulation of nurse cells through phagocytosis (Fig. 2b).

Fertilized oocytes were always brooded to a typical haplosclerid parenchymella larvae (370 ± 43 μm) with a ring of longer cilia near a bare posterior end (Fig. 3). Brooding took place in special brooding chambers ("nurseries") (Fig. 4). TEM examination indicated that larvae contained organized choanocyte chambers. Though no operational canal system was evident at this stage, choanocytes were already morphologically fully developed (Fig. 5). Since there was

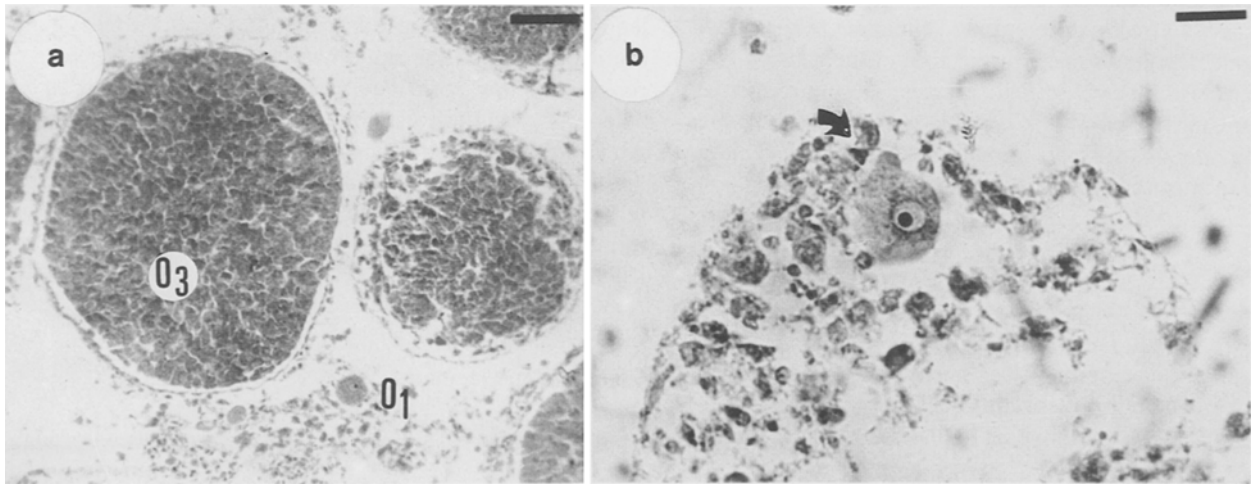


Fig. 2. *Chalinula* sp. Oocytes development. a: Primary oocyte (o_1) next to mature oocyte (o_3). Note cells that encircle oocytes during development; scale bar = 50 μm. b: Young oocyte during process of engulfing a nurse cell (arrow). Note nucleus with prominent nucleolus; scale bar = 12.5 μm

