JOURNAL OF CRUSTACEAN BIOLOGY, 23(4): 890–896, 2003

SUBSTRATE DETERMINANTS AND DEVELOPMENTAL RATE OF CLAW ASYMMETRY IN AMERICAN LOBSTERS, *HOMARUS AMERICANUS*

Jason S. Goldstein and Michael F. Tlusty

(JG) Old Dominion University, Department of Biological Sciences, Hampton Boulevard, Norfolk, Virginia 23529 U.S.A. (jgoldste@odu.edu);
(MT, correspondence), New England Aquarium, Edgerton Research Laboratory, Lobster Rearing and Research Facility, 1 Central Wharf, Boston, Massachusetts 02110-3399 U.S.A. (mtlusty@neaq.org)

A B S T R A C T

A new index was used to describe and quantify claw asymmetry for American lobsters, *Homarus americanus*. Length : width (L:W) ratios were calculated for each claw, and a measure of claw asymmetry (C_a) was computed as the percent reduction in the claw with the smaller L:W ratio. Fourth stage (first benthic stage) lobsters had relatively symmetrical claws, with L:W ratios of 4.3 in the cutter and 4.1 in the crusher claw, and a corresponding C_a value of 5.4%. Hatchery animals that could not differentially exercise one claw maintained a low average C_a value (2.5%). In adult animals, the L:W ratio decreased to a minimum of 2.7 for cutter claws, and 2.0 for crusher claws, giving a C_a value of 25.9%. This method was then used to assess claw development in animals exposed to one of four different natural substrates (cobble, shell, sand, and plant) in a hatchery setting. The development of claw asymmetry is known to be a function of increased exercise in one claw, and substrates allowing for more exercise should exhibit faster, greater asymmetry. It was observed that the overall morphology of claws changes greatly between the sixth and seventh stage. Although no treatment differences were observed at the sixth stage, by the seventh stage, animals subjected to shell substrate yielded significantly more asymmetrical claws. Thus, where lobsters settle can have a dramatic impact on the rate of development of claw asymmetry with potential fitness consequences.

The North American lobster (*Homarus americanus* H. Milne Edwards, 1837) exhibits the largest chelipeds (claws) of any known crustacean. The ''Great Chelae,'' as published by Herrick (1909), exemplifies just one of many early studies examining the structure and function of American lobster claws (also see Smith, 1873, and Templeman, 1935). Although they may comprise less than 5% of the total body weight of early-staged juvenile lobsters, chelae may constitute over 50% of the total body weight of large lobsters (Lang *et al*., 1977). In addition to these dramatic differences, *H. americanus* chelipeds exhibit one of the best-known models of developmental asymmetry in the animal kingdom. Initially, lobster claws are composed of predominately fast-acting muscle fibers (Smith, 1873), but subsequent and regular stimulation of one claw can cause differentiation and development of slow acting, powerful muscles via a physiological feedback loop (Emmel, 1908; Lang *et al*., 1978; Lnenicka *et al*., 1988; Govind and Pearce, 1986, 1989). This resultant ''crusher'' claw becomes stouter and more robust in shape, while the remaining ''cutter'' claw develops to form a more slender contour containing faster responding muscles that yield a significantly reduced strength (Govind and Pearce, 1992). The fourth (post-larval) and fifth (early benthic phase juvenile) developmental stages are essential critical periods for determining claw asymmetry (Emmel, 1908; Govind and Pearce, 1989). Once a crusher claw has been determined, the asymmetric pattern becomes fixed for life, regardless of any future claw autonomy or regeneration (Kent *et al*., 1989). Conversely, a lobster that does not have an opportunity to differentially exercise one claw will display two symmetrical cutter claws, an occurrence very common in hatchery-reared animals. Claw asymmetry is therefore not specifically under genetic control; rather, it is influenced during a small window of time during the critical juvenile period by extrinsic factors (Lang *et al*., 1978).

Knowing that this vital critical period for asymmetric claw development exists, the process is often assumed as being discrete and absolute. However, muscle differentiation is not complete within a single molt and may reflect a certain degree of plasticity (Govind, 1995).

