LARVAL SETTLEMENT AND METAMORPHOSIS OF TWO GREGARIOUS SABELLARIID POLYCHAETES: *SABELLARIA ALVEOLATA* COMPARED WITH *PHRAGMATOPOMA CALIFORNICA*

JOSEPH R. PAWLIK

Scripps Institution of Oceanography; A-008, University of California, San Diego, La Jolla, California 92093, U.S.A.

(Figs. 1–12)

Two sabellariid polychaetes, *Sabellaria alveolata* from European waters and *Phragmatopoma californica* from the west coast of North America, are known from previous work to have larvae that settle and metamorphose preferentially on the cemented sand tubes of conspecific adults. The naturally occurring inducers of larval metamorphosis were recently isolated and identified for *P. californica*. In the present study, larval behaviour of *S. alveolata* and *P. californica* was compared in reciprocal laboratory settlement assays. For both species, metamorphosis occurred to a greater extent on conspecific tube sand than on control sand or on heterospecific tube sand. Extraction of the tube sand of *S. alveolata* with organic solvents diminished its capacity to induce metamorphosis of conspecific larvae, but this capacity was not transferred to the extracts, as was the case for *P. californica*. The substance responsible for the enhanced metamorphosis of *S. alveolata* on conspecific tube sand remains unknown. The free fatty acid (FFA) inducers of larval metamorphosis of *P. californica* either inhibited, or had no effect on, metamorphosis of *S. alveolata*. Both species responded abnormally upon exposure to unnaturally high concentrations of certain (particularly polyenoic) FFAs. Abnormal larval responses of *S. alveolata*, however, did not incorporate behavioural components of normal metamorphosis, as were observed for *P. californica*. FFAs were isolated from the natural tube sand of *S. alveolata* at less than one-tenth the concentration found in the natural tube sand of *P. californica*. The differences between the two species provide further evidence that a very specific mechanism is responsible for the perception of FFAs by the larvae of *P. californica*.

Metamorphosing larvae of *S. alveolata* were much less discriminating in their choice of substrata than were those of *P. californica*. When averaged over all assays performed, 43·7% of the larvae of *S. alveolata* metamorphosed on control sand, whereas only 1·5% of the larvae of *P. californica* did the same. Perhaps for concomitant reasons, a high percentage of the larvae of *S. alveolata* metamorphosed spontaneously in culture vessels shortly after the onset of metamorphic competence, while larvae of *P. californica* postponed metamorphosis indefinitely under the same circumstances.

Agitation of assay dishes containing conspecific tube sand significantly enhanced metamorphosis of *P. californica* but had no effect on larval responses of *S. alveolata*. For both species, the metamorphosis-inducing capacity of sand from recently cemented natural tubes was not significantly different from that of sand from older natural tubes. The basal remnants of damaged or destroyed colonies, therefore, are likely to stimulate larval settlement for both species, a proposition supported by field observations.

INTRODUCTION

The distribution and abundance of most benthic marine invertebrates ultimately result from the recruitment of their planktonic larvae, which may forego

settlement and metamorphosis in the absence of an environment suitable for adult survival. Consequently, there has been considerable interest in the responses of invertebrate larvae to various physical, chemical and biological factors that influence substrate selection (for recent reviews see Burke, 1983; Crisp, 1984; Hadfield, 1986). Several studies have demonstrated that specific chemical cues are required for the initiation of larval settlement, particularly among species closely associated with other organisms, conspecific adults or microbial films (Crisp, 1984; for recent references see Hadfield, 1986; Pawlik & Faulkner, 1986), although the unambiguous identification of these chemical signals has been accomplished in only a few cases (Kato *et al.* 1975; Pawlik, 1986).

Studies of the larval settlement responses of gregarious sabellariid polychaetes have demonstrated a high degree of substrate specificity (Eckelbarger, 1978). These worms construct around themselves tubes of cemented grains of sand. Adult worms have separate sexes and broadcast their gametes on a broadly defined seasonal basis or in response to physical disturbances that result in tube damage (Curtis, 1978; Gruet & Lassus, 1983; Barry, 1987). The resulting larvae feed in the plankton for several weeks to months before attaining a level of maturity at which metamorphosis may take place. Metamorphically competent larvae then tactually explore potential substrata with their tentacles and bodies (Eckelbarger, 1978; Smith & Chia, 1985; Amieva & Reed, 1987; Amieva, Reed & Pawlik, 1987). Settlement (attachment) immediately precedes metamorphosis (the terms are used interchangeably herein), which results in the loss of larval provisional setae, anterior rotation of the larval tentacles and opercular cirri, reduction of the episphere, formation of a primary mucoid tube and initiation of sand-tube construction. Several species within the Sabellariidae are gregarious and form large mounds and reefs of aggregated tubes in the lower intertidal and subtidal along rocky coasts worldwide (for references see Pawlik, 1986; Pawlik & Faulkner, 1987).

Over a span of six decades, Douglas P. Wilson published a definitive series of papers on British sabellariids that provided some of the earliest evidence of delayed metamorphosis and specificity of larval settlement among marine invertebrates. Larvae of the reef-forming species, *Sabellaria alveolata* (L.), were stimulated to settle and metamorphose in greater numbers in response to contact with conspecific adult tubes or with the mucoid tubes of recently metamorphosed juvenile worms (Wilson, 1968*b*). In the absence of these stimuli, larvae could postpone metamorphosis for several weeks. Settlement was enhanced by water motion and the presence of sand grains in suspension, but physical factors generally had little effect on larval responses. The substance responsible for larval metamorphosis did not dissolve in water and was unaffected by drying, but treatment of mucoid tubes with concentrated acid resulted in the loss of the inductive capacity. Sand tubes from a long-dead colony retained some capacity to induce larval metamorphosis, although less so than recently constructed tubes.

The larvae of *Sabellaria spinulosa* Leuckart, a species that forms colonies in the North Sea but is non-gregarious in southern Britain, also settled in response

to the tubes of conspecific adults, but showed little response to the tubes of *S. alveolata* (Wilson, 1970*b*). Larvae of *S. alveolata* preferred to settle on naturally built conspecific tubes rather than on those of *S. spinulosa*, but this response was strangely reversed if the tubes of both species had been constructed by adult worms from beach sand in the laboratory (Wilson, 1970*a*).

Like *Sabellaria alveolata* and *S. spinulosa*, the larvae of *Phragmatopoma californica* (Fewkes), a gregarious sabellariid from the west coast of North America, settle and begin metamorphosis upon contact with the sand tubes of conspecific adults (Jensen & Morse, 1984). Various substrata, including sympatric algae and invertebrates, the tubes of other polychaete species and posterior conspecific tube portions were ineffective or less effective than anterior conspecific tube portions. The metamorphosis-inducing capacity was lost when tube sand was boiled in distilled water. Pawlik (1986) demonstrated that extraction of the tube sand with organic solvents also resulted in loss of the inductive capacity. The capacity was retained in the organic extracts, and the active fraction was isolated and identified as a mixture of free fatty acids (FFAs) ranging from 14 to 22 carbons in length. Not all of the component FFAs of the mixture were effective at inducing larval metamorphosis. Assays of 37 FFA standards and 9 FFA derivatives revealed that the metamorphic response was highly specific, dependent on the length and conformation of the FFA acyl chain and on the presence of the carboxylic acid functional group (Pawlik & Faulkner, 1986).

The study reported herein was undertaken to determine whether settlement and metamorphosis of the larvae of *S. alveolata* could be attributed to the presence of FFAs in conspecific tube sand, as was demonstrated for *P. californica*. The near-equivalence in larval and adult morphologies, tube construction and gregarious habit led to the hypothesis that the larval responses of the two species would be very similar. The inquiry was experimentally addressed in two ways: by assaying the percentage of larval metamorphosis of one species in response to the tube sand of the other, and by following the bioassay-directed extraction and isolation procedures described in Pawlik (1986). Inasmuch as the culture and assay techniques employed by previous investigators differed considerably, this study was additionally seen as an opportunity to compare the larval growth and onset of metamorphosis of the two species under standardized conditions.

MATERIALS AND METHODS
Collection of animals and tube sand

Colonies of adult *Sabellaria alveolata* were collected from Widemouth Bay, Cornwall, England in late January 1986. Blocks of living worm reef were removed from intertidal rocks with a saw and crowbar. The top 5 cm of several blocks was crushed, and the adult worms rinsed away with repetitive washings of sea water. The resulting worm-free tube sand was rinsed in fresh water, frozen and flown to Scripps Institution of Oceanography, La Jolla, California, where it was kept frozen (< -20 °C) for later treatment (see *Extraction and isolation procedures*). Living blocks of reef were packed in a minimal amount of sea water under oxygen and flown to Scripps Institution of Oceanography, where they were maintained in an aquarium supplied with a continuous flow of sand-filtered sea water at 10–15 °C. Adult worms were fed a suspension of cultured phytoplankton

on a twice-weekly basis. Outflow from the holding tank was filtered prior to its return to the sea to prevent the inadvertent introduction of eggs or larvae of *S. alveolata* into the North Pacific. Blocks of worm reef containing adult *Phragmatopoma californica* were collected and maintained as described in Pawlik (1986).

