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# Serotonin Injections Induce Metamorphosis in Larvae of the Gastropod Mollusc *Ilyanassa obsoleta*

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## Abstract:

Bath-applied serotonin (5-HT) induces competent larvae of the marine snail *Ilyanassa obsoleta* to metamorphose. Previously, the mode of action of 5-HT, whether as an external ligand or as an internal neurotransmitter, was unknown. Larvae were injected with $10^{-4}$ *M* 5-HT and other pharmacological agents to provide evidence that serotonergic neurons are necessary for metamorphosis in *Ilyanassa* larvae and that serotonin functions as a neurotransmitter or neuromodulator during this process. About 50% of 5-HT-injected animals metamorphose within 48 hours. Fluoxetine, a 5-HT re-uptake inhibitor, and alpha-methy1-5-hydroxytryptamine (αm5HT), a 5-HT agonist, were also effective inducers of metamorphosis. Gramine (3-[dimethyl-aminomethyl]indole), a 5-HT antagonist, inhibited the inductive activity of 5-HT, while the amino acid gamma-aminobutyric acid (GABA) resulted in rates of morphological restructuring similar to those of controls. Collectively, the results of our experiments support the idea that serotonergic neurons are active during larval metamorphosis of *Ilyanassa* and that 5-HT does not induce metamorphosis by binding to epidermal chemoreceptors.

## Article:

### *Introduction*

Most marine invertebrates spend the early portion of their lives as planktonic larvae, surviving in an environment that is drastically different from the benthic one they will inhabit as adults. Larval metamorphosis is often triggered by physical and biological features of the environment. For many animals, especially molluscs, chemical factors are the most important inducers of metamorphosis (reviewed by Crisp, 1974, 1984; Pawlik, 1992a,b). Several molluscs are known to settle and metamorphose in response to specific environmental compounds (Scheltema, 1961; Hadfield, 1977; Morse and Morse, 1984; Hadfield and Scheuer, 1985; Levantine and Bonar, 1986; Zimmer-Faust and Tamburri, 1994). The oyster *Crassostrea virginica,* whose habitat overlaps with that of the mud snail *Ilyanassa obsoleta* (Fox and Ruppert, 1985), appears to settle in response to a conspecifically derived peptide (Zimmer-Faust and Tamburri, 1994). The natural inducer for *Ilyanassa* larvae is unknown, but like the metamorphic inducers for *Crassostrea* and the nudibranch *Phestilla sibogae* (Hadfield and Sheuer, 1985), it is a small, organic, water-borne molecule (Levantine and Bonar, 1986).

Exogenous neurotransmitters can act as external ligands, mimicking the action of natural metamorphic inducers. For example, larvae of the abalone *Haliotis rufescens* are induced to settle and metamorphose by the neurotransmitter GABA and its structural analogues (Morse *et al.,* 1979; Morse and Morse, 1984; Morse *et al.,* 1984). Like the natural inducer, GABA acts at receptors on the larval epithelium (Trapido-Rosenthal and Morse, 1985; 1986a,b; Wodicka and Morse, 1991).

Exogenous 5-HT is a reliable inducer of metamorphosis in *Ilyanassa,* but its mode of action is unclear (Levantine and Bonar, 1986). Levantine and Bonar suggested that 5-HT acts either at a different site or in a manner different from that of the natural inducer. In their experiments, a low level of metamorphosis in response to mud extract (the natural inducer) was reached in 2 hours. The same metamorphic rate was reached 20 hours later for animals exposed to 5-HT. The difference in timing between these two inducers might reflect

the time required for uptake and internal release of 5-HT or its conversion to another active molecule within the larval nervous system.

The monoamine serotonin is common in both vertebrate and invertebrate nervous systems (reviewed by Kandel *et al.,* 1991; Frazer and Hensler, 1994). Its role as a neurotransmitter and neuromodulator in a wide range of behaviors in molluscs was extensively reviewed by Walker (1986). As examples, serotonin is active in controlling heartbeat rhythm (Liebeswar *et al.,* 1975) and feeding and biting behaviors (Weiss *et al.,* 1978; Kupfermann and Weiss, 1981; Gelperin, 1981; Ram *et al.,* 1981; 1991; Yeoman *et al.,* 1994), and in modulating swimming motor patterns (Parsons and Pinsker, 1989; McPherson and Blankenship, 1991; Satterlie and Norekian, 1995). It also regulates ciliary beating in larval and adult stages of several molluscan species (Gosselin, 1961; Diefenbach and Goldberg, 1990; Diefenbach *et al.,* 1991; Goldberg *et al.,* 1994). Both freshwater and marine molluscs develop serotonergic neurons as embryos and maintain populations of these neurons throughout their lives (Goldberg and Kater, 1989; Koshtoyants *et al.,* 1961; Diefenbach and Goldberg, 1990; Satterlie and Norekian, 1995), although, as discussed below, populations of serotonergic neurons are not necessarily static (Barlow and Truman, 1992).

Serotonin appears to be an endogenous factor that stimulates ciliary beating in nudibranch veligers (Koshtoyants *et al.,* 1961; Coon *et al.,* 1990; Barlow, 1990). Serotonergic neurons in the CNS innervate the velum I—the swimming and feeding organ—and the foot of the nudibranch *Berghia verrucicornis* (Kempf *et al.,* 1991) and of *Ilyanassa* larvae (S. C. Kempf, Auburn Univ., pers. comm.). This discovery, along with Levantine and Bonar's (1986) result showing that metamorphosis in *Ilyanassa* occurs in response to exogenously applied 5- HT, supports the hypothesis that serotonergic neurons and internally active 5-HT are necessary for larval metamorphosis in this species. The role of 5-HT in settlement and metamorphosis in *Ilyanassa* may or may not be a cilio-excitatory one.

Although the activity of serotonergic neurons may be necessary for the induction of metamorphosis, not all of the serotonergic neurons in the larval brain are retained throughout this process. In *Haliotis,* five pairs of 5-HT-immunoreactive (IR) neurons occur in the cerebral gangion by four days after fertilization, and one of these pairs innervates the velum (Barlow and Truman, 1992). This particular neural complement remains stable until metamorphosis, when the pair of velar serotonergic neurons is rapidly lost.

As mentioned above, exogenous neurotransmitters, which are usually small molecules (Erulkar, 1994), may mimic natural compounds by acting at external chemo-receptors (Trapido-Rosenthal and Morse, 1986a,b; Wodicka and Morse, 1991). Larvae can also take up a variety of small molecules from seawater for internal usage (Jaeckle and Manahan, 1989). *Haliotis* larvae can transport amino acids from the environment into their bodies, thereby acquiring energy (Manahan, 1983). Transported compounds can affect larval behavior and could even trigger metamorphosis. The delay in response of *Ilyanassa* to 5-HT in comparison to the natural inducer suggests that 5-HT does not act at epithelial chemoreceptors (Levantine and Bonar, 1986). Instead, exogenous 5-HT may be transported across the larval epidermis and be available to mimic the release of endogenous 5-HT and induce metamorphosis. We injected 5-HT and related pharmacological agents into the hemocoel of competent *Ilyanassa* larvae to determine if serotonergic activity in the nervous system promotes metamorphosis.

