that the coral aragonite actually has a Sr/Ca distribution coefficient similar to that of seawater but that the seawater-like solution from which the coral aragonite is precipitated has a Sr/Ca ratio modified somewhat from that ratio in seawater by the mediating action of the tissue.
14. D. W. Knutson, R. W. Buddemeier, S. V. Smith, *Science* **177**, 270 (1972); J. H. Hudson, E. A. Shinn, R. B. Halley, B. Lidz, *Geology* **4**, 361 (1976); R. E. Dodge, R. C. Aller, J. Thompson, *Nature (London)* **247**, 547 (1975).
15. We thank D. W. Kinsey and P. L. Jokiel for critical comments. Dr. J. A. Philpotts provided mass spectrometry data for intercalibration with the atomic absorption data, and V. A. Greenberg provided advice and assistance on atomic abosrption analytical techniques. Drs. P. W. Glynn, J. A. Marsh, M. Pichon, R. Bak, and R. Kinzie provided materials, respectively, from Panama, Guam, Australia, Curacao, and Tarawa. Logistic support at Midway was provided by the U.S. Navy and the Koral Kings Dive Club. The research was supported by NSF grant OCE 7681599. Contribution Number 566 of the Hawaii Institute of Marine Biology and 962 of the Hawaii Institute of Geophysics.

23 October 1978; revised 24 January 1979

# γ-Aminobutyric Acid, a Neurotransmitter, Induces Planktonic Abalone Larvae to Settle and Begin Metamorphosis

Abstract. *γ-Aminobutyric acid (a simple amino acid and potent neurotransmitter in human brain and other tissues of higher animals) and certain of its congeners rapidly and synchronously induce planktonic larvae of the red abalone,* Haliotis rufescens, *to settle and commence behavioral and developmental metamorphosis. These naturally occurring inducers of algal origin apparently are responsible, in part, for the substrate-specific recruitment, induction of settling, and the onset of metamorphosis of abalone and other planktonic larvae upon specific algae which provide naturally favorable habitats for the young of these species in coastal waters. These observations provide a convenient experimental model for further analysis of the basic molecular mechanisms by which environmental and endogenous factors control the recruitment and development of planktonic larvae. Halogenated organic pesticides significantly interfere with larval settling, as quantified in a new bioassay based upon these findings.*

One of the most highly specialized adaptations to life in the sea has been the evolution of reproductive strategies based upon the planktonic (drifting) dispersion of larvae. Planktonic larvae of many benthic (bottom-dwelling) aquatic species are induced to settle from the water column and begin their genetically programmed metamorphosis to adults by substrate- or environment-specific chemical triggers, the precise nature of which has remained largely obscure (*1*).

To gain a better understanding of the molecular nature of such factors controlling the specific settlement (recruitment) and reproductive efficacy of marine planktonic larvae, to better understand the evolution and distribution of marine species, and to more efficiently control elements of the global protein resource represented by the marine plankton, we have undertaken experiments with larvae of the large marine snail (gastropod mollusk) *Haliotis rufescens*, the California red abalone. Members of this herbivorous genus represent an economically important and protein-rich food resource in many areas of the world (*2*). Gravid adults of the species chosen may be obtained throughout the year; reproduction and early larval development can be controlled conveniently by a simple chemical method (*3*).

Present techniques for commercial and experimental cultivation of abalone under artificial conditions result in high postlarval mortality (*4*). This mortality primarily results from microbial overgrowth as a secondary consequence of an abnormal retardation of metamorphosis and development under artificial conditions; this retardation appears to reflect the absence of some naturally required morphogenetic inducing substance (or substances) (*5*). Accordingly, we have sought to identify naturally required biological inducers of behavioral and developmental metamorphosis of the planktonic larvae and to resolve from these the biochemical entities responsible for the required induction.

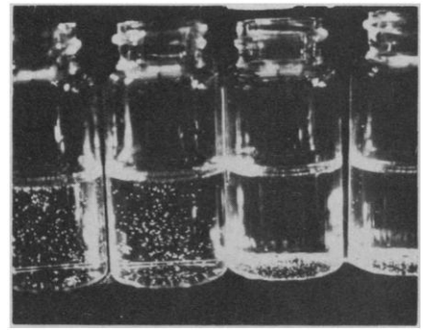

Fig. 1. GABA-dependent induction of behavioral and developmental metamorphosis is demonstrated and conveniently measured in small glass vials (diameter, 2.2 cm) containing portions of 100 to 300 competent, swimming larvae of *H. rufescens* (in 10 ml of filtered seawater at 15°C). GABA was added to the two vials on the right (to a final concentration of 1 m*M*) 3 minutes before the photograph was taken; the two vials on the left received no additions. Microscopic examination (at ×20 to ×40) shows virtually all of the larvae in the presence of GABA to have settled and assumed plantigrade attachment and locomotion on the glass; none are attached to glass in the absence of GABA.