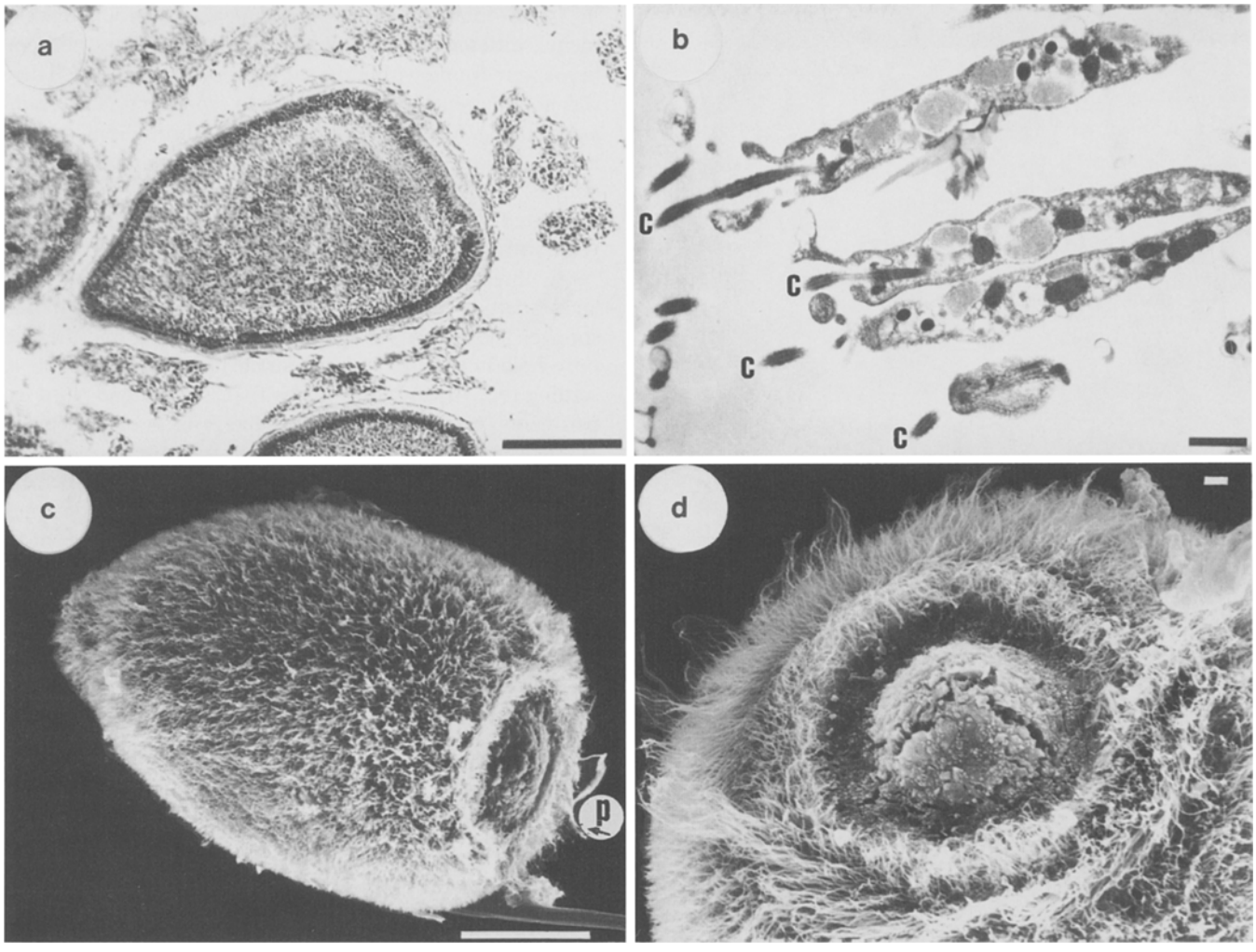


Fig. 3. *Chalinula* sp. a: Longitudinal section through larva still incubated within the parental brooding chamber; scale bar = 1 μm. b: All surface cells of larva are ciliated (c); scale bar = 1 μm. c: Larva covered with cilia, note the posterior end (p) with ring of longer cilia; scale bar = 100 μm. d: Posterior end of larva with central portion denuded of cilia and circumference with ring of long cilia; scale bar = 10 μm

