LARVAL SETTLEMENT AND METAMORPHOSIS OF SABELLARIID POLYCHAETES, WITH SPECIAL REFERENCE TO PHRAGMATOPOMA LAPIDOSA, A REEF-BUILDING SPECIES, AND SABELLARIA FLORIDENSIS, A NON-GREGARIOUS SPECIES

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ABSTRACT

The naturally-occurring inducers of larval settlement and metamorphosis have recently been isolated and identified for the northeast Pacific reef-building sabellariid polychaete, Phragmatopoma californica, and the larval responses of this species compared, in reciprocal laboratory settlement assays, to those of its European counterpart, Sabellaria alveolata. The present study includes the larval behavior of two additional sabellariids from the western Atlantic, P. lapidosa, a reef-building species, and S. floridensis, a non-gregarious species.

Larval responses of P. lapidosa were very similar to those of P. californica. In reciprocal laboratory assays of both species, greater metamorphosis occurred on conspecific than on heterospecific tube sand, but both metamorphosed more frequently on heterospecific tube sand than on control sand. Organic solvent extraction of the sand/cement matrix of tubes of P. lapidosa removed its capacity to induce conspecific metamorphosis. The capacity was retained in the lipid-soluble extract and was recovered as a single fraction by high-performance liquid chromatography. The inducers were identified by gas chromatography as a mixture of free fatty acids (FFAs) ranging from 14 to 22 carbons in length. The mixture contained the same component FFAs as the inductive fraction from the natural tube sand of P. californica, but the relative proportions were different. Of the FFAs found in the naturally-occurring mixture, larval metamorphosis was greatest in response to 16:1 at as low as 1 μg/g sand (surface area = 36 cm²). Metamorphosis-inductive FFAs were isolated from natural tube sand of P. lapidosa at approximately 4-5 μg/g sand, although extraction was believed to be incomplete. In assays of 37 FFA standards and 9 FFA derivatives, metamorphosis of P. lapidosa was dependent on the length and conformation of the acyl chain length and on the presence of a carboxylic acid functional group, as had previously been demonstrated for P. californica. Reciprocal cross-fertilization of gametes of P. lapidosa and P. californica resulted in larvae that developed and metamorphosed normally. Results of this and previous investigations suggest that P. lapidosa and P. californica are geographic races of the same species, and the trinomials Phragmatopoma lapidosa and P. l. californica are proposed for each subspecies.

Larval responses of Sabellaria floridensis resembled those of S. alveolata in three respects: (1) upon reaching maturity, a large percentage of the larval metamorphosed spontaneously in culture vessels, (2) in laboratory settlement assays, a large proportion of the larvae metamorphosed on control sand, and (3) metamorphosis was not enhanced upon exposure to the FFAs that induce metamorphosis of Phragmatopoma larvae. Unlike the larvae of S. alveolata, which settle gregariously, larvae of the non-gregarious S. floridensis did not metamorphose to any greater extent on conspecific tube sand than on control sand. Among sabellariid polychaetes, enhanced settlement on conspecific tube sand may be the only requirement for reef formation.

The Sabellariidae is a family of marine polychaetes that constructs tubes of cemented grains of sand and shell fragments. There are at least six extant genera encompassing more than 50 species, of which approximately two-thirds build solitary tubes at intertidal to abyssal depths worldwide. About 20 species, pre-
specific genus *Gunnera*, construct colonies and reefs of aggregated tubes in the intertidal and subtidal of temperate and tropical coasts in many parts of the world (Achir, 1972; Kirtley, 1974; Pawlik and Faulkner, 1988).

*Phragmatopoma lapidosa*, a gregarious species, occurs along the east coast of Florida from Cape Canaveral south, throughout the Caribbean, and to the southern coast of Brazil (Kirtley, 1974). Reefs of the sand tubes of *P. lapidosa* extend for hundreds of kilometers of coastline (Kirtley and Tanner, 1968), and are thought to be of considerable importance in the sorting, deposition and stabilization of sand (Kirtley, 1967; Multer and Milliman, 1967; Gram, 1968; Kirtley and Tanner, 1968; Mehta, 1973; Gore, 1986). Like coral reefs, colonies of *P. lapidosa* support large and diverse assemblages of associated invertebrates and fishes (Novato and Peres, 1961; Narch and Rodrigues, 1965; Fanta, 1968; Gore et al., 1978). *Sabellaria floridensis* is sympatric over much of the range of *P. lapidosa*, occurring along the coast of the southeastern United States and throughout the Caribbean (Kirtley, 1974). Unlike *P. lapidosa*, *S. floridensis* is a non-gregarious species, usually building solitary or paired tubes on shells and stones resting on sandy or muddy bottoms.