Juvenile abalone (1 to 20 mm) of several species are found in naturally occurring coastal "nursery grounds," consisting of rocks covered with crustose red algae (*5-7*). Small juveniles can be found on these algae in local densities that are orders of magnitude greater than those in surrounding and otherwise similar habitats lacking such algae. Experiments performed under controlled conditions confirm the significance of this association, proving that larvae of *H. rufescens* show preferential settlement (substrate-specific recruitment) on crustose red algae including species of *Lithothamnium, Lithophyllum, Hildenbrandia*, and their close relatives; feeding, metamorphosis, and growth of the newly settled abalones ensue rapidly on these algae and their associated epiphytes (*5, 7, 8*). Under conditions in which larvae exhibit quantitatively reproducible substrate-specific settlement in response to these natural algal inducers, it has been possible to assay and identify simple chemical triggers of this activity.

Uniformly competent swimming larvae of *H. rufescens* are produced in the laboratory by controlled fertilization and cultivation after the peroxide induction of synchronous spawning in male and female adults (*3*). These swimming larvae exhibit substrate-specific settlement in response to the crustose coralline red algae *Lithothamnium* spp. and *Lithophyllum* spp. (Table 1) and the crustose noncoralline red *Hildenbrandia* spp. (*7*); significantly less settling is observed in response to the foliose (branching) coralline red *Bossiella* sp. No settling is observed under the conditions of this experiment when larvae are exposed to a variety of clean inorganic surfaces or those coated with diatoms, bacteria, other algae, or various juvenile invertebrates (Table 1) (*7*).

Extracts of the crustose coralline red algae contain inducers of settling but prove toxic to the larvae in high concentrations; both toxic and settling activities are proportional to the concentration of extract added. Neither denaturation of the extract by boiling nor digestion with proteolytic enzymes inactivates the inducers of settling. As seen in tests performed with a limiting (nontoxic) amount


0036-8075/79/0427-0407$00.50/0  

of extract, these treatments actually enhance the induction of settling by the crude algal homogenate (Table 1). Larvae exposed to additions of extract treated in this manner show significant and rapid settling on the clean glass of the vessel in which they are contained; ciliary swimming ceases immediately, and plantigrade attachment, gliding locomotion, and grazing behavior (characteristic side-to-side scraping of the surface) commence as normally seen on the intact inducing algae. These results suggest that at least one natural inducer of this behavioral metamorphosis is not a protein but is, in fact, released by proteolytic digestion (and, to a lesser extent, by thermal denaturation) of algal protein. An amino acid or prosthetic group (or both) linked to protein is thus implicated.