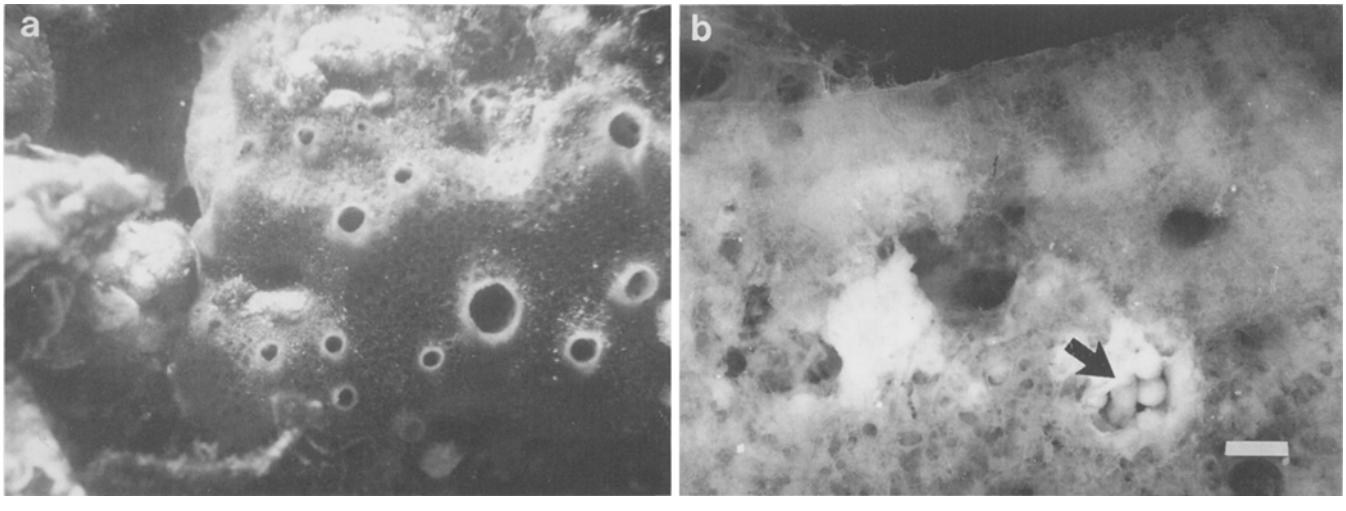
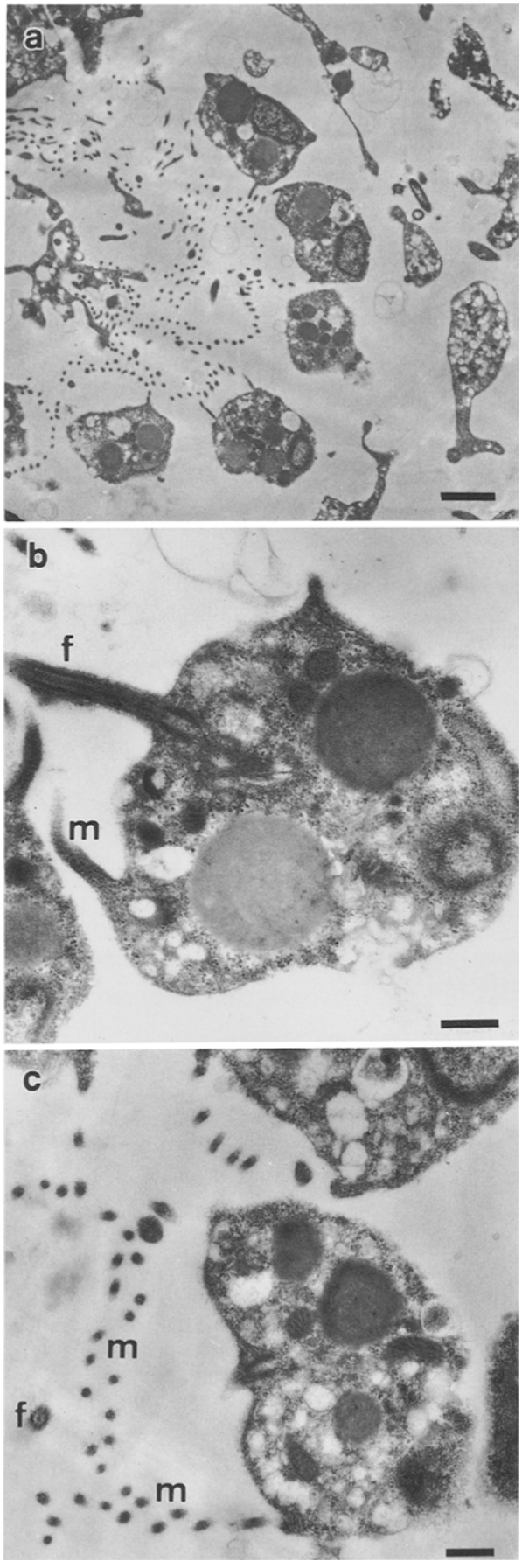


