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A Direct and Active Influence of Gravity on the Behavior of a Marine Invertebrate Larva

Abstract. Larvae of the bryozoans Bugula neritina *and* Bugula stolonifera *exhibit an apparent negative geotaxis under conditions of darkness and constant temperature. This behavior cannot be accounted for by buoyancy since the larvae are negatively bouyant, nor is it a consequence of gradients in the partial pressures of dissolved gases since the response occurs under conditions where the gradient is reversed or when experiments are conducted in chambers with interfaces of only glass and water. Pressure bomb experiments indicate that the behavior is not a barokinesis. Centrifuge experiments, however, showed that larvae of* Bugula stolonifera *orient directly and actively to gravity, while those of* Bugula neritina *have some other measure of geographic up. Since bryozoan larvae lack statocysts, the sensory apparatus mediating the gravity response in* Bugula stolonifera *is still unknown.*

The vertical distributions of planktonic organisms and the settlement patterns of larvae of benthic organisms are determined by various biological and physical environmental cues. Responses to the perception of geographic up and down, generally referred to as geotaxes (*1*), are commonly reported behaviors among planktonic marine invertebrate larvae. The larvae of some marine invertebrates possess either statocysts or statocytes that are thought to function in the detection of gravity (*2*). An apparent geotaxis has been observed both in larvae that are known to have gravity receptors and those that do not, but there is no evidence that the movement is accomplished through an active, direct response to gravity (*3*). Bryozoan larvae lack statocysts (*4*), yet some exhibit an apparent negative geotaxis prior to settlement (*5*). Explanations that may account for this behavior include (i) orientation to the geomagnetic field; (ii) response to a partial pressure gradient of dissolved gases; (iii) positive buoyancy, a passive direct effect of gravity; (iv) high barokinesis, an active indirect effect of gravity; and (v) orientation to a gravitational force, an active direct response to gravity. Two or more of these alternatives acting in concert may account for an observed geotactic behavior. We report that the negative geotaxis of larvae of the bryozoan *Bugula stolonifera* is an active, direct, true gravity response. Although the mechanism of gravity reception is not known, neither positive buoyancy nor high barokinesis can account for the response.

Under laboratory conditions, an apparent negative geotaxis occurs in larvae of the cellularioid cheilostome bryozoans *Bugula neritina* and *B. stolonifera*. We analyzed responses of larvae of both species to four environmental cues that might explain this behavior (*6*).

We did not test for the influence of magnetic fields. It seems unlikely that species such as *B. neritina* and *B. stolonifera*, with wide latitudinal distributions, including in the case of *B. neritina* a distribution that spans the magnetic equator, could effectively use geomagnetic cues to control vertical distribution unless different populations orient at radically different angles to the field.

Orientation along a gradient in partial pressures of gases dissolved in the seawater was discounted on the basis of qualitative observations. First, free-swimming larvae of *B. neritina* (*7*) and *B. stolonifera* distributed themselves near the top of a vessel kept in darkness, even when it was sealed in such a way that gases could only enter from the bottom. Second, larvae of both species settled near the top of a vertical column of water that was kept in darkness, at constant temperature, and sealed to present an equivalent glass-water interface at both ends of the chamber.

The observed distribution of *Bugula* larvae might result from the larvae being positively buoyant. Buoyancy is known to be a contributing factor in determining the vertical orientation and distribution of certain aquatic organisms (*8*). However, *Bugula* larvae were found to be negatively buoyant when swimming was arrested by lowering the temperature or by adding 1 percent Formalin or 1 percent sodium azide to the seawater.