Larval culture

Larval culture procedures are fully detailed in Pawlik (1986). Spawning was initiated in both species by removing worms from their tubes. The spawned eggs of *S. alveolata* do not adhere to glass as do those of *P. californica*, and greater care was required when rinsing them after fertilization. Swimming trochophores hatched after 12–18 h at 20 °C and after 18–24 h at 15 °C. Larvae were transferred at a concentration of approximately 1 larva/ml to 4 l glass jars filled with 3 l of 1 μm-filtered sea water containing 40 mg/l each of streptomycin sulphate and sodium penicillin G and a 1:1 mixture of *Phaeodactylum tricornutum* Bohlin and *Pavlova lutheri* (Droop) at 10^5 cells/ml. Jars were placed in 20 °C or 15 °C constant-temperature baths, 70 cm beneath two 40 W fluorescent lamps set for a 14 h:10 h light:dark cycle. Larval cultures were continuously agitated by motor-driven paddles. Culture jars were cleaned and media changed every other day.

Growth, maturation and metamorphosis of larvae in culture

Cultures of both *Sabellaria alveolata* and *Phragmatopoma californica* were monitored for larval growth, for the onset of metamorphic competence and for metamorphosis in culture vessels at 15 °C and 20 °C over a 40-day period. Newly hatched trochophores were separated from unhatched eggs and uniformly suspended in sea water. The mean number of trochophores in three 1 ml aliquots of the suspension was determined and a volume of suspension containing approximately 4000 larvae was transferred to 3 l of sea water in a 4 l jar. For each species, two such jars were prepared for each temperature (two jars were used in the event that one was broken or spilled during the 40-day period). Larvae were cleaned and fed as described above. At the time of hatching, and every other day thereafter, 10 larvae were removed from each set of treatment jars (5 from each of the two jars for each temperature), narcotized in 0·37 M-MgCl$_2$ and fixed in 2% buffered formalin in sea water. The fixed larvae were examined with a stereomicroscope at 25 × magnification and the body length (exclusive of tentacles) recorded for each.

Beginning on day 13 for the 20 °C cultures and on day 20 for the 15 °C cultures, 30 larvae were removed from each set of treatment jars and assayed in the presence of conspecific tube sand (see *Larval assays*). The percentage of larvae metamorphosing on the tube sand was recorded after 24 h and the unmetamorphosed larvae were returned to the culture jars. Assays for metamorphic competence were discontinued after 50% or more of the larvae metamorphosed in response to conspecific tube sand.

It was observed that a sizeable number of the competent larvae of *S. alveolata* would metamorphose in the culture vessels in the absence of any substrate other than that offered by the containers themselves. This response was quantified for both species by counting the metamorphosed larvae in each set of treatment jars as the jars were cleaned on alternate days. Metamorphosed larvae that had affixed themselves to the bottoms and walls of the glass jars were removed with a gentle spray of sea water, counted and discarded. At the end of the 40-day period, the remaining unmetamorphosed larvae were counted and a running percentage of metamorphosed larvae was determined by dividing the running sum of metamorphosed larvae by the sum of all the larvae that had metamorphosed during the 40-day time-course and the unmetamorphosed larvae that remained at the end of the time-course. The percentage of larvae accounted for at the end of the time-course was computed by dividing the denominator of the above ratio by 4000, the approximate number of trochophores added to each jar at the beginning of the time-course.

Types of sand used in assays

With the exception of experiments involving the use of natural tube sand, all larval assays were performed with 'clean sand' (Ottawa sand, cement-testing quartzite standard, 20–30 mesh, surface area ~ 36 cm^2/g; Fisher Scientific) which had been baked in a muffle furnace at 550 °C for 6 h to remove any organic material. The top 0·5 cm of tube sand from blocks of living worm reef of both *S. alveolata* and *P. californica* was scraped off without damaging the adult worms inside. The

blocks were then placed in flowing sea-water aquaria and covered with clean sand, more of which was added as the worms rebuilt their tubes. After 3–5 days, the newly built, anterior portions of the tubes were removed from the reef block, without damaging the adult worms, by lightly scraping the block surface with a glass rod. Sand tubes were rinsed in deionized water, frozen, lyophilized, broken up by lightly grinding them in a mortar and pestle, and the resulting 'tube sand' was sieved to remove clumps and debris. Clean sand was also kept for 3–5 days in a shallow dish in the same aquaria that contained the reef blocks, and thereby developed similar surface microflora as the sand incorporated into the tubes. This 'microbially filmed control sand' was rinsed, lyophilized and sieved in the same manner as tube sand.

Natural tube sand was used in experiments designed to test whether the inductive capacity of tube sand decreased with tube age. For *Sabellaria alveolata*, 'new natural tube sand' was taken from the frozen collection of the top 5 cm of worm reef and 'old natural tube sand' was derived from the base of a block of living worm reef (at 15 cm depth) that had been maintained in an aquarium for 6 months. For *Phragmatopoma californica*, 'new natural tube sand' was taken from the top 1 cm of a freshly collected reef block and 'old natural tube sand' was derived from the base (at 15–20 cm depth) of the same reef block. Natural tube sand was rinsed in deionized water, frozen, lyophilized and sieved (retained between mesh sizes 25 and 40) before use in larval assays.

Extraction and isolation procedures

A portion of the tube sand of both species was successively extracted for approximately 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.

In an attempt to identify the inducers of larval metamorphosis in *Sabellaria alveolata*, 2–3 kg of frozen, natural tube sand was extracted as described above, with additional extraction in twice-distilled water after extraction in methanol. Extracts were evaporated to dryness (water extract was lyophilized), weighed and redissolved in a known volume of the extraction solvent for application to clean sand. The methanol extract, which appeared to enhance larval metamorphosis, was further partitioned on 30 ml of silica gel by 'flash' chromatography (Still, Kahn & Mitra, 1978) with 50 ml of diethyl ether (fraction 1), 50 ml of 1:1 diethyl ether:methanol (fraction 2) and 100 ml of methanol (fraction 3). The resulting fractions were evaporated and redissolved in the same fashion as the extracts. All solutions were kept tightly capped at -15 °C until use. All extracts and fractions were assayed at 1 mg/g of clean sand.

The free fatty acid (FFA) fraction from the extract of the natural tube sand of *Sabellaria alveolata* was prepared by high-performance liquid chromatography as described in Pawlik (1986). This fraction was esterified by reaction with diazomethane in distilled diethyl ether to obtain the corresponding methyl esters, and the composition of this methyl ester mixture was determined by gas chromatographic analysis as described in Pawlik (1986).

Larval assays

With the exception of choice experiments, 1 g of the sand type to be assayed was weighed out into a disposable, 100 mm diameter, polystyrene petri dish. The dish and sand were rinsed once and then filled with 50 ml of 1 μm-filtered sea water. Into each dish, 30($\pm$2) larvae were transferred. The age of larvae used in assays was 20–30 d after hatching for larvae cultured and assayed at 20 °C or 25–35 d after hatching for larvae cultured and assayed at 15 °C. Dishes were placed on a rocking platform set at 28 cycles/min (unless otherwise specified) for 24 h under the same lighting regimen and temperature employed in larval culture (20 °C, unless otherwise specified). Plates were then examined under a stereomicroscope at 15× magnification and larvae were scored as not metamorphosed (swimming or crawling, no loss of provisional setae or rotation of larval tentacles), metamorphosed (attachment, loss of provisional setae, rotation of larval tentacles, commencement of tube construction), or abnormal (for *P. californica* see Pawlik & Faulkner, 1986; for *S. alveolata* little or no movement, some rotation of provisional setae, no tube formation, sometimes death).

All assays were run with five replicates and the mean percentage of larval response for each assay was determined. With the exception of the choice experiments and assays designed to test the effects of agitation, differences in mean larval metamorphosis were tested with one-way analysis of variance

(ANOVA) performed on arcsin-transformed data. Tukey's Honestly Significant Difference Method (T-method) was employed *a posteriori* to determine which treatments resulted in different mean larval responses at the 0·05 level of significance. For the choice and agitation experiments, the *t* test was used to ascertain the significance of differences between mean larval responses in comparisons of arcsin-transformed data. Statistical methods are fully detailed in Sokal & Rohlf (1981).