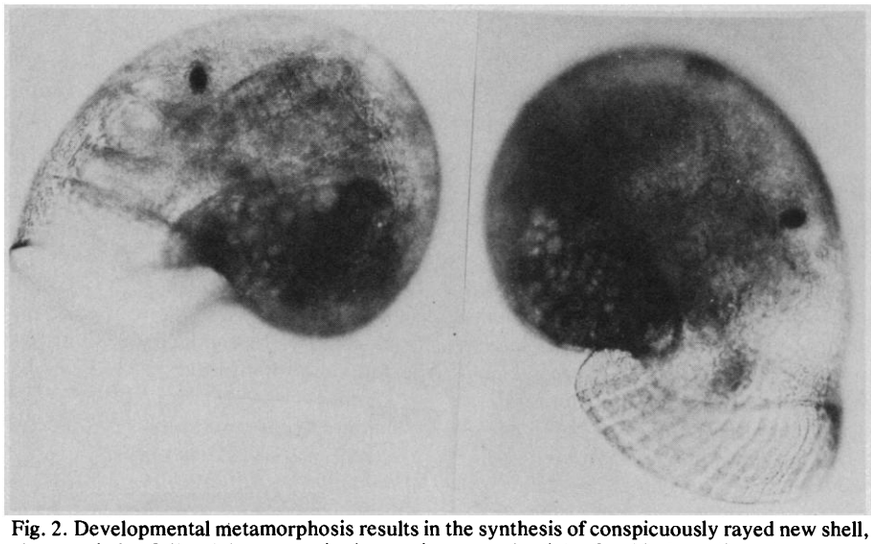

Fig. 2. Developmental metamorphosis results in the synthesis of conspicuously rayed new shell, characteristic of the adult, as seen in the specimen, on the right, fixed in formalin 42 hours after induction by $10^{-6}M$ GABA (pure). The sibling on the left, treated in parallel, received no GABA. The longest dimensions are 210 and 180 $\mu$m, respectively. One of the eyes is visible (upper right) through the shell of each animal.

Table 1. Specificity of induction; extraction and preliminary characterization of inducers. In experiment 1, uniformly healthy abalone larvae cultured and maintained at 15°C (in seawater filtered to exclude 5-$\mu$m particles) were exposed to potential inducers of settling on day 6 after fertilization. Portions of larvae (600 ± 200 per sample) were tested in 300 ml of filtered seawater in glass dishes with or without additions as shown; settling (plantigrade attachment and locomotion) was scored after 12 hours by microscopic examination. *Lithothamnium* and *Lithophyllum*, crustose coralline red algae; *Bossiella*, foliose coralline red alga; diatoms, *Cyclotella*, *Nitzschia*, *Tetraselmis*, and *Cocconeis* spp., tested singly and in mixed cultures; bacteria and microalgae are those cultured from local seawater filtered to exclude 5-$\mu$m particles; *Macrocystis pyrifera*, giant kelp, the preferred food of adult *H. rufescens*. Algae and bacteria were tested at 0.2 to 1.0 g (wet weight) per sample. The extract of *Lithothamnium* was prepared by homogenization in filtered seawater, centrifugation (10,000*g*, for 10 minutes), boiling (100°C for 10 minutes in the dark) and proteolytic digestion (trypsin and pronase B, both at 1 mg/ml, for 16 hours at 20°C in the dark), and boiling to inactivate the proteases. Settling was observed only on the surfaces of the coralline red algae and on the glass of the vessels receiving the treated extract. Mortality of larvae ≤ 1 percent in all samples. In experiment 2, 7-day-old larvae were assayed, in duplicate, in small glass vials (containing 150 ± 50 larvae in 10 ml) as shown in Fig. 1. Additions were all 1 m$M$ as indicated; *p*H was readjusted (with NaOH or HCl) as necessary to yield *p*H 7.8 in all test vials prior to the introduction of larvae. Results were scored after 12 hours at 15°C, as in experiment 1. Other neurotransmitters and effectors tested were L-epinephrine; L-norepinephrine; serotonin; histamine; acetylcholine; choline; and indole-3-butyric, -acetic, -propionic, and -acrylic acids (*7*).

| Inducer | Settled (%) |
|---|---|
| *Experiment 1* | |
| None | 0 |
| *Lithothamnium* sp. and *Lithophyllum* sp. | 82 |
| *Bossiella* sp. | 4 |
| Diatoms | 0 |
| Bacteria and microalgae | 0 |
| *Macrocystis pyrifera* | 0 |
| *Lithothamnium* extract, 2 $\mu$g of protein per milliliter | 2 |
| The same, boiled | 6 |
| The same, protease digested; boiled | 23 |
| *Experiment 2* | |
| None | 0 |
| GABA ($\gamma$-aminobutyric acid) | ≥ 99 |
| $\alpha$-Aminobutyric acid (D- and L-) | 0 |
| $\beta$-Aminobutyric acid (D- and L-) | 0 |
| *n*-Butylamine | 0 |
| *n*-Butyric acid | 0 |
| *n*-Pentanoic acid | 0 |
| Succinic acid | 0 |
| $\gamma$-Guanidinobutyric acid | 0 |
| $\gamma$-Hydroxybutyric acid | 58 |
| $\delta$-Amino-*n*-valeric acid | 89 |
| $\epsilon$-Amino-*n*-caproic acid | 74 |
| L-Glutamic acid | 12 |
| D-Glutamic acid | 0 |
| L-Glutamine | 0 |
| L-Aspartic acid | 0 |
| Other neurotransmitters and effectors | 0 |