Fig. 4. *Chalinula* sp. a: The sponge in its microhabitat. b: Section of sponge with brooding chamber (arrow) full of oocytes, embryos, and larvae; scale bar = 1 mm



no synchronization in the development of reproductive elements, different developmental stages of oocytes and embryos were found within the same brooding chamber. There was no apparent seasonal pattern in reproduction of *Chalinula* sp. (Fig. 6). Spermatic cysts existed throughout the year in the majority of the sponge population (Fig. 6a) and oocytes and embryos, as well as larvae, were also found incubated within the sponge choanosome throughout the year, with only a few exceptions (Fig. 6b–d).

In three different settlement experiments, 50% of the larvae settled within 4.5 h of release from the parental sponge. The first larvae settled 1 h after release and the last, only 7.5 h later (Fig. 7). Most larvae (74%) were capable of settling (72, 70, and 86% in the first, second and third experiments, respectively). During the process of settlement, larvae usually approached the substratum anteriorly. It was noticed that larvae preferred rough surfaces for settlement rather than smooth. Following attachment to the substrate, the process of metamorphosis from the general shape of an oval larva to a flat cap-shaped sessile sponge began and lasted 1 to 6 h. During this process the surface cells reabsorbed the cilia (Fig. 8).

Discussion

Chalinula sp. is a viviparous species similar to other haplosclerid sponges (e.g. Tuzet 1932, Liaci et al. 1973, Elvin 1976, Fell 1976, Ilan and Loya 1990). On the other hand, unlike most haplosclerid sponges, this species is hermaphroditic as found also for *Haliclona oculata* (Wapstra and Soest 1987) and *Niphates* sp., thus verifying that gonochorism is not the rule in the order Haplosclerida (Ilan and Loya 1990).

The size of *Chalinula* sp. spermatic cysts (26 ± 7 μm) is among the smallest known for sponge species which range from 15 to over 200 μm (Reiswig 1983). The size of oocytes is among the largest known, within the range reported for other sponges (Fell 1983).

Prior to this study, the larvae of two other species from the genus *Chalinula* have been studied, one from the temperate waters of New Zealand (Bergquist et al. 1979) and the other from a similar environment on the coast of the Netherlands (Wapstra and Soest 1987). Morphological description of the larvae differ between the two studies. The larvae from New Zealand lack long posterior cilia which are present on the surface of the larvae from the Netherlands. Bergquist et al. (1979), who reported generic differences between haplosclerid larvae, placed "*Chalinula*" in the genus *Reniera*, based on the absence of longer cilia from the posterior end of their larvae in addition to skeletal criteria. However, it

Fig. 5. *Chalinula* sp. Choanocyte chamber found inside a larva. a: Choanocyte chamber; scale bar = 2 μm. b: Choanocyte; note flagellum (f) connection to cell body and microvilli (m) which compose the cell collar; scale bar = 500 nm. c: Choanocyte; note the cross section through the collar's microvilli (m) and flagellum (f); scale bar = 500 nm