To test for a barokinetic response of *Bugula* larvae, we used a pressure bomb in conjunction with a photocell counting device. The experimental device consisted of a rectangular Plexiglas chamber (inside dimensions, 5 by 3 by 3 cm) connected at the inflow to a filtered compressed air supply and at the outflow to a reservoir of distilled water at the base of a 1-m graduated column. The four vertical faces of the chamber were blackened except for a pinhole aperture 1 cm from the floor at the center of each of the two ends through which passed a beam from a fiber-optic light source. Opposite the light was a silicon phototransistor (Radio Shack 276-130) whose current output through a resistor was measured by a single-channel penwriter. Each time a larva crossed the light beam, the penwriter recorded a potential drop

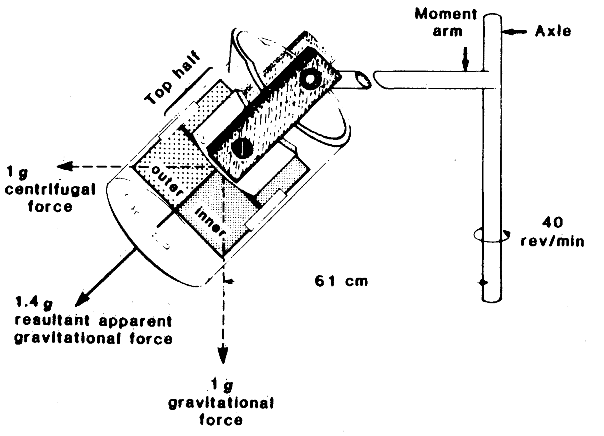

Fig. 1. Schematic illustration of the setup used in centrifuge experiments to test for an active and direct effect of gravity on larval settlement.

associated with interruption of the photocurrent. Because *Bugula* larvae rarely are observed to swim in a straight path in a predictable direction for any appreciable length of time, it was not feasible to measure swimming rates of individual larvae directly.

Larvae were gently transferred with a wide-mouth pipette from the tubs to the pressure chamber, which was adjusted to a volume of approximately 25 ml and clamped shut. Swimming activity was measured at pressures of 0 cm and 100 cm H_2O (73.6 torr) for 5 minutes during each experiment. At the end of each experiment larvae were counted. Average number of photocell counts per larvae per minute were compared between the two treatments with a single-classification analysis of variance. Swimming activity, as measured by frequency of photocurrent interruption, was not significantly different ($P > .10$) between ambient and elevated (100 cm H_2O) pressure conditions (Table 1).

Table 1. Swimming activities of larvae under ambient (0 cm H_2O) and elevated (100 cm H_2O) levels of pressure. Activity was measured as the mean number of photocurrent interruptions per larva per minute ($\overline{\text{ilm}}$); $\overline{\overline{\text{ilm}}}$, $\overline{\text{ilm}}$ averaged over total number of trials.

Trial	Activity ($\overline{\text{ilm}}$) 0 cm H_2O	Activity ($\overline{\text{ilm}}$) 100 cm H_2O	Larvae (N)
	Bugula neritina		
1	0.59	0.52	28
2	0.52	0.53	29
3	0.84	0.84	48
4	0.85	0.71	63
5	0.47	0.38	42
6	0.45	0.38	89
7	0.57	0.56	70
8	0.49	0.37	41
$\overline{\overline{\text{ilm}}}$	0.598	0.536	
	Bugula stolonifera		
1	0.42	0.40	71
2	0.19	0.19	32
3	0.81	0.82	17
4	0.38	0.57	24
5	0.14	0.22	156
6	0.31	0.22	44
$\overline{\overline{\text{ilm}}}$	0.375	0.403	

To evaluate the effect of displacing the apparent gravity vector away from its natural position perpendicular to the earth's surface, *Bugula* larvae were induced to settle on wooden substrata inside 1000-ml Pyrex beakers swung on a low-speed centrifuge (Fig. 1). The centrifuge was designed to swing the buckets out at an angle of 45° from the vertical, producing a resultant gravitational plus centrifugal force (apparent gravitational force) of $1.4g$ at the center of mass of each bucket. The radius (center of mass to centrifuge axle) was 61 cm at equilibrium speed (40 rev/min, provided by a Bodine 652 electric gearmotor). Pieces of basswood (10 by 10 cm) were used as the substrate. They were autoclaved and then incubated in aerated seawater from the same locality as the experimental colonies. After this treatment, which promoted growth of a microbial film that enhances larval settlement, a square was fixed inside each bucket in the plane defined by the moment arm and axle of the centrifuge; larvae were dipped with a small beaker from the tubs containing parent colonies, concentrated in larger beakers, and introduced to the buckets in darkness. The centrifuge was set in motion and left in darkness for 6 hours. The buckets were then removed from the centrifuge and rinsed with three changes of fresh seawater to wash away swimming and crawling larvae.

