

LIGHT ORGAN AND EYESTALK
COMPENSATION TO BODY TILT IN THE LUMINESCENT
MIDWATER SHRIMP, *SERGESTES*
SIMILIS

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SUMMARY

The posterior light organ and eyestalk of the midwater shrimp, *Sergestes similis* Hansen, are capable of 140° of angular movement within the body during pitch body tilt, maintaining the organs at near horizontal orientations. Counter-rotations compensate for 74-80% of body inclination.

These responses are statocyst mediated. Unilateral statolith ablation reduces compensation by 50%. There is no evidence for either homolateral or contralateral control by the single functioning statocyst. Bilateral lith ablation abolishes counter-rotation. Light organ and eyestalk orientations are unaffected by the direction of imposed body tilt.

Bioluminescence is emitted downward from horizontal animals with an angular distribution similar to the distribution of oceanic light. The amount of downward directed luminescence in tilted animals decreases at large angles of body inclination due to less than total compensation by the light organs.

Eye turning towards a light source is induced by upward-directed illumination. The resulting change in eyestalk orientations never amounts to more than 25° . The turning is abolished by bilateral statolith ablation. Downward directed illumination, comparable in intensity to oceanic light, generally does not generate significant eye turning.

Light organ orientations remain unaffected by directional illumination, both before and after bilateral statolith ablation.

The compensatory counter-rotations by the posterior light organ and eyestalk suggest that counter-illumination by *S. similis* remains effective in inclined animals.

INTRODUCTION

A proposed function of bioluminescence by mesopelagic organisms is counter-illumination, whereby an animal utilizes downward-directed luminescence to eliminate its silhouette. According to this scheme, oceanic light which has been reflected or absorbed by the body is replaced by equivalent bioluminescence (Clarke, 1963; Dahlgren, 1916). For counter-illumination to be effective, bioluminescence must

match the physical characteristics of the replaced oceanic light. These include wavelength (Tsuji & Haneda, 1971; Swift, Biggley & Napora, 1977; Herring & Locket, 1978; Young & Mencher, 1980), intensity (Young & Roper, 1976; Case *et al.* 1977; Young *et al.* 1980), and angular distribution (Denton, Gilpin-Brown & Wright, 1972; Herring, 1976; Herring & Locket, 1978). In addition, luminescence should be stimulated only by downward-directed illumination.

Previous studies of bioluminescence in the midwater shrimp, *Sergestes similis*, have demonstrated that it fulfils most of the criteria necessary for counter-illumination (Warner, Latz & Case, 1979). The animal responds only to downward-directed illumination, bioluminescence is sustained, matches the intensity of stimulus illumination, and matches the spectral composition of oceanic light. Preliminary measurements indicated a similar angular distribution of luminescence to that of oceanic light. The luminescent sources, the organs of Pesta (Hansen, 1922), are modified parts of the hepatopancreas and consist of vertically arranged, modified hepatopancreas tubules (Dennell, 1940; Herring, 1981).

While counter-illumination typically is studied in the laboratory with horizontally positioned specimens, in fact many midwater animals have been observed *in situ* in other orientations (Barham, 1970). Observations of *S. similis* from deep submersibles indicate that many individuals maintain inclined body positions, yet are never tilted more than 90° from the horizontal (Omori, 1974; A. Alldredge, E. Barham, B. Robison, personal communications).

For counter-illumination to remain effective in inclined animals, the light organs must be able to compensate for body tilt. The large posterior organ of Pesta in *S. similis* and other sergestid shrimps achieves this by rotation within the body (Omori, 1974). Euphausiid photophores are also capable of angular movement, but this response has only been considered for light stimuli (Hardy, 1962; Land, 1980).

Counter-rotations by the light organs would then serve to maintain downward-directed luminescence. The eyestalks might respond to body tilt in order to keep the eye fixed in space to facilitate perception of the directionality of visual stimuli. Shrimp possess statocysts, which may be expected to control angular movements of the eyestalks and light organs. These organs should be insensitive to visual stimuli, should cease responding upon statocyst removal, and should share similar characteristics with other statocyst-mediated behaviour such as eyestalk movements in crayfish (Hisada, Sugawara & Higuchi, 1969) and lobsters (Schone & Schone, 1967; Schone, 1971), eye movements in carp (Traill & Mark, 1970) and cephalopods (Budelman, 1975; Hartline, Hurley & Lange, 1979; Schone & Budelman, 1970), and righting responses in shrimp (Schone, 1954) and crayfish (Yoshino, Takahata & Hisada, 1980).

The results of the present study indicate that counter-rotations by the posterior organ of Pesta and eyestalk of *Sergestes similis* compensate for imposed body tilt. This behaviour is statocyst mediated and is minimally affected by directional illumination. This suggests that counter-illumination can be effective in inclined animals.

MATERIALS AND METHODS

Adult specimens of *Sergestes similis* were trawled from 25–300 m in the Santa Barbara Basin, near Santa Barbara, California. Animals were placed in chilled sea water and brought into the laboratory within 3 h of collection where they were maintained in 100 l aquaria with flow-through, sand-filtered, chilled sea water (12 °C). Shrimp were tested within 7 days after collection and were not fed.

Measurements of light organ and eyestalk orientations

For testing in a clear acrylic chamber (1.75 × 2.5 × 10 cm), specimens were restrained by a clamp around the abdomen and a brace surrounding the anterior cephalothorax. This immobilized the cephalothorax while allowing free movement of eyestalks, limbs, and pleopods. The chamber was sealed and perfused with chilled sea water at approximately 50 ml/min. It was held on a freely rotating shaft which indicated angle of rotation. The chamber was positioned so that the axis of pitch rotation was centred through the midregion of the restrained animal.

Animals restrained in the chamber were viewed with a Sony video camera fitted with an f/1.8 25 mm lens and extension tube. Orientations were video recorded. Magnification at the video monitor was 5 ×. Illumination was provided by a 60 W incandescent red light positioned 10 cm above and behind the chamber. Observed visual effects on orientations were minimal, as predicted from unpublished data indicating that light exposure during daylight trawling severely reduced the sensitivity of *Sergestes* vision.

Specimens were acclimated to the chamber for 30–60 min following handling. They were then subjected to an angular rotation series about the transverse axis from 0° horizontal to 180° inverted horizontal and back, for both head up and head down tilt directions from the horizontal. This was done by rotating the chamber every 60 s in discrete steps. Increments of 10° were made in the 0–90° range for all experiments. In the 90–180° range, normal animals were rotated in 10° increments, while increments of 30° were used for other experiments. Organ and body positions were videotaped for 55 s following each rotation step. As steady-state orientations were reached a few seconds after rotation, this allowed more than sufficient time for a stable condition to be reached.

Posterior organ of Pesta and the right eyestalk orientations were measured at each rotation angle during stop-frame playback on a video monitor. An equatorial ring of dark pigment on the posterior organ served as an easily visible guide to orientation of the organ. This was done by sighting with an angle finder along the pigment band and reading the inclination with respect to the horizontal. Since the eyestalk was symmetrical in lateral view, a short (0.5 cm) length of black monofilament was glued to its tip as a pointer, permitting use of the angle finder to measure changes in eyestalk orientation.

