

Larval settlement of *Sabellaria cementarium* Moore, and comparisons with other species of sabellariid polychaetes

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Tube-building marine worms of the polychaete family Sabelliidae exhibit a wide range of settlement strategies; the larvae of some species settle with near-absolute specificity on the sandy tubes of conspecific adults to build extensive reefs, while others are not gregarious and construct solitary or paired tubes. *Sabellaria cementarium* forms aggregations in some localities, but it is nongregarious throughout much of its range. Larvae of *S. cementarium* were competent to metamorphose in 25 days at 15°C, much sooner than previously reported and within the range of other sabelliids cultured under the same conditions. In two of three experiments, there were no significant differences in larval settlement on conspecific tube sand versus control sand or tube sand extracted with organic solvents, suggesting that this species does not aggregate in response to a chemical cue. Settlement was not enhanced in response to free fatty acids, naturally occurring inducers of settlement for two reef-forming sabelliids, *Phragmatopoma lapidosa lapidosa* and *P. l. californica*. In reciprocal assays, larvae of *S. cementarium* and *P. l. californica* did not significantly discriminate between conspecific and heterospecific tube sand; yet, where they co-occur, the tubes of the former species are not found in aggregations of the latter. The two species were not interfertile, although one cross resulted in ~14% fertilization with larvae dying shortly after hatching. The processes by which larvae of *S. cementarium* avoid settlement on the tubes of *P. l. californica* (and the reverse for the latter species) remain unclear.

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Les polychètes marines tubicoles de la famille des Sabelliidae font preuve d'un éventail complexe de stratégies de colonisation; les larves de certaines espèces s'établissent selon une spécificité quasi absolue dans les tubes sablonneux d'adultes conspécifiques et construisent de vastes récifs, alors que d'autres n'ont pas de tendance grégaire et construisent des tubes isolés ou pairés. *Sabellaria cementarium* forme des rassemblements en certains endroits, mais est solitaire dans une grande partie de sa répartition. Les larves de *S. cementarium* se sont avérées capables de se métamorphoser en 25 jours à 15°C, ce qui est beaucoup plus rapide qu'on ne l'avait cru à ce jour et cette durée est du même ordre que celle qui prévaut chez d'autres sabelliidés élevés dans les mêmes conditions. Dans deux expériences sur trois, nous n'avons pas constaté de différences significatives entre la colonisation de tubes de sable d'individus conspécifiques et la colonisation de tubes de sable témoins ou de tubes de sable extrait au moyen de solvants organiques; ce résultat indique que la formation de rassemblements n'est pas sous le contrôle de stimulus chimiques. La tendance grégaire n'est pas accentuée par la présence d'acides gras libres, substances reconnues comme des déclencheurs de colonisation chez deux sabelliidés de récifs, *Phragmatopoma lapidosa lapidosa* et *P. l. californica*. Au cours d'expériences réciproques, les larves de *S. cementarium* et celles de *P. l. californica* n'ont pas démontré de tendance significative à la discrimination entre les tubes de sable d'individus conspécifiques et les tubes d'individus hétérospécifiques; cependant, là où les deux espèces cohabitent, les tubes de *P. l. californica* ne se retrouvent pas dans les rassemblements de *S. cementarium*. Les deux espèces peuvent se croiser, bien qu'un croisement ait donné lieu à ~14% de fécondation et que les larves soient mortes peu après l'éclosion. Les mécanismes qui empêchent l'établissement de *S. cementarium* dans des tubes de *P. l. californica* et inversement l'établissement de *P. l. californica* dans les tubes de *S. cementarium* demeurent obscurs.

[Traduit par la rédaction]

Introduction

Sabelliid polychaetes are sedentary marine worms that live in tubes built of cemented grains of sand. Approximately two-thirds of the extant species construct solitary tubes, while the majority of the remaining species aggregate to form colonies and, in some localities, extensive reefs (Kirtley 1974; Pawlik and Faulkner 1988). The growth of colonies and reefs is dependent on the recruitment of planktonic larvae, which settle preferentially on the sand tubes of adult worms (Wilson 1968; review in Pawlik and Faulkner 1988).