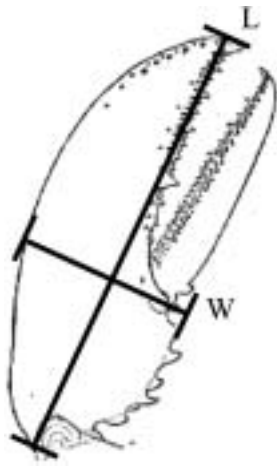

Fig. 1. A cutter claw with the length and width delineated as measured in this study.

For example, histological sectioning of claws shows that by the seventh stage the crusher claw still contains a central band of fast fibers, which is not completely absent until the thirteenth stage (Ogonowski *et al.*, 1980). Because asymmetry is initiated via exercise on one claw, it is likely that once it has begun, subsequent exercise may promote more rapid muscle differentiation leading to greater asymmetry. Ecologically, the determination of claw asymmetry occurs at the ontogenetic shift from a pelagic to a benthic existence. If all substrates are not equally conducive to providing lobsters with an opportunity for sufficient claw exercise, then that habitat in which a lobster settles may profoundly impact morphological and behavioral traits, and ultimately survivorship and fitness. Given these potential habitat interactions, the scope of this study aimed to (1) analyze claw asymmetry as a continual as opposed to discrete process, (2) assess how different nearshore settlement substrate types might influence the development of claw asymmetry using hatchery-reared animals, and (3) determine if there are significant differences in the rate of development between these different substrate types allowing for an assessment of differential changes during the developmental sequence.

Materials and Methods

Animal Source and Rearing

Lobster larvae from two wild-caught egg-bearing females were reared from first to fourth stages in a semi-closed flow-through seawater system (parameters: 17°19°C, 31‰ salinity) at the New England Aquarium's Edgerton Research Laboratory (Boston, Massachusetts, U.S.A.). Larvae were subjected to artificial lighting with a daily cycle of 13 hours of light and 11 hours of dark. Larvae were fed live, nutritionally enriched (SELCO®) brine shrimp (*Artemia* sp.) nauplii three to four times daily up until the lobster larvae's second stage, after which adult frozen brine shrimp were administered twice per day.

Experimental Design and Setup

Upon molting into fourth stage, 60 lobsters were placed into individual 0.47 l plastic containers (dimensions: 14 cm × 9 cm × 9 cm). Animals were randomly assigned among four different substrate treatments ($n = 15$ per treatment group). Substrate treatments chosen were reflective of common nearshore habitat types and consisted of: (1) sand (washed and filtered, 0.5–0.9 mm diameter range); (2) crushed oyster shell (*Crassostrea virginica*, 2.5–6.0 mm chips); (3) cobble (5.0–9.0 mm diameter range); and (4) assorted and mixed clumped aquatic flora (sea lettuce (*Ulva lactuca*), eelgrass (*Zostera marina*), rockweed (*Fucus* sp.), and irish moss (*Chondrus crispus*), average wet weight per clump ≅1.66 g). Enough substrate was used to cover the bottom of each container. Small, smooth 6–8-mm holes were drilled on all sides of each container, including the bottom, to allow for maximum water circulation and removal of uneaten food, wastes, and dissolved organic matter. All 60 containers were distributed randomly into two fiberglass seawater trays (dimensions: 193 cm × 18 cm × 2 cm) within the hatchery's semi-closed flow through seawater system. Containers were rotated once per week in a systematic fashion to minimize position effects within the tray. Physical parameters within the tray were identical to prior larval rearing conditions (i.e., light cycle, temperature range, salinity). A feeding regime consisted of adult frozen brine shrimp once per day. Weekly water samples taken from each tray were analyzed for levels of ammonia, nitrate, pH, and dissolved oxygen. Sea trays were siphoned and cleaned regularly to minimize build-up of solid organic waste products.

