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Effects of variable water motion on regeneration of *Hemipholis elongata* (Echinodermata, Ophiuroidea)

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Abstract. To determine whether increased water motion affects patterns of regeneration in the subtidal burrowing brittlestar *Hemipholis elongata* (phylum Echinodermata), individuals were subjected to laboratory-controlled turbulence conditions. Half of each replicate aquarium experienced oscillatory (wave-like) turbulence while the other half had no turbulence. Individual brittlestars from which arm-tips had been removed were allowed to burrow and to regenerate. Regenerated arm-tip length and weight were tested for differences between organisms in calm and turbulent conditions. Regenerated arm-tip length differed significantly between control and treatment, but arm-tip dry weight and skeleton/tissue ratio of regenerated arm-tips did not. To quantify plasticity in the skeleton, 15 morphological measurements made on the proximal face of vertebral ossicles (using scanning electron microscopy) were integrated as an index of overall ossicle size. We found a significant difference in the overall size index of the vertebral ossicles between treatments, but could not determine which of the measurements contributed most to the difference. The results indicate that regeneration in *H. elongata* is a complex process that can be modified by environmental conditions.

Additional key words: phenotypic plasticity, brittlestar

Phenotypic plasticity has been defined as the differential phenotypic expression of a genotype under various environmental conditions (West 1997). Corals, sponges, and other marine invertebrates have long been known to alter their phenotype in response to such environmental cues as predator damage, water motion, light level, and pressure (Palumbi 1984; Dennison & Barnes 1988; Etter 1988a,b; Bell & Gosline 1997; Trussell 1997a,b; West 1997; Arsenault et al. 2001). Among echinoderms, phenotypic plasticity has been demonstrated in response to food availability (Edwards & Ebert 1991; Levitan 1991), mechanical stimuli (Lewis & Storey 1984; Dafni 1986, 1988; Clements et al. 1994), and pollutants (Dafni 1980; Dafni & Erez 1987). Water motion has been shown to affect the development of the echinoid test (McPherson 1965; Lewis & Storey 1984; Dotan & Fishelson 1985; Dafni 1986). Although Clements et al. (1994) hypothesized that exposure to stronger currents may lead to a higher calcium to tissue ratio in the arms, phenotypic plasticity has not been as thoroughly examined in ophiuroids.

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Differences in composition as a result of cellular processes contribute to the regenerative plasticity of echinoderm skeletons. These differences result from the processes of ionic resorption, reallocation, and repair of extracellular calcified components. Resorption clears away unsuitable stereom (DuBois & Chen 1989) that ultimately may be reallocated to other areas of the body. Resorption of larval spicules during metamorphosis is well documented and the body of literature covering resorption processes in postmetamorphic ossicles of several echinoderm classes is growing (see DuBois & Chen 1989). Evidence of reallocation of resorbed materials for maintenance and repair is documented in the regeneration of broken spines (Heatfield 1971), the reworking of Aristotle's lantern (Ebert 1980), and the thickening of coronal plates (Smith 1980) of echinoids.

Extracellular calcite deposition also occurs in areas of the ophiuroid skeleton subject to increased wear (Byrne 1994; LeClair 1995a; Stewart 1995). Reallocation of resorbed ions and other internal nutrient stores likely play a role in the repair and/or regeneration of ophiuroid arms lost to predation or breakage (Fielman et al. 1991; Stancyk et al. 1994). Most evi-

dence to date points towards a continual turnover and reworking of the echinoderm stereom, although the extent of the dynamic nature of this system has yet to be confirmed (DuBois & Chen 1989). Nonetheless, the results of the aforementioned studies suggest that the various means of cellularly modifying skeletal composition can be expressed in measurable morphological differences.

The ability to continually remodel the stereom for the regeneration of lost tissue is important because the bodies of infaunal brittlestars are routinely cropped by a variety of predators. Moreover, consumption of the exposed arms of infaunal brittlestars is a significant trophic pathway in some soft-sediment habitats (Duineveld & Van Noort 1986; Stancyk et al. 1994; Pape-Lindstrom 1997). Recovery from sublethal predation has been shown to be an important aspect of the life history of these animals (Bowmer & Keegan 1983), and the ability to regenerate lost tissue rapidly is significant to their survival (Stancyk et al. 1994).