Previous reports have demonstrated that settlement of gregarious sabelliids occurs when larvae contact specific chemical signals (metamorphosis is part of the process of settlement, and the two terms are used interchangeably herein; see Pawlik 1990b). Larvae of the New World subspecies *Phragmatopoma lapidosa lapidosa* and *P. l. californica* settle in response to free

fatty acids (FFAs) isolated from organic extracts of the tube sand of adult worms (Pawlik 1986, 1988b). Their responses are highly specific, dependent on the length and confirmation of the acyl chain of the FFA molecule and on the presence of a free carboxylic acid functional group (Pawlik and Faulkner 1986; Pawlik 1988b). However, other sabelliids do not respond to FFAs. Larvae of *Sabellaria alveolata*, a reef-forming species from European waters, preferentially settled on the tubes of conspecific adults, but the techniques that led to the isolation of the chemical cues responsible for settlement of *Phragmatopoma* did not result in the identification of the chemical factors responsible for gregarious settlement of *S. alveolata* (Pawlik 1988a). Moreover, the same FFAs that had induced settlement of *Phragmatopoma* either had no effect on, or inhibited, settlement of *S. alveolata* (Pawlik 1988a).

The settlement responses of nongregarious sabelliids are not as well known. *Sabellaria floridensis* from the Gulf of Mexico and Caribbean occurs in deep water as solitary individuals or clumps of a few tubes on shells or debris resting on bottom sediments. Their larvae showed no preference for

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sand from conspecific tubes over sand from other sources, and again, FFAs that induced settlement of *Phragmatopoma* had little effect on larvae of *S. floridensis* (Pawlik 1988b).

In this study, we investigated the settlement responses of *S. cementarium* Moore, 1906. This species is found intertidally and subtidally throughout the North Pacific (Ushakov 1955; Hartman 1969). Unlike most sabellariids, it forms aggregations in some localities but not in others. In the inshore waters of Washington and British Columbia, it forms small clumps on rocks and shells; near Coos Bay, Oregon, it builds reefs with another sabellariid, *Idanthyrus ornamentatus* (Posey *et al.* 1984); but on the open coast of California, *S. cementarium* is usually found singly or in pairs on rocks or debris. Throughout the southern extension of its distribution in the eastern North Pacific, *S. cementarium* co-occurs with the reef-forming species *P. l. californica*, yet individuals of the former are not found in aggregations of the latter (J. R. Pawlik, personal observation).

Smith and Chia (1985a, 1985b) previously described the larval development and metamorphosis of *S. cementarium*, with an emphasis on larval and juvenile morphology. They reported that larvae of *S. cementarium* did not settle in the absence of sand, but they would metamorphose on sand from an intertidal embayment, sand from the tubes of conspecifics, and sand from the tubes of two heterospecifics, *P. l. lapidosa* and *I. ornamentatus*. However, these experiments were only preliminary; they were not replicated and were run for 18 days prior to the assessment of results.

This report follows investigations of the settlement responses of three gregarious sabellariids, *P. l. californica*, *P. l. lapidosa*, and *S. alveolata*, and one nongregarious species, *S. floridensis* (Pawlik 1986, 1988a, 1988b, 1990a; Pawlik and Faulkner 1986, 1988). The intent of the present investigation was threefold: (i) to compare the rates of larval development of *S. cementarium* with those of previously studied species, using the same culture procedures, (ii) to compare the settlement of *S. cementarium*, which is found in aggregations only under some circumstances, with that of previously studied gregarious and nongregarious species, and (iii) to determine the extent to which larval responses of *S. cementarium* might overlap with those of *P. l. californica*, a reef-forming species that is sympatric with *S. cementarium* over part of its range.

Materials and methods

Clumps of adult *S. cementarium* on rocks and shells were dredged (40–60 m depth) from the waters surrounding the San Juan Islands (particularly from off Rock Point, Lopez Island), Washington, May through July 1989. Living reef chunks of *P. l. californica* were collected near Ladera Street, Point Loma, San Diego, and Dike Rock, La Jolla, California, and transported to the Friday Harbor Laboratories, University of Washington, in January and May 1989. Animals were kept in aquaria with flowing seawater; aquaria containing *P. l. californica* were maintained at 18°C with a submersible heater, while aquaria containing *S. cementarium* remained at ambient seawater temperatures (12–15°C).

Cross-fertilization experiments were performed as a simple assessment of genetic similarity between *S. cementarium* and *P. l. californica* (Pawlik 1988b). For each species in a given experiment, 2000–3000 unfertilized eggs were added to each of three 100 mm diameter polystyrene petri dishes containing 50 mL of filtered (1 µm) seawater (FSW) at 15–18°C. One dish was set aside without addition of sperm to serve as a control. Five drops of undiluted sperm were added to 10 mL FSW, mixed, and five drops of this suspension were added to a dish containing conspecific eggs. The dish was lightly stirred and the addition of sperm was repeated twice after 10-min intervals. Then, the

seawater and sperm suspension in the dish was decanted and replaced with FSW. Cross-fertilizations were undertaken in the same fashion. After 24 h at 15–18°C, the entire contents of each dish were transferred to a 100-mL graduated cylinder that had been wrapped in black electrical tape, and the contents of the cylinder brought up to 100 mL. The cylinders were placed under fluorescent lights and after 10 min, swimming trochophores were distributed near the top of the cylinders and unfertilized eggs sank to the bottom. For each cylinder, trochophores were pipetted into one dish and unfertilized eggs into another. The trochophores were fixed with several drops of 10% Formalin in FSW. Each dish was then placed on a transparent grid of 1-cm squares, and the number of either eggs or trochophores was counted in each of the 10 squares chosen haphazardly. Percentage fertilization was calculated from the mean values of 10 counts. The entire experiment was repeated on 3 separate days.

