The present flow configuration can be used with essentially any fluorescence microscope having incident illumination, whether inverted or upright. Thus, the high speed of the water in the jet (~ 10 m/sec) and the laminar character of the flow on the cover slip allow the system to be oriented in any direction. The great spectral width of the mercury lamp and the large selection of filter combinations available make this type of instrument suitable for a wide variety of stains.

With this flow configuration it is possible for the nonspecialist to assemble, from components that are standard equipment in many laboratories, a flow cytometer that is superior to most with respect to both resolution and simplicity in use and that is capable of making cellular DNA measurements with a CV on par with or better than the best systems commercially available.

Note added in proof: Recently our instrument has consistently yielded DNA histograms with a CV below 1.0 percent (*8*), demonstrating that its resolution is better than this value.

HARALD B. STEEN
TORE LINDMO

Department of Biophysics,
Norsk Hydro's
Institute for Cancer Research,
Montebello, Oslo 3, Norway

References and Notes

1. P. K. Horan and L. L. Wheeless, Jr., *Science* **198**, 149 (1977).
2. W. Göhde, in *Fluorescence Techniques in Cell Biology*, A. A. Thaer and M. Sernetz, Eds. (Springer Verlag, Berlin, 1973), p. 79; J. W. Gray, D. Peters, J. T. Merrill, R. Martin, M. A. Van Dilla, *J. Histochem. Cytochem.* **27**, 441 (1979).
3. P. J. Crosland-Taylor, *Nature (London)* **171**, 37 (1953); M. A. Van Dilla, T. T. Trujillo, P. F. Mullaney, J. R. Coulter, *Science* **163**, 1213 (1969); P. F. Mullaney, M. A. Van Dilla, J. R. Coulter, P. N. Dean, *Rev. Sci. Instrum.* **40**, 1029 (1969).
4. W. A. Bonner, H. R. Hulett, R. G. Sweet, L. A. Herzenberg, *Rev. Sci. Instrum.* **43**, 404 (1972); T. Lindmo and H. B. Steen, *Biophys. J.* **18**, 173 (1977).
5. We are indebted to O. Sørensen for constructing the stabilized power supply.
6. B. Barlogie, G. Spitzer, J. S. Hart, D. A. Johnston, T. Büchner, J. Schumann, B. Drewinko, *Blood* **48**, 245 (1976).
7. The resolution was independent, that is, CV ≤ 1.5 percent, of the angle α between the water jet and the cover slip in the range α = 15° to 30°; the resolution remained better than CV = 3 percent up to α = 90°, that is, vertical incidence. With α = 20°, the distance between the point where the cells hit the cover slip and the optical axis of the microscope could be varied from 200 to 300 μm without affecting the resolution. The resolution was independent of the driving pressure of the water jet over the range 1 to 4 kg/cm². No special preparation of the cover slip was needed. The limit of resolution appeared to be determined primarily by the stability of the light source. With the 25-V d-c power supply stabilized to within ± 1 mV, the peak channel number of a reference sample remained constant within ± 1.5 percent for several hours. The pulse amplifier was operated with a time constant of 2 μsec. Its linearity, as judged from the position of the particle doublet peak, was within 1 percent.
8. T. Lindmo and H. B. Steen, in preparation.

14 November 1978; revised 8 January 1979

Strontium-Calcium Thermometry in Coral Skeletons

Abstract. *The strontium to calcium ratio of skeletal aragonite in three genera of reef-building corals varies as a simple function of temperature and the strontium to calcium ratio of the incubation water. The strontium/calcium distribution coefficients of coral aragonite apparently differ from the corresponding coefficient of inorganically precipitated aragonite. With some care, coral skeletons can be used as recording thermometers.*

Discordant interpretations have been offered to explain the ratio of Sr to Ca in coral skeletons and the variation of that ratio. The extremes of those interpretations may be summarized as follows. (i) Corals precipitate Sr and Ca in the same ratio as these elements occur in seawater (*1*, *2*). (ii) Corals precipitate Sr and Ca in a ratio which varies with taxonomy, metabolism, growth rate, or other biological parameters (*3*, *4*). (iii) Corals precipitate Sr and Ca in a ratio which reflects the seawater ratio modified by a temperature-sensitive Sr/Ca "distribution coefficient," which describes the proportional discrimination of the precipitate for Sr coprecipitation in the aragonite ($CaCO_3$) crystal lattice (*5*).