Because ophiuroids can replace lost tissue rapidly, via the cellular processes described above, they are excellent subjects for the study of regeneration. In addition, the phenotypic plasticity that has been documented for other echinoderm classes suggests that ophiuroids may have the potential to regenerate differently under various environmental regimes. *Hemipholis elongata* SAY 1825 is a suitable candidate for studying this phenomenon because it extends its arms vertically into the water column to feed, as opposed to lying along the surface of the sediment like most infaunal deposit-feeding species, e.g., *Amphipholis gracillima* (= *Microphiopholis gracillima,* Hendler 1995). *H. elongata* is an infaunal ophiuroid (family Ophiactidae) commonly found in subtidal mud bottoms off the southeastern coast of the United States in densities as high as 2,400 individuals m^{-2} (Ruppert & Fox 1988: 70–73; Valentine 1991), as well as off the coasts of Brazil, Cuba, and Panama (Absalao 1990; Hendler 1995). In South Carolina, these brittlestars are routinely found in association with the buried portion of the tubes of the onuphid polychaete worm *Diopatra cuprea* (Ruppert & Fox 1988: 70–73). Adults have a disc diameter ranging 3–12 mm with individual arm lengths up to 10 cm (Beardsley 1997). The ophiuroid buries its disc 2–4 cm beneath the sediment surface and extends 2 or 3 arms into the water column for feeding and respiration (Beardsley & Colacino 1994). Finally, *H. elongata* is found in conditions of fluctuating water motion.

Among environmental variables, water motion may induce various morphological changes in an ossicle. Clements et al. (1994) proposed that a more energetic hydrodynamic regime induced higher skeletal regeneration rates by individuals of *Ophiophragmus filograneus* in planted, compared to natural, seagrass beds. To test this hypothesis, we examined regeneration by a burrowing ophiuroid, *H. elongata,* under laboratory-controlled water motion. The following questions were addressed: (1) does water motion affect the length of regenerated arm-tips, (2) does water motion affect the dry weight (DW) of regenerated arm-tips, and (3) does water motion affect the overall size of vertebral ossicles?

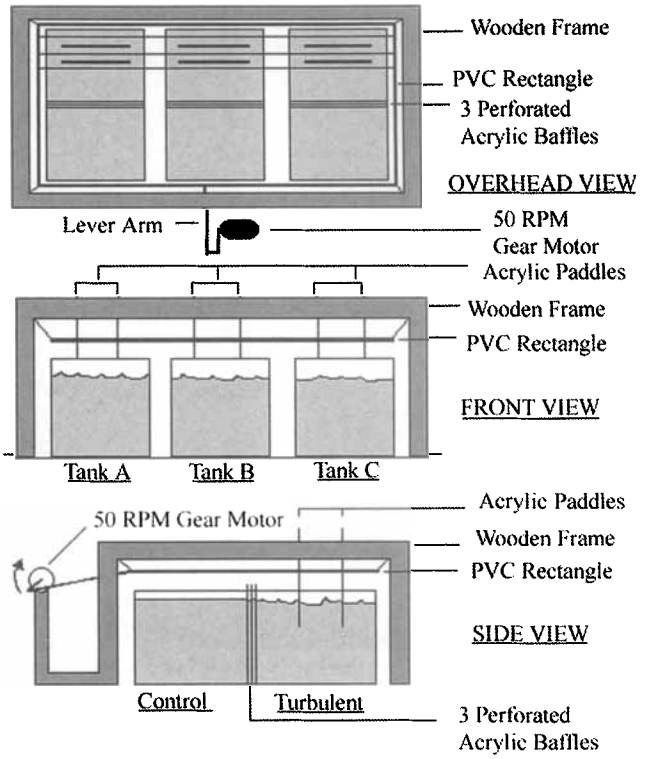

Fig. 1. Diagram of machine set-up. Acrylic baffles are spaced 4 cm apart and each is perforated with half-centimeter drill holes. Dimensions of the PVC rectangle are 2 m × 60 cm × 1.5 cm. The aluminum shaft from the gear motor is 1.5 cm in diameter × 65 cm.

Methods

Individuals (n=60) of *Hemipholis elongata* were collected from Johnson Creek, adjacent to Hunting Island, South Carolina, in spring 1997 and were maintained in laboratory aquariums for ~12 weeks before the experiment. A machine that produced variable water motion via a system of paddles was built and tested (Fig. 1).

The 3 replicate aquariums (each ~115 liters) were divided into control and experimental halves (Fig. 1) by acrylic baffles perforated with drill holes; these restricted water movement to one half of each tank,

while allowing all other water characteristics to remain the same throughout each tank. A filtration unit was placed in one corner of each tank half. To produce variable water motion in one half of each tank, we built a machine consisting of a 50-rpm gear-motor (Grainger, Stk. #4Z538A) and a set of 6 acrylic paddles (2 paddles per tank). The acrylic paddles hung from a large wooden frame into the tanks. Also suspended beneath this wooden frame was a laced PVC rectangle attached to the gear-motor via an aluminum shaft. As the gear motor turned, the PVC rectangle was pushed and pulled, thereby moving the paddles and creating turbulence.