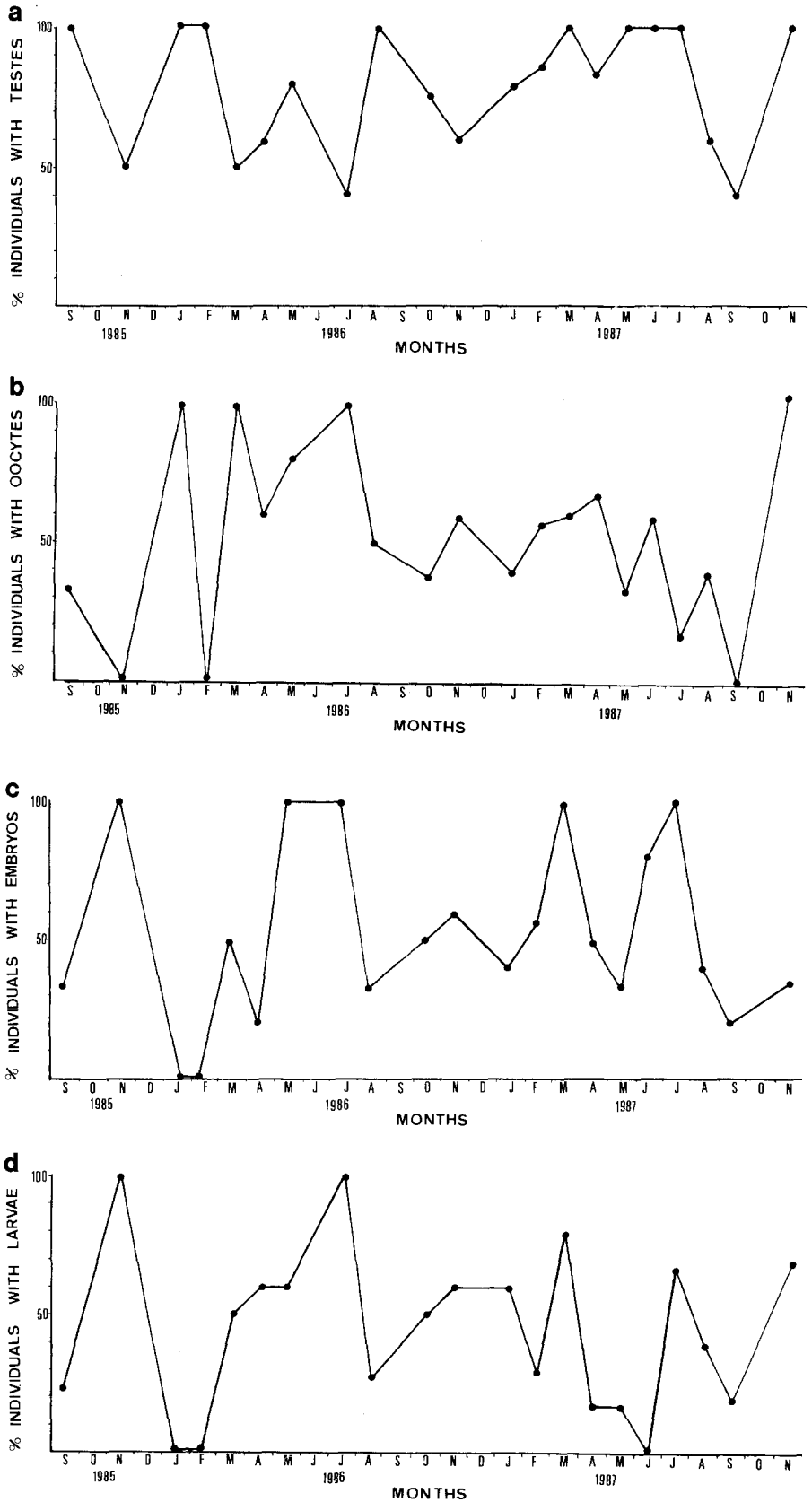


Fig. 6. *Chalinula* sp. Reproduction cycle of a population during 1985 to 1987. Percent of individuals in the population found with a: spermatic cysts (testicular follicles); b: oocytes; c: embryos; and d: larvae