## Materials and Methods

### *Animal culture*

Adult *Ilyanassa obsoleta* (Say, 1922) were kept in the laboratory in well-aerated tanks from which egg capsules were removed twice weekly. Every day capsules were briefly rinsed in 70% ethanol to remove bacteria and were placed in fresh 0.2-$\mu$m-filtered Instant Ocean (FI0). Swimming larvae were removed from the dish and held at 7°C until enough larvae were collected to begin a new culture (Leise, 1996).

Larval *Ilyanassa obsoleta* were cultured in the laboratory from embryos (Collier, 1981) as previously de-scribed (Leise, 1996). Briefly, larval cultures were maintained in an airlift system in a 50-50 mixture of 0.2-$\mu$m-filtered

natural seawater and FIO with penicillin and streptomycin antibiotics (Miller and Hadfield, 1986). Larvae were fed algae daily: young cultures (less than 3 days old) received 40 ml of *Isochrysis galbana, Monochrysis* sp., *Dunaliella tertiolecta,* or *Skeletonema rathbun;* older cultures were also fed *Nannochloropsis* sp. Larval culture medium was changed every 6 days.

### *General experimental protocol*

Experiments in which test solutions were applied to larvae in the bath seawater were conducted in 24-well plastic Falcon tissue culture plates. Ten larvae were added to each well along with 2 ml of the appropriate solution. Each treatment and control group was replicated three times and each experiment was repeated three times, yielding a potential total of 90 individuals. Experimental treatments and control groups for individual experiments were obtained from one culture. Different cultures were used for at least two of the repeated experiments to ensure that results were consistent and did not depend upon the culture used. Results from each experiment were pooled, and the combined results were tested for statistical significance, as described below. The graphs presented in this paper were obtained from these pooled results. Mortality was generally near zero in the bath experiments, and around 5% in the injection experiments. The results reported here include only surviving individuals. Usually, less than 400 larvae were available for experimentation in any particular culture. As a result, in many experiments the number of larvae in the control groups was reduced to maintain sufficient numbers of animals in the experimental treatments. This did not impair the statistical significance of the results.

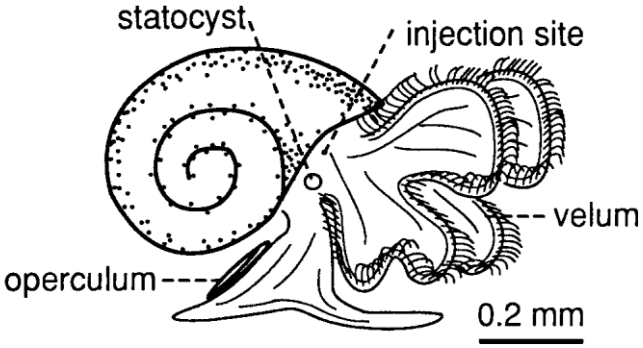


**Figure 1.** Diagram of a competent larva (redrawn from Fretter and Graham, 1962). Injection site is approximately 50 $\mu$m dorsal to the statocyst and probably adjacent to the cerebral ganglia (Lin, 1995).

### *Injection experiments*

Animals that attained a shell length of about 630 $\mu$m or longer were described as competent by Scheltema (1962). In our cultures, 12-40-day-old animals measuring 600-625 $\mu$m were usually competent and were used for all injection experiments. Larvae that were smaller than 600 $\mu$m showed a lower percentage of metamorphosis in response to 5-HT or the natural inducer. Older, larger larvae (>700 $\mu$m) were not useful for injection experiments as a relatively high percentage of these animals metamorphosed spontaneously and in response to FIO (control) injections. This obscured the effects of 5-HT and other experimental compounds.

To facilitate access to the larval hemocoel, larval shells were decalcified by culturing the animals in a low pH and calcium-free artificial seawater bath overnight (Pires and Hadfield, 1993). Larvae were reacclimated to normal seawater (pH 7.9) using several changes of FIO. Prior to injection, larvae were embedded in a 1% wgt/vol solution of low-melting-point agarose (Type VII, Sigma Chemical Co.). Animals were then teased from the gel and pipetted into the experimental chamber. This procedure temporarily impeded ciliary beating and swimming activity so that the larvae lay on the bottom of the dish or swam very slowly and were easily injected. Larvae recovered from this treatment within several hours and swam slowly in the test chamber. Injected animals were less active than uninjected animals, but showed no increase in mortality over the period of most experiments.

Decalcified larvae were injected with a small volume (about 6 nl or about 10% of larval volume) of the test solution using a Picospritzer II (General Valve Corporation) (McCaman *et al.,* 1977). The injection site was approximately 50 $\mu$m dorsal to the statocyst, just under the epidermis. This site was just lateral to the brain and

avoided direct injection into ganglionic neuropils (Fig. 1) (Lin, 1995). Glass micropipettes were pulled on a Flaming/Brown Model P-87 automatic puller (Sutter Instruments).

Following injection, larvae were observed for signs of metamorphosis. Metamorphic induction was considered to have begun when ciliated cells were dropped from the larval swimming organ, or when the velum was dropped in pieces (Fig. 2). Results were scored at 48 hours; a positive result (metamorphosis) was scored only if metamorphosis was complete. Partial metamorphosis (animals retaining mounds of velar supportive cells or ciliated cells from the pre- or post-oral bands) (Pires and Hadfield, 1993) was not considered to be a positive result. Control and experimental animals were taken from the same culture. Positive (5-HT bath) (Levantine and Bonar, 1986) and negative (FIO bath) controls were included in each experiment to determine the capability of that age group for developmental change.

### *Test solutions*

Fluoxetine was a generous gift from the Eli Lilly Re-search Laboratory; αm5HT and 5-HT were purchased from Research Biochemicals International; GABA, gramine, and L-ascorbic acid were purchased from Sigma Chemical Co. Chemical solutions used for injections were $10^{-4}$ *M* (unless otherwise noted) because larval response was usually optimal at this concentration. The optimal response for fluoxetine was seen at $10^{-6}$ *M*. Chemicals were introduced into solution in 1 ml of distilled, deionized water, and then diluted in 99 ml of FIO to achieve a $10^{-4}$ *M* solution. Solutions of $10^{-6}$ and $10^{-8}$ *M* were made by serially diluting aliquots of the $10^{-4}$ *M* solution. The one exception to this procedure involved gramine, which was first dissolved in 1% ethyl alcohol. Injections of 1% ethanol into competent larvae resulted in no significant induction of metamorphosis.