We carried out controlled laboratory experiments to test the field observations which led to the interpretations summarized above (*6*). Our preliminary findings demonstrated that there is a linear relationship between the Sr/Ca ratio in skeletons of reef-building corals and the ambient water temperature at which the precipitation of skeletal aragonite occurred; that the relationship is not directly growth-rate dependent, except insofar as temperature and growth rate are related; and that there are apparent differences in the Sr/Ca ratio among coral taxa.

We have since examined field data in order to validate our laboratory findings, and we report here field and further laboratory results. These results are easily interpreted in terms of a Sr/Ca distribution coefficient which apparently differs from that of aragonite precipitated inorganically from seawater. Moreover, we have revised the absolute values of our initial Sr/Ca ratios on the basis of what we believe to be an accurately determined Sr/Ca ratio for an internal (coral) standard. Our results suggest that the discordant interpretations of the observed Sr/Ca variation are largely attributable to analytical limitations.

Skeletons of the finely branching coral *Pocillopora damicornis* were obtained from the Pacific coast of Panama (*7*). These corals had been stained in their ambient environment with alizarin red S and then left there to grow at temperatures continuously monitored with recording thermographs. Growth after staining was visible as white coral tips above the pink-stained portion of the skeletons. We analyzed the Sr/Ca ratio of the unstained tips of these corals by atomic absorption spectrophotometry (*6*, *8*). The coral Sr/Ca ratios obtained by this procedure were adjusted by comparison with a coral aragonite standard for which a Sr/Ca value has been established by mass spectrometry. Samples of that standard were routinely analyzed with each batch of coral skeletal Sr/Ca samples.

Because between-tip Sr/Ca variations within a single colony of the Panama corals proved not to differ significantly from variations between repeated analyses of single tips, we pooled tips within subsequently analyzed coral colonies with confidence that we were not losing information about intracolony variability. We then assessed other levels of variability in the coral Sr/Ca ratio. Table 1 presents an analysis of variance designed to examine sources of variability among coral collection sites (*9*) (that is, large-scale environmental variation), between paired coral heads from the same site (small-scale environmental variability or biological differences among corals, or both), and among triplicate analyses of ground-up skeleton from individual coral heads (analytical precision). The analysis demonstrates that 81 percent of the total variability (the sum of the squares among collections divided by the total sum of the squares) represents large-scale environmental variation; 16 per-

Table 1. Analysis of variance of *Pocillopora* Sr/Ca ratios ($\times 10^3$) among collections, between corals at a collection site, and among replicate analyses within corals.

Degrees of freedom	Sum of squares	Mean square	F
	Among collections		
6	1.4172	0.2362	40.7 (significant at $P < .001$)
	Between corals		
7	0.0406	0.0058	0.57 (not significant)
	Within corals		
28	0.2840	0.0101	
	Total		
41	1.7418		

0036-8075/79/0427-0404$00.50/0

cent of the variation is analytical. The precision of a single analysis of the Sr/Ca ratio is about 1.0×10^{-4} (the square root of the mean square within the corals). The between-coral variability within sites is insignificant. We conclude that an environmental signal and analytical imprecision account for the observed variation in the Sr/Ca ratio of *P. damicornis*.

We then analyzed these Panama data along with data obtained from the tips of *P. damicornis* colonies collected from well-characterized collection sites at Oahu, Hawaii, Midway, Johnston, Enewetak, and Guam islands (*10*). The analysis thus includes corals from the same species but genetically isolated populations. The Oahu corals include collections from five controlled-temperature experiments (*6, 8*) and three field collection sites. Samples from one to five field collection sites were available for each of the other locations. Because intercolony variability at a collection site had proven to be insignificant relative to the analytical precision (Table 1), we averaged analyses from individual collection sites to improve within-site analytical precision. Figure 1a presents a scatter diagram with the geometric mean regression line (*11*) relating temperatures to the skeletal Sr/Ca ratio of *P. damicornis*. The equation for this line is (Table 2)

$$\text{Sr/Ca} \times 10^3 = 11.01 - 0.071\,T \quad (1)$$

where the temperature T is in degrees Celsius. The Sr/Ca ratio of seawater is approximately 8.6×10^{-3} (*5*), and so Eq. 1 yields the following temperature-dependent distribution coefficient K [Table 2 and (*5*)]:

$$K = 1.27 - 0.0083\,T \quad (2)$$

Corals were grown experimentally for 1 month in seawater at 25°C and with the Sr/Ca ratio of that water elevated by an average of 40 percent above ambient levels by the metered addition of a $SrCl_2$ solution to the seawater flowing through the aquarium. The tips of the coral skeletons were enriched to a Sr/Ca ratio of 12.9×10^{-3}, corresponding to a K of 1.07 and close to the predicted value (Eq. 2) of 1.06. Thus, the skeletal Sr/Ca ratio of *Pocillopora* is directly dependent upon T and the Sr/Ca ratio of seawater. We have examined the effects of other variables on this ratio. Over the past several years, we have grown corals for periods of 1 to 6 months under laboratory conditions of altered light dose and photoperiod, various concentrations of inorganic plant nutrients, lowered salinity, and enhanced water motion; by altering these independent environmental variables, we have altered the coral growth rate. None of the aforementioned environmental variables other than T and the seawater Sr/Ca ratio demonstrably affects the Sr/Ca ratio of the corals. Nor do we find support for the suggestion (*3, 4*) that growth rate directly affects the Sr/Ca ratio of the coral skeleton, although there is a relationship between T and growth (*6, 7*).

We have sufficient field and laboratory data to examine the relationship between T and the Sr/Ca content of two other coral genera (Fig. 1 and Table 2). *Porites* (Fig. 1b) (including *P. compressa* and *P. lobata* from the Pacific Ocean and *P. asteroides* from the Caribbean) shows a relation between T and the Sr/Ca ratio that does not differ significantly from that of *Pocillopora*. Three species of *Porites* from several locations in Pacific Oceania, the Australian Great Barrier Reef, and the Caribbean Sea, thus, are all well characterized by a single regression line. *Montipora verrucosa* (Fig. 1c) yields a regression equation which is somewhat less well defined than those of the other two taxa but which shows significantly ($P < .05$, analysis of covariance) higher Sr/Ca ratios than the other two corals. Limited data for *Acropora*, in the same family as *Montipora*, indicate Sr/Ca ratios close to that of *Montipora*. An average regression slope calculated from Weber's data (*3*) for the T dependence of the Sr/Ca ratio in 22 genera of corals is very close to the relationship we establish (Table 2 and Fig. 1d), although Weber concluded that T did not exert primary control on the Sr/Ca ratio of coral skeletons.

Kinsman and Holland [Table 2 and (*12*)] reported a K value for inorganically precipitated aragonite of

$$K = 1.24 - 0.0045\,T \quad (3)$$

The regression lines describing the K values for coral aragonite as determined by both Weber (*3*) and us are below the

Table 2. Summary of the regression equations for Sr/Ca $\times 10^3$ (R) versus temperature (T) and the temperature-dependent distribution coefficients (K) calculated from these equations.

Data set	R versus T(°C)	Coefficient of determination	Number of observations	K versus T(°C)
Pocillopora damicornis	$R = 11.01 - 0.071T$	0.77	32	$K = 1.27 - 0.0083T$
Porites sp.	$R = 10.94 - 0.070T$	0.71	22	$K = 1.27 - 0.0081T$
Montipora verrucosa	$R = 11.64 - 0.089T$	0.63	18	$K = 1.35 - 0.0104T$
Our coral average	$R = 11.32 - 0.082T$	0.60	72	$K = 1.30 - 0.0094T$
Weber's coral average (*3*)	$R = 10.84 - 0.081T$		1368	$K = 1.26 - 0.0098T$
Inorganic aragonite (*12*)	$R = 10.66 - 0.039T$			$K = 1.24 - 0.0045T$

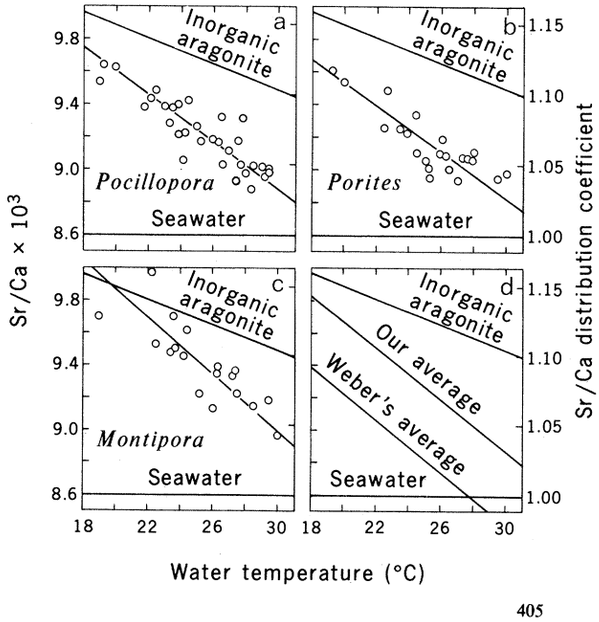

Fig. 1. Relationship between the temperature at and immediately preceding collection and the Sr/Ca ratio of the outermost portions of the skeletons of three coral taxa sampled during this investigation: (a) *Pocillopora*, (b) *Porites*, and (c) *Montipora*, the average fit through those points; and (d) Weber's (*3*) coral average. For comparison, the relationships between temperature and the Sr/Ca ratio of inorganically precipitated aragonite (*12*) and the Sr/Ca ratio of seawater are shown.