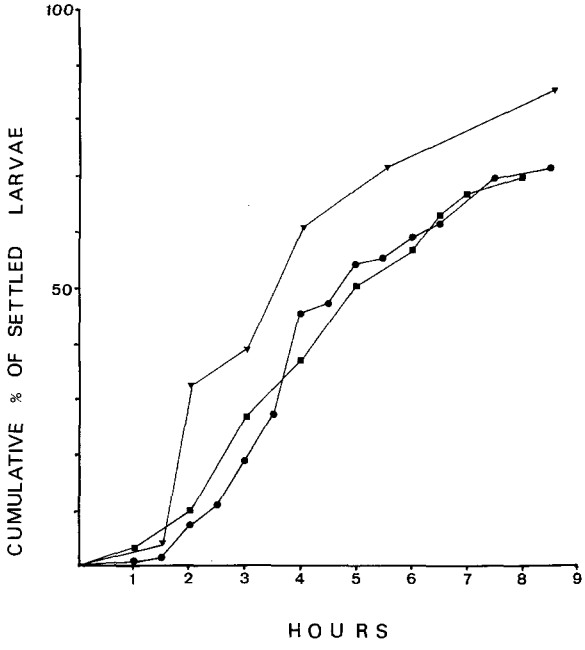


Fig. 7. *Chalinula* sp. Cumulative percent of settled larvae during settlement experiments. Experiments carried out in: (●) June 1988, $n=136$; (■) July 1988, $n=30$; and (▼) September 1988, $n=28$

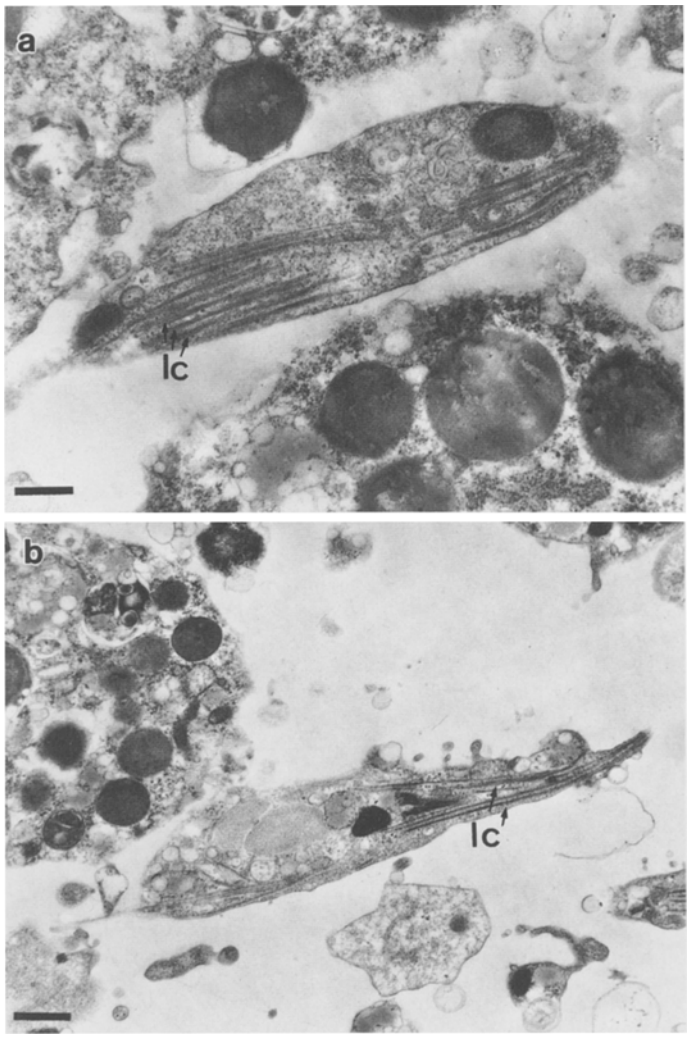


Fig. 8. *Chalinula* sp. Larval cilia during reabsorption. Two surface cells (a, b) reabsorbing larval cilia (lc) during metamorphosis. a: Start of reabsorption; scale bar = 500 nm. b: Later stage after cell growth and accumulation of cilia; scale bar = 1 μm