Methods for larval culture are fully described in Pawlik (1986, 1988a). Newly hatched trochophores were transferred to 4-L glass jars filled with 3 L FSW (final concentration of ~1 larva/mL) containing 40 mg/L each of streptomycin sulfate and sodium penicillin G and a 1:1 mixture of *Phaeodactylum tricornutum* Bohlin and *Pavlova lutheri* (Droop) at ~10⁵ cells/mL. Jars were placed in 15 or 20°C constant-temperature baths beneath a fluorescent lamp set for a 14 h light : 10 h dark cycle. Larval cultures were continuously agitated by motor-driven paddles. Culture jars were cleaned and media changed every other day. Separate jars were maintained to monitor larval growth, the onset of metamorphic competence, and metamorphosis in culture (Pawlik 1988a). At the time of hatching, and every other day thereafter, 10 larvae were removed from jars at each temperature, narcotized in 0.33 M MgCl₂, and fixed in 2% buffered Formalin in FSW. The fixed larvae were examined with a stereomicroscope at 25× magnification and the body length recorded. Beginning on day 20 after hatching, 20–30 larvae from jars at each temperature were added to dishes containing conspecific tube sand and percent metamorphosis was recorded after 24 h (see below). Assays for metamorphic competence were discontinued after 50% or more of the larvae metamorphosed in response to conspecific tube sand.

Tube sand of *P. l. californica* was prepared by covering living reef chunks with a standard sand, allowing the adult worms to incorporate the sand into their tubes, then removing the newly formed portions of the tubes for use in assays (Pawlik 1986, 1988a). Because the tubes in clumps of adult *S. cementarium* are arranged haphazardly, the same techniques could not be used; rather, assays of the tube sand of this species employed natural tube sand from whole, occupied tubes. Adult worms were removed from tubes, tubes were crushed, tube sand was rinsed in deionized water, frozen, lyophilized, and sieved (retained on mesh sizes 25 and 40) before use in larval assays. A portion of the tube sand was successively extracted for ~3 h in each of four solvents or solvent mixtures of increasing polarity: hexane, diethyl ether, 1:1 diethyl ether – methanol, and methanol. Extracted tube sand was then placed under vacuum for 15–20 min to remove all traces of organic solvents. Control sand was prepared by placing shallow dishes of clean standard sand in aquaria containing clumps of adult *S. cementarium* for several days to allow establishment of a microbial film on the sand grains; then the sand was rinsed, lyophilized, and sieved in the same manner as tube sand.

Larval assays were performed as in Pawlik (1988a). FFA standards were adsorbed onto sand grains by spreading organic solvent solutions containing known concentrations of each extract or compound onto clean sand and allowing the volatile solvent to evaporate. For each replicate, 1 g of a given sand type was added to a 60 mm diameter polystyrene petri dish filled with FSW and containing 20–30 larvae. Dishes were placed on a gently rotating platform for 24 h, then examined under a stereomicroscope at 15× magnification, and larvae were scored as not metamorphosed (swimming or crawling, no loss of provisional chaetae), metamorphosed (attachment, loss of provisional chaetae, rotation of larval tentacles, commencement of tube construction), or abnormal (loss of chaetae without attachment or rotation of tentacles, little or no movement or death). The mean percent larval response was determined for three to five replicates. Differences in mean larval metamorphosis were tested with one-way analysis of