line determined by Kinsman and Holland and steeper than their line. The absolute difference between the values of Kinsman and Holland and the coral data might represent minor laboratory calibration differences in the accurate measurement of the Sr/Ca ratio of aragonite. In fact, on the basis of what we believe to be an accurate determination of the Sr/Ca ratio of an internal coral standard, we have revised our own initially determined Sr/Ca values (*6*, *8*) upward by about 13 percent. A 5 percent downward revision of the data of Kinsman and Holland (or further upward revision of our data) would bring their curve largely into the cloud of our data points, but there would still be a slope difference between the line for inorganic aragonite and that of the corals. The coral aragonite Sr/Ca K value converges on the inorganic aragonite K value below the T at which reef-building corals survive. Even with uncertainties about analytical accuracy, the process of coral aragonite formation must differ significantly in its products from the process of inorganic aragonite precipitation. We assume such a difference to be due to biological mediation in the aragonite Sr/Ca precipitation (*5*), although the mechanism remains unresolved at this time (*13*).

The Sr/Ca ratio in skeletons of reef-building corals has the potential to be used as a recording thermometer, if variations in that ratio through the coral skeleton are measured in conjunction with the chronological record preserved in coral density bands (*14*). There are several points of reservation regarding quantitative application of this thermometer.

The range of the reported Sr/Ca ratios in corals is relatively large, we suspect in part because of problems in determining that ratio accurately even within a single laboratory. It is not clear, for example, whether the minor difference between Weber's regression equation (*3*) and ours (Table 2 and Fig. 1d) represents laboratory calibration differences, inaccuracy in his rather coarsely averaged temperature data, taxonomic differences, or some combination of the three. In our laboratory we have overcome the accuracy limitation by routinely comparing analyses with a control standard, well-mixed aragonite powder from a single colony of *Porites*. Our regression equations can only be regarded as "accurate" relative to the Sr/Ca ratio we accept for that standard, although we have gone to some effort to establish the absolute Sr/Ca ratio of that standard. We believe that it is presently impractical to compare absolute coral Sr/Ca ratios or the corresponding T estimates among laboratories without rigorous intercalibration.

The range of T used to examine the relationship between Sr/Ca and T can also introduce significant problems in the use of this thermometer. Goreau (*2*) examined discrete samples of a massive coral (*Montastrea annularis*) which had grown for 2 years in the T range 26.5° to 30°C; he concluded that there was neither a significant T signal in the Sr/Ca ratio nor a significant difference between seawater Sr/Ca and coral Sr/Ca. Were we to restrict our available data set to comparable T, we would derive a skeletal Sr/Ca ratio of about 9.1×10^{-3} (that is, only slightly above that of seawater) and apparently a very weak T dependence in that ratio. With such a restricted data set, we might therefore have also concluded that the coral Sr/Ca ratio shows little relation to T. The absolute offset between our data and Goreau's may represent a real taxonomic difference or another example of interlaboratory differences.

The problem, of course, is that this T range is insufficient, relative to the T coefficient of K and present analytical resolution of the ratio, to permit one to recognize the functional relationship that clearly exists. The corals we have used span much of the survival T range for these taxa (indeed, with relatively localized exceptions, for most other taxa of reef-building corals as well).

The Sr/Ca ratio of the coral taxa examined changes an average of 8×10^{-5} per degree Celsius. The single-analysis precision of our Sr/Ca analysis is about 10^{-4}, so our T resolution based on individual analyses is about 1°C. If the T dependence of K had as gentle a slope as determined for inorganic aragonite (*12*), our T resolution would be about 2°C. We believe that the precision of individual analyses can eventually be improved. In the meantime, replication is the most satisfactory method to gain adequately detailed T resolution from coral Sr/Ca ratios.

The use of skeletal Sr/Ca ratios as a thermometer may be potentially upset by diagenetic alteration of the coral skeletons. That problem needs to be considered, but it should be no more of a practical barrier to sclerothermometry (thermometry based on the use of coral) than it is to isotopic dating of coral skeletons. Some coastal settings may be subjected to altered Sr/Ca seawater ratios from runoff or groundwater inputs. This problem should be minor on most oceanic reefs.