We found that $\gamma$-aminobutyric acid (GABA), a neurotransmitter in higher animals, is a potent inducer of rapid settling found in the active algae (Fig. 1). In the experiment shown, 100 percent of the larvae tested were induced to settle and begin their characteristically snail-like plantigrade attachment, gliding locomotion, and grazing behavior on the clean glass of the test vials within 7 minutes after the addition of 1 m$M$ GABA. Settling is more slowly induced by 1 $\mu M$ GABA (≥ 98 percent in 2 hours); no settling is observed in control portions of larvae receiving no inducer. GABA is active in inducing behavioral metamorphosis at concentrations as low as $\sim 10^{-7}M$, with half-maximal effectiveness (50 percent settling) at $< 10^{-6}M$. True developmental metamorphosis of the larvae also is induced by GABA (Fig. 2). The rapid growth of new shell (consisting largely of the protein conchiolin) becomes clearly visible 24 to 36 hours after the addition of $10^{-7}$ to $10^{-5}M$ GABA ($\sim 10^{-6}M$ optimal); prolonged exposure of larvae to higher concentrations of the free inducer is toxic (*7*). Larvae in culture at 15°C develop competence for GABA-induced settling and metamorphosis (in parallel with competence for induction by the intact coralline algae) between day 6 and day 7 after fertilization, when they have attained well-developed eyespots, sensory cephalic tentacles, and a prominent muscular foot (*7*). GABA is found in high concentration in the crustose coralline red algal extracts; total intracellular concentration within the algae (if unsequestered) is on the order of $\sim 10^{-2}M$. Unusually for this amino acid, however, virtually all of the GABA present is covalently linked to protein or some other macromolecular algal constituent by an amide or ester linkage (*7*).

The accessory photosynthetic pigments responsible for coloration of the coralline red algae, phycoerythrobilin (a linear tetrapyrrole bile pigment) and its specific protein conjugate, phycoerythrin (*9*), contain two identical cyclic imino analogs of GABA within the tetrapyrrole (Fig. 3); both pigments also are potent inducers of settling. Virtually all this pigment in the algae is in the conjugated

form (total intracellular concentration $\sim 10^{-2} M$); both free and conjugated phycoerythrobilin exhibit half-maximal induction of settling at $\sim 10^{-6} M$ and are toxic to the larvae at concentrations $\geq 10^{-5} M$ (*7*). Other linear (for example, bilirubin and biliverdin) and cyclic (hemin and hematin) tetrapyrroles and other proteins (including hemoglobin, which contains the cyclic tetrapyrrole heme group) are inactive over comparable concentrations. Gabaculine, a simple cyclic analog of GABA (Fig. 3), also is a potent inducer of rapid settling, with half-maximal activity observed at $\sim 10^{-6} M$ (*7*).

The structural relationships (Fig. 3) of compounds showing significant activity in the induction of settling (Table 1) (*7*) presumably reflect a map of the apparent stereochemical specificity determinants of some GABA receptor (or receptors) or transport and activation systems in the larvae. These demonstrate an absolute requirement for the primary carboxyl group of GABA and for specific substitution at the $\gamma$ position. Increasing or decreasing the chain length of GABA homologs progressively decreases their activity in the induction of settling; the shorter amino acids, including $\beta$-alanine, alanine, and glycine, thus exhibit apparent affinities for the receptor sites, which may be too weak to be of physiological significance below 1 to 10 m$M$. Glutamic acid in high concentration is slowly active, either by itself or after metabolic decarboxylation to GABA. Only closely related structural homologs or analogs of GABA are active; a variety of other neurotransmitters, amino acids, peptides, and related effectors and metabolites have proved ineffective (Table 1 and Fig. 3) (*7*).

GABA and phycoerythrobilin are the most potent inducers of settling that we have yet found in active red algal extracts, although their role in the induction of settling on the intact algae and the possible roles of other inducers remain to be elucidated. Both GABA and phycoerythrobilin are present primarily as conjugates of proteins or other macromolecules in very high concentrations within the inducing algae; although it is possible that enzymatic or other hydrolysis within the animal may be a necessary precursor to active induction, we find no evidence for release of chemical inducer by larvae settled on the algae (*7*).