Claw Symmetry Quantification

Individual pairs of claws were photographed and measured for each animal at both stage six and seven. A representative digital photograph was taken using a video monitor in tandem with a Machintosh® Quadra 840AV linked with an Olympus SZH10 research stereo dissecting microscope at 10× magnification. Claw symmetry was measured by comparing the length : width ratios of each claw (Fig. 1). The length and width of each claw was then measured directly from the photograph. The length was the longest point from the proximal end of the claw to the distal tip. Width was measured as the span of a line perpendicular to the length line at the point where the dactyl joins the pollex. These values were used to compute a L:W ratio for each claw, with one ratio (R_u) being larger than the other (R_r). Claw symmetry (C_a) was then calculated as the percent reduction of R_r, and was expressed by:

$$C_a = (1 - (R_r/R_u)) \cdot 100$$

Perfectly symmetrical claws would exhibit little to no reduction ($C_a = 0\%$) of one claw ($R_r = R_u$). Functionally, both claws would contain similar muscle morphology and respond as cutter claws (Govind, 1995). Asymmetrical

animals would demonstrate a reduction of one claw (R_r) with this claw being the crusher claw. The C_a values for asymmetrical animals have not been measured prior to this study. For purposes of comparison, the claws of known symmetrical and asymmetrical animals were also assessed as reference animals. Fourth-stage animals ($n = 12$) were measured to assess initial claw dimensions. A random sample of one-year-old animals ($n = 15$) from our hatchery were visually identified as being symmetrical (hatchery animals in featureless habitat often possess symmetrical claws), and then measured. We also measured small, legal-sized males (SLSM, $n = 15$, carapace length (CL) range 115–125 mm); large, legal-sized males (LLSM, $n = 10$, CL range 147–183 mm); and legal-sized females (LSF, $n = 14$, CL range 111–137 mm) from Nova Scotia, Canada, that were being held at a local commercial wholesaler. The claw measurements of these five groups of reference animals were used to compare the rate and magnitude of claw asymmetry to those of each experimental treatment.

Statistical Analyses

This experiment was initially analyzed as a two-way repeated measures ANOVA with the two treatment factors being substrate type and animal stage. The claw asymmetry data failed normality tests primarily because of a bimodal distribution given that C_a values were much larger during the seventh stage compared to the sixth. Transforming the data failed to correct for this normality difficulty, and therefore the data were ranked, and the two-way repeated measures ANOVA was conducted on ranked data. However, because of mortalities as well as difficulties in interpreting some photographs, including blurred images and nondistinct morphological features, our data set did not include a complete set of photographs for each animal for the two subsequent months. Therefore, separate one-way ANOVAs were conducted on the sixth and seventh stage data to assess substrate main effects. The C_a values are ratios, and while ratios are supposed to be square root transformed (Zar, 1984), in all cases, such a transformation did not alter the interpretation of the results, increase the normality of the data, or increase the power of the test. Thus, these main effect analyses and all subsequent reported values were taken from untransformed data. Values reported in text are means $\pm$ 95% confidence intervals.

Results

Fourth-stage lobsters were already beginning to exhibit claw asymmetry, as their C_a values were 5.4 $\pm$ 1.3. Claws of these animals were long and narrow, with L:W ratios of $R_u = 4.4 \pm 0.3$ and $R_r = 4.1 \pm 0.3$. As postulated, animals that were raised in featureless containers should not develop claw asymmetry. This was the case even after a year's time ($C_a = 2.5 \pm 1.0$), although claws were becoming relatively wider ($R_u = 3.4 \pm 0.1$, $R_r = 3.3 \pm 0.1$). In comparison, wild adults demonstrated a significant L:W reduction in the crusher claw. The C_a values were approximately 25%, and did not further decrease with increasing male size (SLSM, $C_a = 25.8 \pm 1.6$; LLSM, $C_a = 25.7 \pm 1.8$, t-test, $P > 0.80$). However, males were more asymmetrical than females (LSF $C_a = 21.3 \pm 3.4$, t test, $P < 0.025$). The L:W ratios were smallest in males, with $R_u = 2.7 \pm 0.1$, and $R_r = 2.0 \pm 0.1$.

