

PATTERNS OF LIMB LOSS IN THE BLUE CRAB, *CALLINECTES SAPIDUS* RATHBUN, AND THE EFFECTS OF AUTOTOMY ON GROWTH

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ABSTRACT

A substantial proportion of the blue crab (*Callinectes sapidus* Rathbun) population in a subestuary of the Chesapeake Bay was either missing or regenerating at least one limb in 1986 (24.8%) and 1987 (18.8%). Most crabs were missing a single cheliped; loss of four or more limbs was rare. Between June and November 1987, laboratory experiments were conducted in which one cheliped or both chelipeds and two pereopods were removed from blue crabs to examine the long-term effect of autotomy on growth and molting frequency. Crabs with all appendages intact served as controls. Loss of a single cheliped did not alter the molt increment, percent wet weight increase, or molting frequency of crabs compared to animals experiencing no limb loss. Multiple limb loss, however, significantly reduced the molt increment and percent weight increase in the first post-autotomy molt but did not affect the duration of the intermolt. By the second molt following autotomy, molt increments for crabs missing four limbs did not differ significantly from those of intact or singly autotomized animals. Regenerating limbs were 85-88% of the lengths of undamaged, contralateral limbs in the first molt following autotomy. Up to three molts were required to regenerate 100% of the contralateral limb length. Removal of the major, crusher cheliped in both single and multiple autotomy treatments resulted in crabs possessing two minor, cutter claws after regeneration. Crabs failed to regenerate a distinct crusher even after three molts following autotomy. Although multiple autotomy reduced the molt increment in laboratory experiments, the rarity of severe limb loss in the Rhode River indicates that growth in the population is not affected greatly by autotomy.

Autotomy refers to the reflex severance of a limb or other body part at a preformed breakage plane (Wood and Wood, 1932; Bliss, 1960; McVean, 1982). Many taxa (e.g., lizards and ophiuroids) can autotomize varying fractions of body parts (Sheppard and d'A. Bellairs, 1972; Wilkie, 1978; Bowmer and Keegan, 1983) and are capable of continuous regrowth of the missing structure (Congdon et al., 1974). Crustacean autotomy responses differ in that autotomy involves loss of the entire limb. Furthermore, because growth in crustaceans is a discontinuous process, appendages can be replaced only by molting. The cost of autotomy for crustaceans, therefore, could be high since these animals must survive one or more molt cycles before an appendage can be regenerated completely.

Crustacean growth is dependent upon the duration of the intermolt (molt interval) and the size increase at each molt (molt increment) (Hartnoll, 1982). The diversion of metabolic resources to regenerate autotomized appendages obviously could affect growth, and limb autotomy has been shown to reduce size increase at the molt in *Cancer pagurus* (Bennett, 1973), *Hemigrapsus oregonensis* and *Pachygrapsus crassipes* (Kuris and Mager, 1975), *Uca pugilator* (Hopkins, 1982), and *Callinectes sapidus* (Ary et al., 1987). Effects of limb loss appear to be additive, such that the molt increment decreases proportionally as increasing numbers of limbs are lost (Bennett, 1973; Kuris and Mager, 1975).

The effect of limb autotomy on the molt interval is complex and depends on the stage in which the limbs were lost and the number of limbs removed. Multiple limb loss before a critical preparatory stage (probably early proecdysis, D₀; Skinner and Graham, 1972) either hastened the next ecdysis (Skinner and Graham, 1970;

1972; Fingerman and Fingerman, 1974; Hopkins, 1982) or had no effect on the molt interval (Ary et al., 1987), while limb autotomy between D_0 and apolysis, D_1 , lengthened the cycle in *Gecarcinus lateralis* (Skinner, 1985). Autotomy after stage D_1 seemed to have no effect on the timing of the molt; however, missing limbs were not regenerated (Skinner, 1985). As few as two missing limbs triggered a precocious ecdysis in *Uca pugilator* (Fingerman and Fingerman, 1974); whereas, at least five limbs had to be autotomized to shorten the molt interval in *G. lateralis* (Skinner and Graham, 1972).