To approximate the intensity of the water motion normally encountered by infaunal brittlestars in their natural habitat, the rate of dissolution of wintergreen flavored Lifesavers® was measured in the field (Koehl & Alberte 1988). Previously weighed Lifesavers® were placed in the water column for 5 min during falling and slack tides at the level that the brittlestars extend their arms. The partially dissolved Lifesavers® were weighed and percent dissolution was calculated. To calibrate the machine, a similar Lifesaver® dissolution test was performed in the turbulent halves of each tank. Calculated percent dissolution (mean ± standard deviation) for the falling tide was 81 ± 4% and that for the slack tide was 62 ± 7%. The machine was set to allow for ~70% dissolution of a Lifesaver® in 5 min (roughly 16–17 paddle strokes/min). By comparison, Lifesavers® placed in the control halves of the tanks dissolved ~40% in 5 min. Turbulence, as measured by Lifesaver® dissolution, differed significantly between treatments ($p<.0001$). The paddles produced a primarily oscillatory movement of water, best described as a "variability of water motion" (D. Miller, pers. comm.).

All 60 animals were anesthetized with a 1:1 mixture of 35 ppt $MgCl_2$ and seawater, and the oral frame of each individual was measured to the nearest 0.1 mm according to the method of Singletary (1980). The total length of each arm and its position relative to the madreporite was recorded. After measurement, ~2 cm was cut from the tip of each arm. Each animal was placed in a 6.5-cm diameter PVC core and covered with sieved, homogenized sediment from the collection site. Randomly selected cores ($n=10$) were placed in either the control or experimental side of each tank for a total of 30 control and 30 experimental animals.

Brittlestars were allowed to regenerate for 40 days. So that each animal experienced every microhabitat encountered by all other individuals within each tank half, cores were rotated every 4 days. Every 8 days, the paddles were stopped to allow all individuals an equal opportunity to feed. Uniform dispersal of food was achieved by pipetting 0.1 g of a ground mixture of Tetra-Min® directly into the bubble stream of each filtration unit. After 1 h, the excess food was siphoned from the bottom, the small volume of water removed during that process (~5% of total volume) was replaced with fresh seawater, and the paddles were restarted.

Temperature and salinity were maintained at 20°C and 34–35‰ and were checked daily. Dissolved oxygen was measured weekly. The entire experiment was run in darkness except during the 1–2 h required to manually rotate the PVC cups and clean the filters (every 4th day), and to feed the animals (every 8th day). Running the experiment in this manner not only maximized the foraging activity of each brittlestar (unpubl. obs.), but also minimized any potential variation in lighting between treatments.

After the 40-day regeneration period, brittlestars were removed and anesthetized, and the total arm length and regenerated arm-tip length were measured. A two-way analysis of variance (ANOVA: SAS Institute, Inc. 1989) with 3 tanks and 2 treatments was used to test the hypothesis that increased water motion causes regenerated arm-tips to be shorter relative to a control.

All regenerated brittlestar arm-tips were cut on the proximal side of the regeneration scar with a scalpel, categorized by treatment, tank, and individual, and were then randomly assigned for use in 1 of 3 procedures. For each animal, 2 arm-tips were weighed, 2 arm-tips were used for scanning electron microscopy (SEM), and the remaining arm-tip was removed and preserved in 1 of 3 ways (70% alcohol, 3% buffered glutaraldehyde, or frozen) for future study.

Two arm-tips per individual (60 per treatment) were transferred to pre-weighed aluminum pans and dried to constant weight in a drying oven at 37°C for 24 h. Total dry weight was determined to the nearest 0.01 mg using a Mettler AG245 balance. The tips were then ashed in their pans in a Lindberg muffle furnace at 400°C for 4 h. Skeletal weight (= ash weight) was determined and tissue weight was derived by subtracting skeletal weight from total dry weight. A two-way analysis of variance (ANOVA) was used to test the hypothesis that increased water motion causes the dry weight of regenerated arm-tips to be greater relative to a control. Weight ratios for skeleton: total, tissue: total, and skeleton: tissue were calculated and Kruskal-Wallis one-way ANOVA's were used to test for significant differences between the ratios.

To prepare them for SEM, arm-tips were cleaned of tissue in a 7% sodium hypochlorite solution for 1–3 h, and then rinsed with de-ionized water (see LeClair 1995b). To increase sample size while minimizing var-

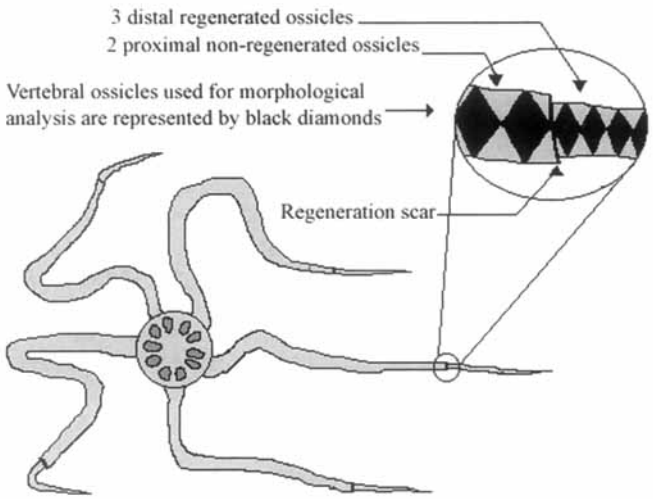

Fig. 2. Diagram of brittlestar depicting the locations of ossicles used for morphological analysis: 2 original, non-regenerated ossicles immediately proximal to the scar and 3 newly regenerated ossicles immediately distal to the regeneration scar.