Claw asymmetry was statistically significantly different between sixth and seventh stage animals (two-way repeated measures ANOVA, $F_{1,38} = 24.25$, $P < 0.001$, $1-\beta = 0.999$, $\alpha = 0.05$), and treatments (two-way repeated measures ANOVA, $F_{3,46} = 2.77$, $P < 0.05$, $1-\beta = 0.428$, $\alpha = 0.05$). Animals photographed twice exhibited, on average, an 8.3 $\pm$ 3.1 increase in C_a. Animals reared on cobble had the lowest C_a values, whereas animals reared on shell had the greatest C_a values (Tukey's HSD Test, $q = 3.90$, $P < 0.04$) (Fig. 2). Animals on sand or with plants were intermediate to, and statistically similar to, these two extremes (for all other pair-wise comparisons, Tukey's HSD Test, $q < 2.99$, $P > 0.15$). The month $\times$ treatment interaction term was not statistically significantly different (two-way repeated measures ANOVA, $F_{3,38} = 1.05$, $P > 0.3$, $1-\beta = 0.057$, $\alpha = 0.05$), suggesting that all treatments exhibited the same rate of change. However, because claw asymmetry increased in all treatments, any treatment response difference between months may have been masked. Thus, main effects were analyzed to determine if differences existed between substrate treatments for sixth and seventh stage animals.

The measurements of claw asymmetry for sixth stage experimental animals did not exhibit statistically significant differences between treatments ($F_{3,42} = 0.494$, $P < 0.69$, Fig. 2). The C_a in these animals (10.37 $\pm$ 1.34, $n = 46$) was intermediate to the values observed for hatchery and wild animals. The R_u was not different from the fourth stage value of 4.4, while the crusher claw was already beginning to get wider (Table 1). In seventh stage animals, statistically significant differences in C_a were observed between treatments ($F_{3,42} = 3.476$, $P < 0.025$, $1-\beta = 0.569$, $\alpha = 0.05$, Fig. 2). As with the results from the repeated measures ANOVA, animals reared in a shell substrate demonstrated the greatest reduction of R_r ($C_a = 22.73 \pm 3.56$), whereas those reared on cobble exhibited the least amount of reduction in R_r ($C_a = 13.57 \pm 411$, Tukey's HSD Test, $q = 4.514$, $P < 0.02$). The C_a for animals reared with plants or on sand exhibited values intermediate to and not statistically different from these two extremes (for all other pair-wise comparisons, Tukey's HSD Test, $q < 2.67$, $P > 0.24$, see Fig. 2). Changes in C_a were a reflection