The fact that the most potent and abundant inducers identified in the algal extract (GABA and phycoerythrobilin) are both covalently complexed to protein or other macromolecules (or both) and, therefore, not freely extracted is sufficient to explain our observation that the activity of the crude algal extract is greatly enhanced by proteolytic digestion or thermal denaturation (Table 1). These findings also confirm and explain our biological observations indicating that the active, intact crustose coralline red algae do not contain or release significant quantities of freely diffusible inducing substances. Thus we see (i) no chemotactic behavior of competent larval abalones with respect to the inducing algae; (ii) no induction of "heterologous settling" (that is, settling on adjacent nonalgal surfaces, as induced by the addition of free GABA); (iii) no induction of settling by water in which the intact algae were incubated for 16 hours at 15°C; and (iv) no induction of settling by active algae separated from the larvae by a semipermeable dialysis membrane (*7*). Chemotaxis, heterologous settling, or induction under these conditions might be expected if settling were induced by a freely diffusible substance normally released from the algae. The algae "trap" the larvae but do not lure them or induce them to settle from afar. Apparently, direct contact with, and possibly exploratory ingestion of, the inducing algal substrate is required (*7*). This contact is provided by the searching behavior of the competent larvae, which swim toward the water surface, temporarily cease swimming, sink to the bottom, and then resume upward swimming within a matter of seconds or minutes; this repeated rising and sinking, coupled with oceanic drifting, presumably provides an efficient means by which the competent larvae continually inspect and directly sample the bottom until suitable (inducing) substrates are found.

Species specificity of alga-induced set-

Table 2. GABA-dependent bioassay detects sensitivity to pesticides at sublethal concentrations. Assay of GABA-dependent settling was performed as in Fig. 1, in the presence or absence of added pesticides (nominal concentration, 0.1 ppm). Mortality and interference in settling were quantified after 15 hours at 15°C.

| Pesticide* | Mortality (%) | Interference in settling (%) |
|---|---|---|
| None | 0 | 0 |
| DDT | 0 | 100 |
| Methoxychlor | 0 | 100 |
| Dieldrin | 11 | 100 |
| 2,4-D | 0 | 7 |

*DDT, 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane; methoxychlor, 1,1,1-trichloro-2,2-bis(*p*-methoxyphenyl)ethane; 2,4-D, (2,4-dichlorophenoxy) acetic acid.

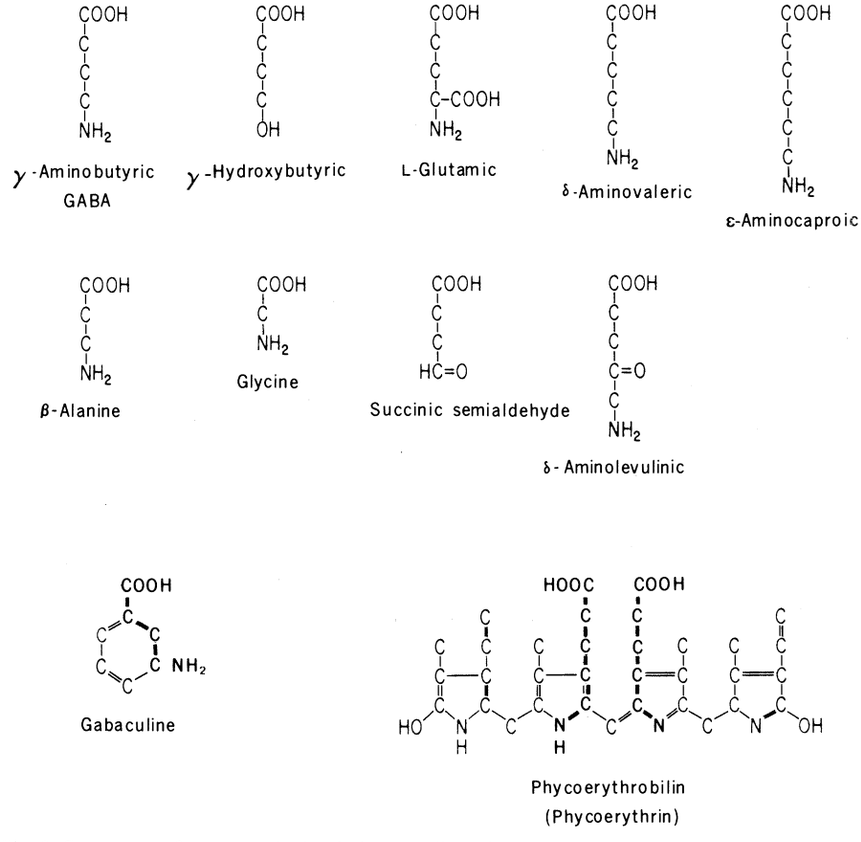


Fig. 3. Structures of compounds that induce settling of competent *H. rufescens* larvae, showing homologous relations between active inducers. Methylene hydrogen atoms have been omitted [from the data in Table 1 and (*7*)].