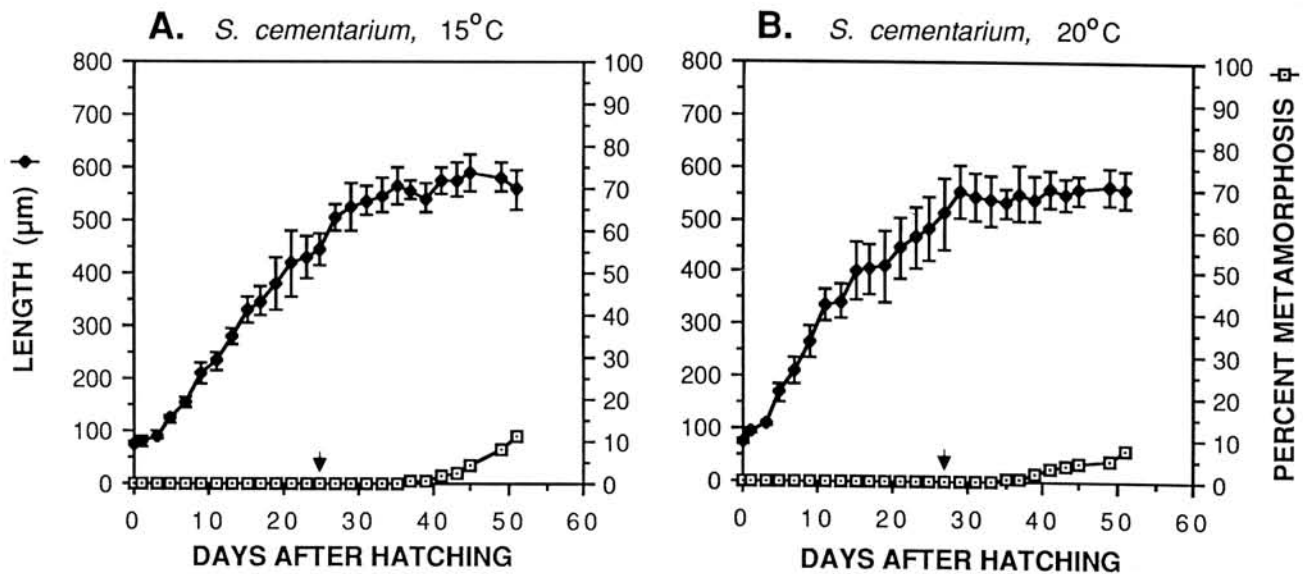


FIG. 1. Growth, maturation, and cumulative percent metamorphosis of larvae of *Sabellaria cementarium* in culture at (A) 15°C and (B) 20°C. Vertical bars represent one standard deviation above and below mean larval length ($N = 10$). The arrow indicates the day on which 50% or more of a sample of 20–30 larvae settled in assays of conspecific tube sand.

variance (parametric ANOVA) performed on arcsine-transformed data. Tukey's honestly significant difference method (T method) was employed *a posteriori* to determine which treatments resulted in different mean larval response at the 0.05 level of significance (Sokal and Rohlf 1981).

Results

Data on larval growth, the onset of metamorphic competence, and larval metamorphosis in culture jars for *S. cementarium* at 15 and 20°C are presented in Fig. 1 and Table 1. A full account of the ontogenetic changes in the morphology of larvae of *S. cementarium* is presented in Smith and Chia (1985a, 1985b). Developmental rates were faster for *S. cementarium* in this study than had been previously observed: Smith and Chia (1985a) reported the onset of competence in 5–6 weeks, with settlement occurring in 6–8 weeks at 10–14°C; in this study, 50% of assayed larvae settled after 25 days (3.6 weeks) at 15°C. Larvae were also considerably larger at competence, measuring $444 \pm 30 \mu\text{m}$ after 25 days and reaching a maximum mean length of $591 \pm 35 \mu\text{m}$ after 45 days at 15°C, whereas Smith and Chia (1985a) reported that metamorphosing larvae measured $\sim 350 \mu\text{m}$ after 6–8 weeks at 10–14°C.

Sabellaria cementarium and *P. l. californica* were not inter-fertile (Fig. 2). Although the cross of eggs of *P. l. californica* with sperm of *S. cementarium* resulted in mean of 13.9% swimming trochophores, these larvae swam abnormally and died within a few days of hatching. *Phragmatopoma lapidosa californica* and *S. alveolata* were previously found not to be inter-fertile (Pawlik 1988a) as were *P. l. californica* and *S. floridensis* (Pawlik 1988b). Trochophores that swam normally were produced from the fertilization of eggs of *S. floridensis* with sperm of *S. alveolata*, but the reciprocal cross did not result in fertilized eggs (Pawlik 1988b). The two subspecies of *Phragmatopoma* were fully inter-fertile; this, in part, resulted in their synonymization (Pawlik 1988b).