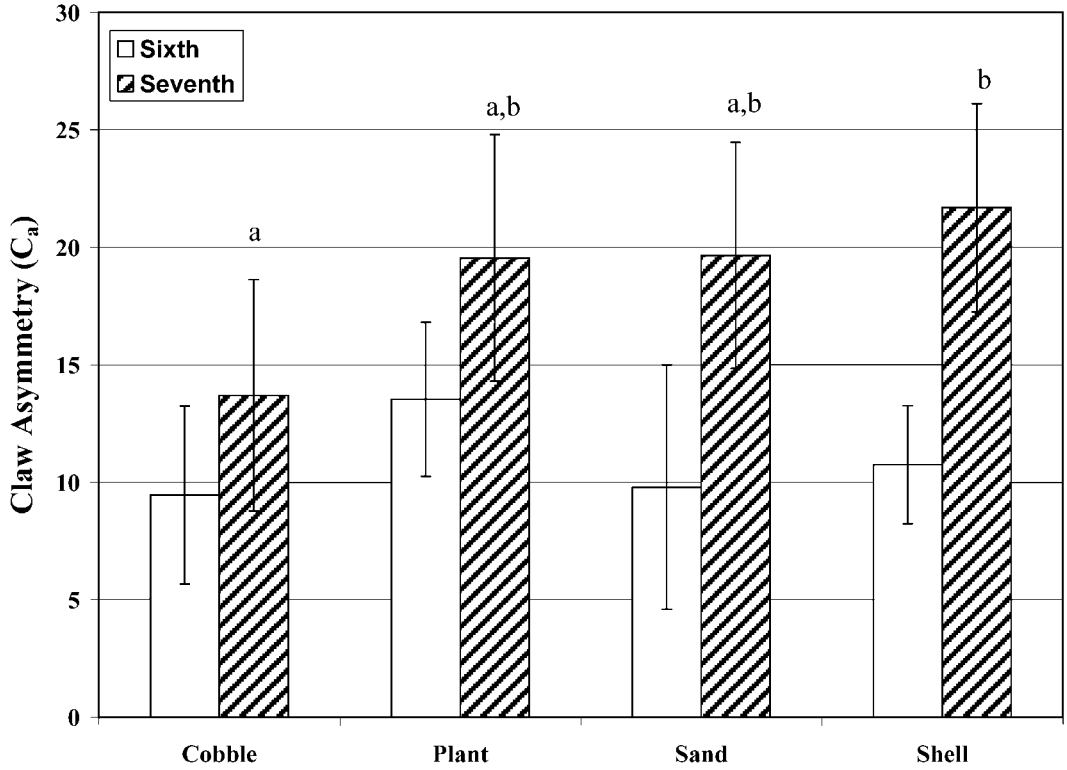

Fig. 2. Values for C_a for sixth and seventh stage animals reared in a hatchery on each of four different substrates. Results are from independent analyses of sixth and seventh stage data. Significance for the seventh stage animals are listed above the bars, and similar letters denote statistically equivalent treatments.

of claws increasing in width as R_r was more reduced than R_u (Table 1).

Discussion

A new index of claw morphology, the percentage of reduction in the L:W ratio of the crusher claw relative to the cutter claw, was used as an index of claw asymmetry. The only other index used to assess claw asymmetry prior to this study was crusher volume (Aiken and Waddy, 1980). Measuring depth was not feasible in this study because of the intricacies and complications of using microscopic photoimaging on small, predominantly two-dimensional claws. Length and width were simple to photograph because they exist in the same plane, and it was difficult to accurately hone the focal plane on the point of maximum claw depth. In addition, the torque put on twisting a claw to take a depth measurement would occasionally break off the claw of the animal and render the claw useless for further measurements of growth. One additional benefit of a L:W ratio is it is a dimensionless number, and thus no effort would need to be expended on calibrating images. These traits make L:W ratios a more conservative and precise measure of claw asymmetry and better for the study of small animals because of its noninvasive technique.

Utilizing this new index, we observed that development of claw asymmetry was not a discrete process, because claw morphology continuously changed as individual animals matured. Typical L:W ratios of claws began at approximately 4.5 and decreased to a value of 2.7 in the cutter and 2.0 in the crusher as the animal approached 0.5 kg. The C_a values for did not decrease as animals grew past a legal size, indicating that claw asymmetry asymptoted ($C_{\bar{a}}$) at a value of approximately 25%. Although this study did not assess the age at which $C_{\bar{a}}$ arises, we hypothesize that it occurred before animals were one year old. In this study, $C_{\bar{a}}$ fell within the 95% confidence interval for the mean C_a value of seventh-stage animals in the shell treatment. Ogonowski *et al.* (1980) observed that the reduction in the mass of slow acting muscle

Table 1. L:W ratios for cutter (R_u) and crusher (R_r) claws for the reference and experimental animals. Values are averages, confidence intervals, and the sample size

Experimental substrates	Sixth stage		Seventh stage	
	R_u	R_r	R_u	R_r
Cobble	4.34	3.94	4.29	3.69
	0.15	0.18	0.26	0.22
	$n = 11$		$n = 12$	
Plant	4.53	4.00	4.76	3.82
	0.15	0.20	0.24	0.24
	$n = 13$		$n = 9$	
Sand	4.59	4.15	4.31	3.51
	0.19	0.23	0.23	0.15
	$n = 10$		$n = 12$	
Shell	4.67	4.17	4.41	3.40
	0.13	0.14	0.15	0.17
	$n = 12$		$n = 13$	

fibers was complete by the thirteenth stage. Although claw differentiation becomes fixed during the fifth stage, claw morphology continues to change beyond this, but quickly plateaus at a relatively young age (see hypothetical curves in (Fig. 3).