Settled larvae were allowed to develop either 1 (*B. neritina*) or 2 (*B. stolonifera*) days to facilitate counting. Ancestrulae on the inner and outer (with respect to the centrifuge axle) rectangular halves of the squares were counted and tested against a 50–50 distribution with a replicated goodness-of-fit G test. It was expected that if negative geotaxis depended on gravity, or a function of gravity, equal numbers of larvae would settle on the inside and outside halves. If there existed some independent measure of geographic up, larvae would settle preferentially on the outside half, which contains the uppermost part of the substratum when the buckets are pivoted out at speed.

Larvae of *B. neritina* settled predominantly on the outside half of each square (Table 2), and over 90 percent of the larvae settled within 2 cm of the top edge of each square. These findings indicate that *B. neritina* larvae do not reorient in the presence of a modified gravity vector and, therefore, have some measure of geographic up other than gravity. In the case of *B. stolonifera*, however, the numbers of larvae that settled on inside and outside halves of the squares did not differ significantly in five out of six replicate trials (Table 2), and over 72 percent settled within the top halves. These results support the hypothesis that *B. stolonifera* larvae orient by an active direct response to gravity.

Our studies on the mechanisms that underly the apparent negative geotaxes displayed by two species of *Bugula* larvae permit two conclusions. In the case of *B. stolonifera*, larval settlement patterns can be redistributed from geographic up to become oriented with respect to an artificial gravitational force, but larvae do not respond indirectly to gravity by varying swimming rate with hydrostatic pressure. This true gravity response occurs in larvae without a known receptor for gravity detection. In the case of *B. neritina*, larval settlement patterns cannot be explained by buoyancy, barokinesis, or an active direct gravity response. The mechanism of the apparent geotaxis exhibited by *B. neritina* larvae is not known.

In summary, our findings indicate that

Table 2. Settlement frequencies of larvae on inside and outside halves of wood squares in the gravity experiment. Statistical significance of difference between observed and equal distributions on inside and outside halves was tested by a G test.

Trial	Larvae (N) Inside	Larvae (N) Outside	P
	Bugula neritina		
1	12	312	$<< .005$
2	274	621	$<< .005$
3	123	592	$<< .005$
	Bugula stolonifera		
1	197	192	$> .5$
2	232	276	$> .1$
3	1118	1076	$> .5$
4	698	913	$< .005$
5	221	208	$> .5$
6	360	375	$> .5$

gravity has an active and direct role in influencing larval biology in at least *B. stolonifera*, but that fundamental differences in responses to environmental cues exist between it and its congener *B. neritina*.

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References and Notes

1. Although geotaxis in a restricted sense means an active oriented response to gravity, the term is sometimes used to describe any light-independent vertical movement in the water column, including responses to environmental cues such as temperature gradients or geomagnetic fields. For convenience we shall adopt the broader definition of geotaxis and use "gravity response" to signify geotaxis in the restricted sense. Thus, barokinesis, or modulation of swimming rate by changes in hydrostatic pressure, coupled with an active or passive vertical orientation is considered an apparent geotaxis although it is mediated only indirectly by gravity.
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6. Colonies of *B. neritina* were collected in the vicinity of Los Angeles, Calif., in April 1982 and shipped to the Museum of Comparative Zoology where they were maintained at 12°C in tubs of aerated seawater from off Nahant, Mass. Colonies of *B. stolonifera* were collected from Woods Hole and Onset, Mass., in August 1982 and maintained at 20°C in tubs of aerated seawater from the collection sites. Larvae were obtained by illuminating the colonies with a 500 W photolamp; colonies were kept in darkness at all other times. Experiments on *B. neritina* larvae were conducted at 12°C in the seawater from off Nahant and those on *B. stolonifera* larvae were conducted at 20°C in water from the collection sites.
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Social Stress and Atherosclerosis in Normocholesterolemic Monkeys