For assays of extracts, fractions or standards, 150 μl of a solution containing the desired amount of each substance was spread on to 1 g of clean sand on glassine weighing paper. Control sand was treated with solvent alone. After the solvent had evaporated, control and treated sands were separately added to disposable polystyrene petri dishes, which were then placed in a chamber under vacuum for 10–15 min to remove any trace of solvent.

Free fatty acid (FFA) standards were assayed at 1·0 mg/g sand and at 300 and 100 μg/g sand. FFA derivatives were assayed at 1·0 mg/g sand. FFAs and FFA derivatives were purchased from Sigma Chemical Co. (St Louis, MO) and Nu Chek Prep Inc. (Elysian, MN) at the highest available purity. Double-bond placement of the unsaturated FFA standards is detailed in Pawlik & Faulkner (1986). FFA derivatives used in assays were the monoglyceride, methyl ester, alcohol and alcohol acetate ester of 16:1 and these four derivatives plus the n-alkene of the 18:2 series.

Choice experiments similar to those run by Wilson (1970a) were conducted for comparative purposes. For each assay, a diametric line was marked on to the bottom of a polystyrene petri dish and 0·5 g of one of two sand types were placed in each hemicircle. Thirty larvae of each species were offered a choice between clean sand and conspecific tube sand and between conspecific tube sand and heterospecific tube sand. Assay dishes were not agitated. After 24 h at 20 °C, and under the same lighting regimen as employed in larval culture, counts were made of the number of metamorphosed larvae on the sand on each side of the dish. Five replicates were performed for each choice experiment. Additionally, clean sand and conspecific tube sand were also assayed separately to compare the two assay methods.

RESULTS

Growth, maturation and metamorphosis of larvae in culture

Data on larval growth, the onset of metamorphic competence and larval metamorphosis in culture for both *S. alveolata* and *P. californica* cultured at 15 °C and 20 °C are presented in Fig. 1 and Table 1.

Effect of organic solvent extraction of tube sand on larval metamorphosis of Sabellaria alveolata

The percentage metamorphosis of *S. alveolata* in response to microbially filmed control sand, extracted conspecific tube sand and unextracted conspecific tube sand is presented in Fig. 2 for larvae cultured and assayed at 20 °C. There were significant differences in mean larval responses among treatments (ANOVA, $F_{2, 12} = 25\cdot77$, $P < 0\cdot001$). *A posteriori* analysis (T-method, $\alpha = 0\cdot05$) revealed that the mean percentage of metamorphosed larvae was significantly greater in response to unextracted tube sand than to control sand or to extracted tube sand. Extracted tube sand induced greater metamorphosis than did control sand.

Responses of the larvae of one species to the tube sand of the other

A comparison of larval responses of *S. alveolata* and *P. californica* to microbially-filmed control sand, conspecific tube sand and heterospecific tube sand is shown in Fig. 3 for larvae cultured and assayed at 20 °C. There were significant differences in mean larval responses among treatments for both species
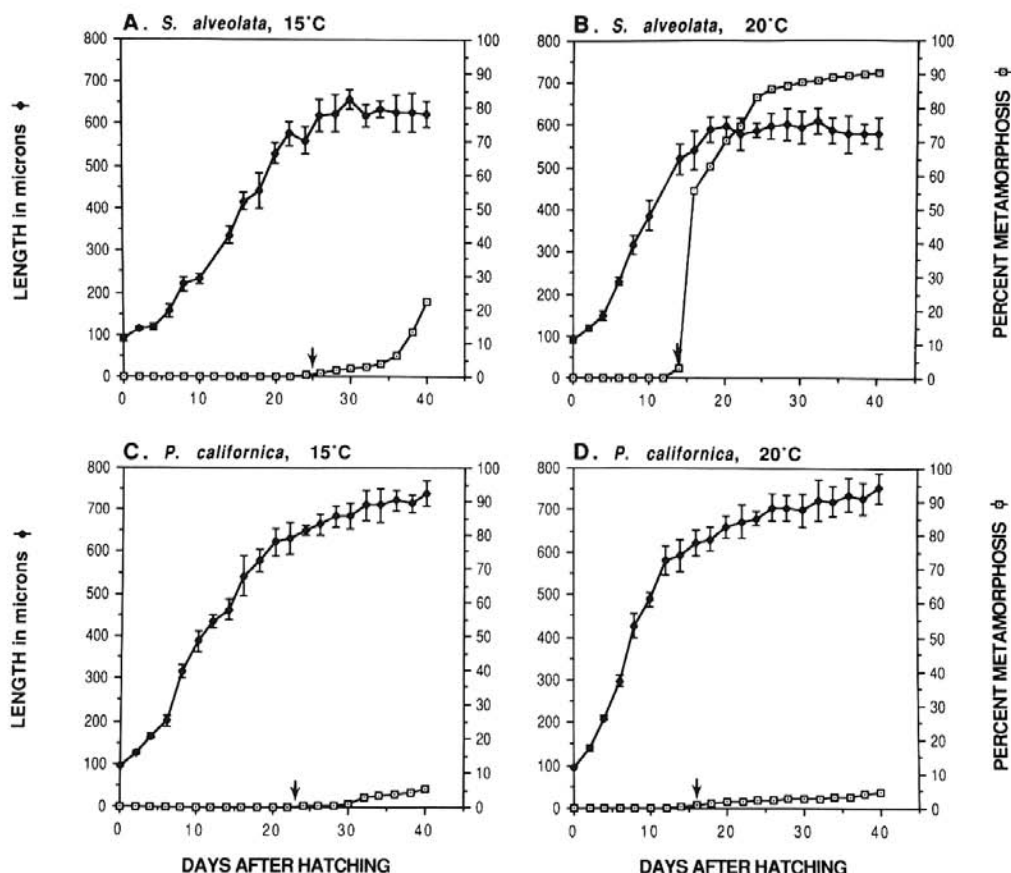
Fig. 1. Growth, maturation, and cumulative percentage metamorphosis of larvae of *Sabellaria alveolata* and *Phragmatopoma californica* in culture. Vertical bars represent one standard deviation about the mean larval length ($n = 10$). The arrow indicates the day on which 50% or more of the larvae metamorphosed in assays of conspecific tube sand. (A) Larvae of *S. alveolata* cultured at 15 °C. (B) *S. alveolata* cultured at 20 °C. (C) Larvae of *P. californica* cultured at 15 °C. (D) *P. californica* cultured at 20 °C.

Table 1. *Data on the growth, maturation and metamorphosis of larvae of* Sabellaria alveolata *and* Phragmatopoma californica *in culture at 15 and 20 °C*

	S. alveolata		P. californica	
	15 °C	20 °C	15 °C	20 °C
Maximum mean length (MML)	658 μm	610 μm	740 μm	754 μm
Time to MML	30 d	32 d	40 d	40 d
Time to metamorphic competence	25 d	14 d	23 d	16 d
Percentage metamorphosis in culture	22·5%	90·4%	5·3%	4·7%
Percentage accounted for at end of time-course	34%	50%	47%	42%

($F_{2, 12} = 51·16$, $P < 0·001$, for *S. alveolata*; $F_{2, 12} = 47·63$, $P < 0·001$, for *P. californica*). For both species, conspecific tube sand induced greater metamorphosis than did control sand or heterospecific tube sand. Whereas the percentage metamorphosis of *S. alveolata* was greater in response to control sand than to the
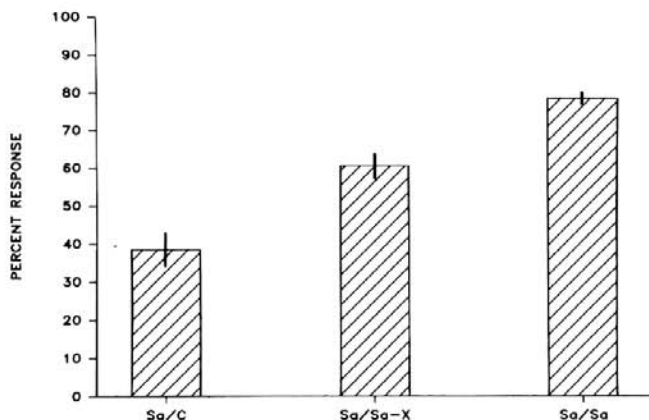
Fig. 2. Mean percentage metamorphosis ($\pm$ S.E., $n = 5$) of larvae of *S. alveolata* in response to microbially filmed control sand (Sa/C), extracted conspecific tube sand (Sa/Sa-X) and conspecific tube sand (Sa/Sa). Larvae cultured and assayed at 20 °C. Non-metamorphosis made up the balance of larval response for each treatment.
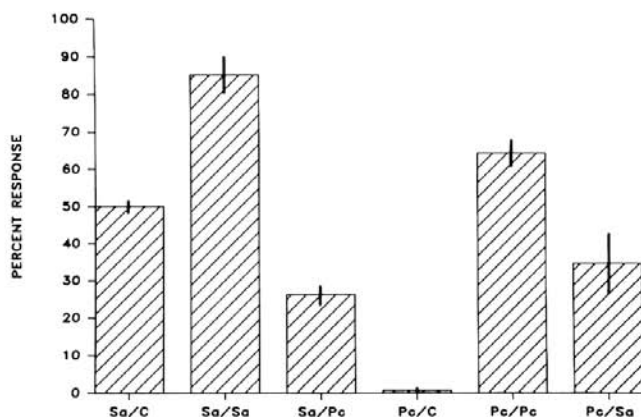