Data were analyzed with 2-way or 3-way chi-square tests (Sprinthall, 1994). For cells with low expected frequencies, a Yates correction was performed to ensure that the chi-square was not inflated (Sprinthall, 1994). Percentage data were normalized using an arc-sin transformation before analysis (Rohlf and Sokal, 1981), but were transformed back to percentages for graphing. Error bars are standard deviations of the mean. Graphs were produced with the Statgraphics software program (Manugistics, Inc.).

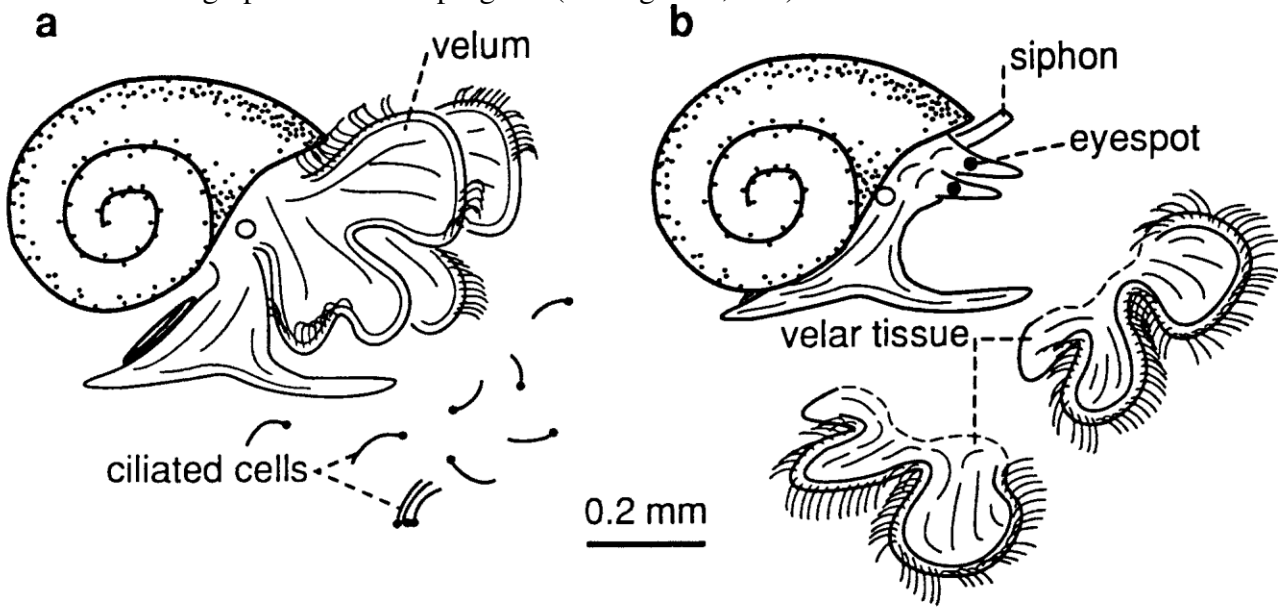


**Figure 2.** (a) Diagram of a larva at the beginning of metamorphosis. Ciliated cells are being individually dropped from the velum. (b) Drawing of a newly metamorphosed juvenile. The velum has been lost in large pieces (modified from Fretter and Graham, 1962).

## Results

Injection and bath-application of 5-HT induced significantly more metamorphosis than did FIO controls (Fig. 3). The results of 5-HT injections were also statistically different from those produced by bath-application of 5-HT. Fluoxetine, a 5-HT reuptake inhibitor (Kandel, 1991; Frazer and Hensler, 1994; Ramamoorthy *et al.,* 1993), and am5-HT, a 5-HT agonist (Walker, 1986; Chaouche-Teyara *et al.,* 1993), also induced significant levels of metamorphosis compared to controls (Figs. 4, 5). The results of $10^{-6}$ *M* fluoxetine injections did not

differ statistically from those seen in the 5-HT bath condition. Injections of $10^{-4}$ *M* fluoxetine induced less metamorphosis than injections of $10^{-6}$ *M* fluoxetine *(cf.* Figs. 4, 6). When $10^{-4}$ *M* fluoxetine and 5-HT are injected together there is an additive effect (Fig. 6). Gramine, a 5-HT antagonist (Zhang and Harris-Warrick, 1994), did not promote metamorphosis when injected alone, and when injected along with 5-HT it resulted in decreased rates of metamorphosis (Fig. 7). To determine if these results were indeed specific to serotonergic neurons and their influence on larval physiology, GABA was injected as a negative control. Injections of this neurotransmitter did not induce significant levels of metamorphosis (Fig. 8).

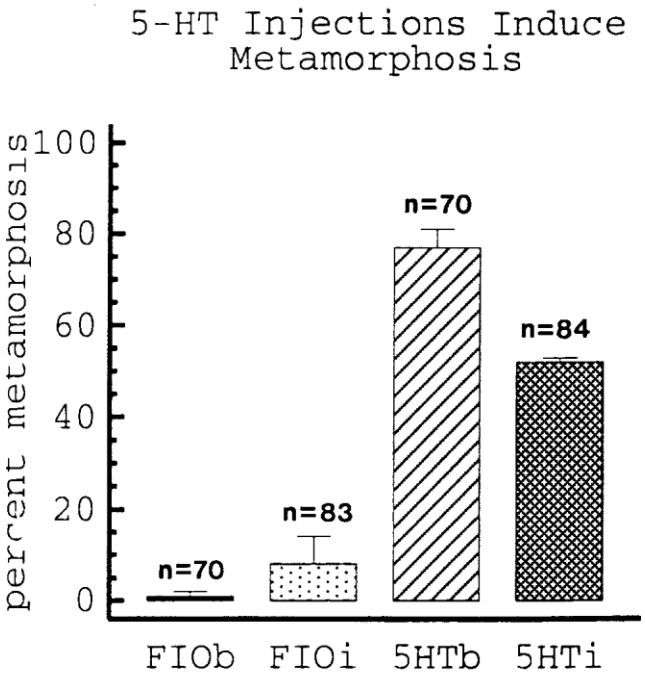


**Figure 3.** Injections of $10^{-4}$ *M* 5-HT (5HTi) induced metamorphosis in about 50% of larvae in comparison to FIO injections (FIOi) which induced an average of 8% metamorphosis ($\chi^2_{.01(1)} = 31.69$). Larvae were scored after 48 hours in all experiments. Comparison of 5-HT bath (5HTb) and 5HTi showed a statistically significant difference between these two treatments ($\chi^2_{0.1(1)} = 11.02$). Control conditions using bath-applied 5-HT (5HTb) and bath FIO (FIOb) induced 77% and 1% metamorphosis, respectively.