The percentage of larvae of *S. cementarium* that metamorphosed in response to microbially filmed control sand, conspecific tube sand extracted in a series of organic solvents, and unextracted conspecific tube sand, for larvae reared and assayed at both 15 and 20°C, is presented in Fig. 3. There were no

TABLE 1. Data on the growth, maturation, and metamorphosis of larvae of *Sabellaria cementarium* in culture at 15 and 20°C

	15°C	20°C
Maximum mean length (μm)	591	563
Time to maximum mean length (days)	45	49
Time to metamorphic competence (days)	25	27
% metamorphosis in culture	11.4	7.2
% accounted for at end of time course	62.4	44.0

		♀	
♂	--	Pc	Sc
		0.0±0.0	0.0±0.0
	Pc	91.8±8.0	0.1±0.1
	Sc	13.9±9.4	77.8±17.3

FIG. 2. Mean percent fertilization (\pm SD, $N = 3$) for reciprocal crosses of the gametes of *Phragmatopoma lapidosa californica* (Pc) and *Sabellaria cementarium* (Sc). The first row is a control, i.e., no sperm were added.

significant differences in mean larval responses among treatments for assays conducted at either temperature (ANOVA, 15°C: $F_{[2,12]} = 3.6$, $P > 0.05$; 20°C: $F_{[2,12]} = 1.4$, $P > 0.05$).

There were significant differences in mean larval metamorphosis among treatments in reciprocal assays of both *S. cementarium* and *P. l. californica* (Fig. 4; *S. cementarium*: $F_{[2,12]} = 5.8$, $P < 0.025$; *P. l. californica*: $F_{[2,12]} = 28.7$, $P < 0.001$). *A posteriori* analysis (T method, $\alpha = 0.05$) indicated that the mean percentage of metamorphosed *S. cementarium* was significantly greater in response to conspecific tube sand than to microbially filmed control sand. There was no difference in

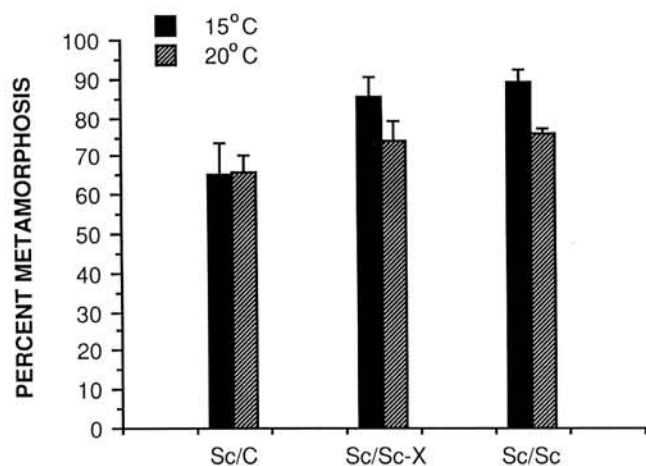


FIG. 3. Mean percent metamorphosis (\pm SE, $N = 5$) of larvae of *Sabellaria cementarium* in response to microbially filmed control sand (Sc/C), extracted conspecific tube sand (Sc/Sc-X), and conspecific tube sand (Sc/Sc) for larvae cultured and assayed at 15 and 20°C.

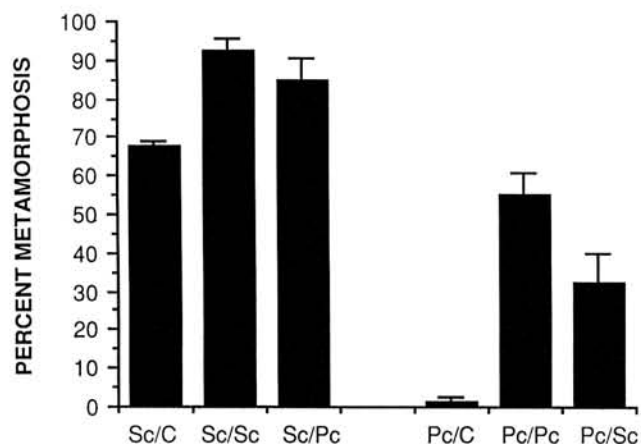


FIG. 4. Mean percent metamorphosis (\pm SE, $N = 5$) of larvae of *S. cementarium* in response to microbially filmed control sand (Sc/C), conspecific tube sand (Sc/Sc), and tube sand of *P. l. californica* (Sc/Pc) and of larvae of *P. l. californica* in response to microbially filmed control sand (Pc/C), conspecific tube sand (Pc/Pc), and tube sand of *S. cementarium* (Pc/Sc). Larvae were cultured and assayed at 18–20°C.

response to control sand versus heterospecific tube sand or between the two types of tube sand for *S. cementarium*. For larvae of *P. l. californica*, there was greater metamorphosis on both conspecific and heterospecific tube sand than on control sand, but there was no significant difference in larval response toward either type of tube sand. The significant difference in settlement of *S. cementarium* in response to conspecific tube sand versus control sand (Fig. 4) is contrary to the results of earlier experiments (Fig. 3), likely because of the very low variance in percentage metamorphosis on control sand. The magnitude of this difference, however, is not similar to that of other sabellariids that settle gregariously (*P. l. californica*, Pawlik 1986, 1988a; *S. alveolata*, Pawlik 1988a; *P. l. lapidosa*, Pawlik 1988b).