The statocysts, at the base of the antennules, are transparent organs of 1 mm diameter containing a secreted lith attached to sensory hairs (Cohen, 1955). Body tilt shifts the lith to bend mechanosensory hairs (Schone, 1968; Schone, 1975). Gravity detection has commonly been experimentally impaired in crustaceans by

statocyst removal (e.g. Clark, 1896; Neil, 1975*a*), but a less injurious and quite sufficient method was simply to cut into the statocyst dorsal wall and extract the lith.

Rotation series were performed on normal specimens, on specimens following both unilateral and bilateral statolith ablation, and on two types of controls. In one control (sham), one statocyst was incised without lith removal. In the second control, a similar incision was made in the left antennule approximately 1 mm from the statocyst.

Preliminary experiments indicated that the results of rotation sequences were independent of each other, with prior rotation experience of an animal being unimportant. Orientations of the light organ and eyestalk from one rotation sequence to the next depended only on statocyst condition. Most animals were subjected to one or two rotation sequences. Some, however, were tested in a progression of three sequences: (1) in the normal condition, (2) after unilateral statolith ablation, and (3) after bilateral lith ablation.

Results were expressed as orientation of either the posterior light organ or the eyestalk, with respect to the horizontal plane, for each angle of body inclination. In some cases measurements were presented as deviation from the body angle. For statistical analysis, data over a 180° range of body tilts centred about 0° horizontal were pooled from all rotation sequences of a given experiment, and linear regressions calculated. Subsequent analysis of computed *F* values verified that the relationship between organ orientation and angle of body tilt within this range was indeed linear. Student's *t* tests and an *F* test were used to compare slopes, elevations, and variances from different experiments.

Directional light effects

Two preliminary experiments examined the interaction of gravitational and visual cues in determining eyestalk and light organ orientations. One considered bright light illumination of light maintained animals, while the second involved dim light with dark-maintained animals.

For both experiments, specimens were restrained against a transparent acrylic rod in an acrylic chamber (7 × 10 × 15.5 cm) by two loops of monofilament, one of which passed around the cephalothorax while the other held the abdomen. The chamber was sealed and perfused with chilled sea water. The rod was connected to a bearing shaft which, when turned, resulted in body inclinations about the transverse axis.

Stimulus illumination at 475 nm was provided by a monochromator with tungsten light source, and transmitted by a glass fibre optic light guide before passing through a 1 mm aperture at a distance of 5 cm from the centre of the axis of rotation of the animal. Light intensities were regulated by neutral density filters and measured by a United Detector Technology Inc. 40 × Optometer. Stimulus duration was controlled by a mechanical shutter.

Responses of restrained animals were viewed with a Panasonic newvicon (red sensitive) video camera with *f*/1.8 lens and recorded on videotape. Illumination for the camera was generated by a 25 W red incandescent source filtered to 700 nm.

(Wratten 89 B filter) at 10 cm from the specimen. The eyes of dark-adapted animals were insensitive to this background illumination (personal observations).

Animals were subjected to alternating 15 s intervals of darkness and light stimulation in an increasing-intensity series. Eyestalk and light organ orientations were measured from the videotape record 10 seconds into each interval. For each stimulus intensity, the difference of the response to light exposure from the dark orientation was determined.

Bright-light effects

Day-trawled, light-maintained animals were dark adapted for at least 2 h prior to testing. Stimulus intensities ranged from 2.9×10^{-5} to $2.9 \times 10^{-1} \mu\text{W} \cdot \text{cm}^{-2}$, several orders of magnitude brighter than oceanic light at depth (Clarke, 1966). Normal animals in horizontal, vertical-up and vertical-down body positions were illuminated from below with the light stimulus. Eyestalk orientations of six animals were measured.

Dim-light effects

Specimens were trawled at night, recovered and sorted under dim red light, and thereafter maintained in constant darkness and handled under dim red light only. Normal animals and those with bilaterally ablated statoliths were stimulated with both upward- and downward-directed illumination while in horizontal, vertical-up and vertical-down body positions. Stimulus intensities ranged from 5.3×10^{-6} to $5.3 \times 10^{-3} \mu\text{W} \cdot \text{cm}^{-2}$ and were comparable with oceanic light intensities at depth in the Santa Barbara Basin (Clarke, 1966). Eyestalk and posterior light organ responses of five animals were determined.

Measurements of angular distributions of luminescence

Specimens were restrained against a transparent acrylic rod in a rotation chamber as described above. Animals were positioned so that the axis of pitch rotation was centred through the hepatopancreas. Steady bioluminescence was induced by squeezing the eyestalks.

Bioluminescence was detected by an EMI 9701-B photomultiplier operating at -800 V and fitted with an electro-mechanical shutter. The photomultiplier, located beneath the chamber, was positioned 5 cm from the animal and had an unobstructed view of the ventral surface of the body over a 180° range centred on the horizontal. The acceptance angle at the photocathode was 46° . The photomultiplier signal was led through a Keithley 427 Current Amplifier to a Grass 79D Polygraph.

Specimens in the chamber were acclimated in the dark for 60 min following handling. They were then subjected to body rotations about the transverse axis by manually turning the shaft holding the restrained animal at constant velocity from the vertical head-up position to the vertical head-down position and vice versa. For each animal, 5–8 scans were made. A foot switch activated at 10° increments correlated angle of body tilt with the bioluminescence trace.

At each 10° increment of angle of body tilt, the level of bioluminescence was determined and corrected for dark current. For each scan, the values at each rotation

angle were standardized to the maximal value. Scans displaying significant changes in the baseline intensity of luminescence were discarded.

When normal animals were rotated about the transverse axis, the three largest organs of *Pesta* performed compensatory counter-rotations to maintain near horizontal orientations and the amount of downward directed bioluminescence as a function of angle of body tilt was measured. Measurements of the true angular distribution of light emission from the light organs were made from animals with both statoliths removed. Since no compensatory movements by the light organs occurred in these specimens, rotating an animal in front of a fixed photomultiplier was equivalent to rotating the photomultiplier about a fixed animal.

For each condition, standardized data from each scan were pooled and expressed as relative bioluminescence. For each angle of body tilt, the standardized mean and standard deviation were calculated. The distributions were compared to Tyler's calculated angular distribution of oceanic light (Denton *et al.* 1972) by use of the Kolmogorov-Smirnov Two-Sample Test (Zar, 1974).

RESULTS

Orientations of posterior light organ and eyestalk

The orientations of the posterior organs of *Pesta* and right eyestalks of restrained specimens of *Sergestes similis* were determined by the angle of body inclination and the condition of the statocysts. In animals with detection of gravity partially or completely impaired, orientations at each inclination differed from normal.

The restraint imposed by the experiments was well tolerated. Two normal specimens exhibited unchanged responses of the organs of *Pesta* after 25 h of restraint in the chamber. Most experimental animals remained in the chamber for only a few hours.

Responses of posterior light organ

When an animal initially in a horizontal position was inclined about the transverse axis, the posterior organ of *Pesta* counter-rotated to remain nearly horizontal. Steady state was reached within several seconds following body rotation. This compensatory response by the posterior light organ occurred for both head-up and head-down body tilts (Fig. 1A-C). Instead of assuming the same angle as the body at 50° from the horizontal, the organs are shifted only 15-20°. These counter-rotations were absent after bilateral statolith ablation (Fig. 1D-F). The organ of intact animals was observed to compensate for body roll, but this response was not quantified.