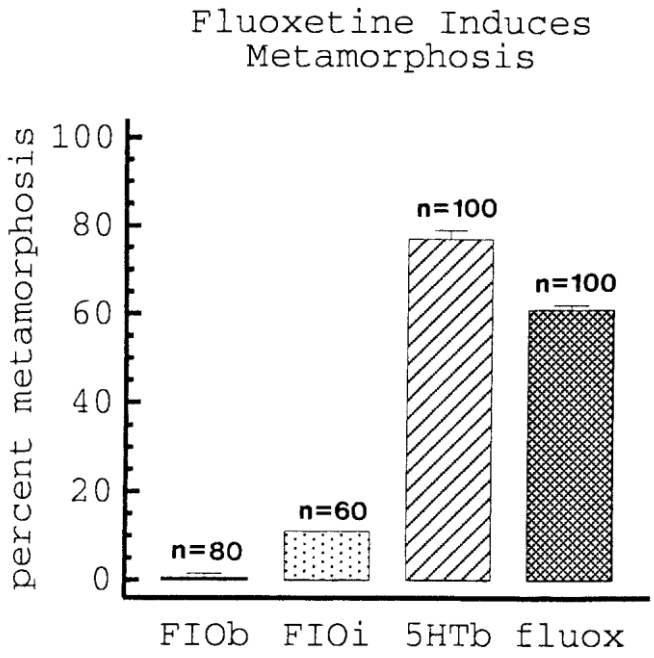


**Figure 4.** $10^{-6}$ *M* fluoxetine injections (fluox) induced 61% metamorphosis. Statistical analysis comparing $10^{-6}$ *M* fluoxetine injections with FIO injections showed a highly significant effect ($\chi^2_{.01(1)} = 34.67$). The fluoxetine treatment was not significantly different from the 5HTb condition ($\chi^2 = 2.42$; accept $H_0$).

## Discussion

To partially test the hypothesis that serotonin and serotonergic neurons are directly involved in metamorphosis, behaviorally relevant amounts of 5-HT were injected into competent *Ilyanassa* larvae. Injections induced a high level of metamorphosis, suggesting that serotonin may be normally released as a neurotransmitter during metamorphosis. Because metamorphosis was considered to have occurred only if the loss of the velum and development to the juvenile state were completed within the experimental period, percentages of metamorphosis reported in this study may be somewhat less than those of other authors. Even with these more rigorous criteria, the results of our injection experiments were nonetheless significant.

Many compounds can be oxidized by seawater, losing or changing potency and thus acting in unexpected ways within larvae (Pires and Hadfield, 1991). Metamorphosis in response to bath-applied 5-HT could reflect the activity of this neurotransmitter within the larval nervous system or the activity of some unidentified oxidized product of this compound. Direct injection of 5-HT into the hemolymph eliminates the confounding factor inherent in bath-application studies, although it does not eliminate the possibility that serotonin is metabolized within larvae and that it is some metabolite, rather than serotonin, that was active during our experiments. Still, these experiments allow us to further define the locus of 5-HT activity in the larval nervous system.

5-HT plays numerous roles in molluscan nervous systems (Walker, 1986) and major groups of serotonergic neurons have been identified in several gastropod taxa (Koshtoyants *et al.,* 1961; Walker 1986; Jahan-Parwar *et*

*al.,* 1987; Goldberg and Kater, 1989; Diefenbach and Goldberg, 1990; McPherson and Blankenship, 1991; Fitzgerald and Carew, 1991). For example, 5-HT regulates neurite extension and growth-cone motility in the freshwater snail *Helisoma trivolvis* (Price and Goldberg, 1993); stimulates motor neurons in *Aplysia californa* (Ram *et al.,* 1991); plays an important role in molluscan peripheral tissues, especially the heart (Walker, 1986); and heightens motor activity in embryonic gastropods in a developmentally sensitive manner (Koshtoyants *et al.,* 1961; Diefenbach *et al.,* 1991). In these young animals exogenous serotonin increases ciliary activity during early embryonic stages, then sensitivity to 5-HT decreases to an intermediate level. Eventually, ciliary beating alternates with long pauses and contraction of the velar lobes (Koshtoyants *et al.,* 1961). Results from these studies suggest that 5-HT is an endogenous factor partially responsible for controlling the characteristic ciliary beating that occurs during embryonic development in many gastropods.

Full metamorphosis in the prosobranch *Ilyanassa* is similar to the partial metamorphosis of *Phestilla sibogae* seen in response to hydrogen peroxide and catecholes (Pires and Hadfield, 1991). The first sign of metamorphosis is the loss of velar metachronal rhythm (Bonar, 1973). This loss may be due to the uncoupling of gap junctions at the bases of the preoral ciliated cells. If the velar ciliated cells are separated from the central nervous system (CNS), coordinated ciliary arrests are abolished and the cilia show continuous metachronal beating (Mackie *et al.,* 1976). Studies by Koshtoyants *et al.* (1961) revealed the cilio-excitatory role of 5-HT, but electrophysiological studies have shown that spikes generated by electrical stimulation of the velar cells elicit ciliary arrests (Mackie *et al.,* 1976; Arkett, 1988), indicating that 5-HT is not responsible for cessation of ciliary beating. Serotonin is thus unlikely to be the neurotransmitter at neurociliary junctions but may act at extrasynaptic receptors (Mackie *et al.,* 1976). In *Ilyanassa,* 5-HT injections induce a high percentage of metamorphosis within 48 hours, but we do not know if the larval response to this injection involves a cilio-excitatory action of 5-HT, a modulatory effect on extra-synaptic receptors near the ciliated epithelium (Mackie *et al.,* 1976), activity on a separate set of cells—perhaps within the CNS, or a combination of these effects.

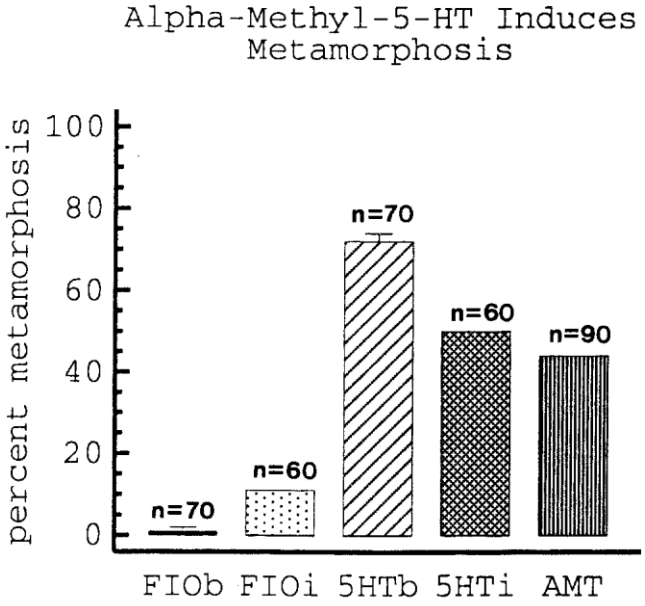


**Figure 5.** Injections of alpha-methyl-5-HT (AMT) induced 44% metamorphosis. This 5-HT agonist was as effective as 5-HT. FIOi induced 11% metamorphosis in this set of experiments. Metamorphic induction was significantly higher for AMT than for FIOi animals ($\chi^2_{.01(1)} = 17.97$).