Larval responses of *S. cementarium* to FFAs of variable chain length and unsaturation at two concentrations are shown in Fig. 5. Naturally occurring FFAs induce metamorphosis of *P. l. lapidosa* and *P. l. californica* (Pawlik 1986, 1988b). At 1 mg FFA/g sand (Fig. 5A), none of the FFAs enhanced settlement

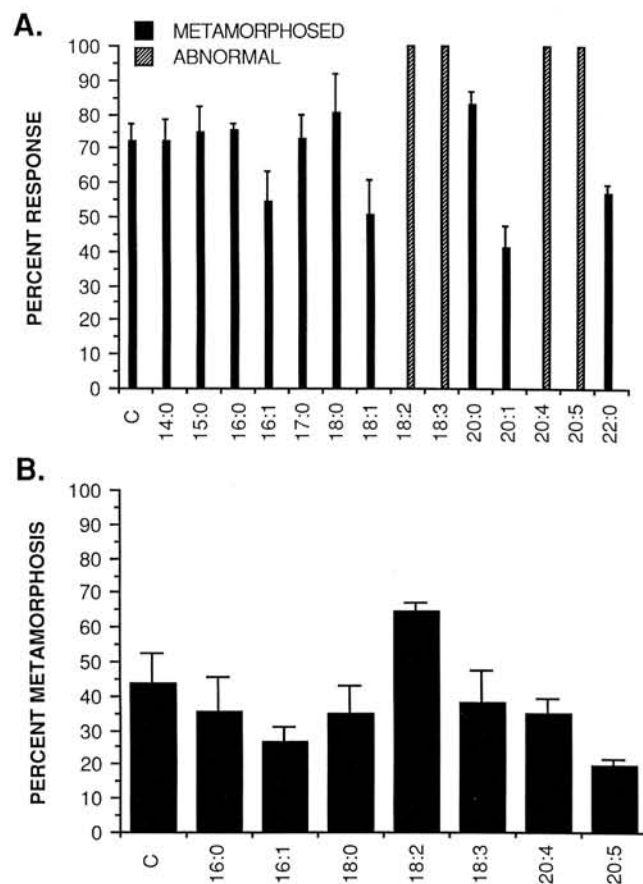


FIG. 5. Mean percent response (\pm SE) of larvae of *S. cementarium* to clean sand treated with solvent alone (C) and clean sand treated with free fatty acids (FFAs). The number of carbon atoms in the FFA molecule precedes the colon and the number of double bonds follows. (A) 1 mg FFA/g sand, $N = 3$; (B) 100 μ g FFA/g sand, $N = 5$. Larvae were cultured and assayed at 18–20°C.

over levels occurring on control sand, and there were no significant differences among the treatments that did not induce an abnormal response ($F_{[10,22]} = 2.2$, $P > 0.05$). The polyenoic acids 18:2, 18:3, 20:4, and 20:5 were toxic to larvae at 1 mg/g sand. Unlike the responses of *P. l. lapidosa* or *P. l. californica*, toxic responses of *S. cementarium* did not incorporate any of the components of normal metamorphosis (Pawlik 1988b). At FFA concentrations of 100 μ g/g sand (Fig. 5b), there were significant differences in mean larval metamorphosis among treatments ($F_{[7,32]} = 2.7$, $P < 0.025$), but none of the differences were between control sand and sand treated with any of the FFAs.

Discussion

Development time to metamorphic competence was several days longer for *S. cementarium* than for most other sabellariids that have been raised under comparable conditions (Pawlik 1988a, 1988b). Previous studies were conducted at Scripps Institution of Oceanography, La Jolla, California, whereas the present study was undertaken at Friday Harbor Laboratories, Friday Harbor, Washington. Algal cultures used as food for larvae grew more slowly at Friday Harbor than had previously been experienced at La Jolla, even though the same techniques were used. It seems likely that nutritionally poorer phytoplankton used as food for *S. cementarium* larvae might have resulted in slower growth. Larvae of *P. l. californica* were also observed

to grow slower at Friday Harbor Laboratories, but after completion of this study, a different algal culture medium was used (Alga-Gro Concentrate, Carolina Biological Supply Co.), and larval growth of *P. l. californica* returned to the rate reported in previous studies (Pawlik 1988a).