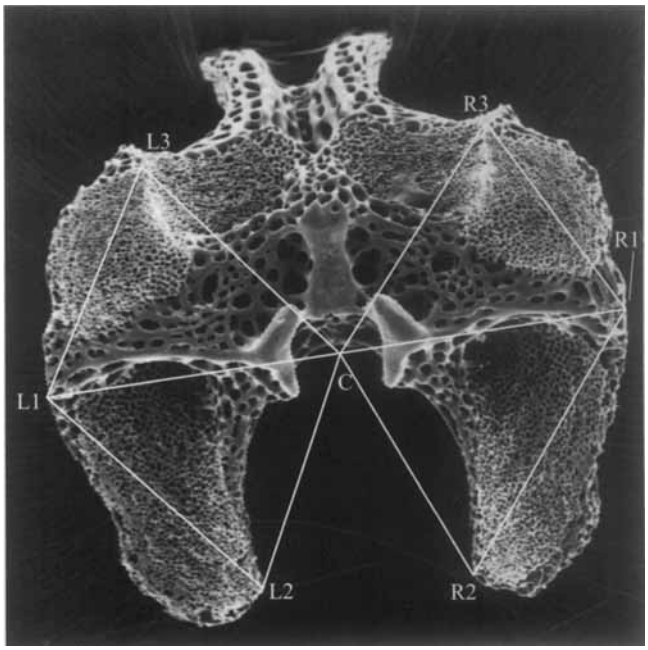

Fig. 3. Proximal face of regenerated vertebral ossicle depicting morphological measurements 1–15. Measurements 1–9 are linear and 10–15 are of area. Using the area measurement tool in Scion Image, we measured the area of the fine-pored stereom of each fossa (10–13), the area of the articular condyle (C) and the coarse-pored stereom (14), and the total area of the ossicle face (15). The measurements are abbreviated as follows: W = width, L = left, R = right, F = fossa, O = oral, B = aboral, T = lateral, M = medial, D = diagonal, A = area, C = condyle, S = stereom, and X = ossicle. 1: L1-R1 = W—Width; 2: L1-L2 = LOFTD—Left oral fossa lateral; 3: R1-R2 = ROFTD—Right oral fossa lateral; 4: L1-L3 = LBFTD—Left aboral fossa lateral; 5: R1-R3 = RBFTD—Right aboral fossa lateral; 6: C-L2 = LOFMD—Left oral fossa medial; 7: C-R2 = ROFMD—Right oral fossa medial; 8: C-L3 = LBFMD—Left aboral fossa medial; 9: C-R3 = RBFMD—Right aboral fossa medial; 10: LOFA—Left oral fossa; 11: ROFA—Right oral fossa; 12: LBFA—Left aboral fossa; 13: RBFA—Right aboral fossa; 14: CSA—Condyle stereom; 15: XA—Entire ossicle face.

iation in ossicle dimensions, 5 vertebral ossicles per arm tip (300 per treatment) were systematically selected to include the 2 immediately proximal and 3 immediately distal to the regeneration scar (see Fig. 2). These ossicles were dissected from the vertebral series, transferred to plastic multi-well plates, and allowed to air-dry overnight.

Dried ossicles were affixed to aluminum stubs on their distal faces and were sputter-coated with gold for 2 min. The proximal face of each ossicle was oriented orthogonally and was magnified to between 80× and 200× using a Hitachi S-2500Δ scanning electron microscope. A TIF-8 format computer image was captured via Irridium computer software and each image was analyzed with a beta-version of ScionImage image analysis computer software (a PC-based version of the Macintosh platform's NIH-Image available at http://www.scioncorp.com). To calibrate the ScionImage software, an image of a 30 μm polystyrene bead was taken at several magnifications and its diameter was measured.

To characterize the dimensions of the proximal face of each ossicle, 15 morphological measurements were taken from the proximal face of the ossicle in an image (Fig. 3). A multivariate analysis of variance (MANOVA: SAS Institute, Inc., 1989) and discriminant analysis (SAS Institute, Inc., 1989) were used to test the hypothesis that increased water motion causes the proximal face of the vertebral ossicle to be larger relative to a control. The MANOVA was used to test the quantitative effects of treatment whereas discriminant analysis was used to determine which variables contributed most to any differences that may have been found by the MANOVA. MANOVA and discriminant analysis are appropriate models to use in this analysis because the 15 morphological measurements are not independent. MANOVA can detect small differences among measurements that would not be detected by other statistical tests, e.g., separate ANOVAs, which require independent responses. Still, a separate ANOVA for each of the 15 morphological measurements was performed as a follow-up procedure. All statistical analyses performed in this study used an alpha value = .05.