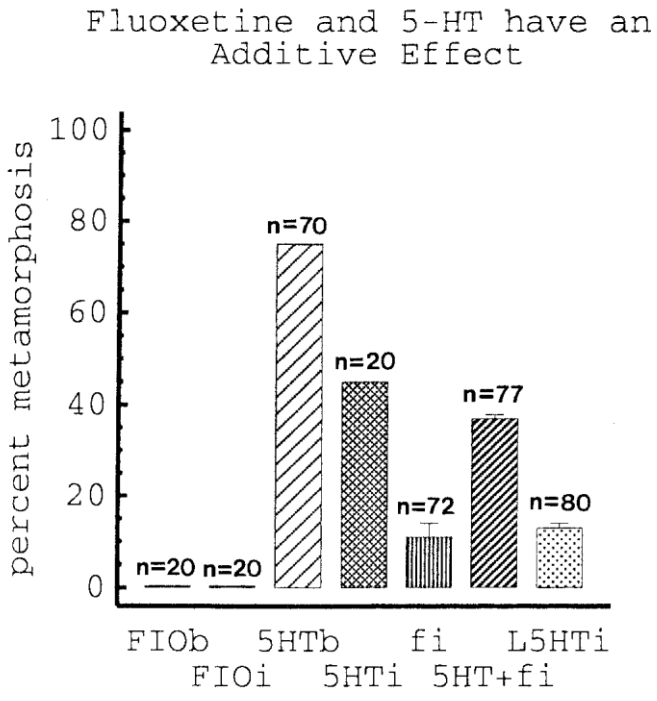


**Figure 6.** When $10^{-8}$ *M* 5-HT (L5HTi) and $10^{-4}$ *M* fluoxetine (fi) are injected in combination (5HT + fi) there is an additive effect in comparison to injections of these compounds alone ($\chi^2_{.01(2)} = 23.16$). $10^{-8}$ *M* 5-HT injections resulted in significantly less metamorphosis than injections of $10^{-4}$ *M* 5-HT ($\chi^2_{.01(2)} = 9.76$).

Both *Ilyanassa* and *Phestilla* lose their vela early in metamorphosis. *Phestilla* ingests its velar ciliated cells and incorporates the remaining supportive cells into the cephalic epidermis (Bonar and Hadfield, 1974). *Ilyanassa* may lose the velum *in toto* (Scheltema, 1962), or ciliated cells may drop off individually or in clumps (Couper

and Leise, 1994). Like *Phestilla, Ilyanassa* metamorphoses a number of hours after initial exposure to an inducer (Bonar and Hadfield, 1974; Levantine and Bonar, 1986). The reason for this long delay is unknown.

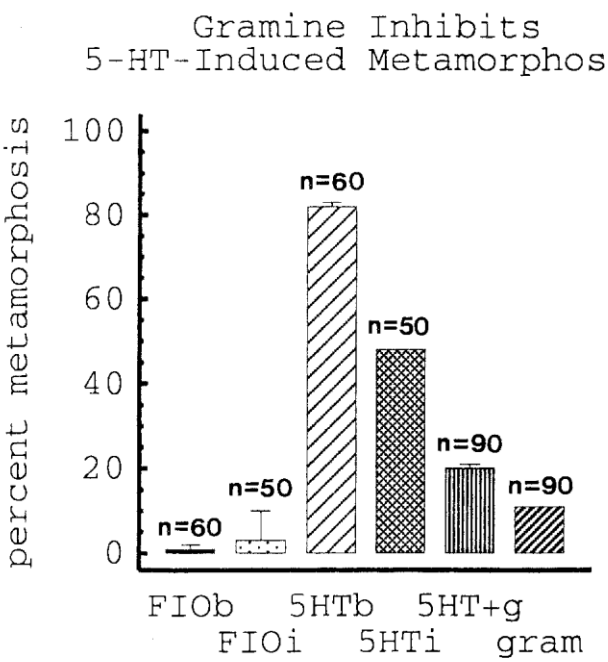


**Figure 7.** The 5-HT antagonist gramine reduces 5-HT-induced metamorphosis from 48% to 20%. A 3-way chi-square analysis comparing levels of metamorphosis induced by injections of gramine (gram), gramine + 5-HT (5HT+g), and 5-HT alone, showed that this reduction is significant ($\chi^2_{.01(2)} = 26.27$).

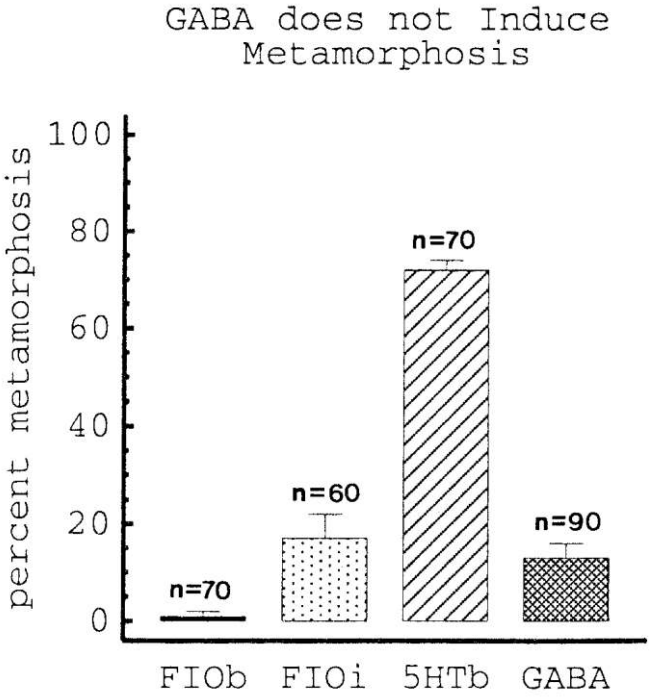


**Figure 8.** The amino acid GABA was used as a negative control. Results of GABA injections showed no increase in metamorphosis in comparison to FIOi controls, suggesting that metamorphosis is specific to 5-HT or 5-HT analog ($\chi^2 = 0.24$; accept $H_0$).