The present account is the fourth in a series describing studies of the chemical basis for the initiation of larval settlement among sabellariid polychaetes (Pawlik, 1986; 1988; Pawlik and Faulkner, 1986). Larvae of gregarious sabellariid settle and metamorphose with a high degree of specificity on the cemented sand tubes of the adult worms (reviewed in Pawlik, 1986; 1988). Organic solvent extraction of the tube sand of *P. californica*, a reef-building species from the west coast of North America, greatly diminished the capacity of the tube sand to induce settlement of conspecific larvae in laboratory assays. The inductive capacity was retained, however, in the lipid-soluble extract, and from it, a single, highly active fraction was isolated. Analysis of this fraction revealed a mixture of free fatty acids (FFA) ranging from 14 to 22 carbons in length, some of which were effective in inducing larval settlement and metamorphosis at naturally-occurring concentrations. Subsequent assays of 37 FFA standards and 9 FFA derivatives demonstrated that the metamorphic response was highly specific, dependent upon the length and conformation of the acyl chain and on the presence of a free carboxylic acid functional group. Surprisingly, *Sabellaria alveolata*, a reef-building species from European waters, exhibited very different settlement behavior than did *P. californica* (Pawlik, 1988). In reciprocal assays, metamorphosis of both species occurred to a greater extent on conspecific tube sand than on heterospecific tube sand. Extraction of the tube sand of *S. alveolata* diminished its capacity to induce metamorphosis of conspecific larvae, but the capacity was not transferred to the organic extracts, and a lipophilic inducer could not be isolated or identified. The FFAs that induced metamorphosis of the larvae of *P. californica* were either ineffective at enhancing metamorphosis of *S. alveolata* or inhibited the response. Moreover, FFAs were present in the natural tube sand of *S. alveolata* at less than 1/10 the concentration at which they were found in natural tube sand of *P. californica*. Larvae of *S. alveolata* metamorphosed with much less discrimination in their choice of substrata than did those of *P. californica*, and a high percentage settled spontaneously in culture vessels. While the larval settlement behavior of *P. californica* appeared well suited for colony and reef formation, this was much less apparent for *S. alveolata*. The investigation reported herein is an extension of the previous comparisons of sabellariid larval settlement behavior to include those of *P. lapidosa* and *S. floridensis*. Although the larval development of both species was described in detail by Eckelbarger (1976; 1977; 1978), no experimental assessments were made of substrate preferences at the time of settlement. The settlement responses of the two species were hypothesized to show greater similarity to those of their respective congeners. It was additionally hoped that differences in the larval behavior of the non-gregarious *S. floridensis*, as compared with that of *S. alveolata*, might better explain reef formation by the latter.

**Materials and Methods**

Blocks of living colonies of *P. lapidosa* were collected intertidally from Seminole Shores, St. Lucie Inlet, Martin County, Florida, April 1985, and from the north jetty of Fort Pierce Inlet, St. Lucie County, Florida and Sailfish Point, Martin County, Florida, September 1985. Colonies were collected subtidally from Pointe Dunkerque, south coast of Martinique, West Indies, August 1985 and June 1986. Worm-free tube sand was obtained from crushed blocks of red reef from the Fort Pierce Inlet and Sailfish Point collections of September 1985 and 1986. Collection, transport and maintenance of reef blocks and production of worm-free tube sand were the same for *P. lapidosa* as for *P. californica* and *S. alveolata* (Pawlik, 1986; 1988). Specimens of *S. floridensis* were obtained, in individual or paired tubes on bivalve shells or sand dollar tests, from the Gulf Specimen Company, Panama, Florida, May 1985 and June 1986.