tling presumably reflects discrimination of more than simple GABA and phycoerythrobilin moieties, which may be common to many red algae. Extended receptor-specificity discriminants may include recognition of the algal species-specific proteins to which the small inducers are fully complexed. Recognition of other constituents and properties also may play some role in the natural process of substrate selection, although we have experimentally excluded any significant involvement of the larval visual system (*7*).

The physiological GABA-dependent induction of behavioral and developmental metamorphosis (Figs. 1 and 2) affords a convenient measure for quantifying and defining other factors that may influence, or be required for, the success of these processes. Thus, for example, the GABA-dependent induction of settling provides a rapid bioassay for the presence of interfering pollutants, including a variety of halogenated organic pesticides (Table 2) (*5*). Results of these assays indicate that settling (and thus, reproductive success) is far more sensitive to such environmental stress than is the simple viability of the planktonic larvae.

We have shown here that settlement and genetically programmed behavioral and developmental metamorphosis of planktonic molluscan larvae can be induced by a simple amino acid decarboxylation product (used as a neurotransmitter and neurohormone in human brain and other animal tissues) and by certain of its analogs, produced by specific recruiting algae. These compounds constitute the simplest and best characterized molecular structures thus far identified with activity controlling the rapid settlement and development of any marine planktonic larvae. Similar amino acid decarboxylation products (the biogenic amines, with neurotransmitter, neurohormone, and hormonal activities in higher animals) have been found to regulate gene expression in the evolutionarily antecedent unicellular eukaryotes (*10*). Thus it may be expected that GABA and other amino acid–derived effectors and related simple molecules specialized for signal transduction and regulation will be found to mediate interspecific control of larval dispersion, settlement, and metamorphosis in other marine and freshwater species. We have found recently that planktonic larvae of the chiton *Mopalia muscosa* (another herbivorous mollusk) are induced to settle and undergo behavioral metamorphosis specifically by GABA and by intact *Lithothamnium* and *Lithophyllum* spp. Conversely, larvae of other molluscan (such as carnivorous *Conus*) species that have proved unresponsive to these algae are not induced to settle by GABA or its congeners.

The lock-and-key mechanism of stereochemically specific induction of settling and the related interactions between abalones and their crustose coralline red algal hosts apparently reflect the intimate and adaptive coevolution of these planktonically dispersed animal herbivores and their algal substrates (*7*). Evolutionary perfection of this mechanism proceeds via continued selection for reproductive advantage in both the abalone and its (inducing) algal host. As a consequence of these mutualistic interactions, the animal is provided with required, potent, and specific inducers of settling and metamorphosis, which in turn ensure that larval commitment and development will commence only in suitable microhabitats. The algae and their epiphytes also provide the animals with preferred foods capable of supporting rapid development and growth (*5, 7*) and a source of pigmentation that is incorporated as highly effective camouflaging coloration in the developing shell (*6, 8*). The inducing algae benefit reciprocally from the nondestructive shallow grazing of the animals specifically recruited to their surfaces. As shown by Adey (*11*) for the case of another gastropod mollusk (limpet) association with *Lithothamnium*, such grazing, continuously freeing the algal epithallium from fouling epiphytes, can be an absolute requirement for continued algal growth.

The system described here provides a convenient experimental model for further analysis of the basic molecular mechanisms by which environmental and endogenous factors control the recruitment and development of planktonic larvae. Initial experiments with this system suggest that cyclic adenosine monophosphate and calcium may directly mediate the induction by GABA which we have observed (*7*). Practical applications of algal- and GABA-induced settling and metamorphosis for improved economic efficiency of molluscan cultivation, reseeding, management, and genetic improvement of cultivars for food production have been suggested (*3, 5*). In addition, the GABA-dependent bioassay provides a highly sensitive, rapid, and reproducibly quantitative indicator of the stress imposed upon marine environments, organisms, and their productivity by halogenated organic pesticides and other pollutants from industrial, agricultural, and urban activities (*5, 7*). Finally, it is possible that the cross-reactivity observed between porphyrin bile pigments (and certain of their precursors and metabolites) and complementary neurotransmitter-type (GABA) receptors may in some measure explain the involvement and degeneration of autonomic and central nervous system neurons characteristic of severe porphyria in mammals (*12*).