The deviation of the posterior light organ orientation from tilt angle followed a sinusoidal function, with maximum values at vertical body positions (Fig. 2A). When the body was horizontal (0°) the light organ was also horizontal. Rotation angles up to 90° from the horizontal generated light-organ orientations which deviated only slightly from the horizontal. For 90° of head-down body tilt, the organ was tilted approximately 20° (Fig. 2B). Such compensation to body tilt through counter-rotation of the organ occurred within a region which included a full 90° range of body tilt in both rotation directions, from vertical head-up through horizontal t

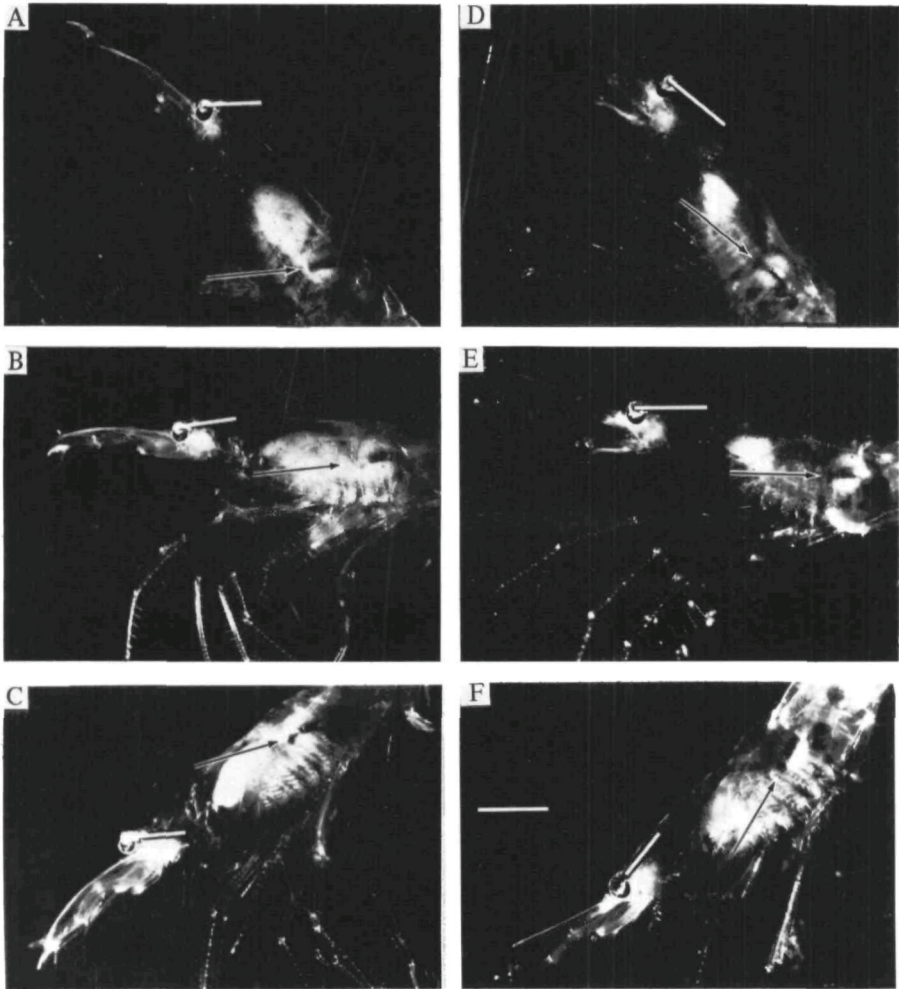


Fig. 1. Specimens of *Sergestes similis* in different body inclinations. Orientation of the posterior organ of Pesta, at the posterior end of the cephalothorax, is revealed by inclination of the equatorial pigment band (arrow). Eyestalk orientation is seen by means of a pointer glued to the end of the eyestalk. Compensatory responses in normal animals in (A) head-up, (B) horizontal, and (C) head-down body positions. Responses of animal after bilateral statolith ablation in (D) head-up, (E) horizontal, and (F) head-down body positions. Compensatory movements are absent. Scale bar in (F) = 4 mm.

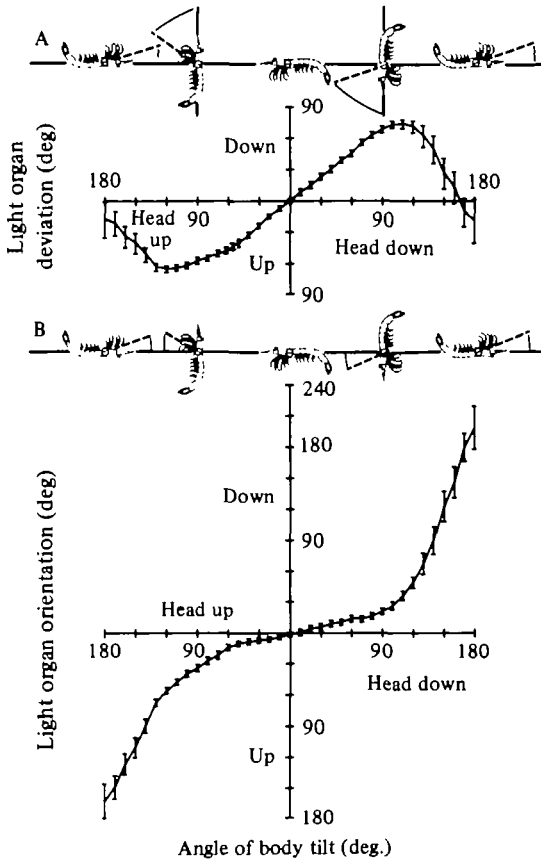


Fig. 2. Responses of posterior light organs of normal animals to 360° of body tilt. (A) Mean deviation with standard error of light-organ orientation from angle of rotation at each angle of body tilt. (B) Mean orientation with standard error of organ orientation with respect to horizontal at each rotation angle. Graphical representations of body configurations are located above appropriate axis positions.

vertical head-down. Beyond this range, for body inclinations of 90–180°, where the animal was rotated into inverted body positions, the organ was unable to undergo additional counter-rotations. First it moved passively with the body, and then at the largest angles of body tilt it relaxed towards the inverted horizontal position. This meant that the mean organ rotation increased more than that of the body rotation increment (Fig. 2B). This probably resulted from a combination of two factors: (1) release from the compensatory counter-rotations exhibited at smaller body inclinations, and (2) relaxation towards the inverted organ position.

The counter-rotations of the posterior light organ were effective in maintaining near-horizontal positions over a wide range of body inclinations (Fig. 2B). Observations of animals maintained at 40° body inclinations for 30 min gave no evidence for habituation.