Cross-fertilization experiments were performed as a simple assessment of relatedness between species. Female worms, bearing lavender or pink abdominal setigers, and male worms, with white to yellow abdominal setigers, were removed from their tubes (2-4 of each), segregated and rinsed twice in flowing seawater. Females were allowed to spawn their gametes into dishes containing 200 ml of 1-μm-filtered seawater (FSW) at 20°C. Males placed on a glass surface shed dry (undiluted) sperm. For each species in a given experiment, 2,000-3,000 unfertilized eggs were added to 50 ml of FSW in 8-cm diameter covered dishes. One dish was set aside without addition of sperm to serve as a control. Five drops of dry sperm were added to 10 ml of FSW and five drops of this suspension were added to a dish containing conspecific eggs. The dish was lightly stirred and addition of sperm repeated twice after 10-min intervals. The seawater overlaying the eggs in the dish was then decanted and replaced with 50-ml FSW. At the same time as self-fertilizations were performed, cross-fertilizations were undertaken in the same fashion. Dishes were placed on a rocking platform at 28 cycles/min at 20°C. After 24 h, unfertilized eggs of *P. californica* and *P. lapidosa* remained stuck to the bottom of the glass dish, while the hatched, swimming trophophores were in suspension. The trophophores were decanted into a separate dish and fixed with several drops of 10% formalin in a transparent grid of 1 cm squares, and the number of either eggs or trophophores counted in each of 10 squares chosen haphazardly. Percentage fertilization was calculated from the mean values of 10 counts. Unfertilized eggs of *S. floridensis* and *S. alveolata* did not adhere to glass. After 24 h, the entire contents of each dish were transferred to a 100-ml graduated cylinder and up to 100 ml. After 10 min 60 cm beneath 40 watt fluorescent lamps, swimming trophophores were pipetted on the top of the water-filled cylinder and unfertilized eggs sank to the bottom. Trophophores were pipetted into one dish and the unfertilized eggs transferred to another. Percentage fertilization was determined as previously described.

Methods for larval culture, for determination of larval growth, maturation and metamorphosis and for production of the types of sand used in assays were those detailed in Pawlik (1988). Inasmuch as *S. floridensis* is non-gregarious, assays of the tube sand of this species employed natural tube sand from Florida, DC. The sand was rinsed with distilled water and oven-dried on aluminum foil (retained on mesh sizes 25 and 40) before use in larval assays. Extraction, isolation and identification of lipid soluble components of the natural tube sand of *P. lapidosa* were undertaken as described in Pawlik (1986; 1988). The diethyl ether: methanol extract was separated by flash chromatography and high-performance liquid chromatography (HPLC) to yield six components: one fraction comprising the material adsorbed onto the flash column and subsequently eluted, four HPLC fractions, and one fraction composed of waste material not collected during HPLC fractionation.

Larval assays were performed as in Pawlik (1988). Larvae used in assays were cultured at 20°C and assayed at the same temperature. All extracts, fractions and standard sperm were added directly to the sand grains by spreading organic solvent solutions containing known concentrations of each substance onto clean sand and removing the volatile solvent under a vacuum. Unless otherwise indicated, all assays were run with 5 replicates (30 ± 2 larvae per replicate) and the mean percentage of larval response for each assay calculated. Differences in mean larval metamorphosis were determined 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 larval responses at the 0.05 level of significance (Sokal and Rohlf, 1981). For situations in which the conditions for parametric testing were not met (e.g., zero variance for one or more treatments), the non-parametric Kruskal-Wallis test (KWT) was performed in lieu of a one-way ANOVA (Conover, 1980).
RESULTS

Cross-fertilization Experiments.—Interfertility was employed as a simple indicator of the relatedness of Phragmatopoma californica and P. lapidosa (A, B, C), and Sabellaria floridensis, P. californica and S. alveolata (D, E). *Cross not performed in this study; P. californica and S. alveolata were previously found not to be interfertile (Pawluk, 1988).

![Table showing the results of cross-fertilization experiments](image)

Figure 1. Percentage fertilization in reciprocal crosses of the gametes of Phragmatopoma californica and P. lapidosa (A, B, C), and Sabellaria floridensis, P. californica and S. alveolata (D, E). *Cross not performed in this study; P. californica and S. alveolata were previously found not to be interfertile (Pawluk, 1988).

Growth, Maturation and Metamorphosis of Larvae in Culture.—Data on larval growth, the onset of metamorphic competence and larval metamorphosis in culture for both P. lapidosa and S. floridensis cultured at 15°C and 20°C are presented in Figure 2 and Table 1.

Effect of Organic Solvent Extraction of Tube Sand of P. lapidosa on Conspecific Larval Metamorphosis.—The percentage metamorphosis of larvae of P. lapidosa in response to microbially-filmed control sand, conspecific tube sand extracted in organic solvents and unextracted conspecific tube sand is presented in Figure 3. There were significant differences in mean larval responses among treatments (ANOVA, $F_{2,12} = 81.57, P < 0.001$). A posteriori analysis (T-method, $\alpha = 0.05$)

![Graph showing growth and metamorphosis](image)

Figure 2. Growth, maturation, and cumulative percentage metamorphosis of larvae of Phragmatopoma lapidosa and Sabellaria floridensis 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 P. lapidosa cultured at 15°C. (B) P. lapidosa cultured at 20°C. (C) Larvae of S. floridensis cultured at 15°C. (D) S. floridensis cultured at 20°C. indicated that the mean percentage of metamorphosed larvae was significantly greater in response to unextracted tube sand than to control or to extracted tube sand. There was no difference in larval response to control sand versus extracted tube sand.