Finally, to account for size variation among the brit-

Table 1. Regenerated arm-tip length and weight parameters. All data are mean ± standard deviation.

	Length (mm)	% Regenerated		n
Control	12.1 ± 6.0	60.5 ± 30.0		131
Turbulent	9.2 ± 5.7	46.0 ± 28.5		141
p-value	p<.0001			
	Tissue (mg)	Skeleton (mg)	Total (mg)	n
Control	0.38 ± 0.18	0.99 ± 0.55	1.37 ± 0.68	53
Turbulent	0.21 ± 0.11	0.52 ± 0.34	0.73 ± 0.39	56
p-value			p<.0001	
	Tissue: Total	Skeleton: Total	Skeleton: Tissue	n
Control	0.30 ± 0.12	0.70 ± 0.12	2.85 ± 1.43	53
Turbulent	0.33 ± 0.16	0.67 ± 0.16	2.77 ± 1.64	56
p-value		p=.695 for each ratio		

tlestars, each individual's oral frame diameter was initially placed in the MANOVA as a quantitative covariate that represented a fixed, known source of variability. However, the complexity and high degree of nesting in this MANOVA model prevented SAS from utilizing this value. To compensate, a correlation analysis was performed between the oral frame diameter and each of the 15 morphological measurements in both proximal and distal ossicles.

Results

The initial size of animals, based on oral frame diameter, was approximately equal. The group means for oral frame diameter, an indicator of the overall size of each individual, ranged from 2.96 ± 0.35 mm (mean ± standard deviation) to 3.40 ± 0.36 mm. An ANOVA found no significant differences between groups (sum-of-squares 1.197, df=5, mean-square = 0.239, F-ratio = 2.136, p=.075). The results of the correlation analysis showed no correlation between oral frame diameter and the recorded measurements of ossicle morphology; all r-values for proximal and distal ossicles were <0.26. This indicated that, within the size range used, the overall size of the individual has no effect on the parameters measured at the end of the arm. However, this does not preclude the possibility of overall animal size affecting the development of some other, unmeasured parameter.

The group means for arm length before cutting ranged from 67.9 ± 15.9 mm (mean ± standard deviation) to 77.1 ± 16.5 mm (6 groups: 3 tanks, 2 treatments). An ANOVA found a significant difference between the group means for arm length (sum-of-squares 2451.320, df=5, mean-square = 490.264, F-ratio = 2.420, p=.036). A Tukey HSD multiple comparison test attributed the results of the ANOVA to a significant difference between only one Control group and one Turbulent group; there were no significant differences among the other group means.

Analysis of randomly chosen regenerated arm-tips indicated that the number of regenerated ossicles per arm-tip for individuals was 44 ± 7.8 in the Control (n=30) and 40.3 ± 9.0 in the Turbulent treatment (n=30), with no significant difference (p=.335).

Nonetheless, the mean lengths of the regenerated arm-tips (Table 1) support the hypothesis that increased water motion causes the regenerated arm-tips to be shorter than in the control. The ANOVA demonstrated that regenerated arm-tip length differed significantly between treatments (sum-of-squares = 9719.238, df=271, F-value = 2.25, p<.0001). In contrast, data on skeleton, tissue, and total weight and on weight ratios (Table 1) did not support the hypothesis that increased water motion causes the total weight of regenerated arm-tips in Turbulent conditions to be greater than in the Control. On the contrary, mean weights of the regenerated arm-tips in the Control, for both tissue and skeletal components, were nearly twice those of regenerated arm-tips in the Turbulent treatment (Table 1). An ANOVA demonstrated that regenerated arm-tip dry weight was significantly higher for the Control (sum-of-squares = 43.322, df=108, F-value = 3.86, p<.0001). Also, Kruskal-Wallis one-way ANOVA tests found no significant difference between the treatments for tissue: total, skeleton: total, and skeleton: tissue weight ratios (p=.695 for each ratio, and Mann Whitney U test statistic = 1548.5 for ratios incorporating tissue weight, and 1419.5 for skeleton: total).

The data did not support the hypothesis that increased water motion causes the overall size of vertebral ossicles (as estimated from measurements of the

Table 2. MANOVA and individualized parameter ANOVA p-values. See Fig. 3 for parameter definitions.