The response to a rotation sequence was dependent on the condition of the statocysts. Fig. 3 shows a series of three rotation sequences performed on one

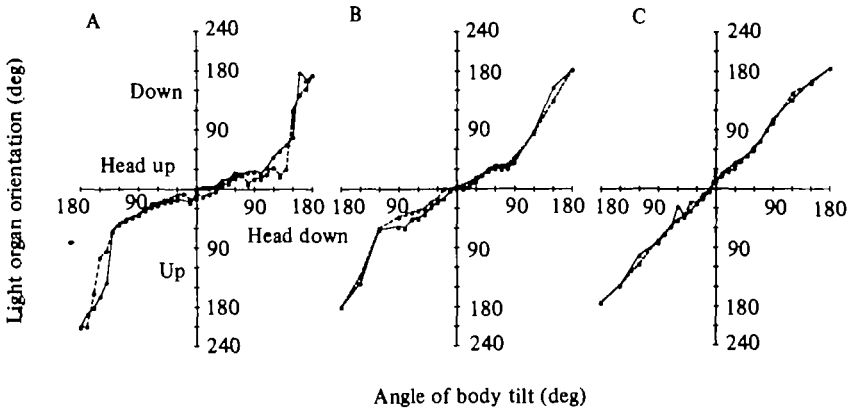


Fig. 3. Effect of statolith ablation in one animal on light organ orientation with respect to body tilt. Solid line represents measurements from horizontal to inverted horizontal, and dashed line represents the opposite. (A) Normal animal. (B) Left statolith removed. (C) Both statoliths removed.

specimen. In the normal condition (Fig. 3A), counter-rotations by the posterior light organ resulted in near-horizontal orientations for body angles up to 120° both up and down. This is apparent as the flattened region centred about the origin. While counter-rotations still occurred after removal of the left statolith (Fig. 3B), they were reduced in magnitude. Thus, the region of compensation was reduced in range and less flattened. When the same animal was tested with both statoliths removed, compensatory counter-rotations were virtually absent. The light organ remained in a fixed position relative to the body (Fig. 3C).

The direction of rotation had no effect on light-organ orientation. There were no significant differences in orientations during rotations away from or towards the horizontal (Fig. 3). Thus the light-organ orientation depended on the particular angle of body tilt, not the direction in which that angle was approached.

All experiments involved body angles from the horizontal to the inverted horizontal, and *vice versa*. Since body inclinations from vertical head-down through horizontal to vertical head-up represented the inherent range of body tilts of free-swimming animals in nature, further data analysis considered this 180° spread only. A single linear regression was calculated for data within this range for each statocyst condition, as the direction of rotation did not affect the light-organ orientations. The results are presented in Fig. 4 and the regression analysis appears in Table 1. The *y*-intercepts of the linear equations for each regression were equivalent to the orientations of the light organ at the horizontal body position. Since the intercepts ranged from -6.0 to 1.1° (Table 1), the alignment of the organ was approximately horizontal when the body was horizontal. Therefore, the various experimental treatments had no significant effect on the initial alignment of the light organ and, consequently, *y*-intercepts (*t* test, $P > 0.05$).

Since *y*-intercepts were nearly identical, the relevant index for each experiment was the slope of the regression. Based on regression analysis, a slope of 0 indicates

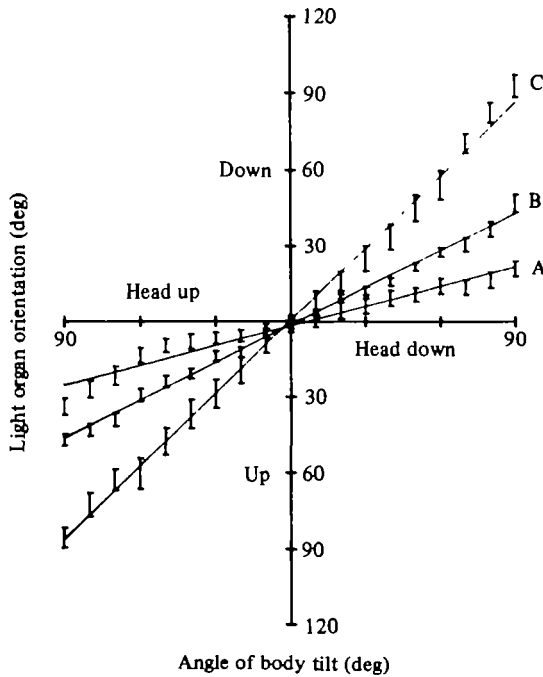


Fig. 4. Effect of statolith removal on posterior light organ orientation in all animals for range of body tilts corresponding to *in situ* inclinations. Mean orientation with standard error with respect to horizontal at each angle of body tilt, with calculated linear regression. (A) Normal animals. (B) Unilateral statolith ablation. (C) Bilateral lith ablation.

total compensation (light organ orientation independent of angle of body tilt), wherein the counter-rotations by the light organ maintained the organ in a horizontal position for all angles of body tilt. On the other hand, a slope of 1 indicates that no counter-rotations occurred (orientation dependent on tilt angle). Under restrained conditions in the laboratory, rotation sequences with normal animals generated a slope of the regression of 0.26 (Table 1). For every 10° of body rotation, from 90° up to 90° down body positions, the light organ only rotated 2.6°.

For animals with both statoliths removed, the slope of the regression was 0.96 (Table 1). This value signified that counter-rotations were absent; for every 10° of body tilt the organ rotated 9.6°. The lack of counter-rotations was not due to fatigue, as the slope of the regression from animals without statoliths, tested last in a series of three rotation sequences, revealed no significant difference ($t = 1.35$, $P > 0.1$) when compared to that from animals tested first.

Removal of one statolith yielded an intermediate slope of 0.50 (Table 1), indicating that some counter-rotation still occurred, though reduced in magnitude, even though the ability to detect gravity was partially impaired. This response was probably not due to acute trauma associated with lith removal, because an individual with one statolith removed showed no apparent differences in organ orientations at 1 and 12 h following the procedure. The partial compensation to body tilt instead seemed associated with reduction of coherent sensory input or to interference from random

Table 1. *Regression analysis of posterior light organ and eyestalk orientations*

(Data were from a 180° range of body tilts, from vertical head up to vertical head down, corresponding to the range of observed body inclinations in free-swimming animals. A slope of 0 indicated complete compensation to body tilt, while a slope of 1 indicated no compensation. Slopes are significantly different [*t* test, $P < 0.001$] unless otherwise stated.)

Condition	Linear equation†	<i>N</i>	Variance	Correlation coefficient‡ (<i>r</i>)
Posterior light organ				
Normal	$y = 0.26x - 1.5$	387	167.8	0.72
Unilateral statolith ablation§	$y = 0.50x - 1.2$	675	169.8	0.90
Bilateral statolith ablation	$y = 0.96x + 1.1$	524	590.0	0.92
Sham incision into one statocyst§	$y = 0.53x - 6.0$	160	132.5	0.93
Control incision into base antennule	$y = 0.36x - 0.9$	160	63.9	0.92
Eyestalk				
Normal	$y = 0.20x + 2.8$	388	68.0	0.79
Unilateral statolith ablation (pooled)	$y = 0.38x + 2.1$	358	190.8	0.83
Homolateral	$y = 0.37x + 7.4$	160	83.7	0.91
Contralateral	$y = 0.38x - 2.2$	198	237.3	0.80
Bilateral statolith ablation	$y = 0.93x + 1.6$	400	659.6	0.89

† All *F* values of the regressions are significant ($P < 0.05$).