Reciprocal Assays of the Larvae of P. lapidosa and P. californica.—There were significant differences in mean larval metamorphosis among treatments in reciprocal assays of both P. lapidosa and P. californica (Fig. 4; $F_{2,12} = 59.05, P < 0.001$ for P. lapidosa; $F_{2,12} = 137.19, P < 0.001$ for P. californica). For both species, there was greater metamorphosis on conspecific tube sand than on either microbially-filmed control sand or heterospecific tube sand, and there was greater metamorphosis on heterospecific tube sand than on control sand.

Responses of the Larvae of P. lapidosa to Lipophilic Isolates of Natural Tube Sand.—The percentage larval response of P. lapidosa to organic solvent extracts of natural conspecific tube sand (Sailfish Point collection) is shown in Figure 5. A mean of 93.1% of the larvae metamorphosed in response to the 1:1 diethyl
ether: methanol extract. A small percentage of larvae responded abnormally to the diethyl ether: methanol extract; these larvae rotated their larval tentacles and opercular cirri anteriorly, shed their provisional setae or held them forward, and moved sluggishly. There were no significant differences between larval responses to control sand and any of the other extracts (KWT, $H_{11} = 7.4, P > 0.05$).

HPLC traces of the diethyl ether: methanol extract of the natural tube sand of *P. lapidosa* exhibited the same peaks as the trace presented in Pawlik (1986: fig. 1b) for fractionation of the extracts of the tube sand of *P. californica*. Mean larval responses to the fractionated components of the extract are shown in Figure 6. Assay of fraction 2 resulted in a mean of 85.3% metamorphosis and 12.0% abnormal response, the latter described previously. There were no significant differences in mean larval response to control sand and sand treated with any of the other fractions.

The larvae of *P. lapidosa* and *P. californica* were exposed to 1 mg extract/g sand of fraction 2 from extracts of the natural tube sand of each species in reciprocal assays (Fig. 7). The proportion of larvae undergoing normal versus abnormal metamorphosis was very similar for larvae of *P. lapidosa* in response to both the conspecific fraction and the heterospecific fraction. The same was not true for larvae of *P. californica*; there was greater mean abnormal response on exposure to fraction 2 from extracts of tubes of *P. lapidosa* than in response to the conspecific fraction.

**Composition of Fraction 2 from Extracts of the Tube Sand of *P. lapidosa*.**

The results of gas chromatographic analysis of fraction 2 derived from extracts of five samples of natural tube sand of *P. lapidosa* are presented in Table 2. The yield of fraction 2 ranged from 10.7 to 18.3 μg/g tube sand with a mean of 13.8 μg/g tube sand ($N = 3$).

**Responses of the Larvae of *P. lapidosa* to FFA Standards and FFA Derivatives.**

Results of assays of the larvae of *P. lapidosa* in response to FFA standards of variable chain length and unsaturation at six concentrations are presented in Figure 8. At 1 mg FFA/g sand (Fig. 8A), larval response to sand treated with fully saturated FFAs was not different than response to control sand. The cis isomer of 16:1 (palmitoleic acid) induced 72.3% metamorphosis, while the trans isomer (palmitoleadic acid) induced no response at all. Assay of 18:3g, 18:4, 20:4, 20:5, 22:4 and 22:6 at 1 mg/g sand resulted in 100% larval mortality. Abnormal responses were otherwise as previously described.

For assays of FFAs at 300 μg/g sand (Fig. 8B), there were significant differences in mean larval metamorphosis among the four treatments in which there were no abnormal responses (KWT, $H_{11} = 55.28, P < 0.001$). There was greater
Figure 5. Mean percentage metamorphosis (± SE, N = 5) of larvae of *P. lapidosa* 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/MethOH), methanol extract (MethOH) and distilled water extract (H2O) of natural conspecific tube sand.

Figure 6. Mean percentage response (± SE, N = 5) of larvae of *P. lapidosa* to clean sand treated with solvent alone (C) and clean sand treated with 1 mg/g of HPLC-fractions 1–4, waste from HPLC fractionation (Waste) and material adsorbed onto flash column (Adsorb).