Parameter	Treatment	Tank	Individual
MANOVA	0.0057	0.1290	0.0001
ANOVAs			
1: W	0.6257	0.1989	0.2058
2: LOFTD	0.5973	0.4023	0.0828
3: ROFTD	0.7513	0.3760	0.0805
4: LBFTD	0.3313	0.2960	0.2196
5: RBFTD	0.3837	0.2849	0.2363
6: LOFMD	0.8564	0.5834	0.0130
7: ROFMD	0.9269	0.4428	0.0169
8: LBFMD	0.3758	0.3552	0.2058
9: RBFMD	0.3005	0.3099	0.2670
10: LOFA	0.7060	0.3744	0.2700
11: ROFA	0.6812	0.2763	0.3697
12: LBFA	0.3233	0.2908	0.3702
13: RBFA	0.3442	0.3003	0.4278
14: CSA	0.6105	0.1451	0.2615
15: XA	0.5236	0.2269	0.3328

Table 3. Discriminant Analysis "Within Canonical Structure" (CAN1) values. See Fig. 3 for parameter definitions.

Parameter	Treatment CAN1	Individual CAN1	Arms CAN1
1: W	−0.0627	−0.0001	0.7365
2: LOFTD	−0.0679	0.0817	0.6665
3: ROFTD	−0.0407	0.0766	0.6870
4: LBFTD	−0.1254	0.0227	0.6200
5: RBFTD	−0.1123	0.0180	0.6103
6: LOFMD	0.0233	0.1395	0.4227
7: ROFMD	−0.0118	0.1409	0.4285
8: LBFMD	−0.1141	0.0124	0.7060
9: RBFMD	−0.1336	0.0093	0.6926
10: LOFA	−0.0485	0.0412	0.5467
11: ROFA	−0.0529	0.0048	0.5692
12: LBFA	−0.1277	0.0072	0.4646
13: RBFA	−0.1223	0.0061	0.4425
14: CSA	−0.0656	0.0278	0.5355
15: XA	−0.0822	0.0074	0.5312

proximal face) to be greater for regenerated arm-tips in the Turbulent condition than in the Control. Six classifying variables were used to identify an ossicle: treatment (control or turbulence), tank (n=3), brittlestar individual (n=10/group), arm position (n=5/brittlestar), proximal (non-regenerated) or distal (regenerated) ossicle, and ossicle position (proximal 1 or 2, distal 1, 2, or 3). These 6 classifying and one interaction term (Treatment × Proximal/Distal) were tested for significant differences using the MANOVA (Table 2). We found no tank effect and no effect due to ossicle position. Although the MANOVA detected significant differences for treatment, brittlestar individual, arm position, proximal/distal ossicle, and the interaction of treatment × proximal/distal ossicle, none of the "Within Canonical Structure" values in the discriminant analysis were above |0.4| for treatment or individual (Table 3); therefore, discriminant analysis could not clarify which of the parameters was contributing most to these differences.

Because the results of the MANOVA and discriminant analysis were ambiguous, the data were more closely examined by producing a separate ANOVA for each of the 15 morphological measurements. Analysis of the p-values for these ANOVAs indicated that there was no significant difference between Turbulent and Control for any of the parameters, i.e., there was no difference between the individual values of each measurement for Turbulent and Control ossicles (Table 2). Thus, in contrast to the results of the MANOVA, the per-measurement ANOVAs indicated no statistically significant difference between ossicles (see Discussion).

As expected, all regenerated distal ossicles were smaller than regenerated proximal ones. The experiment was terminated at 40 days, although the arm-tips had not yet reached their original length, because at this time the regenerated tissue is still pale and unpigmented, thereby allowing for easy identification of the regeneration scar. Although the MANOVA found statistically significant differences in ossicle measurements between the Control and Turbulent treatments (Table 4), note that their means do not appear markedly different and the standard deviations are large (see Discussion).

Discussion

This research supports the proposition that water motion affects regeneration and growth patterns in the ophiuroid *Hemipholis elongata.* Brittlestars subjected to variable water motion regenerated arm-tips that were significantly shorter and lighter (total dry weight) than the regenerated arm-tips of Control individuals. However, the arm-tip composition (skeleton: tissue ratio) did not significantly differ between treatments, indicating that the ophiuroids were not regenerating more robust, more heavily calcified arms in turbulent conditions.

These results contrast with those of Clements et al. (1994), whose data suggested that arm regeneration of *Ophiophragmus filograneus* under the potentially heavier wave action and greater biomechanical stress of replanted seagrass beds resulted in longer, heavier arms with more skeletal material than in their coun-

Table 4. Morphological measurements of distal (regenerating) Control and Turbulent ossicles (mean ± standard deviation). See Fig. 3 for parameter definitions.