Differences in the rate of change in claw asymmetry were not observed within animals (no significant interaction term in the repeated measures ANOVA). However, a substrate treatment difference was observed in seventh stage animals (the second month's photographs) but not in sixth stage animals. The difference is that all animals were not photographed in both months. Thus, the sample size for the analysis of rate change was smaller than that for either month. While direct evidence for treatment effects on the rate of claw asymmetry development are lacking, the significant treatment differences in the second month were suggestive that the rate of claw asymmetry development is variable. The shell treatment, which resulted in the greatest asymmetry in seventh stage animals, was most likely correlated to the amount of exercise animals were provided via their crusher claw. Many animals used the shell for cover, and thus were likely manipulating shell fragments as they were seeking cover (Goldstein and Noetzli, personal observation). Other treatments did not afford this opportunity. Cobble substrate, characterized predominantly by round, smooth surfaces, for example was found to preclude lobsters from moving and manipulating it as effectively.

The importance of claw asymmetry in lobsters cannot be underestimated. Herrick (1909) measured 2433 individuals and observed only three symmetrical animals, demonstrating that symmetrical lobsters are rare in nature. Postlarval lobsters settle out of the water column at roughly the same time this claw differentiation occurs. Thus, benthic habitat structure and composition influences the rate of claw development. This scenario may further act to impact survivorship if there is a benefit to being more asymmetric at an earlier age. There has been little discussion in the literature of the adaptive significance of claw asymmetry. In general, asymmetrical claws may be beneficial in fighting contests, gathering food, or attracting mates. If greater claw asymmetry leads to a higher probability of winning intraspecific agonistic encounters, then claw asymmetry would benefit young animals because they are shelter-restricted (Lavalli, 1991), and individuals gain access to shelter through aggressive encounters. Although Vye *et al.* (1997) found no relationship between the presence or absence of claw symmetry and the probability of winning an aggressive interaction, this has not been tested on recently settled fourth to seventh stage animals. Adaptive significance may also function in gaining access to a wider variety of foods. An individual with greater claw asymmetry should be able to open up a wider range of mussels and other hard-bodied prey than a similarly sized individual with more symmetrical claws. This needs further research, as shelter-restricted lobsters do filter feed (Lavalli, 1991) and thus may not be as dependent on the strength of the crusher claw as older animals. Finally, claw size is important in lobster reproduction (Stein, 1976). The claws of adult males are larger than those of females, likely a result of sexual competition (Stein, 1976). However, the time between the development of claw asymmetry and the onset of maturity is too great to suggest any reproductive

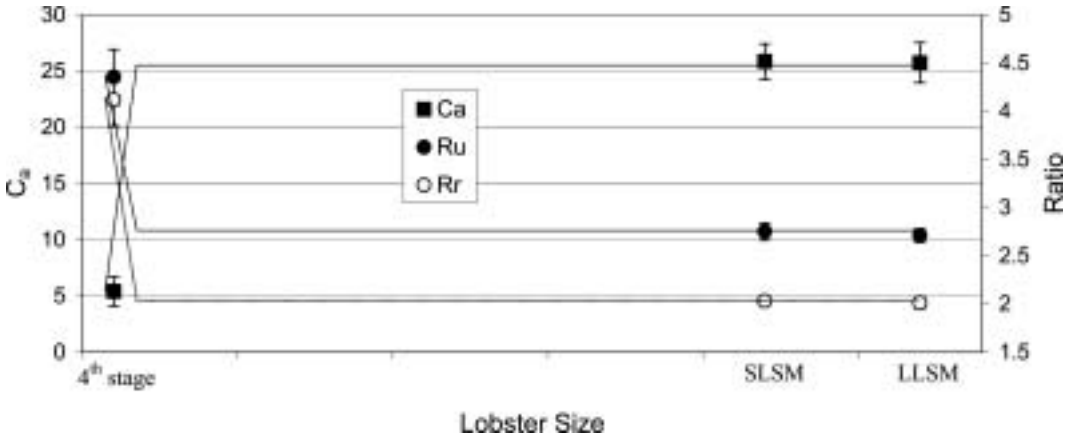

Fig. 3. Values for C_a (left y-axis), and R_u and R_r (right y-axis) for fourth stage hatchery-reared animals and small and large legal-sized males (SLSM and LLSM). The functions are hypothetical and further discussed in the text.

benefit of precocious asymmetrical claw development.