Figure 7. Mean percentage metamorphosis (± SE) of larvae of *P. lapidosa* in response to clean sand treated with solvent alone (Pi/C), clean sand treated with 1 mg/g of conspecific HPLC-fraction 2 (Pi/PiF2) and clean sand treated with 1 mg/g of fraction 2 from the tube sand of *P. californica* (Pi/PiF2) and of larvae of *P. californica* in response to clean sand treated with solvent alone (Pi/C), clean sand treated with 1 mg/g of conspecific fraction 2 (Pi/PiF2) and clean sand treated with 1 mg/g of fraction 2 from the tube sand of *P. lapidosa* (Pi/PiF2).

metamorphosis in response to 14:1, 15:1, 16:1, 18:2, 18:3, 18:3g, 18:4 and 20:4 than to control sand. The same was true for assays of FFAs at 100 µg/g sand (Fig. 8C), which resulted in no abnormal larval responses (ANOVA, *F*15,64 = 10.97, *P* < 0.001). Mean larval metamorphosis in response to 15:1, 16:1, 18:3, 18:3 g, 20:4 and 20:5 was greater than in response to control sand. At 50 µg/g sand (Fig.

Table 2. Percentage composition of free fatty acid (FFA) fractions isolated from extracts of the natural tube sand of *P. lapidosa* as determined by gas chromatography. For each FFA, the number of carbon atoms precedes the colon, the number of double bonds follows (br = branch- and straight-chain FFAs present).

<table>
<thead>
<tr>
<th>FFAs detected</th>
<th>Fort Pierce</th>
<th>Saltfish 1</th>
<th>Saltfish 2</th>
<th>Martinique 1</th>
<th>Martinique 2</th>
<th>H.</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td>4.6</td>
<td>4.6</td>
<td>6.1</td>
<td>2.5</td>
<td>5.2</td>
<td>4.6</td>
</tr>
<tr>
<td>15:0 br</td>
<td>5.0</td>
<td>2.1</td>
<td>3.9</td>
<td>3.6</td>
<td>2.1</td>
<td>3.3</td>
</tr>
<tr>
<td>16:2, 16:1</td>
<td>6.8</td>
<td>3.7</td>
<td>7.4</td>
<td>11.7</td>
<td>12.0</td>
<td>8.3</td>
</tr>
<tr>
<td>16:0</td>
<td>26.7</td>
<td>16.5</td>
<td>28.1</td>
<td>34.6</td>
<td>36.1</td>
<td>28.4</td>
</tr>
<tr>
<td>17:0 br</td>
<td>10.0</td>
<td>5.5</td>
<td>3.6</td>
<td>5.6</td>
<td>1.7</td>
<td>5.3</td>
</tr>
<tr>
<td>18:3, 18:2, 18:1</td>
<td>7.8</td>
<td>7.7</td>
<td>9.6</td>
<td>8.3</td>
<td>10.5</td>
<td>8.8</td>
</tr>
<tr>
<td>18:0</td>
<td>14.5</td>
<td>9.8</td>
<td>12.7</td>
<td>10.4</td>
<td>10.8</td>
<td>11.6</td>
</tr>
<tr>
<td>19:0</td>
<td>2.1</td>
<td>0</td>
<td>1.8</td>
<td>1.9</td>
<td>0</td>
<td>0.8</td>
</tr>
<tr>
<td>20:5</td>
<td>5.1</td>
<td>5.0</td>
<td>6.3</td>
<td>6.8</td>
<td>6.3</td>
<td>5.9</td>
</tr>
<tr>
<td>20:4, 20:3, 20:2</td>
<td>8.1</td>
<td>6.4</td>
<td>8.6</td>
<td>6.8</td>
<td>6.3</td>
<td>5.9</td>
</tr>
<tr>
<td>22:4, 22:3, 22:2</td>
<td>8.7</td>
<td>38.0</td>
<td>11.7</td>
<td>7.1</td>
<td>9.3</td>
<td>15.0</td>
</tr>
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</table>
(KWT, $H^2_8 = 22.09, P < 0.01$), with greater metamorphosis in response to 16:1, 18:2, 18:3, 20:4 and 22:6 than in response to control sand. At 1 µg/g sand (Fig. 8F), 16:1 induced greater larval metamorphosis than did control sand ($H^2_5 = 7.12, P < 0.05$).

The results of assays of 16:1 and 18:2 and derivatives of these FFAs at 1 µg/g sand are presented in Figure 9. Percentage metamorphosis was much greater in response to the FFAs. There were significant differences in mean larval metamorphosis among the 10 treatments in which there were no abnormal responses ($H^2_7 = 18.02, P < 0.05$). Larval metamorphosis was greater in response to the monoglyceride of 18:2 and to the alcohols of both 16:1 and 18:2 (cis-9-hexadecen-1-ol and cis-9,12-octadecadien-1-ol) than in response to control sand.