The Sr/Ca thermometer does not presently appear to be limited by random or otherwise inexplicable biological variability, although there is evidence for taxonomic differences in coral Sr/Ca ratios (*3*, *4*). Bulk coral samples may not accurately reflect long-term mean water T; rather, the corals will bias their bulk composition toward conditions of maximum growth rate. Detailed samples within annual density bands (*14*) should yield reliable chronological T traces.

Corals sample the Sr/Ca ratio of seawater in a biologically mediated but quantitatively simple fashion that is responsive to T and the Sr/Ca ratio of that water. Utilization of corals as recording thermometers is feasible but must be approached with some caution.

S. V. SMITH
Hawaii Institute of Marine Biology, University of Hawaii, Post Office Box 1346, Kanoehe 96744
R. W. BUDDEMEIER
R. C. REDALJE
J. E. HOUCK
Hawaii Institute of Geophysics, University of Hawaii, Honolulu 96822

References and Notes

1. R. C. Harris and C. C. Almy, Jr., *Bull. Mar. Sci. Gulf Caribb.* **14**, 418 (1964).
2. T. J. Goreau, *Proc. R. Soc. London Ser. B* **196**, 291 (1977); *Proc. 3rd Int. Coral Reef Symp.* **1**, 425 (1977).
3. J. N. Weber, *Geochim. Cosmochim. Acta* **37**, 2173 (1973).
4. G. Thompson and H. H. Livingston, *Earth Planet. Sci. Lett.* **8**, 439 (1970).
5. D. J. J. Kinsman, *J. Sediment. Petrol.* **39**, 486 (1969). The distribution coefficient (K) is defined to be the molar Sr/Ca ratio in the aragonite precipitate divided by the equivalent ratio in the precipitating solution. It is a measure of the discrimination of the precipitate for Sr or Ca.
6. J. E. Houck, R. W. Buddemeier, S. V. Smith, P. L. Jokiel, *Proc. 3rd Int. Coral Reef Symp.* **2**, 425 (1977).
7. P. W. Glynn and R. H. Stewart, *Limnol. Oceanogr.* **18**, 367 (1973); P. W. Glynn, *J. Mar. Res.* **35**, 567 (1977). Dr. Glynn furnished samples of corals described in these papers for our analysis.
8. J. E. Houck, thesis, University of Hawaii (1978).
9. A "collection site" is defined here to be a geographic location with its own particular collection time and collection temperature.
10. The collection sites were deliberately chosen to avoid or minimize diel variations in water temperatures. Temperatures were measured either periodically with hand-held thermometers or continuously with in situ thermographs at or near the sites for several days previous to sample collection. The Midway corals were stained in the field to ensure that they were calcifying at the recorded water temperatures (19°C). It was anticipated, from available data (*7*), that these corals might not be calcifying; they were. In all cases the recorded field temperature should be within about ± 0.5°C of the growth temperature of the corals sampled.
11. W. E. Ricker, *J. Fish. Res. Board Can.* **30**, 409 (1973).
12. D. J. J. Kinsman and H. D. Holland, *Geochim. Cosmochim. Acta* **33**, 1 (1969).
13. Our preliminary data show that Sr/Ca and Ba/Ca are enriched in coral tissue relative to either ambient water or coral skeleton [see also T. H. Flor and W. S. Moore, *Proc. 3rd Int. Coral Reef Symp.* **2**, 555 (1977)]. The sites of aragonite precipitation therefore probably are exposed to a solution Sr/Ca ratio that is modified somewhat from the seawater Sr/Ca ratio. It might thus be

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.

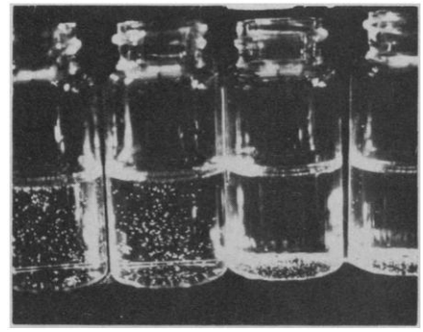

Fig. 1. GABA-dependent induction of behavioral and developmental metamorphosis is demonstrated and conveniently measured in small glass vials (diameter, 2.2 cm) containing portions of 100 to 300 competent, swimming larvae of *H. rufescens* (in 10 ml of filtered seawater at 15°C). GABA was added to the 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