Parameter	Control	Turbulent
1: W	243.39 ± 83.61 μm	239.05 ± 63.12 μm
2: LOFTD	132.61 ± 36.23 μm	130.01 ± 32.64 μm
3: ROFTD	132.19 ± 35.04 μm	132.03 ± 32.90 μm
4: LBFTD	113.49 ± 50.15 μm	107.28 ± 34.87 μm
5: RBFTD	112.59 ± 48.66 μm	106.38 ± 36.15 μm
6: LOFMD	114.30 ± 21.78 μm	115.67 ± 25.84 μm
7: ROFMD	114.67 ± 22.24 μm	114.11 ± 25.43 μm
8: LBFMD	146.54 ± 48.12 μm	141.08 ± 38.12 μm
9: RBFMD	144.28 ± 47.41 μm	137.48 ± 36.47 μm
10: LOFA	6870.03 ± 5025.88 μm^2	6992.74 ± 3678.75 μm^2
11: ROFA	6878.22 ± 5114.00 μm^2	6976.63 ± 3672.61 μm^2
12: LBFA	3944.55 ± 6492.32 μm^2	3176.99 ± 3161.46 μm^2
13: RBFA	3823.74 ± 6120.01 μm^2	3092.25 ± 3243.31 μm^2
14: CSA	20155.89 ± 11859.45 μm^2	19098.24 ± 8156.61 μm^2
15: XA	41422.98 ± 33963.17 μm^2	39336.85 ± 21019.58 μm^2

terparts in natural seagrass beds. In the study of Clements et al. (1994), entire arms were removed and were regenerated, whereas in our 40-d laboratory study, only arm-tips were removed and regenerated. The contrasting results of the two studies may reflect differing developmental strategies in ophiuroids replacing arm-tips vs. entire arms. Alternatively, the present study may have artificially altered regeneration patterns by controlling the natural interplay of various environmental parameters (e.g., temperature, salinity, food accessibility) that may selectively bolster skeletal development during periods of increased hydrodynamic activity.

The skeleton: tissue ratios determined in this study fall within the range of values (0.3–5.75) derived from data of Clements et al. (1994) and of two other studies that have reported total, skeletal, and soft-tissue regeneration for infaunal brittlestars. Stancyk et al. (1994) observed *Amphipholis gracillima* regenerating in a highly organic mud flat. Fielman et al. (1991) investigated regeneration of the same species in the laboratory-controlled absence of particulate and dissolved food sources. The skeleton: tissue weight ratios obtained in this study for *H. elongata* (Table 1) are most similar to those published by Stancyk et al. (1994) for the regenerated arm-tips of *A. gracillima* (2.47 ± 0.11 and 2.51 ± 0.05, mean ± SE).

Differences in arm-tip length

The impetus for this study was an analysis of variation in ossicle morphology in response to variations in hydrodynamic regime. To address this issue, we used SEM and image analysis to characterize ossicle structure; because SEM produces a 2-dimensional image from a 3-dimensional structure, aspects of a sample with much morphological relief, e.g., ossicle length, cannot be measured with great accuracy. Therefore, only the flattest surface of each ossicle, the proximal face, was measured. This aspect of the ossicle has also been examined in several earlier studies (Gage 1990; Dahm 1993; Stewart 1995; Wilding & Gage 1995; LeClair 1996). However, none of the ANOVAs for any of the 15 ossicle measurements detected significant differences between treatments.

Nonetheless, brittlestars in the Turbulent treatment regenerated arm-tips that were significantly shorter than those of their Control counterparts. Because the regenerated arm-tips of Control and Turbulent individuals did not differ significantly in the number of regenerated ossicles (~40 each), the recorded difference in overall regenerated arm-tip length was probably due to differences in the lengths of individual regenerated ossicles, which we did not measure. Given that the proportional overall arm-tip length difference was relatively large, great accuracy may not have been needed to detect differences in individual ossicle lengths and a future study designed to examine this issue would be relatively straightforward to accomplish.

Energy limitations might explain the shorter regenerated arm-tip lengths found in brittlestars in the Turbulent treatment. If these animals spent more energy to maintain a feeding or respiratory posture than did the Controls, they would have had less energy left for regeneration. Unlike other burrowing ophiuroids that ventilate their burrows using undulations of the buried arms (Woodley 1975), individuals of *H. elongata* hold their arms up in the water column and rhythmically contract the tube feet of these exposed arms to force

oxygenated red blood cells through the water vascular system to the buried parts of the body (Christensen & Colacino 2000). They also have a higher rate of respiration than do amphiurids from the same habitat (Christensen & Colacino 2000), indicating that it may be more costly to hold arms erect in the water column.

Clements et al. (1994) proposed that food availability might influence the rate of calcification in *O. filograneus*. Several studies with echinoids (Lewis & Storey 1984; Lewis et al. 1990; Edwards & Ebert 1991; Levitan 1991) provide strong support for this hypothesis. However, although turbulent flow would greatly increase transport of particulate matter such as food (Vogel 1994), the present study eliminated food availability as a variable by providing uniform food amounts and allowing all animals an equal opportunity to feed under no-flow conditions. Our results showed no differences in regenerated arm-tip composition between treatments, supporting the idea that differences in food availability or quality may play a large role in determining composition of regenerated structures.