**Effect of Organic Solvent Extraction of Tube Sand of Sabellaria floridensis on Conspecific Larval Metamorphosis.** — The percentage metamorphosis of larvae of *S. floridensis* in response to microbially-filmed control sand, extracted natural conspecific tube sand and unextracted natural conspecific tube sand is shown in Figure 10. There were no significant differences in mean larval responses among the three treatments (ANOVA, $F_{2,10} = 2.63, P > 0.05$).

**Reciprocal Assays of the Larvae of S. floridensis with P. californica and S. aequivalata.** — There were no significant differences in assays of the larvae of *S. floridensis* on microbially-filmed control sand, natural conspecific tube sand and
heterospecific tube sand (Fig. 11; ANOVA, $F_{x,20} = 0.205, P > 0.05$). For larvae of both *P. californica* and *S. alveolata*, there was greater metamorphosis on conspecific tube sand and on natural tube sand of *S. floridensis* than on control sand ($F_{x,8} = 64.31, P < 0.001$ for *P. californica*; $F_{x,8} = 8.10, P < 0.05$ for *S. alveolata*). There was no difference in mean larval metamorphosis on conspecific tube sand versus tube sand of *S. floridensis* for larvae of either *P. californica* or *S. alveolata*.

**Responses of the Larvae of *S. floridensis* to FFA Standards.** —The responses of the larvae of *S. floridensis* to FFAs of variable chain length and unsaturation at three concentrations are presented in Figure 12. At 1 mg FFA/g sand (Fig. 12A), there were no differences in mean larval metamorphosis in response to control sand versus any of the FFAs that did not elicit an abnormal response ($F_{x,8} = 0.01, P > 0.05$). The same was true for assays of FFAs at both 300 and 100 μg/g sand (Fig. 12B, $F_{x,8} = 0.64, P > 0.05$; Fig. 12C, $F_{x,8} = 0.77, P > 0.05$, respectively). Assays of 18:2, 18:3, 20:4 and 20:5 at 1 mg/g sand resulted in larval mortality, and at 300 μg/g sand, abnormal responses to these FFAs were manifested as sluggish movements and some loss of provisional setae, but without any other components of normal metamorphosis.

**DISCUSSION**

*Phragmatopoma lapidosa* and *P. californica* both Metamorphose in Response to Naturally-occurring FFAs. — Overall, the larval responses of *Phragmatopoma lapidosa* at the time of settlement and metamorphosis were very similar to those of *P. californica* (Pawlak, 1986; 1988). Larvae of *P. lapidosa*, like those of *P. californica*, were highly discriminatory in choosing a substrate upon which to metamorphose: although 40–50% of the larvae of *P. lapidosa* metamorphosed in response to conspecific tube sand (Figs. 3, 4), for all assays performed in the present study, the mean of means was $1.0 ± 0.8\%$ metamorphosis onto clean or microbially-filtered control sand ($N = 11$). This compares with 60–90% metamorphosis of larvae of *P. californica* in response to conspecific tube sand and 1.5 ± 1.7% metamorphosis in response to control sand ($N = 11$; Pawlak, 1986; 1988).

Organic solvent extraction of the tube sand of *P. lapidosa*, like that of *P. californica*, completely eliminated its capacity to induce conspecific larval metamorphosis. The inductive capacity was retained in the lipophilic extract, and a mixture of FFAs was isolated as the component that induced metamorphosis. As with *P. californica* (Pawlak and Faulkner, 1986), larvae of *P. lapidosa* responded with a high degree of specificity to FFA standards: larval response was dependent on the presence of at least one cis double bond in the molecule, conservation of molecular shape with increasing acyl chain length by the addition of cis double bonds, and the presence of a free carboxyl group. Larvae of both species responded abnormally to high concentrations of some FFAs (particularly polyenoic acids), but these responses included many of the behavioral components of normal meta-
morphosis, including loss of provisional setae, anterior rotation of larval tentacles and opercular cirri, and reduction of the episphere.

The differences in the larval responses of *P. lapidosa* and *P. californica* were more intriguing than the similarities. By the end of 40 days of development, over 30% of the larvae of *P. lapidosa* had metamorphosed in culture at 20°C as compared with only 5% of the larvae of *P. californica*, suggesting that *P. lapidosa* is less able to postpone metamorphosis than is *P. californica* (Pawlik, 1988). Yet, both species were equally discriminating in their choice of settlement substrata in laboratory assays (Fig. 4).