Fig. 3. Mean percentage metamorphosis ($\pm$ S.E., $n = 5$) of larvae of *S. alveolata* in response to microbially filmed control sand (Sa/C), conspecific tube sand (Sa/Sa) and tube sand of *P. californica* (Sa/Pc), and of larvae of *P. californica* in response to microbially filmed control sand (Pc/C), conspecific tube sand (Pc/Pc) and tube sand of *S. alveolata* (Pc/Sa). Larvae cultured and assayed at 20 °C.

tube sand of *P. californica*, larvae of *P. californica* metamorphosed to a greater extent on tube sand of *S. alveolata* than on control sand. This result prompted assay of the larvae of *S. alveolata* in the presence of extracted tube sand of *P. californica* (Fig. 4). Although there were significant differences in mean larval responses among treatments ($F_{2, 12} = 7.89$, $P < 0.01$), there was no significant difference in the percentage metamorphosis of *S. alveolata* in response to extracted versus unextracted tube sand of *P. californica*.

The experiments resulting in Figs. 2 and 3 were repeated with larvae cultured and assayed at 15 °C, with the addition of an assay of the larvae of *P. californica* in response to extracted conspecific tube sand. The results of these assays are shown in Fig. 5. There were significant differences in mean larval responses
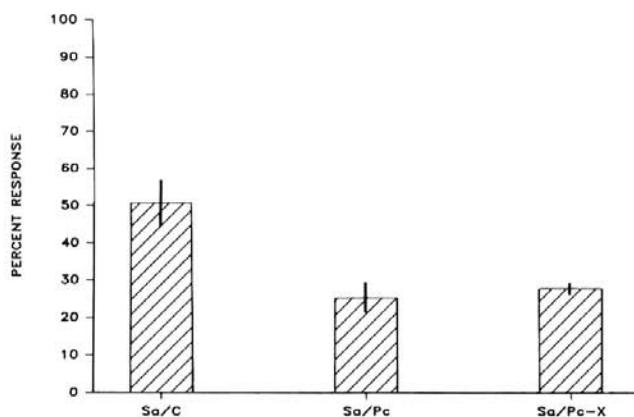
Fig. 4. Mean percentage metamorphosis ($\pm$ S.E., $n = 5$) of larvae of *S. alveolata* in response to microbially filmed control sand (Sa/C), heterospecific tube sand (Sa/Pc) and extracted heterospecific tube sand (Sa/Pc-X). Larvae cultured and assayed at 20 °C.
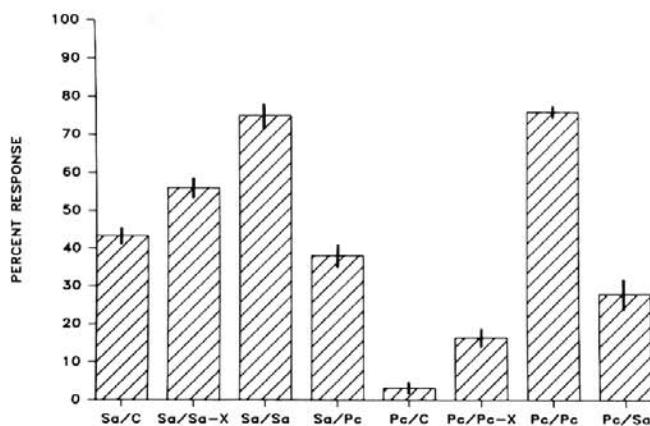

Fig. 5. Mean percentage metamorphosis ($\pm$ S.E., $n = 5$) of larvae of *S. alveolata* in response to microbially filmed control sand (Sa/C), extracted conspecific tube sand (Sa/Sa-X), conspecific tube sand (Sa/Sa) and heterospecific tube sand (Sa/Pc), and of larvae of *P. californica* in response to microbially filmed control sand (Pc/C), extracted conspecific tube sand (Pc/Pc-X), conspecific tube sand (Pc/Pc) and heterospecific tube sand (Pc/Sa). Larvae cultured and assayed at 15 °C.

among treatments for both species ($F_{3, 16} = 26\cdot38$, $P < 0\cdot001$, for *S. alveolata*; $F_{3, 16} = 61\cdot47$, $P < 0\cdot001$, for *P. californica*). Larvae of *S. alveolata* metamorphosed to a greater extent on conspecific tube sand than on control sand, extracted conspecific tube sand or tube sand of *P. californica*. There was no significant difference in larval responses to control sand and either extracted conspecific tube sand or heterospecific tube sand.

At 15 °C the larvae of *P. californica* also metamorphosed to a significantly greater extent on conspecific tube sand than on control sand, extracted conspecific sand or tube sand of *S. alveolata*. There was greater metamorphosis on both heterospecific tube sand and on extracted conspecific tube sand than on control sand.
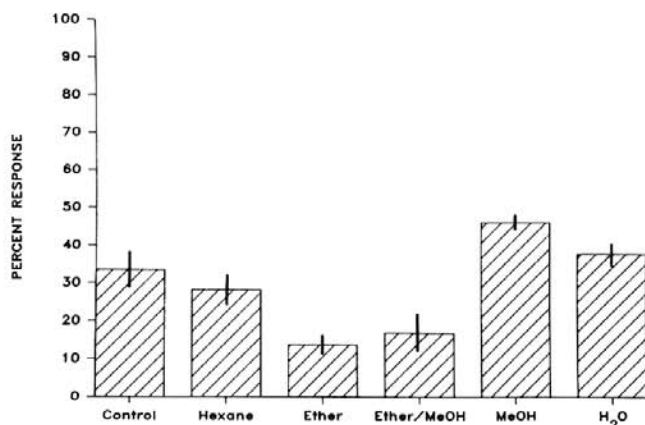
Fig. 6. Mean percentage metamorphosis ($\pm$ S.E., $n = 5$) of larvae of *S. alveolata* in response to clean sand treated with solvent alone (control) and clean sand treated with 1 mg/g of the hexane extract (hexane), diethyl ether extract (ether), 1:1 diethyl ether:methanol extract (ether/MeOH), methanol extract (MeOH) and distilled water extract (H_2O) of natural conspecific tube sand.
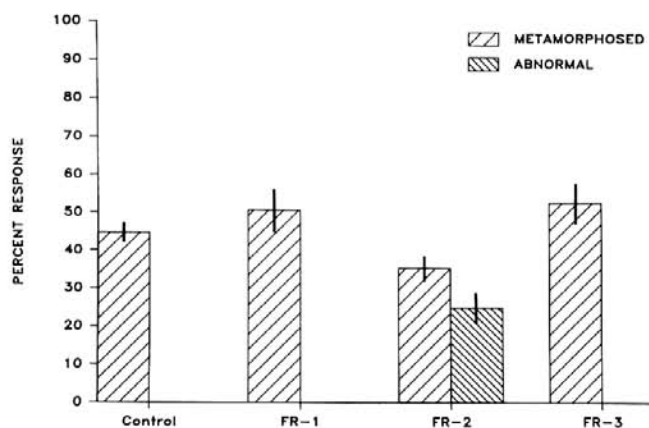

Fig. 7. Mean percentage response ($\pm$ S.E., $n = 5$) of larvae of *S. alveolata* to clean sand treated with solvent alone (control) and clean sand treated with 1 mg/g of fractions 1–3 isolated from the methanol extract of natural conspecific tube sand.

Responses of the larvae of Sabellaria alveolata *to organic solvent extracts of conspecific tube sand*

The percentage metamorphosis of the larvae of *S. alveolata* in response to organic solvent extracts of natural conspecific tube sand is shown in Fig. 6. There were significant differences in mean larval responses among treatments ($F_{5, 24} = 8{\cdot}22$, $P < 0{\cdot}001$). Mean percentage metamorphosis was greater in response to control sand than to sand treated with the diethyl ether extract of natural conspecific tube sand. There was no significant difference between larval responses to control sand and any of the other extracts.