‡ All *r* values are significant ($P < 0.01$; Zar, 1974).

||§ Slopes not significantly different (*t* test, $P > 0.05$).

signals from the damaged organ. Thus, the slope of the regression for each experiment was additively correlated with the condition of the two statocysts. Removal of both statoliths generated a slope approximately double that when only one lith was removed, which in turn was double the slope for normal animals (Table 1). The slopes of these three experiments were all significantly different from each other (*t* test, $P < 0.001$).

The results of the sham experiment (incision into one statocyst without removing the lith) were similar to lith removal (Table 1). The slope of the regression for the sham experiment, 0.53, was not significantly different from the slope of 0.50 for the experiment when one statolith was removed ($t = 1.8$, $P > 0.05$). Perforation of the wall of the statocyst presumably injured mechanosensory hairs (Cohen, 1955).

The trauma associated with the control experiment affected the responses by the experimental animals. An incision made in the base of one antennule, near the statocyst, led to a noticeable loss of body fluids. While the resulting slope of the regression for the control experiment, 0.36 (Table 1), was significantly larger than the slope for the normal condition ($t = 5.8$, $P < 0.001$), it was also significantly smaller than the slope for the experiment in which one statolith was removed ($t = 9.3$, $P < 0.001$). Even though the light organs from control animals which had suffered blood loss compensated less to body tilt than did those of normal animals, they did respond better than animals with partially impaired gravity detection.

Normal animals exhibited different patterns for up and down body tilts. When the range of rotation angles previously analysed was divided into two subranges, 0–90° head-up and 0–90° head-down, the slopes of the calculated regressions, 0.32 and 0.23, respectively, were significantly different ($t = 2.1$, $P < 0.05$). This difference was not explained by responses in the 0–70° range of body tilts, as the mean organ orientations for each of the tilt angles showed no significant differences between the up and down directions (t test, $P > 0.05$). However, the angles of orientation at 80 and 90° head-up body tilts were significantly greater than those at 80 and 90° head-down positions (t test, $P < 0.05$). This signified that counter-rotations by the light organ at 80 and 90° head-up body tilts were smaller in magnitude. While the light organs could effectively compensate to body tilts up to the vertical-down position, the response to head-up tilts was restricted to a range of 70° inclinations.

The anterolateral pair of organs of *Pesta* behaved similarly to the posterior organ, performing similar compensatory counter-rotations and exhibiting an identical statocyst dependence on orientation. Their small size (0.5 × 1.5 mm), however, precluded quantification.

Responses of eyestalk

In response to angular rotations of the body about the transverse axis, the eyestalk counter-rotated to retain a near-horizontal position. This behaviour occurred for both up and down body tilts (Fig. 1A–C), and was absent in animals lacking statoliths (Fig. 1D–F). The counter-rotations were completed within a few seconds following body rotation. These compensatory movements did not habituate over several minutes and are probably maintained for an extended period.

Rotation sequences performed on normal animals generated eyestalk responses analogous to those of the posterior light organ. The deviation of the eyestalk angle from the body angle followed a sinusoidal curve with maximum values at vertical body positions (Fig. 5A). Compensatory counter-rotations maintained the eyestalk at near-horizontal orientations over a 180° range of body tilts centred at the horizontal (Fig. 5B). For example, at vertical body positions the eyestalk was inclined only 17–21° from the horizontal. Unlike the posterior light organ, eyestalk counter-rotations were equally effective for up and down body tilts (t test, $P > 0.05$). Beyond the range of compensation, every 10° of body tilt resulted in a 20° change in the eyestalk orientation. At the inverted horizontal body position, the eyestalks were also inverted and horizontal (Fig. 5B).

Compensatory counter-rotations by the eyestalk were statocyst dependent. Fig. 6 shows the pattern of eyestalk responses with a rotation series performed on a single individual. Body tilts of 90° or less generated compensatory counter-rotations which resulted in near-horizontal eyestalk alignment in the normal specimen (Fig. 6A). Unilateral statolith removal reduced the amount of compensation within this range (Fig. 6B), while bilateral statolith removal abolished counter-rotations (Fig. 6C).

Results from all animals demonstrated the dependence of eyestalk orientation upon statocyst condition. These are shown in Fig. 7 for body inclinations included in the range of *in situ* body positions. The compensatory counter-rotations performed by the eyestalks of normal animals were effective in maintaining near-horizontal

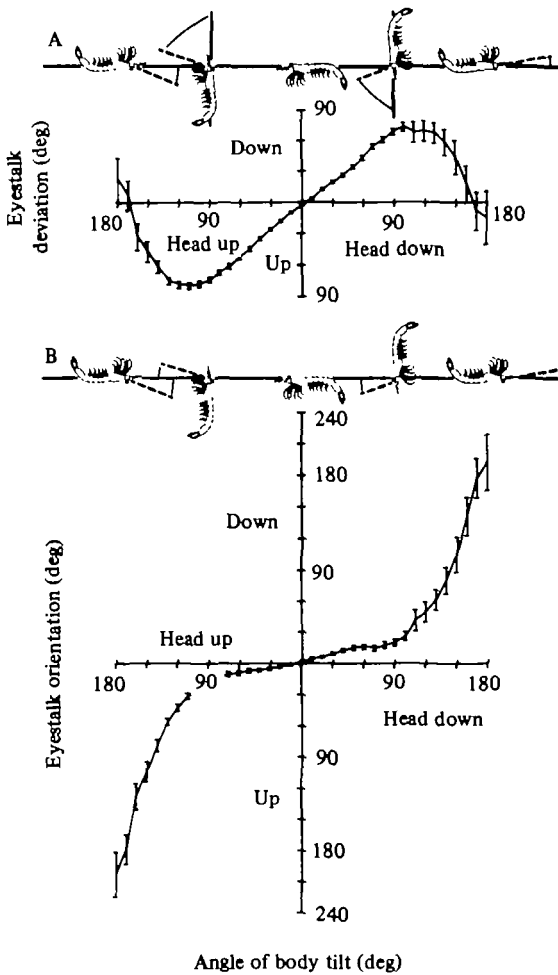


Fig. 5. Responses of eyestalk of normal animals to 360° range of body tilt. As in Fig. 2.

alignment even for large angles of body tilt. For example, when the body was inclined vertically either head-up or head-down, the eyestalk was only rotated 17–21°. In animals with one statolith removed, compensatory counter-rotations still occurred, even though they were reduced in magnitude. In general, the mean eyestalk rotation for each angle of body tilt was approximately double that for normal animals. At the vertical body positions, mean eyestalk rotations were 33–41°. When both statoliths were removed, compensatory counter-rotations were absent, and the mean eyestalk rotation for each angle of body tilt was essentially equivalent to the body tilt (Fig. 7).