In summary, the creation of a L:W index of claw asymmetry allows for a greater insight into habitat effects and recruitment processes on the development of morphological traits in American lobsters. This will be an important step for future lobster prospects, both scientific and commercial. A claw asymmetry index will allow for an objective analysis of claw shape, and subsequent research on rates of asymmetric development and the adaptive significance of claw asymmetry. It will be important to assess whether increased rates of development of asymmetry in postlarval animals carries over to adults, or if claw asymmetry compensates later in life. Such work will have future implications for understanding mating, feeding, and agonistic behavioral studies as well as for understanding stock enhancement programs. Many enhancement programs used symmetrical claws as a marker for hatchery-reared animals. However, if symmetrical animals have a lower survivability than asymmetrical animals, then stock enhancement programs using this technique may be predisposed to failure.

Acknowledgements

The authors thank James Hook Lobster Co., Boston, Massachusetts, for the use and access of their animals, R. Cooper for assistance in digitizing pictures, and C. Noetzli, who provided assistance in collecting data.

Literature Cited

Aiken, D. E., and S. L. Waddy. 1980. Reproductive Biology. Pp. 217–219 *in* J. S. Cobb and B. F. Phillips, eds. The Biology and Management of Lobsters. Academic Press, New York.

Emmel, V. A. 1908. The experimental control of asymmetry at different stages in the development of the lobster.—Journal of Experimental Zoology 5: 471–484.

Govind, C. K. 1995. Muscles and their innervation. Pg. 291–310 *in* J. R. Factor, ed. Biology of the Lobster, *Homarus americanus*. Academic Press, New York.

———, and J. Pearce. 1986. Differential reflex activity determines claw and closer muscle asymmetry in developing lobsters.—Science 233: 354–356.

———, and ———. 1989. Critical period for determining claw asymmetry in developing lobsters.—Journal of Experimental Zoology 249: 31–35.

———, and ———. 1992. Mechanoreceptors and minimal reflex activity determining claw laterality in developing lobsters.—Journal of Experimental Biology 171: 149–162.

Herrick, F. H. 1909. Natural history of the American lobster.—Bulletin of the U.S. Bureau of Fisheries 29: 149–408.

Kent, K. S., J. Pearce, C. Gee, and C. K. Govind. 1989. Regenerative fidelity in the paired claw closer muscles of lobsters.—Canadian Journal of Zoology 67: 1573–1577.

Lang, F., C. K. Govind, and W. J. Costello. 1978. Experimental transformation of muscle fiber properties in lobster.—Science 201: 1037–1039.

———, ———, ———, and S. I. Greene. 1977. Developmental neuoethology: changes in escape and defensive behavior during growth of the lobster.—Science 197: 682–685.

Lavalli, K. L. 1991. Survival and growth of early-juvenile American lobsters, *Homarus americanus*, through their first season while fed diets of mesoplankton, microplankton, and frozen brine shrimp.—Fishery Bulletin (U.S.) 89: 61–68.

Lnenicka, G. A., J. A. Blundon, and C. K. Govind. 1988. Early experience influences the development of bilateral asymmetry in a lobster motoneuron.—Developmental Biology 129: 84–90.

Ogonowski, M. M., F. Lang, and C. K. Govind. 1980. Histochemistry of lobster claw closer muscles during

development.—Journal of Experimental Zoology 213: 359–367.

Smith, S. I. 1873. The early stages of the American lobster (*Homarus americanus*).—Transactions of the Connecticut Academy of Arts and Sciences 2: (2)351–381.

Stein, R. A. 1976. Sexual dimorphism in crayfish chelae: functional significance linked to reproductive activities.—Canadian Journal of Zoology 54: 220–227.

Templeman, W. 1935. Local differences in the body proportions of the lobster, *Homarus americanus*.—Journal of the Biological Board of Canada 1: 213–226.

Vye, C., J. S. Cobb, T. Bradley, J. Gabbay, A. Genzi, and I. Karplus. 1997. Predicting the winning or losing of symmetrical contests in the American lobster *Homarus americanus* (Milne-Edwards).—Journal of Experimental Marine Biology and Ecology 217: 19–29.

Zar, J. H. 1984. Biostatistical Analysis. Prentice-Hall Inc., Englewood Cliffs, New Jersey.

RECEIVED: 10 June 2002.
ACCEPTED: 18 June 2003.