The marked variability in development times reported for larval sabellariids is undoubtedly attributable to different rearing techniques (review in Pawlik 1988b). For other sabellariids cultured at similar temperatures, development time to metamorphic competence has exhibited the following ranges: for *S. alveolata*, 25 days (Pawlik 1988a) to 6–32 weeks (Wilson 1968); for *S. floridensis*, 17 days (Pawlik 1988b) to 18–27 days (Eckelbarger 1977); for *P. l. lapidosa*, 14–30 days (Eckelbarger 1976) to 3–4 weeks (Mauro 1975); for *P. l. californica*, 16 days (Pawlik 1988a) to 18–25 days (Eckelbarger 1977). Clearly, development times for sabellariids are very flexible and caution should be exercised when trying to explain observed differences (cf. Mauro 1975). Although it seems that both temperate (*S. alveolata*, *S. cementarium*) and subtropical to tropical (*S. floridensis*, *P. l. lapidosa*, *P. l. californica*) sabellariids have comparable developmental rates under comparable culture conditions, with larvae attaining competence to metamorphose in 15–30 days, some temperature-dependent differences may exist. Larvae of the tropical species *S. floridensis* did not become competent to metamorphose over the course of a 40-day experiment when reared at 15°C but did so in 17 days at 20°C (Pawlik 1988b). Larvae of temperate *S. cementarium* became competent sooner and grew larger at 15 than at 20°C, although initial growth rates were faster at the higher temperature.

Sabellaria cementarium exhibited surprisingly low levels of metamorphosis in culture (Fig. 1, Table 1), much lower than those observed for *S. alveolata* and *S. floridensis* and on a par with *P. l. californica* (Pawlik 1988a, 1988b). There seems to be little correlation between the ability of sabellariid larvae to delay settlement in laboratory culture and their gregarious settlement to form reefs in nature (see Pawlik 1988b, p. 58). *Sabellaria alveolata*, which forms reefs in European waters, metamorphosed in culture more readily than the less gregarious *S. cementarium* (Pawlik 1988a); the opposite would have been expected if the delay of settlement were to play an important role in reef formation.

Adult *S. cementarium* were fully gravid throughout the months that experiments were run (spring–fall). Smith and Chia (1985a) stated that most sabellariids spawn all their gametes during a restricted breeding season (polytelic reproduction); this is probably not the case for *P. l. lapidosa* (Eckelbarger 1976; Pawlik 1988b), *P. l. californica* (Pawlik 1986; Barry 1989), or *S. alveolata* (Pawlik 1988a), and may not be the case for *S. cementarium*. Fertilization of eggs of *S. cementarium* in the laboratory proved difficult at times: over 80% of the males removed from tubes yielded nonmotile sperm. This problem had not been encountered for any other sabellariid species (Pawlik 1986, 1988a, 1988b). Previous attempts to study the settlement behaviour of *S. cementarium* in southern California failed for lack of viable gametes (J. R. Pawlik, personal observation). It was also discovered that the gametes of *P. l. californica* were rendered infertile if the adults were kept in seawater below 14°C for any length of time. This condition persisted even if the adults were returned to 20°C for several weeks; after a month, normal fertilization occurred again. The cause of this phenomenon is unclear, inasmuch as the affected sperm were fully motile and the eggs appeared normal. This

apparent temperature constraint may play an important role in restricting the northern distribution of *P. l. californica*.

Previous cross-fertilization experiments with sabellariids revealed that the two reef-building species of *Phragmatopoma* from the western Atlantic and eastern Pacific were completely interfertile, prompting their synonymization (Pawlik 1988b). *Phragmatopoma lapidosa californica* was not interfertile with either *S. alveolata* or *S. floridensis* and virtually no trochophores hatched from these reciprocal crosses (Pawlik 1988a, 1988b), so it is surprising that one of the crosses of *P. l. californica* with *S. cementarium* resulted in ~14% hatching, even if the trochophores developed abnormally. Smith and Chia (1985a) found that cross-fertilization of gametes of *S. cementarium* with those of another sabellariid, *I. ornamentatus*, resulted in swimming larvae that died shortly after hatching. If mixed populations of adult *P. l. californica* and *S. cementarium* were to spawn synchronously in nature, they might incur reduced fecundity because of the interaction of their gametes. Although the two species have overlapping geographical distributions, there is no evidence that they are found in close enough proximity for this to occur.