The elevated, although not significantly greater, levels of larval metamorphosis in response to the methanol extract prompted its further separation and assay (Fig. 7). There was no significant difference between the mean percentage
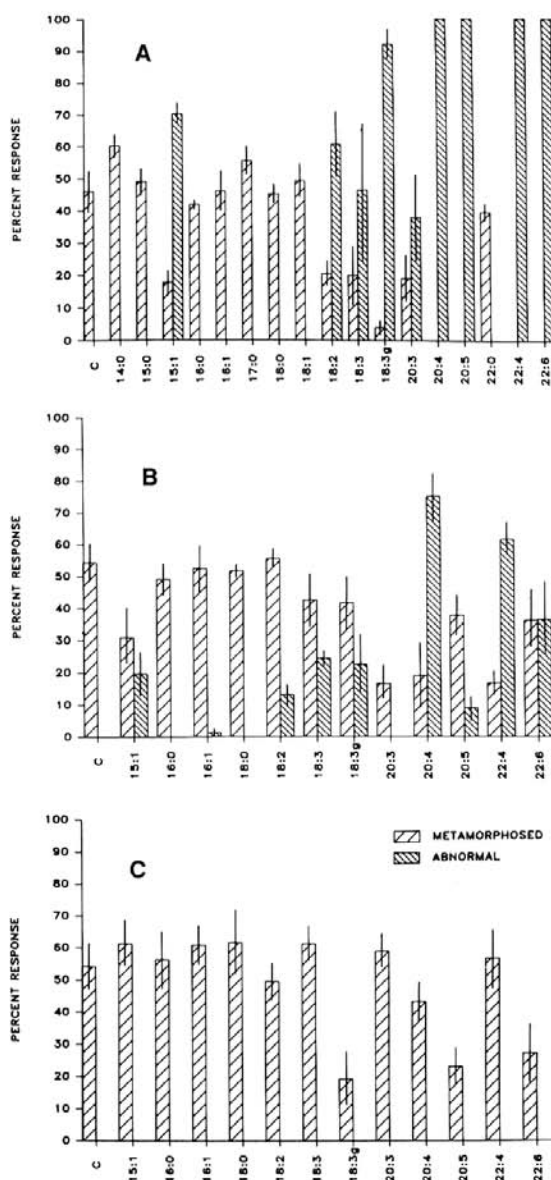
Fig. 8. Mean percentage response ($\pm$S.E., $n = 5$) of larvae of *S. alveolata* to clean sand treated with solvent alone (c) and clean sand treated with free fatty acids (FFAs). The number of carbon atoms in the FFA molecule precedes the colon, the number of double bonds follows. (A) 1 mg FFA/g sand. (B) 300 μg FFA/g sand. (C) 100 μg FFA/g sand.

metamorphosis in response to control sand and either fractions 1 or 3 isolated from the methanol extract ($F_{2, 12} = 0.622$, $P > 0.05$). Larval response to fraction 2 was split between normal metamorphosis and an abnormal response, the latter entailing sluggish movements of the larvae.
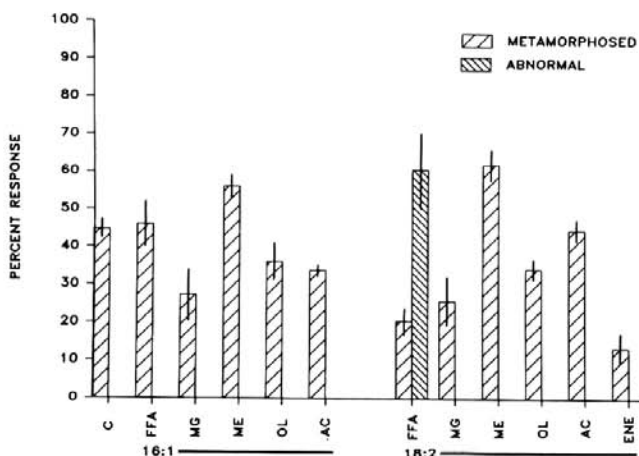
Fig. 9. Mean percentage response ($\pm$ S.E., $n = 5$) of larvae of *S. alveolata* to clean sand treated with solvent alone (C) and clean sand treated with the FFA, monoglyceride (MG), methyl ester (ME), alcohol (OL) and alcohol acetate ester (AC) of 16:1 and these five plus the *n*-alkene of 18:2 (ENE) at 1 mg/g sand.

Responses of the larvae of Sabellaria alveolata to FFAs and FFA derivatives

The responses of the larvae of *Sabellaria alveolata* to FFAs of variable chain length and unsaturation at three concentrations are presented in Fig. 8. In the standard shorthand notation for FFAs, the number of carbon atoms in the molecule precedes the colon and the number of double bonds follows. At 1 mg FFA/g sand (Fig. 8A), there was no significant difference in mean larval metamorphosis in response to control sand as compared to sand coated with any of the fully saturated FFAs (14:0, 15:0, 16:0, 17:0, 18:0, 22:0) or to 16:1 or 18:1 ($F_{8, 36} = 1.75$, $P > 0.05$). The polyenoic acids and 15:1 induced abnormal responses at 1 mg/g sand: 20:4, 20:5, 22:4 and 22:6 caused 100% larval mortality, whereas abnormal responses to 15:1, 18:2, 18:3, 18:3g and 20:3 indicated a moribund appearance.

At 300 μg/g sand (Fig. 8B) there were significant differences in mean larval metamorphosis among the four treatments in which there was no abnormal response ($F_{3, 16} = 12.30$, $P < 0.001$). Larval responses to sand treated with 16:0, 16:1 and 18:0 were not significantly different from response to control sand. Control sand induced a greater percentage of larval metamorphosis than sand treated with 20:3. Abnormally responding larvae moved sluggishly but were not dead.

Assays of FFAs at 100 μg/g sand (Fig. 8C) resulted in no abnormal larval response. There were significant differences in mean larval responses among treatments ($F_{12, 52} = 3.85$, $P < 0.001$). The percentage of larvae metamorphosing in response to control sand was greater than in response to sand treated with 18:3g. Mean larval responses to all other FFAs were not significantly different from responses to control sand.

The results of assays of 16:1, 18:2 and derivatives of these FFAs at 1 mg/g

sand are shown in Fig. 9. There were significant differences in mean larval metamorphosis among the 11 treatments in which there was no abnormal response ($F_{10, 44} = 7.46$, $P < 0.001$). Assay of the alkene of 18:2, cis-9,12-octadecadiene, resulted in significantly less larval metamorphosis than in response to control sand. Mean larval response to all other FFAs and FFA derivatives was not significantly different from the mean response to control sand.

Table 2. *Percentage composition of free fatty acid (FFA) fractions isolated from extracts of the natural tube sand of* S. alveolata *and* P. californica *determined by gas chromatography*

	S. alveolata	P. californica*
FFAs detected		
14:0	2·0	4·9
15:0 br	5·2	2·0
16:2, 16:1	6·2	15·6
16:0	16·8	16·6
17:0 br	10·1	3·0
18:3, 18:2, 18:1	10·1	12·1
18:0	11·6	6·4
19:0	2·7	—
20:5	6·9	23·5
20:4, 20:3, 20:2	12·1	4·7
22:4, 22:3, 22:2	16·6	6·4
Phthalates	—	4·9†

For each FFA the number of carbon atoms precedes the colon, the number of double bonds follows (br = branch- and straight-chain FFAs present).
* Data from Pawlik (1986).
† Phthalate esters present as contaminants (see Results).

FFA content and composition of the tube sand of Sabellaria alveolata

Two samples of natural tube sand from reef blocks of S. *alveolata* were fully extracted and the FFAs isolated. The concentrations of FFAs in the two samples were 0·94 μg/g sand and 0·88 μg/g sand (mean of 0·91 μg/g sand). The mean composition of the two FFA fractions is given in Table 2, with comparative data for the same fraction from the tube sand of P. *californica* (Pawlik, 1986). Phthalate esters, which were detected in the FFA fractions of P. *californica* (Pawlik, 1986), were subsequently found to contaminate the diethyl ether solution of diazomethane used to convert FFAs to methyl esters prior to gas chromatographic analysis. Phthalates were not detected in FFA fractions that had been treated with an uncontaminated diazomethane solution.