Because of the variable effect that autotomy has on molt frequency, the ultimate impact of limb loss on individual growth is uncertain. It is possible that reductions in postmolt size resulting from autotomy might be compensated over several molt cycles by shortened intermolt periods. Few studies, however, have followed the effects of limb loss on the molt increment, molt interval, or final sizes of crabs for more than one molt cycle (but see Kuris and Mager, 1975; McConaugha and Costlow, 1980; Hopkins, 1982). Such information is ecologically important, because differences in relative body size are known to be critical in determining the outcome of encounters between predator and prey (Fox, 1975; Botsford and Wickham, 1978; Polis, 1981; Werner and Gilliam, 1984; Kurihara and Okamoto, 1987; Reaka, 1987; Stein et al., 1988). Smaller body size can reduce an individual's ability to compete for refuge (O'Neill and Cobb, 1979) and mates (Sekkelsten, 1988) and decrease reproductive output, since fecundity is positively correlated with body size in crustaceans (Reaka, 1979; Hines, 1982).

The importance of autotomy for a population, in terms of growth, will depend on the frequency of limb loss among its members, the effect of commonly observed autotomy patterns on molt increment and interval, and the rapidity with which limbs are regenerated and size deficits eliminated. Past experiments have not been designed to reflect autotomy patterns observed in natural populations, in part, because few data exist concerning the natural incidence of limb loss in crustacean populations (Needham, 1953; Edwards, 1972; Bennett, 1973; McVean, 1976; McVean and Findlay, 1979; Durkin et al., 1984; Shirley and Shirley, 1988). The following study was conducted to examine the long-term effects of autotomy on growth of *Callinectes sapidus* Rathbun and relate these findings to patterns of limb loss observed in natural populations in a subestuary of the Chesapeake Bay, Maryland. Specifically, a laboratory experiment was designed to determine the relative effects of single versus multiple limb loss over several molts in terms of: 1) size increase per molt, 2) duration of the molt interval, 3) number of molts needed to regenerate a limb equivalent in length to an undamaged, contralateral limb, 4) comparative regenerative capabilities of different limb types, and 5) survivorship. Finally, patterns of claw dimorphism in regenerating chelipeds of captive blue crabs were observed in an effort to interpret morphological configurations found in the field.

METHODS

Frequency of Autotomy and Regeneration.—Between July and November 1986 and May and November 1987, *C. sapidus* were sampled from the Rhode River, Maryland (38°51'N, 76°32'W) to determine the frequency of autotomy and regeneration in natural populations. The Rhode River is a shallow (maximum depth = 4 m), low salinity (4–15‰) body of water that empties into the central Chesapeake Bay (Hines et al., 1987a; 1987b). Blue crabs were collected twice monthly in upstream and downstream nets of a fish weir spanning a small tributary (Muddy Creek), as well as monthly by replicate otter trawls in the Rhode River (Hines et al., 1987a; 1987b for detailed description of sampling methods). Crabs were measured for: 1) carapace width (i.e., the distance between lateral spine tips), 2) sex, 3) type and side of any missing or regenerating limbs, 4) lengths of limb buds, regenerating limbs, and contralateral, intact limbs, and 5) side of the major, crusher cheliped. Crusher claws were identified

by the large, molariform tooth found proximally on the dactyl, while cutter claws possessed smaller, sharper dactyl teeth. Limb length was considered to be the distance from the autotomy plane in the basi-ischial segment to the dactyl tip of a fully extended limb. Crabs that possessed an unscarred stump wound, indicating possible injury caused during collection, were not included in later analyses.

Growth.—LABORATORY PROCEDURES AND EXPERIMENTAL DESIGN. An experiment designed to test for the effects of limb autotomy on growth was conducted between 15 June and 3 November 1987 in aquaria held in laboratory seawater tables at the Smithsonian Environmental Research Center in Edgewater, Maryland. Twenty-six, 40-liter aquaria were filled with sand to a depth of 10 cm, and then each was divided into three, equally-spaced compartments using plastic partitions (i.e., open-celled ceiling panelling). Seawater, pumped from the Rhode River, was supplied continuously to aquaria which were aerated by air stones. Temperatures and salinities in the aquaria reflected ambient conditions in the Rhode River. Average water temperatures ranged from 28°C in late July to 23°C in late September before declining to 14°C by mid-October. Salinities varied between 9 and 12‰. Photoperiod approximated a 14:10 h light : dark cycle during summer months.