Regression analyses of these data quantified the differences between the three experimental conditions (Table 1). The slope of 0.20 calculated from the regression for rotation sequences on normal animals signified that for every 10° of body tilt the eyestalk only rotated 2°. This situation was comparable to that for the posterior

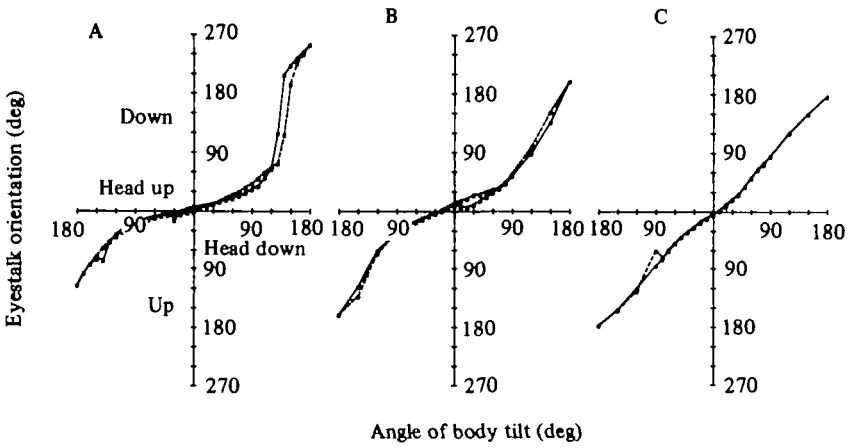


Fig. 6. Effect of statolith ablation in a single animal on eyestalk orientation with respect to horizontal at each angle of body tilt. As in Fig. 3.

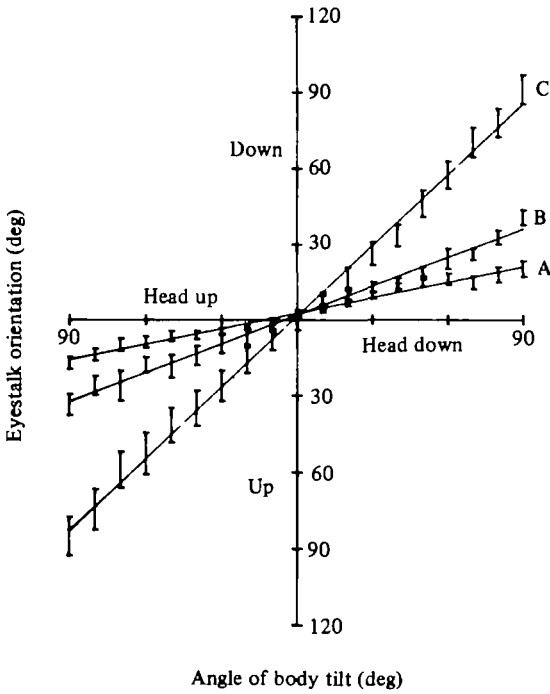


Fig. 7. Effect of statolith ablation on eyestalk orientation for all animals. As in Fig. 4.

light organ, even though the regressions were significantly different (t test, $P < 0.001$). The ability of the eyestalk to counter-rotate did not differ between up and down body tilts (t test, $P > 0.05$). The condition of the statocysts did not affect the resultant y -intercepts of the regressions (t test, $P > 0.05$). However, statolith removal did significantly change the slopes (t test, $P < 0.001$). When one statolith was removed, the calculated slope, 0.38 , was approximately double that for the experiment with normal animals. Thus, removal of one statolith diminished the magnitude of counter-rotations by one half. The effects of contralateral and homolateral lith ablation were not significantly different ($t = 0.55$, $P > 0.50$). The experiment in which both statoliths were removed generated a slope of the regression of 0.93 . This situation approached the model relationship in which a slope of 1.0 represents total absence of compensation. In this case, the eyestalk rotated 9.3° for every 10° of body tilt.

Directional light effects

Bright-light stimulation

The response to upward-directed illumination was a rotation of the eyestalks about the longitudinal axis towards the light source. Based on stop-frame video analysis, the latency of the response after stimulation was 350 ± 136 ms (mean \pm standard deviation, $n = 8$), and the new eyestalk position was maintained for the duration of the light stimulus, after which it returned to the previous dark orientation. The magnitude of rotation was intensity dependent, such that higher stimulus intensities generated more rotation; the apparent decrease in magnitude of rotation at the highest intensity was due to rapid habituation of the response (Fig. 8). The greatest rotation was only 37° (mean of 23°), while in previous experiments involving body tilt the eyestalks turned up to 80° .

Dim-light stimulation

The orientation of the posterior light organ was unaffected by downward-directed or upward-directed (Fig. 9) illumination at all stimulus intensities. This was observed for all three body positions tested. Loss of gravitational input through bilateral statolith ablation did not render the light organ position susceptible to directional light cues.

The eyestalk orientations of normal animals positioned horizontally were unaffected by downward-directed illumination at all intensities tested. While there was no rotation of the eyestalk under these conditions, the eyestalks did exhibit an upward-lifting response at high intensities.

Animals in the vertical-up body position did not exhibit eyestalk turning in response to downward-directed illumination. A similar lack of behaviour by animals tilted head down was observed at low light intensities. Only at high stimulus intensities did eyestalk turning occur. Turning was away from the light source and may have been an avoidance response.

The eyestalks of normal animals in the horizontal body position did not turn in response to upward-directed illumination (Fig. 10). However, eyestalks of animals in vertical up and down body positions turned up to 20° towards the light. Partial habituation occurred at the highest stimulus intensity.

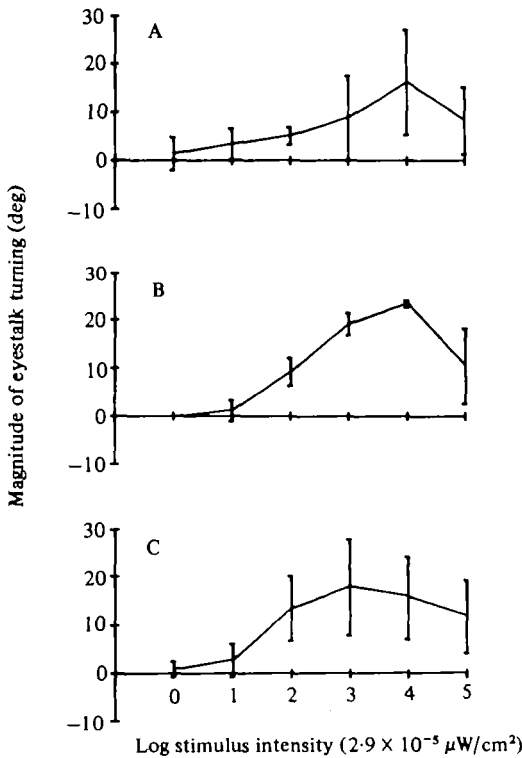


Fig. 8. Eye-turning of eyestalk to upward directed bright illumination. Mean magnitude with standard deviation of turning towards the light stimulus is expressed as a function of each stimulus intensity. (A) Horizontally positioned animals. (B) Vertical head-down body position. (C) Vertical head-up position.