Supplementary assays: substrate choice experiments and the effects of agitation and tube age

To provide a more complete comparison of the results of the present study with those of previous authors, assays were performed to determine whether choice experiments differed considerably in their outcome and to assess the effect of agitation and sand-tube age on larval response. Results of assays in which larvae
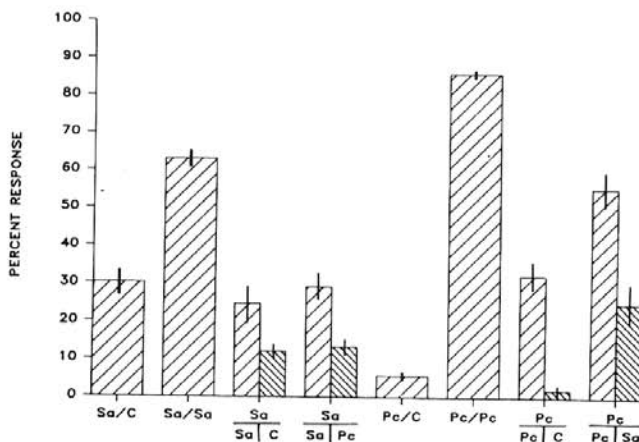
Fig. 10. Mean percentage metamorphosis ($\pm$ S.E., $n = 5$) of larvae of *S. alveolata* in response to clean sand (Sa/C), conspecific tube sand (Sa/Sa), a choice between conspecific tube sand and clean sand in the same dish (Sa/Sa/C) and a choice between conspecific tube sand and heterospecific tube sand in the same dish (Sa/Sa/Pc), and of larvae of *P. californica* in response to clean sand (Pc/C), conspecific tube sand (Pc/Pc), a choice between conspecific tube sand and clean sand in the same dish (Pc/Pc/C) and a choice between conspecific tube sand and heterospecific tube sand in the same dish (Pc/Pc/Sa). Assays run without agitation.
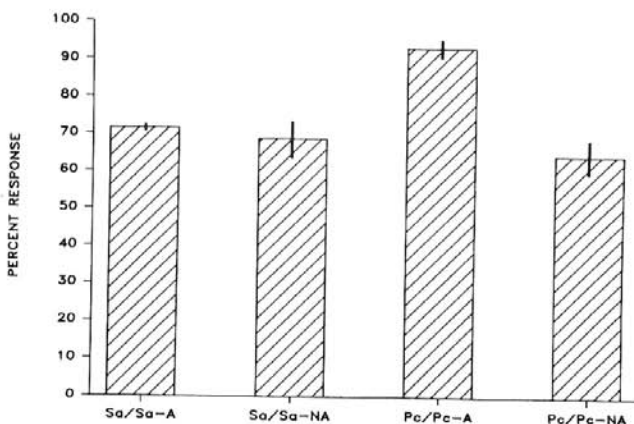

Fig. 11. Mean percentage metamorphosis ($\pm$ S.E., $n = 5$) of larvae of *S. alveolata* in response to conspecific tube sand in agitated assay dishes (Sa/Sa-A) and non-agitated assay dishes (Sa/Sa-NA), and of larvae of *P. californica* in response to conspecific tube sand in agitated assay dishes (Pc/Pc-A) and non-agitated assay dishes (Pc/Pc-NA).

were given a choice of two sand types present in the same assay dish are shown in Fig. 10. Assays of clean sand and conspecific tube sand assayed alone were run as controls. Given a choice between 0·5 g each of conspecific tube sand and clean sand in the same dish, a significantly greater percentage of larvae of *S. alveolata* metamorphosed on conspecific tube sand (*t* test, $P < 0·05$). Similarly, conspecific tube sand was chosen in preference to tube sand of *P. californica* ($P < 0·01$). Larvae of *P. californica* metamorphosed to a greater extent on conspecific tube sand than on clean sand ($P < 0·001$), and chose conspecific tube sand rather than tube sand of *S. alveolata* ($P < 0·01$).

There was no significant effect of agitation on the metamorphosis of larvae of *S. alveolata* exposed to conspecific tube sand (Fig. 11, $P > 0.05$). Under similar circumstances, larvae of *P. californica* metamorphosed to a greater extent in agitated assay dishes than in stagnant ones ($P < 0.01$).

Results of assays comparing new natural tube sand with sand from older tubes are presented in Fig. 12. Responses of larvae to clean sand and to conspecific tube
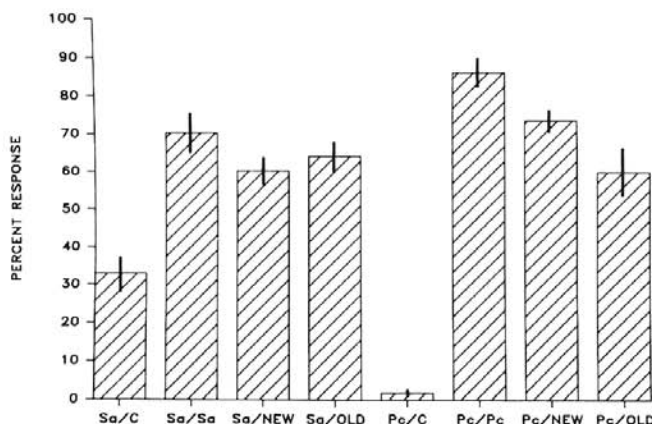

Fig. 12. Mean percentage metamorphosis ($\pm$ S.E., $n = 5$) of larvae of *S. alveolata* in response to clean sand (Sa/C), conspecific tube sand (Sa/Sa), new natural tube sand (Sa/NEW) and old natural tube sand (Sa/OLD), and of larvae of *P. californica* in response to clean sand (Pc/C), conspecific tube sand (Pc/Pc), new natural tube sand (Pc/NEW) and old natural tube sand (Pc/OLD).

sand (from tubes built of clean sand by adult worms in aquaria) are also included. There were significant differences in mean larval responses among treatments for both species ($F_{3, 16} = 9.18$, $P < 0.001$, for *S. alveolata*; $F_{3, 16} = 75.00$, $P < 0.001$, for *P. californica*). For *S. alveolata* there was no significant difference in larval responses to conspecific tube sand, new natural tube sand or old natural tube sand, but responses to each of these were greater than the response to control sand. For *P. californica*, larval metamorphosis on conspecific tube sand was greater than on old natural tube sand and control sand, but there was no significant difference in larval response to conspecific tube sand versus new natural tube sand. Larval responses to new natural tube sand and to old natural tube sand were not significantly different, but both were greater than larval response to control sand.

DISCUSSION

Sabellaria alveolata *and* Phragmatopoma californica: *allopatric counterparts with different metamorphic responses*

Sabellaria alveolata constructs reefs along the coasts of southern Britain (Wilson, 1971, 1974) and France (Dollfus, 1960; Gruet, 1986), south to Spain (Anadón, 1981) and the Mediterranean (Rivosecchi, 1961). *Phragmatopoma*

californica has a comparably broad range, forming reefs from the northern California coast south to Panamá (Kirtley, 1974). Both are primarily warm-temperate species (Briggs, 1974). Owing to their geographical separation it was not surprising to discover that the two species were not interfertile. Reciprocal cross-fertilizations of the gametes of the two species resulted in negligible numbers of abnormally swimming larvae (10–20 from several thousand eggs), while contemporaneous self-fertilizations produced 70–100% normally swimming larvae. Nevertheless, *S. alveolata* and *P. californica* are very similar morphologically, both as larvae and as adults, and form tube reefs that are indistinguishable. The striking differences in the metamorphic responses of the larvae of the two species are probably rooted in their genetic dissimilarity.

There are clear differences in the predisposition of the larvae of the two species to metamorphosis in the absence of materials suitable for tube construction. A considerable proportion of the larvae of *S. alveolata* metamorphosed spontaneously in culture jars. At 20 °C the percentage of metamorphosed larvae rose to over 50% of the total within two days of metamorphic competence (Fig. 1 B). At 15 °C there was a 10-day delay after the onset of competence, but thereafter the percentage of metamorphosed larvae began to rise abruptly (Fig. 1 A). This trend most likely would have continued past day 40, as evidenced by the numbers of metamorphosed larvae in jars that were cultured past that time but were not part of the time-course experiment. In contrast, larvae of *P. californica* in culture at both 15 and 20 °C metamorphosed at low levels after the onset of metamorphic competence (Fig. 1 C, D). There was no substantial increase in the proportion of metamorphosed larvae in cultures of *P. californica* that had been maintained for several months. The larvae of *S. alveolata*, therefore, appear less able to delay the onset of metamorphosis than do larvae of *P. californica*.

Culture temperature had a substantial effect on the time required to reach metamorphic competence for both species. A difference of 5 degrees in temperature nearly doubled the time to metamorphic competence for larvae of *S. alveolata*. The mean summer sea-surface temperatures within the distributions of both species range from about 15 °C in the north to about 26 °C in the south (Sverdrup, Johnson & Fleming, 1942). The culture temperatures used in the present study were, therefore, well within the span likely to be encountered by larvae in nature.