There were further discrepancies between the two species in the lowest effective concentration of FFAs required to induce larval metamorphosis, in the mean concentration of FFAs isolated from natural tube sand, and in the relative composition of the natural FFA mixtures. In assays of FFA standards, larvae of *P. lapidosa* metamorphosed on sand treated with 16:1 to a significantly greater extent than on control sand at as little as 1 μg/g sand, whereas the lowest effective FFA concentration for inducing metamorphosis of *P. californica* (also on 16:1) was 10 μg/g sand (Pawlik, 1986). This increased sensitivity to FFAs appears to be balanced by both lower overall concentrations of FFAs in the tube sand of *P. lapidosa* and lower proportions of the FFAs that induce metamorphosis, relative to non-stimulatory FFAs, in the natural FFA mixture. The mean concentration of FFAs from the tube sand of *P. californica* was 35 μg/g sand (Pawlik, 1986); the mean concentration isolated from tube sand of *P. lapidosa* was 13.8 μg/g sand. The composition of fraction 2 isolated from natural tube sand of *P. lapidosa* had lower proportions of the most abundant inducers identified from fraction 2 of *P. californica*, 16:1, 18:2 and 20:5 (Pawlik, 1986), although the FFA mixture from tube sand of *P. lapidosa* had a higher proportion of stimulatory 22-carbon polyenic acids (Table 2).

If inductive FFAs are secreted by the adult worms onto tube sand, the observed variability in the composition of the FFA mixture may reflect differences in the lipid composition of the phytoplankton upon which the adult polychaetes feed (cf. see Sargent and Whittle, 1981). Despite these differences, stimulatory FFAs were isolated from the natural tube sand of *P. lapidosa* at sufficient concentrations to induce metamorphosis: approximately 1/5 of the mixture was composed of stimulatory FFAs, or approximately 4–5 μg/g sand.

As with the extraction and isolation of FFAs from the tube sand of *P. californica*, the FFA concentration values determined in the present study are probably low estimates of the actual concentrations of FFAs perceived by larvae in nature. The proteinaceous cement secreted by the adult worms to form sand tubes may bind FFAs in a manner analogous to the binding of FFAs and other lipid-soluble compounds within the kerogen matrix of sedimentary rocks (Hoering and Abelson, 1965; Huxley et al., 1984). The likelihood of underestimating the actual concentration of stimulatory FFAs present on the surface of the sand tubes is further discussed in Pawlik (1986).

If the larvae of *P. lapidosa* and *P. californica* both metamorphose in response

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Figure 12. Mean percentage response (±SE, N = 3) of larvae of *S. ferridens* 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.
to the same suite of FFAs, and these FFAs can be isolated from natural tube sand at effective concentrations, why was there greater metamorphosis in response to conspecific tube sand than to heterospecific tube sand for larvae of both species? There are several plausible explanations of the observed specificity that are neither mutually exclusive nor exhaustive. The demonstrated differences in the FFA compositions from extracts of the sand/cement matrix of the two species may account for the specificity. Subtle changes in the proportions of volatile pheromones have been shown to elicit very different behavioral responses in many insect species (Prestwich, 1985). It is also possible that chemical cues other than FFAs play a role in the larval settlement of these two species, and variability in these signals are responsible for greater metamorphosis on conspecific tube sand.

In the present study, larval growth of *P. lapidosa* in culture was considerably faster than that reported by Mauro (1975; 3–4 weeks at 22–25°C), and within the range reported by Eckelberger (1976; 14–30 days at 21–23°C). Eckelberger (1976) found that less than 50% of the eggs of *P. lapidosa* fertilized at 15°C developed into trochophores that were alive 48 h later, although it remains unclear whether low temperature adversely affected fertilization or subsequent development. As reported herein, development of *P. lapidosa* at 15°C took approximately 70% longer than at 20°C (Table 1; Fig. 2A, B), but the percentage of larvae accounted for at the end of the time-course was nearly the same at both temperatures (Table 1). Mauro (1975: 392) attributed the wide gap in the development rates he observed for *P. lapidosa* (3–4 weeks at 22–25°C) and those of *S. alveolata* reported by Wilson (1968; 6 weeks or more at ~15°C) to culture temperature differences or to “species differences unrelated to the temperature of the medium.” However, standardized procedures employed in the culture of both species (Pawlik, 1988 and present study) have revealed similar maturation rates between species at both 15°C and 20°C. Lower culture temperature affected larval growth and development of *S. floridensis* to a greater degree than any of the other sabellariids studied. Larvae of *S. floridensis* grew very slowly at 15°C and did not attain metamorphic competence within the 40 day time-course, but grew more than twice as fast at 20°C and 50% or more were competent to metamorphose by day 17. Although the distributions of *S. floridensis* and *P. lapidosa* overlap to a considerable extent, the effect of temperature on the development of the latter was much less remarkable (Fig. 2A, B). A comparison of the rates of larval development in culture for the 4 species discussed herein is compiled in Table 3 from data present here and in previous studies. The wide disparities in development rates reported in these studies are further discussed in Pawlik (1988).