was also synonymized with the genera *Haliclona* (Soest 1980) and *Acervochalina* (Weerdt 1986, Wapstra and Soest 1987) or claimed to be a valid genus (Boury-Esnault and Lopes 1985). Wapstra and Soest (1987) reported *Acervochalina loosanoffi* larvae as having a ring of longer cilia at the posterior pole, unlike the description given by Fell (1976). It seems that larval morphology may not contribute greatly to the debate over the taxonomic position of the genus *Chalinula,* although the present study strengthens Weerdt's revision.

The phenomenon of a sponge species which always broods its oocytes in clusters to the larval stage, in special brooding chambers, was first reported for *Niphates* sp. from the Red Sea (Ilan and Loya 1990). We report here on another species from the same region, *Chalinula* sp., which also always has brooding chambers. In order to examine whether this is a more common phenomenon than previously imagined, some Australian haplosclerid sponges were studied at the Australian Museum. One of the species studied, *Haliclona finitima* (G.5434) collected at Watsons Bay, Port Jackson, N.S.W., Australia, also contained brooding chambers. Histological preparations revealed that the specimen originally presumed to contain gemmules, was actually filled with embryos and larvae clustered in large, irregularly organized brooding chambers. This finding of brooding chambers in three species belonging to two closely related families Haliclonidae and Niphtidae (Soest 1980), suggests that this type of brooding chamber might be common among members of the order Haplosclerida.

It appears that the population of *Chalinula* sp. at Eilat reproduces all the year around. The male gametes are present throughout the year in most of the sponge population (Fig. 6). Larvae and embryos were absent only in January and February 1986, during which only three individuals were sampled. Hence, a low percentage of reproductive individuals during these months may not be representative of the entire population. The continuous reproduction of *Chalinula* sp. makes it possible for its larvae to settle on vacant space opened on the reef throughout the year. This is in strong contrast to the seasonal pattern of reproduction demonstrated by the sympatric sponge *Niphates* sp. which has no larvae during winter (Ilan and Loya 1990). It would be interesting to study whether *Chalinula* sp. larvae are more capable of competing with the fast growing turfs and macroscopic noncalcareous algae during their winter bloom (Benayahu and Loya 1977) than are larvae of *Niphates* sp., or whether they avoid competition by settling on open substrates.

Several factors reflect the preparation for rapid settlement provided by the maternal assistance during brooding. Among these factors are the large size of spawned larvae

relative to oocytes of oviparous species (Ilan and Loya unpublished data), the high percentage of their settlement (74%) and the short time they spend swimming (1 to 6 h). The readiness for settlement is further indicated by the choanocyte chambers within the larvae which can function soon after metamorphosis, following creation of the canal system.

The period a *Chalinula* sp. larva spent swimming was also much shorter then that reported for *Niphates* sp. (2 to 3 d) which demonstrate a different strategy. *Chalinula* sp. larvae are produced all year around, then settle and metamorphose shortly after release. This enables them to find newly opened spaces on the reef throughout the year. On the other hand, *Niphates* sp. larvae, which are produced seasonally, avoid winter algae and have a longer period of dispersal (Ilan and Loya 1990). As a consequence, their larva can disperse throughout a wider range and search for an appropriate place to settle which may lead to a higher genetical mixing. Both *Chalinula* sp. and *Niphates* sp., breed over a long period, in contrast to the situation in stony corals from the same region which reproduce during only a few nights of the year (Shlesinger and Loya 1985). Lengthening the spawning period enables sponges to overcome the potential threat of losing a year's reproduction if unfavorable conditions occur during a short reproduction period. By means of these two different strategies, which share only certain traits, the two species have became most abundant in the shallow waters of the coral reefs of Eilat.

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