Settlement responses of *S. cementarium* appear to be quite similar to those of *S. floridensis* from the Gulf of Mexico and Caribbean (Pawlik 1988b). *Sabellaria floridensis* is a nongregarious species; unlike *S. cementarium*, it is not known to form aggregations in some parts of its range, yet both are indiscriminate in their settlement on available sand. Contrarily, *S. alveolata*, a reef-forming species from European waters, shows a significant preference for conspecific tube sand over control sand (Pawlik 1988a). Extraction of the tube sand of *S. alveolata* diminished its capacity to induce settlement (Pawlik 1988a), but this was not the case for either *S. floridensis* (Pawlik 1988b) or *S. cementarium*. These results lend further support to the hypothesis that “facultative gregariousness” (i.e., the formation of aggregations in some localities, with a nongregarious habit over the remainder of the geographic range; Pawlik and Faulkner 1986) of species like *S. cementarium* and *S. vulgaris* is under hydrodynamic control, and that the supply of larvae, rather than a specific response to adult tube sand, plays the greatest role in colony formation for these species.

Larvae of neither *S. cementarium* nor *P. l. californica* showed a significant aversion for heterospecific tube sand. This supports the previous observations that larvae of *S. cementarium* would settle as readily on tube sand of *P. l. lapidosa* and *I. ornamentatus* as on conspecific tube sand (Smith and Chia 1985a). Similar results were also reported for larvae of *S. floridensis*, which settled in reciprocal assays on tube sand of *P. l. californica* and *S. alveolata* (Pawlik 1988b). The current result is particularly interesting, however, because *S. cementarium* and *P. l. californica* have overlapping distributions. Based on the data shown in Fig. 4, the two species might be expected to form mixed aggregations. However, in southern California, specimens of *S. cementarium* were never found in intertidal reefs of *P. l. californica*, although solitary tubes containing *S. cementarium* could easily be found under intertidal boulders (J. R. Pawlik, personal observation). Although specimens of *S. cementarium* used in this study were not from southern California, estimates of the planktonic life-span of sabellariid larvae range from 2 to 11 months (Wilson 1971; Barry 1989), so it seems unlikely that populations in the southern extent of the range would have been sufficiently isolated to evolve different settlement behaviours.

It is unclear why *S. cementarium* and *P. l. californica* do not

form mixed aggregations in nature. Certainly, selective pressures would tend to favour the avoidance of heterospecific settlement, or at least of heterospecific spawning, given the fatal interaction of the gametes of the two species. Differences in the locations of competent larvae in the water column may serve to separate the two species at the time of settlement. Adult *S. cementarium* are most abundant at greater depths (>20 m), whereas *P. l. californica* appear to prefer intertidal and shallow subtidal habitats. Larvae may partition themselves as a function of water depth by responding to light, temperature, or pressure. Grosberg (1982) found that the vertical zonation of two species of intertidal adult barnacles was directly reflected in the vertical distribution of their larvae in the water column and in the pattern of their subsequent settlement. Hydrodynamic cues may also be important. Colonies and reefs of *P. l. californica* are very distinctive in their formation, quite unlike the clumps of tubes formed by *S. cementarium*, and larvae may differentially respond to the patterns of flow over these different aggregations.

A similar situation may exist for the interaction between *S. cementarium* and another sabellariid, *I. ornamentatus*. These two species co-occur from Alaska to northern California (Hartman 1969) and were reported by Posey *et al.* (1984) to have formed a sizable reef near Coos Bay, Oregon. Smith and Chia (1985a) also found *I. ornamentatus* mixed in among *S. cementarium* collected from off San Juan Island, Washington. They noted that both species produced ripe gametes at the same time and that cross-fertilization resulted in larvae that died soon after hatching. Smith and Chia (1985a) suggested that reproductive isolation of these two species may occur by temporal separation of spawning or hybrid inviability. Interestingly, Posey *et al.* (1984) found a distinct zonation pattern of the two species in the subtidal reef they studied: the upper reef was almost entirely made up of tubes of *S. cementarium* and the lower reef of *I. ornamentatus*. Unfortunately, no *I. ornamentatus* were encountered in collections for the present study.

Specific FFAs are responsible for the natural induction of settlement of *P. l. californica* and *P. l. lapidosa* (Pawlik 1986, 1988b; Pawlik and Faulkner 1986); the same FFAs are ineffective at inducing settlement of three *Sabellaria* species: *S. alveolata* (Pawlik 1988a), *S. floridensis* (Pawlik 1988b), and *S. cementarium* (this study). Moreover, the concentration of FFAs in tube sand of *P. l. californica* and *P. l. lapidosa* was more than an order of magnitude greater than that found in tube sand of *S. alveolata* (Pawlik 1988a, 1988b). Such a clear distinction between these two genera of sabellariids suggests that the responses of *Phragmatopoma* larvae to FFAs are part of a highly specific chemosensory mechanism for recognizing the presence of adult conspecifics.

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