Immature male ($N = 53$) and female ($N = 25$) blue crabs ranging from 35 mm to 73 mm in carapace width (mean carapace width ± 1 SD = 56.8 ± 8.9 mm) were collected from the Rhode River from mid to late June. Only intact crabs with no evidence of regenerating limbs were used. A single crab was assigned randomly to each aquarium compartment. All crabs were measured for: 1) side of the major cheliped, 2) carapace length (i.e., the distance immediately posterior to the epistomial spine to a point just before the first abdominal segment) and carapace width to the nearest 0.1 mm using vernier calipers, 3) wet weight to the nearest 0.01 g after blotting excess water, and 4) molt stage. Molt stages were determined by examining the propodus of the fifth pereopod (i.e., the paddle-shaped swimming leg) for evidence of epidermal retraction (Van Engel, 1958; Johnson, 1980).

All crabs were left with appendages intact for their first molt in captivity. Within 3 days of hardening subsequent to this equilibration molt (i.e., early stage C; Johnson, 1980), crabs were subjected to one of the following two treatments: 1) single loss (-1 limb), in which a single right, crusher cheliped was removed from an animal, or 2) multiple loss (-4 limbs), in which the right and left chelipids, the right first walking leg (second pereopod), and left swimming leg (fifth pereopod) were removed. A third, control group experienced no limb loss. Each aquarium held one representative from each of the three treatment groups ($N = 26$ crabs/treatment group), and treatments were assigned randomly to crabs within an aquarium. The three treatments ran concurrently.

Limbs for both autotomy treatments were removed over an 18-day period in July, during which time, aquarium water temperatures rose only slightly from 26°C to 28°C. Autotomy was induced by crushing the merus of the appropriate limb with pliers. Crabs in the multiple autotomy treatment were forced to autotomize limbs in rapid succession. Lengths and wet weights of autotomized limbs were recorded. All crabs were measured weekly for carapace lengths and widths, wet weights, molt stage, sexual maturity, presence or absence of the crusher claw, and lengths of limb buds, regenerating limbs, and contralateral, intact limbs. Crabs approaching ecdysis were examined every 2 days for evidence of molting. Each crab was fed a single fish every other day. Salinity and water temperature were measured each week. Crabs were monitored for up to four molt cycles (i.e., one pre- and three post-autotomy molts).

Decreases in sample sizes between the first and fourth molts were due to mortality (20 crabs), escape from aquaria (14 crabs), loss of additional limbs (17 crabs), and lack of a fourth molt (13 crabs) prior to termination of the experiment. The majority (72%) of these losses occurred over the final two molts. Sources of mortality included loss of air flow in two aquaria, disease, and attack by other crabs that managed to climb the barrier separating compartments. Mortality did not vary significantly among treatment groups (G -test, $P > 0.9$, $df = 2$).

STATISTICAL ANALYSES. Carapace lengths, widths, limb lengths, and wet weights were obtained by averaging measurements taken during anecdyosis (stage C; Johnson, 1980). Only wet weights and sizes of limb buds increased significantly during the intermolt period. Variances for all factors (e.g., carapace length) were homogeneous within all four molts (F -max test; Sokal and Rohlf, 1981), but not between all molts due to differences in sample size; therefore, one-way statistical models were used to compare treatment means within molts, only. Residuals were distributed normally. One-way analysis of variance (ANOVA) models were used to compare percent growth increments [i.e., (postmolt value - premolt value)/pre molt value $\times 100$] among treatments. Percentages were arcsine transformed prior to conducting ANOVAs to ensure homoscedasticity (Sokal and Rohlf, 1981). For clarity, back-transformed data are presented in all tables and figures. In addition to examining percent size increases at each molt, postmolt body sizes for each treatment group were compared within molts using one-way analysis of covariance (ANCOVA). ANCOVA adjusted the postmolt value for differences in the covariate (i.e., the premolt value), thereby increasing the precision with which treatment differences could be detected (Steel and Torrie, 1980). Tests for homogeneity of slope of the regression lines were conducted for each molt; no significant interactions were found (two-way ANOVA, $P > 0.05$). Linear contrasts were