Endogenous 5-HT may activate targets postsynaptic to the chemosensory neurons that mediate a larva's response to the natural inducer. Injections of 5-HT may mediate either the settlement response, which includes the cessation of ciliary beating, or the metamorphic response (loss of the velum) or both. Irrespective of its causative role in metamorphosis, if 5-HT is active as a neurotransmitter or neuromodulator, then 5-HT and related pharmacological agents should affect the metamorphic process. We tested several relevant compounds in bath-application and injection experiments in an attempt to support the idea that 5-HT has an internal mode of action. The results support the hypothesis that serotonergic neurons need to be activated for metamorphosis to proceed.

The compound am5-HT is a 5-$HT_2$ agonist in vertebrate nervous systems (Chaouche-Teyara *et al.,* 1993) and a 5-HT agonist in invertebrate nervous systems (Walker, 1986). Although receptor classification is not identical for molluscs and vertebrates, structure-activity studies using 5-HT and chemical analogs indicate that the preferred form of the ligand for activation of any particular 5-HT receptor is probably similar for different species (Walker, 1986). As was expected, αm5-HT induced rates of metamorphosis similar to those seen with 5-HT injections. These results support the idea that 5- HT, and not a metabolite, is active internally as a neurotransmitter in *Ilyanassa.*

Gramine selectively antagonizes activity generated by 5-HT application in the crab stomatogastric ganglion (Zhang and Harris-Warrick, 1994). When $10^{-4}$ *M* gramine was injected into *Ilyanassa* along with $10^{-4}$ *M* 5-HT, induction of metamorphosis was half of that seen with injections of 5-HT alone. This decreased level of metamorphosis indicates that gramine inhibited the action of 5-HT in *Ilyanassa* larvae. Whether gramine and 5-HT compete for the same binding site is unknown.

GABA is a common molluscan neurotransmitter (Osborne, 1971) and can have mixed excitatory and inhibitory effects (Kerkut and Walker, 1961). It induces metamorphosis in *Haliotis* by mimicking the actions of the natural inducer at external chemoreceptors (Trapido-Rosenthal and Morse, 1985, 1986a,b; Barlow, 1990; Wodicka and Morse, 1991). Injection of GABA provided a negative control for our study.

In summary, 5-HT was dearly the most powerful metamorphic inducer of the compounds tested.

The synaptic effects of 5-HT are terminated by the binding of its molecules to specific transporter proteins (re-uptake) in serotonergic terminals; this mechanism is prevented by selective 5-HT reuptake inhibitors like fluoxetine, which are used as effective antidepressants for humans (Kandel, 1991; Ramamoorthy *et al.,* 1993; Frazer and Hensler, 1994; Barondes, 1994). Fluoxetine acts to increase the availability of 5-HT at the synapse in a number of mammalian nervous systems (Sprouse *et al.,* 1993; Biegon *et al.,* 1993), although Sloley *et al.* (1993) provided evidence that this effect may not occur in all vertebrates. Uptake mechanisms for 5-HT occur in brains of the snail *Helix aspersa* and the squid *Loligo pealeii* (Osborne *et al.,* 1975; Feldman and Dowdall, 1973) and are probably widespread in molluscs. As anticipated, fluoxetine injections induced levels of metamorphosis similar to those of 5-HT injections. Fluoxetine injections also induced levels of metamorphosis that were not statistically different from those seen in 5-HT bath conditions. However, 5-HT injections produced rates of metamorphosis that were statistically different from 5-HT bath-application rates. Injected 5-HT may not be available for an extended time in the nervous system of *Ilyanassa.* Injecting a 5-HT reuptake inhibitor could allow 5-HT to persist in the larval nervous system for a longer time, enabling it to exert its metamorphic effects to a greater extent.

The additive effect seen with injections combining $10^{-8}$ *M* 5-HT (which by itself induces low levels of metamorphosis) and $10^{-4}$ *M* fluoxetine corroborates that serotonin and serotonergic neurons are involved in metamorphosis. It is unclear whether 5-HT acts in the nervous system of *Ilyanassa obsoleta* larvae as a CNS neurotransmitter or as a neuromodulator, but the results of this study provide evidence that it is an endogenous compound that plays an important role in metamorphosis.

## Literature Cited


Arkett, S. A. 1988. Development and senescence of control of ciliary locomotion in a gastropod veliger. *J. Neurobiol.* 19: 612-623.

Barlow, L. A. 1990. Electrophysiological and behavioral responses of larvae of the red abalone *(Haliotis rufescens)to* settlement-inducing substances. *Bull. Mar. Sci.* 46: 537-554.

Barlow, L. A., and J. W. Truman. 1992. Patterns of serotonin and SCP immunoreactivity during metamorphosis of the nervous system of the red abalone, *Haliotis rufescens. J. Neurobiol.* 23: 829- 844.

Barondes, S. H. 1994. Thinking about Prozac. *Science* 263: 1102- 1103.

Biegon, A., C. A. Mathis, S. M. Hanrahan, and W. J. Jagust. 1993. [125IP-Iodo-6-nitroquipazine: a potent and selective ligand for the 5-hydroxytryptamine uptake complex. II. *In vivo* studies in rats. *Brain Res.* 619: 236-246.

Bonar, D. B. 1973. An analysis of metamorphosis in *Phestilla sibogae* Bergh 1905 (Gastropoda, Nudibranchia). Ph.D. dissertation, University of Hawaii.

Bonar, D. B., and M. G. Hadfield. 1974. Metamorphosis of the marine gastropod *Phestilla sibogae* Bergh (Nudibranchia: Aeolidacea). *Tiss. Ce1110:* 153-165.

Chaouch-Teyara, K., B. Fournier, M. Safar, and H. Dabire.1993. Vascular and cardiac effects of alpha-methyl-5-HT and DOI are mediated by different 5-HT receptors in the pithed rat. *Eur. J. Pharmacol.* 250: 67-75.

Collier, J. R. 1981. Methods of obtaining and handling eggs and embryos of the marine mud snail *Ilyanassa obsoleta.* Pp. 233-246 in *Marine Invertebrates. Laboratory Animal Management.* National Academic Press, Washington, DC.

Coon, S. L., W. K. Fitt, and D. B. Bonar. 1990. Competence and delay of metamorphosis in the Pacific oyster *Crassostrea gigas. Mar. Biol.* 106: 379-387.

Couper, J. M., and E. M. Leise. 1994. 5-HT injections induce metamorphosis in larvae of the gastropod mollusc *Ilyanassa obsoleta. Am. Zool.* 34(5): 101A

Crisp, D. J. 1974. Factors influencing the settlement of marine invertebrate larvae. Pp. 177-265 in *Chemoreception in Marine Organ-isms,* P. T. Grant and A. M. Mackie, eds. Academic Press, New York.

Crisp, D. J. 1984. Overview of research on marine invertebrate larvae, 1940-1980. Pp. 103-126 in *Marine Biodeterioration: An Inter-disciplinary Study. J.* D. Costlow and R. C. Tipper, eds. Naval Institute Press, Annapolis.