Abstract. *Socially stressed adult male cynomolgus monkeys (*Macaca fascicularis*) fed a low fat, low cholesterol diet developed more extensive coronary artery atherosclerosis than unstressed controls. Groups did not differ in serum lipids, blood pressure, serum glucose, or ponderosity. These results suggest that psychosocial factors may influence atherogenesis in the absence of elevated serum lipids. Psychosocial factors thus may help explain the presence of coronary artery disease (occasionally severe) in people with low or normal serum lipids and normal values for the other "traditional" risk factors.*

The initiation and progression of coronary artery atherosclerosis is often associated with increased concentrations of lipids in the serum (*1*). Despite this association, many individuals develop severe atherosclerotic lesions while having low serum lipid concentrations, and others develop far more atherosclerosis than would be expected on the basis of a modest elevation of serum lipids (*2*). Work with animal models suggests that some of this variability may be explained by the influence of hypertension and immunologic injury to arteries (*3, 4*). Yet, much additional variability in atherosclerosis lesion extent remains unexplained, suggesting the existence of other pathogenetic mechanisms among normocholesterolemic individuals. In recent years, psychosocial variables have been linked increasingly to ischemic heart disease in human beings (*5*) and psychosocial manipulations have been shown to exacerbate atherosclerosis in cholesterol-fed cynomolgus monkeys, rabbits, and swine (*6–8*). At present, though, it is unclear whether psychosocial manipulations are capable of promoting atherogenesis in normocholesterolemic animals and, by implication, in human beings with low or normal serum cholesterol concentrations. The purpose of the present investigation was to provide an initial test of this hypothesis. Our results demonstrate that socially stressed monkeys fed a low fat, low cholesterol diet developed more extensive intimal lesions in the coronary arteries than control animals living under unstressed conditions. Moreover, the differences in lesion extent observed here were not associated with elevations or group differences in serum lipids, blood pressure, serum glucose, or ponderosity.

The experimental animals were 30 male, cynomolgus monkeys (*Macaca fascicularis*), imported as adults from Malaysia and the Philippine Islands. They were assigned to two experimental conditions (designated the "stressed" and "unstressed" conditions), and within each condition ($N = 15$), the monkeys were divided randomly into three, five-member groups. During the study, all groups were housed separately in identical pens measuring 2.0 by 3.2 by 2.5 m. The experiment lasted 21 months, after which all animals were killed and necropsied.

Throughout the study the monkeys were fed a "prudent" diet, modeled on the current recommendations of the American Heart Association; this diet contained almost no cholesterol (0.05 mg of cholesterol per calorie) and was low in saturated fats (*9*). Blood samples for determination of total serum cholesterol and high-density lipoprotein cholesterol (HDLC) concentrations were taken approximately once per month over the course of the study. Other physiologic variables associated with atherosclerosis were measured at regular intervals; these variables included systolic and diastolic blood pressure (bimonthly), fasting serum glucose concentration (semiannually) and ponderosity (the ratio of body weight to body length) (semiannually). All monkeys were sampled in the morning, under ketamine restraint and following a suitable fast (*10*).

To create a significantly stressful social environment, we periodically altered group memberships in the stressed condition by redistributing animals among the three affected groups. The monkeys were redistributed once every 12 weeks in the first year of the study and once every 4 weeks in the following 9 months. Unlike the stressed animals, group memberships among monkeys assigned to the unstressed, or control condition, remained constant throughout the 21-month experiment.

Reorganization of groups was selected as a means of inducing stress in the present study because previous reports had indicated that introduction of strangers fosters a high degree of social instability in macaques (*11*). In an attempt to further enhance competition and social uncertainty, an ovariectomized female