Other biomechanical considerations relevant to turbulent fluid flow may have contributed to the differences found in arm-tip length. As previously proposed, turbulent flow would increase the physical forces on the arms of brittlestars (Clements et al. 1994). Also, because turbulent flow may obscure any hydromechanical signals of nearby predators (Fields & Yen 1997), brittlestars could be at greater risk of predation and may not feed optimally under turbulent conditions. In our study, this inhibitory effect of turbulence may have persisted through the no-flow feeding sessions, resulting in an unanticipated difference in feeding between treatments. The characteristics of fluid flow near the surface of the arm, and especially through and around arrays of spines, bumps, and dips would differ under turbulent flow as well (Cheer & Koehl 1988). This factor—coupled with the fact turbulent flow would increase transport not only of food but also of dissolved substances such as O_2, dissolved organic matter (DOM), and wastes (Vogel 1994)—suggests that fluid flow in the Turbulent treatment may have imposed a unique set of biomechanical conditions quite different from those in the Control. Turbulent brittlestars may have altered their arm-tip regeneration processes in response to these factors.

Variation among individual brittlestars

A significant difference was found in the MANOVA among individuals (statistically nested within Treatment and Tank, Table 2), indicating that—based on the morphological measurements of the vertebral ossicles—the individuals used in this study were not equal. This result is surprising because it seems to run counter to those of an ANOVA test on baseline oral-frame diameters, which determined that the brittlestars were not significantly different in overall size ($p=.075$). However, even though the oral-frame diameters were not correlated with the 15 morphological measurements (all r-values were >0.26), significant differences in overall individual size (not reflected in oral-frame diameter) may have been responsible for the significant difference detected by the MANOVA.

In addition, further examination of the parameter-specific ANOVAs for individual brittlestars indicates that although the MANOVA indicated a significant difference between treatments, it was not detectable among the individual parameters ($p>.05$ except for numbers 6 and 7, which are measurements based on a morphological feature, C in Fig. 3, that may lie outside the calcified ossicle; see Table 2). The nonsignificance of the parameter-specific ANOVAs is not surprising given the large standard deviations around the mean morphological measurements (Table 4). These extreme standard deviations reflect the fact that there was considerable serial variation in ossicle dimensions within and between the arms of an individual brittlestar, as well as among all brittlestars of a particular treatment group. In consideration of these data, the significant result of the MANOVA is probably meaningless and may simply reflect the fact that the absolute value of each morphological measurement mean is slightly higher in the Control than in the Turbulent treatment.

Variation within individual brittlestars

The results of this study indicate that an individual's regenerated ossicles may be significantly different among arms ($p\leq.0001$ for all 15 measurements). This result suggests that these animals may preferentially hold some arms in the water column, while keeping others in the burrow. Although A.B. Christensen (pers. comm.) has observed *H. elongata* alternating arms, no data have been recorded for periodicity. The ossicles in the buried arms, if not exposed to the treatment condition, may have developed differently. Differences among arms of a single individual are intriguing because previous studies have held that the 5 arms of adult ophiuroids are more or less the same (Reese 1966; Dobson 1988). However, Turner (1974) found that 2 nonadjacent arms of juveniles of *O. filograneus* developed more rapidly than the other 3 arms and proposed that this developmental feature permitted earlier burrowing while maintaining the ability to feed at the surface. Fielman et al. (1991) also observed inter-arm differences within individuals that regenerated the

arms in the absence of nutrients. Our results suggest that causes of inter-arm variation, especially in filter-feeding and burrowing ophiuroids, need further study.

Two additional important aspects of ossicle structure that may vary among arms, yet were not measured in this study, are stereom porosity and trabecular thickness. Ophiuroids can change the delicate structure of their ossicles by extracellular resorption of unsuitable stereom and deposition in other areas of the skeleton (DuBois & Chen 1989). Individuals subjected to increased water motion could reinforce their ossicles by increasing the width of the coarse or fine trabeculae. Altering ossicle development in this manner could produce an ossicle with a denser, stronger, and less porous stereom that could be expected to weigh more or have a higher skeleton:tissue weight ratio. Although this does not appear to have been the case, measurement of stereom porosity and trabecular thickness might have elucidated subtle differences in ossicle structure.

Conclusion

The results of this study indicate that *H. elongata* displays moderate phenotypic plasticity in response to increased water motion, although differences between animals subjected to turbulent and control conditions were smaller than expected, based on the results of the study by Clements et al. (1994) on *O. filograneus*. Nonetheless, infaunal brittlestars may be useful organisms with which to monitor environmental degradation or improvement. Measurement of the rate and pattern of arm regeneration under different environmental stresses or toxic conditions may provide a means to assess sublethal effects of habitat modification. Further study may account for the various environmental and physiological factors that shape the morphology in regenerating ophiuroids.

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