Diefenbach, T. J., and J. I. Goldberg. 1990. Postembryonic expression of the serotonin phenotype in *Helisoma trivolvis:* comparison between laboratory-reared and wild-type strains. *Can. J. Zool.* 68: 1382-1389.
Diefenbach, T. J., N. K. Koehncke, and J. I. Goldberg. 1991. Characterization and development of rotational behavior in *Helisoma* embryos: role of endogenous serotonin. *J. Neurobiol.* 22: 922-934.
Erulkar, S. D. 1994. Chemically mediated synaptic transmission: an overview. Pp. 181-208 in *Basic Neurochemistry: Molecular, Cellular, and Medical Aspects,* 5th ed., G. *J.* Siegel, ed. Raven Press, New York.
Feldman, J. L., and M. J. Dowdall. 1973. 5-hydroxytryptamine: an uptake mechanism in synaptosomes from the optic lobe of squid *(Loligo pealeii). Biol. Bul1145:* 432-433.
Fitzgerald, K., and T. J. Carew. 1991. Serotonin mimics tail shock in producing transient inhibition in the siphon withdrawal reflex of *Aplysia. J. Neurosci. 11:* 2510-2518.
Fox, R. S., and E. E. Ruppert. 1985. *Shallow-water Marine Benthic Macroinvertebrates of South Carolina: Species Identification, Com-munity Composition and Symbiotic Associations.* University of South Carolina Press, Columbia.
Frazer, A., and J. G. Hensler. 1994. Serotonin. Pp. 283-308 in *Basic Neurochemistry: Molecular, Cellular, and Medical Aspects,* 5th ed. G. *J.* Siegel, ed. Raven Press, New York.
Fretter, V., and A. Graham. 1962. Larval forms. Pp. 448-476 in *British Prosobranch Molluscs.* Royal Society, London.
Gelperin, A. 1981. Synaptic modulation by identified serotonin neurons. Pp. 288-304 in *Serotonin Neurotransmission and Behavior.* B. L. Jacobs and A. Gelperin, eds. MIT Press, Cambridge.
Goldberg, J. I., and S. B. Kater. 1989. Expression and function of the neurotransmitter serotonin during development of the *Helisoma* nervous system. *Devel. Biol.* 131: 483-495.
Goldberg, J. I., N. K. Koehncke, K. J. Christopher, C. Neumann, and T. J. Diefenbach. 1994. Pharmacological characterization of a serotonin receptor involved in an early embryonic behavior of *Hell-soma trivolvis. J. Neurobiol.* 25: 1545-1557.
Gosselin, R. E. 1961. The cilioexcitatory activity of serotonin. *J. Cell. Comp. Physiol.* 58: 17-25.
Hadfield, M. G. 1977. Chemical interactions in larval settling of a marine gastropod. Pp. 403-413 in *Marine Natural Products Chemistry.* D. J. Faulkner and W. H. Fenical, eds. Academic Press, New York.
Hadfield, M. G., and D. Scheuer. 1985. Evidence for a soluble metamorphic inducer in *Phestilla:* ecological, chemical and biological data. *Bull. Mar. Sci.* 37: 556-566.
Jaeckle, W. B., and D. T. Manahan. 1989. Feeding by a "nonfeeding" larva: uptake of dissolved amino acids from seawater by lecithotrophic larvae of the gastropod *Haliotis rufescens. Mar. Biol.* 103: 87-94.
Jahan-Parwar,B., K.-S. Rozsa, J. Salanki, M. L. Evans, and D. O. Carpenter. 1987. *In vivo* labeling of serotonin-containing neurons by 5,7-di hydroxytryptamine in *Aplysia. Brain Res.* 426: 173-178.
Kandel, E. R. 1991. Disorders of mood: depression, mania and anxiety disorders. Pp. 867-883 in *Principles of Neural Science,* E. R. Kandel, J. H. Schwartz, and T. M. Jessell, eds. Elsevier, New York.
Kandel, E. R., J. H. Schwartz, and T. M. Jessell (eds.). 1991. *Principles of Neural Science,* 3rd ed. Elsevier, New York.
Kerkut, G. A., and R. J. Walker. 1961. The effects of drugs on the neurones of the snail, *Helix aspersa. Comp. Biochem. Physiol.* 3: 143-160.
Kempf, S. C., A. Saini, and A. Jones. 1991. The ontogeny of neuronal systems expressing SCP-like and serotonin-like antigens in *Berghia verrucicornis. Soc. Neurosci. Abstr.* 17: 1356.
Koshtoyants, Kh. S., G. A. Buznikov, and B. N. Manukhin. 1961. The possible role of 5-hydroxytryptamine in the motor activity of embryos of some marine gastropods. *Comp. Biochem. Physiol.* 3:21)- 26.
Kupfermann, I., and K. R. Weiss. 1981. The role of serotonin in arousal of feeding behaviour of *Aplysia.* Pp. 255-287 in *Serotonin Neurotransmission and Behavior,* B. L. Jacobs and A. Gelperin, eds. MIT Press, Cambridge.
Leise, E. M. 1996. Selective retention of the fluorescent dye DASPEI in a larval gastropod mollusc after paraformaldehyde fixation. *Micros. Res. Tech.* 33: 496-500.
Levantine, P. L., and D. B. Bonar. 1986. Metamorphosis of *Ilyanassa obsoleta:* natural and artificial inducers. *Am. Zool.* 26: 14A.
Liebeswar, G., J. E. Goldman, J. Koester, and E. Mayeri. 1975. Neural control of circulation in *Aplysia. III:* Neurotransmitters. *J. Neurophysiol.* 38: 767-779.