Perhaps as a result of their limited ability to delay metamorphosis, larvae of *S. alveolata* metamorphosed to a greater extent on clean or microbially filmed sand than did larvae of *P. californica*. For larvae of *S. alveolata* assayed in all the experiments performed in the present study, the mean of the means was $43 \cdot 7 \pm 8 \cdot 3\%$ metamorphosis in response to microbially filmed and clean control sand ($n = 12$). For all assays performed with larvae of *P. californica* in Pawlik (1986), Pawlik & Faulkner (1986) and in the present study, the mean of the means was $1 \cdot 5 \pm 1 \cdot 7\%$ metamorphosis in response to control sand ($n = 11$). At both assay temperatures, the larvae of *S. alveolata* were less discriminating in their choice

of settlement substrates than those of *P. californica*. This difference added to the difficulty of identifying the substance responsible for the enhanced metamorphosis of *S. alveolata* on conspecific tube sand.

Although organic-solvent extraction of conspecific tube sand resulted in a significant drop in its capacity to induce metamorphosis of *S. alveolata* (Figs. 2, 5), the attenuation was not as distinct as that observed under similar circumstances for *P. californica* (Pawlik, 1986). Furthermore, the organic solvent extracts (Fig. 6) and fractions derived from these extracts (Fig. 7) induced no greater metamorphosis of *S. alveolata* than did clean sand, implying that the extraction process may have partially destroyed, rather than liberated, the inductive substance. In contrast, metamorphosis of *P. californica* on extracts and fractions of conspecific tube sand was significantly greater than on control sand, a result that led to the isolation and identification of the inducers of larval metamorphosis for that species (Pawlik, 1986).

There was no enhancement of metamorphosis of *S. alveolata* in response to the FFAs, which were identified as the naturally occurring inducers of metamorphosis of *P. californica* (Fig. 8 and Pawlik, 1986). At 1 mg/g sand, larvae of *S. alveolata* died in response to the same polyenoic FFAs that killed larvae of *P. californica*. However, non-fatal, abnormal responses to FFAs at 1 mg/g and 300 μg/g sand differed between the two species. Abnormal responses of *P. californica* incorporated many of the behavioural components of normal metamorphosis, including loss of provisional setae and rotation of larval tentacles (Pawlik & Faulkner, 1986). Although the behavioural components of normal larval metamorphosis are the same in both species, larvae of *S. alveolata* responding abnormally to the presence of high concentrations of certain FFAs retained their setae and did not rotate their tentacles forward, but became moribund. At 100 μg/g sand, a concentration at which there was no abnormal response in either species, metamorphosis of *P. californica* was enhanced in response to 16:1, 18:2, 18:3, 18:3g, 20:4, 20:5, 22:4 and 22:6 (Pawlik, 1986; Pawlik & Faulkner, 1986), but metamorphosis of *S. alveolata* in response to these FFAs was either not significantly different or lower (e.g. 18:3g) than in response to control sand (Fig. 8C). Briefly, then, the FFAs that induced metamorphosis of *P. californica* had no effect on, or inhibited, metamorphosis of *S. alveolata*. Furthermore, although some FFAs (particularly polyenoics) induced abnormal responses in both species when assayed at high concentrations, these responses included components of normal metamorphosis for larvae of *P. californica* but not for larvae of *S. alveolata*.

FFAs were present in extracts of the natural tube sand of *S. alveolata*, but at concentrations less than an order of magnitude lower than those found in the natural tube sand of *P. californica* (Pawlik, 1986). In addition, the composition of the FFA mixture from natural tube sand of *S. alveolata* was different from that of *P. californica* (Table 2), with higher levels of some branch-chain and fully saturated FFAs and lower levels of 16:1 and 20:5, which comprise the most

abundant inducers of larval metamorphosis in the FFA mixture from the natural
tube sand of *P. californica* (Pawlik, 1986). Branch-chain FFAs are believed to
have a bacterial origin (Shaw, 1974).

Some of the FFAs present in the mixture isolated from the tube sand of
S. alveolata inhibited metamorphosis of conspecific larvae at low concentrations
and induced abnormal responses at higher concentrations (e.g. 18:3g, 20:4,
20:5; Fig. 8). Thin-layer chromatography revealed that FFAs were present in
the ether, ether–methanol and methanol extracts of tube sand of *S. alveolata*,
which may explain the significant attenuation of larval metamorphosis in response
to the ether extract (Fig. 6). Further fractionation of the methanol extract resulted
in concentration of the FFAs into fraction 2, assay of which resulted in some
abnormal response (Fig. 7).

The presence of FFAs in the tube sand of *S. alveolata*, however low the
concentration, may explain the enhanced larval metamorphosis of *P. californica*
on tube sand of *S. alveolata* as compared with control sand (Figs. 3 and 5). The
considerably higher concentration of FFAs present in the tube sand of
P. californica might also explain the inhibition of metamorphosis of *S. alveolata*
in response to tube sand of *P. californica* relative to control sand at 20 °C (Fig. 3).
The latter hypothesis was tested by extracting the tube sand of *P. californica* prior
to assay, thereby removing the potentially inhibitory FFAs. There was, however,
no significant effect of extraction with organic solvents (Fig. 4). There was also
no difference in larval response of *S. alveolata* to control sand versus hetero-
specific tube sand when assays were run at 15 °C (Fig. 5).

The greater than 10-fold higher concentration of FFAs in the natural tube sand
of *P. californica* relative to the concentration in the natural tube sand of
S. alveolata is highly suggestive, although circumstantial, evidence for the
production of FFAs by adult *P. californica*. In a similar vein, the disparity in
larval responses between the two species suggests that the FFA-induced settle-
ment and metamorphosis of *P. californica* is mediated by a specific mechanism,
whether by a receptor, by an enzyme, or by alteration of the larval surface
membrane structure. In any case, larvae of *P. californica* respond to specific FFAs
(Pawlik & Faulkner, 1986) at concentrations at which they occur in natural tube
sand (Pawlik, 1986), and the response is not shared by a confamilial allopatric
cognate, *S. alveolata*.

Comparisons with the results of earlier studies

In southern California, adult *Phragmatopoma californica* are gravid with viable
gametes throughout the year. Larval recruitment occurs at low levels much of
the year, with increased levels in the spring and particularly large peaks
attributable to spawning induced by storm damage (Barry, 1987). Wilson (1968a)
working in Plymouth, England, successfully spawned adult *Sabellaria alveolata*
collected from the Cornish coast during several months of the year, but obtained
the best fertilizations during midsummer months coinciding with the period of
natural spawning. Worms collected in the field after this time were spent of
gametes. Gruet & Lassus (1983) performed comprehensive studies of the

reproductive cycle of *S. alveolata* collected along the coast of France near Nantes. They found mature oocytes in adult worms throughout the year, with only a brief period in the autumn when most of the worm coeloms were devoid of gametes. Adult *S. alveolata* from reef chunks collected in late January 1986 from the coast of Cornwall and transported to southern California for use in the present study were fully gravid from the time of the first induced spawning, one week after arrival, until late October 1986, the time of this report. Spawned gametes produced healthy larvae throughout this period.

Differences in larval culture techniques are undoubtedly responsible for the striking differences in the rates of larval growth and onset of metamorphosis of *S. alveolata* observed in the present study as compared with those of Wilson (1929, 1968*a*, 1970*a*). At 15 °C Wilson obtained metamorphically competent larvae in 7–8 weeks after fertilization, 6 weeks at the earliest. Larvae were most frequently cultured on a diet of flagellates in unstirred vessels, yielding considerable variability in larval growth and maturation (Wilson, 1968*a*). In the present study, larvae were very uniform in size and stage of development throughout the time-course experiments, with metamorphic competence reached in 25 d at 15 °C and 14 d at 20 °C. Mean larval length of *S. alveolata* cultured by Wilson (1968*a*) was ~500 μm, with a maximum of up to ~700 μm. The mean length of mature larvae cultured at 15 °C in the present study was ~625 μm, with a maximum of ~690 μm. Eckelbarger (1977) cultured *P. californica* in stirred vessels on a mixture of flagellates, and observed metamorphosis between 18 and 25 days after fertilization at 21–23 °C and after 34–39 days at 17–18 °C. This compares with 16 days after hatching at 20 °C and 23 days at 15 °C, as reported herein. The combination of diet, slow agitation and frequent water changes was probably responsible for the rapid and uniform larval growth observed in the present study. The 1:1 mixture of the diatom *Phaeodactylum tricornutum* and the flagellate *Pavlova lutheri* employed herein was chosen from several monocultures and mixtures of phytoplankters commonly used in invertebrate mariculture as the best suited for growth of larvae of *Phragmatopoma californica* (M. R. Amieva, unpublished results). Widely divergent maturation rates have been reported in several laboratory studies of sabellariid larval development (see reviews in Eckelbarger, 1975, 1977). Mauro (1975) attributed the differences in the pre-metamorphic development rates of *P. lapidosa* he cultured and *S. alveolata* cultured by Wilson (1968*a*) to either culture temperature or genetic differences. Wilson (1968*b*) surmised that the long pre-competent period and wide variability in growth rates he observed were adaptive for larval dispersal in a species with a limited adult habitat. Although it now appears that these are not fixed developmental traits, it is likely that the pre-competent period and variability of growth observed by Wilson are better assessments of larval growth under natural conditions, especially in the light of the considerable spatial and temporal heterogeneity of natural phytoplankton communities (Steele, 1978).