**Settlement of *S. floridensis* is Nonspecific: Larval Behavior Otherwise Similar to that of *S. alveolata.*—The results of this study indicate that *S. floridensis* and *S. alveolata* are closely related species, although certainly not as closely related as *P. lapidosa* and *P. californica.* Eggs of *S. alveolata* were not fertilized by sperm of *S. floridensis,* but the reciprocal cross resulted in normally-swimming trochophores (Fig. 1D, E). Both *S. floridensis* and *S. alveolata* exhibited high levels of non-specific metamorphosis: for all assays performed in the present study, the mean of means was 54.4 ± 8.5% metamorphosis of *S. floridensis* in response to clean or microbially-filmed control sand (N = 5), as compared to 43.7 ± 8.3% metamorphosis of *S. alveolata* (N = 12; Pawlik, 1988). Levels of spontaneous metamorphosis of *S. floridensis* in culture at 20°C were intermediate between those of *S. alveolata* and *P. lapidosa* under identical conditions (Fig. 2B, D and Pawlik, 1988). Metamorphosis was not enhanced in the presence of FFAs for either species of *Sabellaria* (Fig. 12; Pawlik, 1988). The abnormal responses of larvae of *S. floridensis* upon exposure to polyenoic FFAs, like those of *S. alveolata,* lacked any similarity to normal metamorphosis, in contrast to the abnormal responses of larvae of both species of *Phragmatopoma.*

In agreement with the present investigation, Eckelberger (1977) reported that more than half of the larvae of *S. floridensis* in some of his cultures metamorphosed in the absence of tube-building materials. He also observed that some recently metamorphosed larvae, in the presence of sand grains, would crawl around culture dishes for several days before starting tube construction. Once sand tubes were built, some of these juveniles continued to crawl on their tentacles, bearing sand tubes detached from the substrate. In the present study, post-larval behavior of this kind was occasionally observed in assay dishes, but it occurred no more frequently than in assays of *S. alveolata.*

It is not clear why high percentages of larvae of both *P. californica* and *S. alveolata* metamorphosed in response to the natural tube sand of *S. floridensis* (Fig. 11). Adult *S. floridensis* may secrete substances along with their tube cement (e.g., FFAs) that induce metamorphosis of these other species, but to which conspecific larvae are insensitive. Perhaps the more advanced, reef-forming sabellariids have appropriated compounds present in the tubes of their non-gregarious predecessors for use as chemical cues of gregarious larval settlement. Unfortunately, because *S. floridensis* is non-gregarious, insufficient natural tube sand could be obtained to attempt the isolation of whatever compounds might be present that induce metamorphosis of *P. californica* and *S. alveolata.*

Inasmuch as most of their responses are so similar, why do larvae of *S. alveolata* settle gregariously and form reefs, while larvae of *S. floridensis* do not? Comparison of the larval behavior of two reef-building species demonstrated that *P. californica* is well adapted for gregarious settlement, but the same could not be said for *S. alveolata* (Pawlik, 1988). Larvae of *P. californica* responded with nearly absolute specificity to conspecific tube sand and, in its absence, could delay metamorphosis for an indefinite period of time. Larvae of *S. alveolata* preferred to metamorphose on conspecific tube sand, but a large portion would metamorphose on control sand or on the walls of culture vessels. Unlike the larvae of either of these colony-
It seems likely that the two subspecies of *P. lapidosa* descended from a continuous population of a single species that existed prior to the formation of the isthmus of Panama, 3.1–3.6 million years ago (Keigwin, 1978). Separation of the two populations since that time has probably resulted in the slight differences in adult morphology, larval development and metamorphic specificity observed for the two forms. The remaining two species in the genus *Phragmatopoma*, *P. attenuata* and *P. moerchi* (and synonyms of the latter, including *P. virgini* and *P. peruenensis*; Hartman, 1944; Kirtley, 1974), both from the Pacific coast of South America, probably share the same ancestry and fall within the same species complex. Both *P. attenuata* and *P. moerchi* settle gregariously to form reefs and differ from each other and from the two subspecies of *P. lapidosa* only in minor aspects of the morphology of their opercular setae (Hartman, 1944; Kirtley, 1974). *Phragmatopoma* may be a monospecific genus, but further consolidation awaits comparison of the interstitiality, larval development and settlement behavior of *P. attenuata* and *P. moerchi*.

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