Lin, M.-F. 1995. Gangliogenesis and morphogenesis of NADPH-diaphorase activity in the prosobranch gastropod *Ilyanassa obsoleta.* Master's Thesis, University of North Carolina Greensboro.
Mackie, G. O., C. L. Singla, and C. Thiriot-Quievreux. 1976. Nervous control of ciliary activity in gastropod larvae. *Biol. Bull.* 151: 182-199.
Manahan, D. T. 1983. The uptake and metabolism of dissolved amino acids by bivalve larvae. *Biol. Bull.* 164: 236-250.
McCaman, R. E., D. G. McKenna, and J. K. Ono. 1977. A pressure system for intracellular and extracellular ejections of picoliter volumes. *Brain Res.* 136: 141-147.
McPherson, D. R. and J. E. Blankenship. 1991. Neural control of swimming in *Aplysia brasiliana* III. Serotonergic modulatory neurons. *J. Neurophysiol.* 66: 1366-1379.
Miller, S. E., and M. G. Hadfield. 1986. Ontogeny of phototaxis and metamorphic competence in larvae of the nudibranch *Phestilla sibogae* Bergh (Gastropoda: Opistobranchia). *J. Exp. Mar. Biol. Ecol.* 97:95-112.
Morse, A. N. C., and D. E. Morse. 1984. Recruitment and metamorphosis of *Haliotis* larvae induced by molecules uniquely available at the surfaces of crustose red algae. *J. Exp. Mar. Biol. Ecol.* 75: 191-215.
Morse, D. E., N. Hooker, H. Duncan, and L. Jensen. 1979. γ-amino-butyric acid, a neurotransmitter, induces planktonic abalone larvae to settle and begin metamorphosis. *Science* 204: 407-410.
Morse A. N. C., C. A. Froyd, and D. E. Morse. 1984. Molecules from cyanobacteria and red algae that induce larval settlement and metamorphosis in the mollusc *Haliotis rufescens. Mar. Biol.* 81: 293- 298.
Osborne, N. N. 1971. Occurrence of GABA and taurine in the nervous systems of dogfish and some invertebrates. *Comp. Gen. Pharmacol.* 2: 433-438.
Osborne, N. N., L. Hiripi, and V. Neuhoff. 1975. The *in vitro* uptake of biogenic amines by snail *(Helix pomatia)* nervous tissue. *Bio-them. Pharmacol.* 24: 2141-2148.
Parsons, D. W., and H. M. Pinsker. 1989. Swimming in *Aplysia brasiliana:* behavioral and cellular effects of serotonin. *J. Neurophysiol.* 62: 1163-1176.
Pawlik, J. R. 1992a. Induction of marine invertebrate larval settlement: evidence for chemical cues. Pp. 189-236 in *Ecological Roles of Marine Natural Products, V* . *J.* Paul, ed. Comstock Publishing, Ithaca, NY.
Pawlik, J. R. 1992b. Chemical ecology of the settlement of benthic marine invertebrates. *Oceanogr. Mar. Biol. Annu. Rev.* 30:273-335.
Pires, A., and M. G. Hadfield. 1991. Oxidative breakdown products of catecholamines and hydrogen peroxide induce partial metamorphosis in the nudibranch *Phestilla sibogae* Bergh (Gastropoda: Opisthobranchia). *Bio/. Bull.* 180: 310-317.
Pires, A., and M. G. Hadfield. 1993. Responses of isolated vela of nudibranch larvae to inducers of metamorphosis. *J. Exp. Zool.* 266: 234-329.
Price, C. J., and J. I. Goldberg. 1993. Serotonin activation of a cyclic AMP-dependent sodium current in an identified neuron from *Helisoma trivolvis. J. Neurosci.* 13: 4979-4987.
Ram, J. L., U. A. Shukla, and G. S. Ajimal. 1981. Serotonin has both excitatory and inhibitory modulatory effects on feeding muscles in *Aplysia. J. Neurobiol.* 12: 613-621.
Ram, J. L., F. Zhang, and L-X. Liu. 1991. Contraction, serotonin-elicited modulation, and membrane currents of dissociated fibers of *Aplpia* buccal muscle. *Biol. Bull.* 180: 276-283.
Ramamoorthy, S., A. L. Bauman, K. R. Moore, H. Han, T. Yang-Feng, A. S. Chang, V. Ganapathy, and R. D. Blakely. 1993. Antidepressant- and cocaine-sensitive human serotonin transporter: molecular, cloning, expression, and chromosomal localization. *Proc. Natl. Acad. Sci. USA* 90: 2542-2546.
Rohlf, F. J., and R. R. Sokal. 1981. *Statistical Tables,* pp. 54-59. W. H. Freeman, New York.
Satterlie, R. A., and T. P. Norekian. 1995. Serotonergic modulation of swimming speed in the pteropod mollusc *Clione limacina. III.* Cerebral neurons. *J. Exp. Biol.* 198: 917-930.
Scheltema, R. S. 1961. Metamorphosis of the veliger larvae of *Nassarius obsoletus* (Gastropoda) in response to bottom sediment. *Biol. Bull.120:* 92-109.
Scheltema, R. S. 1962. Pelagic larvae of New England intertidal gastropods. *Trans. Am. Microsc. Soc.* 81: 1-11.
Sloley, B. D., S. Orikasa, and A. A. Boulton. 1993. Catabolism of intracerebroventricularly injected 5-hydroxytryptamine in mouse: effect of coinjection of tryptamine and several pretreatments. *Can. J. Pharmacol.* 71: 201-204.
Sprinthall, R. C. 1994. *Basic Statistical Analysis.* Allyn and Bacon, Boston.

Sprouse, J. S., D. R. McCarty, and M. W. Dudley. 1993. Apparent regional differences in 5-HT 1 A binding may reflect [3H18-0H-DPAT labeling of serotonin uptake sites. *Brain Res.* 617: 159-162.
Trapido-Rosenthal, H. G., and D. E. Morse. 1985. L-α,ω-diamino acids facilitate GABA induction of larval metamorphosis in a gastropod mollusc *(Haliotis rulescens). J. Comp. Physiol. B* 155: 403- 414.
Trapido-Rosenthal, H. G., and D. E. Morse. 1986a. Availability of chemosensory receptors is down-regulated by habituation of larvae to a morphogenetic signal. *Proc. Natl. Acad. Sci. USA* 83: 7658- 7662.
Trapido-Rosenthal, H. G., and D. E. Morse. 1986b. Regulation of receptor-mediated settlement and metamorphosis in larvae of a gastropod mollusc *(Halioti.s rulescens). Bull. Mar. Sci.* 39: 383-392.
Walker, R. J. 1986. Transmitters and modulators. Pp. 279-452 in *The Mollusca.* Vol. 9. *Neurobiology and Behavior,* Part. 2. K. M. Wilbur, ed. Academic Press, New York.
Weiss, K. R., J. L. Cohen, and I. Kupfermann. 1978. Modulatory control of buccal musculature by a serotonergic neuron (metacerebral cell) in *Aplysia. J. Neurophysiol.* 42: 791-803.
Wodicka, L. M., and D. E. Morse. 1991. cDNA sequences reveal mRNAs for two Ga signal transducing proteins from larval cilia. *Biol. Bull.* 180: 318-327.
Yeoman, M. S., A. W. Pieneman, G. P. Ferguson, A. Ter Maat, and P. R. Benjamin. 1994. Modulatory role for the serotonergic cerebral giant cells in the feeding system of the snail, *Lymnaea.* I. Fine wire recording in the intact animal and pharmacology. *J. Neurophys.* 72: 1357-1371.
Zhang, B., and R. M. Harris-Warrick. 1994. Multiple receptors mediate the modulatory effects of serotonergic neurons in a small neural network. *J. Exp. Biol.* 190: 55-77.
Zimmer-Faust, R. K., and M. N. Tamburri. 1994. Chemical identity and ecological implications of a waterborne, larval settlement cue. *Limnol. Oceanogr.* 39: 1075-1087.