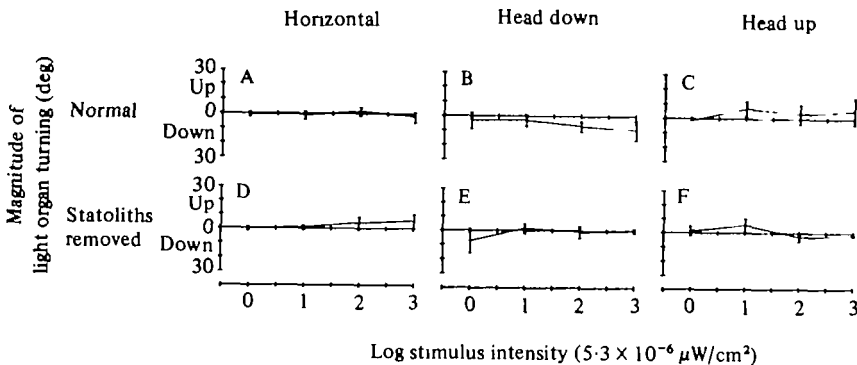


Fig. 9. Effect of upward-directed dim illumination on changes in light-organ orientation. Mean magnitude of change with standard deviation is expressed as function of stimulus intensity. Positive values represent upward turning while negative values signify downward turning. Normal animals in (A) horizontal, (B) vertical head-down, and (C) vertical head-up body positions. Animals without statoliths in (D) horizontal, (E) vertical head-down, and (F) vertical head-up body positions.

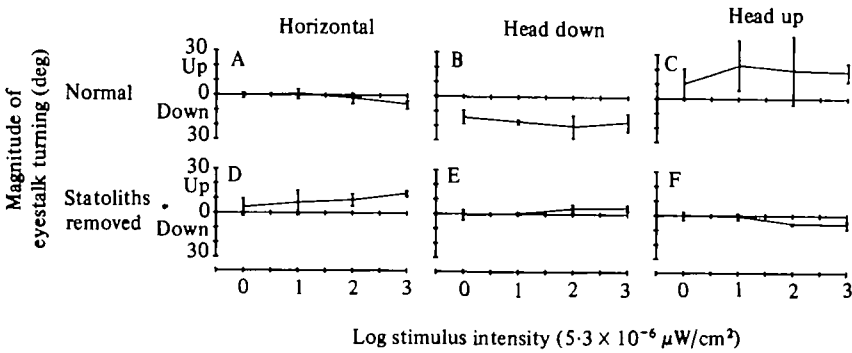


Fig. 10. Effect of upward-directed dim illumination on changes in eyestalk orientation. As in Fig. 9.

Bilateral statolith ablation eliminated eyestalk turning (Fig. 10) except for high intensity upward-directed stimulation of horizontally positioned animals. High-intensity upward-directed stimulation of horizontally positioned animals. High and attempted tail flipping were also observed. In any case, at low stimulus intensities, no significant amounts of turning occurred. Generally, the elimination of gravitational cues in the determination of eyestalk orientations did not result in greater turning towards directional illumination. In fact, most eyestalk turning appeared to be statocyst dependent.

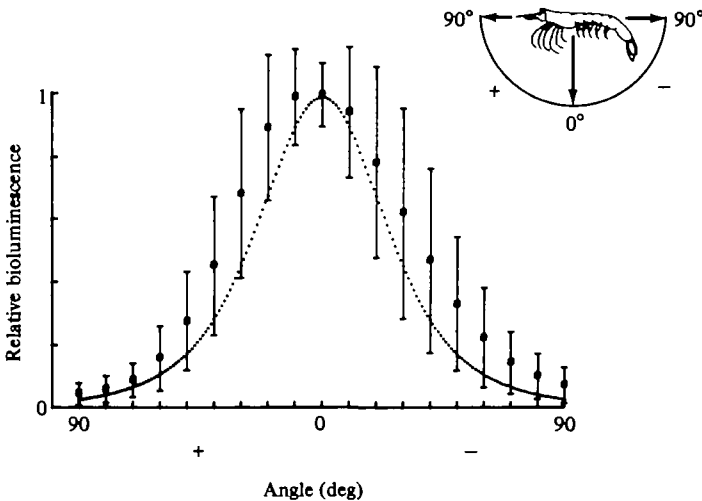


Fig. 11. Angular distribution of bioluminescence measured about the transverse axis. Light emission was induced by squeezing the eyestalks of animals without statoliths. Recording geometry is diagrammed at upper right. For each angle of body tilt, the standardized mean (closed circle) with standard deviation represents 38 scans of luminescence from 6 animals. Dotted line represents calculated angular distribution of oceanic light (see text).

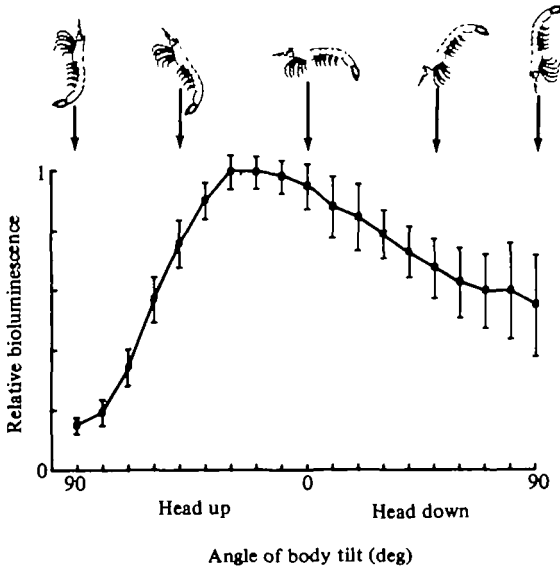


Fig. 12. Distribution of downward-directed luminescence for normal, inclined animals. Light emission was induced by squeezing the eyestalks. Diagrams depict the measurement configuration. For each angle of body tilt, the standardized mean with standard deviation of downward-detected luminescence represents 25 scans from 4 animals.

Bioluminescence emission pattern

The angular distribution of bioluminescence measured about the transverse axis was highly directional (Fig. 11). Maximum light output was downward, with minimum emission horizontally. The angular distribution of luminescence was similar to the calculated angular distribution of oceanic light at a depth of 250 m (Denton *et al.* 1972). While the distribution of luminescence appeared slightly broader, the two distributions were not significantly different (Kolmogorov-Smirnov, $D = 0.053$, $P > 0.05$).

Since maximal light emission was downward and the light organ in a horizontal animal was oriented horizontally, bioluminescence was emitted normal to the ventral surface of the light organs. Therefore, the amount of downward-directed luminescence in normal animals would be predicted to be a function of the light organ orientation. This correlation is demonstrated in Fig. 12. Downward-directed light emission was greatest for body positions close to the horizontal. At greater inclinations, there was a decrease in downward-emitted luminescence. This was more pronounced for head-up body tilts. When inclined 90° up, only 15% of maximal emission was still projected downward, while at the 90° down body position, luminescence was still 56% of maximum. This significant difference ($t = 11.69$, $P < 0.001$) was explained by the more effective compensation by the light organ to head-down body tilts.

DISCUSSION

The posterior light organ and eyestalk of *S. similis* exhibited similar statocyst-mediated behaviour, sharing the following characteristics: (1) similar range of angular movement, (2) orientation determined by angle of body tilt and not direction of body rotation, (3) similar compensatory abilities, (4) additive effect of statolith removal on magnitude of counter-rotations, and (5) essentially complete absence of compensatory responses after bilateral statolith ablation.

Both organs exhibited a 140° range of angular movement, compensating for transverse axis body tilt by maintaining near-horizontal orientations. Counter-rotations of the posterior light organ had previously been observed in *S. similis* and other sergestid shrimps (Omori, 1974). The range of eyestalk movements is greater than reported for other animals (Table 2). Euphausiid photophores and eyes are capable of 180° of angular movement, although in response to directional illumination and not body tilt (Hardy, 1962; Land, 1980).