The tendency of metamorphically competent larvae of *S. alveolata* to metamorphose in culture was also observed for the larvae of *S. floridensis* by Eckelbarger (1977). In several experiments Wilson noted that the larvae of

S. alveolata would metamorphose in agitated culture dishes in the absence of suitable tube-building materials (Wilson, 1968 *b*, pp. 391, 392, 394). One explanation for the particularly high levels of metamorphosis of *S. alveolata* in culture may be that the larvae of *S. alveolata* respond more strongly to inducers secreted by recently metamorphosed juvenile worms, and therefore the metamorphosis of just a few on to the sides and bottom of the culture jar results in a cascade of larval metamorphosis. To a limited extent, this response does occur for larvae of both *S. alveolata* (Wilson, 1968 *b*) and *P. californica* (Pawlik, 1986), and undoubtedly accentuated the observed results, especially for *S. alveolata* cultured at 20 °C (Fig. 1 B). Nevertheless, the results of the time-course experiments are believed to be an accurate representation of the relative inability of *S. alveolata* to delay metamorphosis, primarily because all metamorphosed larvae were removed from culture jars every other day. Wilson found that larvae of *S. alveolata* required several days in the presence of metamorphosed juveniles before gregarious metamorphosis occurred (Wilson, 1968 *b*, p. 394; 1970 *a*, p. 26), and daily removal of metamorphosed larvae did not inhibit further metamorphosis (Wilson, 1968 *b*, p. 421).

As previously demonstrated by Wilson (1968 *b*, 1970 *a*), the results presented herein confirm that the larvae of *S. alveolata* metamorphose to a greater extent on conspecific tube sand than on alternative substrata. Wilson similarly observed considerable levels of metamorphosis in response to clean sand (e.g. Wilson, 1968 *b*, p. 402). In addition to experiments in which individual sand types were offered to larvae, Wilson ran assays in which larvae were offered small piles of several different sand types, frequently arrayed in complex patterns, all in the same assay dish (Wilson, 1970 *a*, p. 11). He recognized that the latter experiments had certain flaws, however, in that larvae did not evenly distribute themselves in the assay dishes (Wilson, 1970 *a*, p. 28). In the present study a simplified version of Wilson's free-choice experiments was run employing only two piles of differing sand types, one on either side of the assay dish. Unlike Wilson's experiments, which were run in total darkness, assay dishes were illuminated for 14 of 24 h in the present study. Results of these assays for larvae of both *S. alveolata* and *P. californica* (Fig. 10) were similar to those run with solitary sand types, but the overall percentage of larvae responding was somewhat lower and the variability in the results of the assays greater, possibly as a result of the uneven distribution of larvae in the non-agitated assay dishes. Wilson (1970 *a*, p. 29) observed similar reductions and variability in larval responses and ascribed them to variable growth and age of the larvae used in the experiments.

Wilson (1968 *b*) found that agitation generally resulted in enhanced levels of metamorphosis of *S. alveolata*, although he did not stir the dishes in which he ran many of his assays. Results of the present study indicate that agitation has little effect on the metamorphosis of *S. alveolata*, but that it enhances metamorphosis of *P. californica* (Fig. 11). There was greater variability of larval response in assay dishes that were not agitated, again possibly the result of the uneven distribution of larvae in unagitated dishes.

The question of whether natural tube sand retains its capacity to induce metamorphosis over time is of primary importance in understanding whether the remnants of dead or destroyed sabellariid reefs will be recolonized by larval recruits. Wilson (1970a) demonstrated that a greater proportion of the larvae of S. alveolata metamorphosed in response to conspecific tube sand from a living colony than in response to tube sand from a 'long-dead' colony, but that there was greater metamorphosis on old tube sand than on clean shore sand. In the present study, old and new natural tube sand of S. alveolata were equally effective at inducing metamorphosis (Fig. 12). The old natural tube sand was taken from the base of a living colony that had been maintained in an aquarium for 6 months. Tubes at the base of the colony were occluded with sand grains deposited by the worms living above and had not been in contact with worms for at least as long as the colony had been in the aquarium. The tube sand from the 'long-dead' colony used by Wilson was undoubtedly older, inasmuch as no living worm was present in the area and the remnant tubes were partly overgrown with algae (D. P. Wilson, personal communication).

Jensen & Morse (1984) reported that the capacity of the posterior portions of tubes (at a depth of more than 5 cm from the colony surface) of P. californica to induce larval metamorphosis was much less than that of the anterior tube portions (at a depth of 5 cm or less). In their assays, a mean of 44% of the larvae metamorphosed on anterior tubes but only 4% metamorphosed on posterior tube portions after 60 h. In contrast, the results of the present study demonstrate that tube sand taken from a depth of 15–20 cm below the surface of the colony was not significantly less effective at inducing metamorphosis than was tube sand from the top 1 cm of the colony, although sand from tubes built by adult worms in the laboratory induced significantly greater metamorphosis than old natural tube sand (Fig. 12). The latter results are consistent with field observations. Colonies of P. californica in the lower intertidal are frequently destroyed by waves and wave-borne debris during winter storms, leaving only uninhabited remains of the reef base behind. Within a few months' time, however, large numbers of larvae have metamorphosed on many of the ruined colonies and have begun constructing new tubes on the old remnants (cf. Gruet, 1986). The relative decline in larval response of P. californica to conspecific tube sand of greater age (Fig. 12), however, may indicate the gradual degradation of inductive FFAs over time.

Larval settlement and reef formation

Based on the laboratory results of this and previous studies, gregarious settlement and reef construction is an understandable consequence of the larval behaviour of Phragmatopoma californica. Larvae of P. californica have a very specific, nearly 'all or nothing' response to conspecific tube sand and, in its absence, have the capacity to postpone metamorphosis for a seemingly indefinite period of time. Metamorphosis occurs on the edges of occupied colonies or on the remnants of dead or damaged colonies, resulting in the enlargement or repopulation of the aggregates. For genetic reasons or otherwise, only a very small

proportion of larvae are unable to delay metamorphosis or are unspecific in their choice of settlement substrates. While the majority of these 'errant' solitary settlers die upon metamorphosing in unsuitable habitats, a small percentage metamorphose in an appropriate environment for adult survival and their tubes form the foundations for new colonies.

A similar scenario is not apparent for *Sabellaria alveolata*. Indeed, one might predict a predominantly non-gregarious lifestyle from the data presented herein. Larvae of *S. alveolata* metamorphose in greater numbers in response to conspecific tube sand, but a considerable proportion are not particularly discriminating in their choice of substrate and appear to be unable to delay metamorphosis for very long after competence is reached. Non-specific metamorphosis was particularly rapid at the higher culture temperature, prompting the prediction that *S. alveolata* may be least likely to form colonies in warmer seas. Yet *S. alveolata* constructs massive aggregations throughout its range, including the southernmost Mediterranean extension (Rivosecchi, 1961). Of course, larvae of *S. alveolata* from the southern end of its distribution may have different responses from those used in the present study. In addition, larval behaviour may be different under less than optimal natural conditions. Low or patchy phytoplankton abundance would lengthen larval development time and increase developmental variability, outcomes similar to those observed by Wilson (1929, 1968*b*). Concomitant effects might be a greater delay of metamorphosis after attaining competence and greater substrate selectivity. Other factors, both biotic and abiotic, may also play a role in affecting gregarious settlement of *S. alveolata*.

Other species of *Sabellaria* form aggregations in part, but not all, of their geographic ranges. *Sabellaria spinulosa* builds reefs in the North Sea, but is non-gregarious off the coast of Britain (Hagmeier & Kandler, 1927; Linke, 1951; Wilson, 1970*b*). *Sabellaria vulgaris* forms solitary tubes over most of its range (east coast of North America), yet constructs patch reefs in Delaware Bay (Curtis, 1978). Similarly, *S. cementarium*, which is non-gregarious throughout the North Pacific, builds reefs in an embayment on the Oregon coast (Posey, Pregnall & Graham, 1984). As previously suggested (Pawlik & Faulkner, 1987), a trade-off between the delay of larval metamorphosis and substrate specificity appears likely for these species. Retention or concentration of larvae under particular hydrodynamic conditions, such as those found in embayments, may shift the balance towards gregarious settlement. Similar physical factors may influence gregarious settlement of *S. alveolata*, inasmuch as some of the largest reefs formed by this species are situated in bays and estuaries (Dollfus, 1960; Gruet, 1971, 1986; Horne, 1982).

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