DANIEL E. MORSE
NEAL HOOKER
HELEN DUNCAN
LLOYD JENSEN

*Marine Science Institute*
and *Department of Biological Science,*
*University of California,*
*Santa Barbara 93106*

**References and Notes**

1. D. J. Crisp, in *Chemoreception in Marine Organisms*, P. T. Grant and A. M. Mackie, Eds. (Academic Press, New York, 1974), p. 177.
2. K. W. Cox, *Calif. Fish Game* **118**, 1 (1962).
3. D. E. Morse, H. Duncan, N. Hooker, A. Morse, *Science* **196**, 298 (1977); in *Proceedings of the U.N. Symposium, CICAR-II, Caracas, July 1976; FAO Fish. Rep.* **200**, 291 (1976); D. E. Morse, N. Hooker, A. Morse, in *Proc. World Maricult. Soc.*, **9**, 543 (1978).
4. D. L. Leighton, in *Proceedings, Symposium of the Latin American Aquaculture Association, Maracay, Venezuela, November, 1977*, in press; H. Kan-No, in *Proceedings First International Conference on Aquaculture Nutrition*, W. Shaw, Ed. (University of Delaware, Newark, 1975) p. 195; T. Shibui, *Bull. Iwate Fish. Exp. Station* **2**, 1 (1972); T. Imai, *Venus* **25**, 159 (1967).
5. D. E. Morse, *Univ. Calif. Sea Grant College Program Annual Report*, W. Powell, Ed. (Univ. of California Institute of Marine Resources, Santa Barbara, 1978), p. 71; ———, N. Hooker, L. Jensen, H. Duncan, *Proc. World Maricult. Soc.*, in press.
6. D. R. Crofts, *Haliotis* (University Press of Liverpool, Liverpool, 1929), p. 17; S. A. Shepherd, *Aust. J. Mar. Freshwater Res.* **24**, 217 (1973): M. Tegner, in preparation.
7. Analyses of biological specificity, mechanisms, and behavioral metamorphosis involved in settling, as well as biochemical analyses of receptor specificity and mechanisms, have been assembled (D. E. Morse, L. Jensen, N. Hooker, A. Cameron, M. Tegner, in preparation).
8. F. Clogston, unpublished data.
9. Phycoerythrin and phycoerythrobilin were purified from *Lithothamnium* spp. by the methods of C. Brooks and E. Gantt, *Arch. Mikrobiol.* **88**, 193 (1973); W. Rudiger and P. O'Carra, *Eur. J. Biochem.* **7**, 509 (1969); D. J. Chapman, W. J. Cole, H. W. Siegelman, *J. Am. Chem. Soc.* **89**, 5976 (1967); P. O'Carra and C. O'hEocha, *Phytochemistry* **5**, 993 (1966).
10. C. T. Roberts, Jr., and D. E. Morse, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 1810 (1978).
11. W. H. Adey, *Phycologia* **12**, 111 (1973).
12. Preliminary accounts of these findings have been reported in *Proceedings of the International Symposium on Geogeography* (Univ. of New Zealand Press, Auckland, in press) and in *Proceedings of the Western Society of Naturalists Symposium, Tacoma Wash.*, D. Montgomery, Ed. (Univ. of Washington Press, Seattle, 1978), p. 17.
13. We wish to acknowledge the assistance of K. McCollum with amino acid analyses of algal extracts, Drs. M. Tegner and A. Cameron with observations of recruitment, and S. Anderson with observations and collection of abalones. Financial support was provided by the Marine Science Institute of the University of California, Santa Barbara, and the NOAA Sea Grant program under grant 04-6-158-44021-R/NP-1A; special thanks are given to Drs. Henry Offen and James Sullivan, directors of these agencies, and to Drs. Cadet Hand, Wallis Clark, Fred Conte, and Jens Sorensen for their assistance and support of this work. We also thank Drs. Neal Holmes, Beatrice Sweeney, and James Case for their helpful suggestions in the preparation of this manuscript.

23 October 1978; revised 24 January 1979