The body positions at which counter-rotations were effective included the full range of inclinations in free-living individuals of *S. similis* observed *in situ* from research submersibles (Omori, 1974; A. Alldredge, E. Barham, B. Robison, personal communications), from the vertical head-up through horizontal to the vertical head-down body positions. Within this range, counter-rotations by the light organ and eyestalk compensated for 76–80% of imposed body tilt. The eyes of other animals compensate less (Table 2).

Light organ and eyestalk orientations in *S. similis* were dependent on magnitude of body inclination regardless of tilt direction. Other studies, however, have been characterized by asymmetric responses. Opposite directions of approach to a particular body position produce discrepancies in eyestalk orientations of $20\text{--}60^\circ$ (Hisada *et al.* 1969; Budelman, 1975; Neil, 1975*a*).

The effect of statolith ablation was additive. Removal of one statolith caused a 50% reduction in the magnitude of compensatory counter-rotations. In this condition, compensatory eyestalk responses were equally controlled by either the homolateral or contralateral statocyst, unlike *Nautilus*, where the compensatory response of each eye is under the control of the homolateral statocyst (Hartline *et al.* 1979), or the mysid *Praunus*, in which each statocyst is responsible for eyestalk movement in one direction only (Neil, 1975*b*). Removal of both statoliths abolished compensation. This response is shared by all statocyst-mediated systems involving eye or eyestalk counter-rotations (Table 2). Thus, gravitational cues detected by the statocysts provided the information necessary for determining light organ and eyestalk orientations in the dark.

Light organ orientations, which resulted from statocyst-mediated counter-rotations, were unaffected by directional illumination comparable to oceanic light intensity and wavelength. In addition, they remained unaffected by directional illumination after bilateral statolith removal. Thus, gravitational cues from the statocysts provided essentially all the information necessary for establishing light organ orientations.

While eyestalk orientations were generally unaffected by downward-directed

Table 2. Survey of eye counter-rotation†

Animal	Range of angular movement for 360° body rotation (deg)	Condition of statocysts			Range considered for analysis (max. body tilt from horiz. in deg.)	Reference
		Normal	Unilateral ablation	Bilateral ablation		
Shrimp, <i>Sergestes similis</i> <i>Nautilus</i>	140	0.20	0.38	0.93	90	Present study Hartline <i>et al.</i> 1979
	90	0.50-0.60	0.50‡ 1.0§	1.0	40	
	85	0.50	—	—	65	Budelman, 1975 Traill & Mark, 1970 Hisada <i>et al.</i> 1969
76	0.45	0.60	1.0	80		
85	0.58 0.65	0.68 —	0.84 —	90 90		
Crayfish, <i>Procambarus</i> Spiny lobster, <i>Panulirus</i>	—	0.74	—	—	30	Schone, 1971
	—	0.83	—	—	45	Schone & Schone, 1967 Budelman, 1975
	30	0.78 0.83	— 0.93	1.0 1.0	45 80	

† Expressed as amount of eye movement per unit transverse axis body rotation. Linear regression calculated for eye orientations with respect to horizontal over indicated range of body tilt. When necessary, data were first converted from eye deviation from body angle.

‡ Contralateral.

§ Ipsilateral.

illumination, eye turning of up to 25° was induced by both bright and dim upward-directed illumination. While the eyestalks rotated towards the light source, the magnitude of rotation was insufficient to point the eye directly towards the source of illumination. Therefore, directional illumination was not as effective a cue as gravity in determining eyestalk orientations, as found in other studies (Traill & Mark, 1970; Neil, 1975a). While eyestalk movements in *S. similis* were mainly statocyst mediated, euphausiids, which lack known balance organs, use light direction as a sufficient orientational cue. Land (1980) found that euphausiid eyes track a very bright light accurately over a 180° range. Other crustaceans lacking known balance organs, however, orientate to gravity in the dark (Sulkin, 1973), and the cladoceran *Daphnia magna*, which exhibits a geotactic response (Grosser, 1953) and eye tracking of light (Ringelberg, 1964), also performs eye counter-rotations in the dark to compensate for body tilt (Jander, 1975). Therefore, the possibility remains that euphausiids may also display compensatory eye movements in response to body tilt.

Servo-control theory can be applied to the two feedback systems involved in determining eyestalk orientations (Hisada *et al.* 1969; Neil, 1975a). The statocyst control system is open-loop, as eyestalk movements do not affect statocyst activity. The visual control system is closed-loop since visual information from the eye acts as negative feedback to the compensatory movements. If the visual control system is inhibited by statocyst activity, then the elimination of gravitational input should increase the contribution of visual cues in determining eye orientations (Neil, 1975a).

Contrary to this hypothesis, bilateral statolith ablation in *S. similis* did not increase the magnitude of eyestalk turning. This indicated that the release of the visual control system from inhibition by the statocysts, if such inhibition were present, had not occurred. In fact, eyestalk turning by tilted animals towards upward-directed illumination was eliminated. Statocysts devoid of the statolith exhibit spontaneous neural activity (Cohen, 1955; Takahata & Hisada, 1979). Such statocyst activity may have maintained inhibition of the visual control system. In other studies (Traill & Mark, 1970; Neil, 1975a), removal of both statocysts resulted in an enhanced orientational effect of visual input, even though the change was not as large as expected.

Similar light organ and eyestalk movements, even in agitated animals, suggest closely linked neural control of eyestalk and light organ. However, the two systems in *S. similis* can be uncoupled, as in tilted animals stimulated with upward-directed illumination. In this case, eyestalk turning was not accompanied by light organ turning. Thus, the eyestalk control system may involve a visual component not present in the light organ control system, allowing the eyes to fixate a light source while the light organ orientation was maintained according to gravitational cues.

Bioluminescence in horizontal animals was emitted vertically downward, projecting normal to the ventral surface of the light organ. The angular distribution of luminescence was similar not only to the distribution of oceanic light (Denton *et al.* 1972) but also to the angular distributions of emitted luminescence from other midwater animals (Denton *et al.* 1972; Herring, 1976; Herring & Lockett, 1978). Total compensation by the light organ of tilted animals would be expected to result

in equal intensities of downward-directed luminescence at all body inclinations. However, as counter-rotations compensated for only 74% of the imposed body tilt, the amount of downward-detected luminescence decreased at larger body inclinations. Animals luminescing in response to visual stimulation, instead of the mechanical stimulus utilized, may compensate for this by an increased output.

The counter-rotations performed by the light organ and eyestalk would serve important functions during counter-illumination by *S. similis* by compensating for the effects of body tilt. First of all, bioluminescence from the light organs would be maintained in approximately a downward direction. Compensatory movements by the eyestalks would stabilize the visual field (Milne & Milne, 1965). Not only does this facilitate neural processing of visual information (Hartline *et al.* 1979), but it may also allow for detection of polarized light by tilted animals (Hisada *et al.* 1969). In addition, since *S. similis* does not counter-illuminate to upward-directed light (Warner *et al.* 1979), eyestalk counter-rotations preserve up and down directionality, and guarantee that downwelling oceanic light, the proper stimulus for counter-illumination, will be detected as downward directed, perhaps preventing maladaptive luminescence.

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