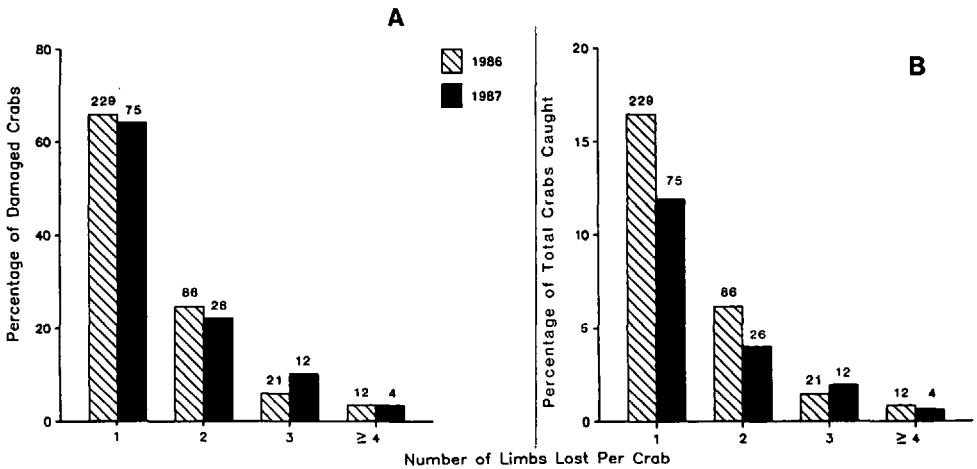


Figure 1. The number of limbs missing or regenerating per crab expressed as: A) a percentage of damaged crabs ($N = 348$ in 1986 and 117 in 1987), and B) a percentage of total crabs caught ($N = 1,400$ in 1986 and 622 in 1987) in the Rhode River for 1986 and 1987. Number of damaged crabs per category is given above each bar. In 1A, between-year differences among damaged crabs for relative numbers of autotomized limbs were not significant ($P > 0.5$).

used to make a priori comparisons of final least-square (i.e., adjusted) mean sizes for the treatments in ANCOVA and unadjusted mean percent growth increments in ANOVA. A 95% level of significance was used in all analyses. All statistical analyses were performed using Statistical Analysis Systems (SAS) software (SAS Institute, 1981).

RESULTS

Frequency of Autotomy and Regeneration.—Data presented here are part of a long-term sampling effort designed to determine how the frequency of autotomy varies both temporally and spatially in the Rhode River; however, only findings that are pertinent to the growth experiments are presented. Of 1,400 crabs collected in the Rhode River in 1986, 24.8% were missing or regenerating at least one limb. In 1987, 18.8% of the 622 crabs collected showed similar evidence of autotomy. The difference in the frequency of autotomy and regeneration between years was significant (G -test, $P < 0.005$, $df = 1$).

The most common form of autotomy among damaged animals (i.e., those animals missing or regenerating limbs) involved loss of only a single limb (ca. 65% in both years; Fig. 1A). Severe multiple autotomy, in which animals lost four or more limbs, occurred in less than 4% of the damaged crabs (Fig. 1A) and less than 1% of the total catch (Fig. 1B). No crab was found to be missing or regenerating more than six limbs. Although comparatively rare, multiple autotomy occurred more often than would have been expected based on a binomial distribution in which the probability of loss of each of 10 limbs was the same (G -test, $P < 0.001$, $df = 2$). The relative frequencies of damaged animals missing or regenerating either one, two, three, four, or more limbs did not differ significantly between years (G -test, $P > 0.5$, $df = 3$; Fig. 1A).

Different limb types were not lost with equal frequency in either 1986 or 1987 (G -test, $P < 0.001$, $df = 4$). Chelipeds comprised approximately 50% of the damaged limbs; whereas, the fifth pereopods showed the lowest incidence of autotomy (Fig. 2). No differences in autotomy frequencies were found between

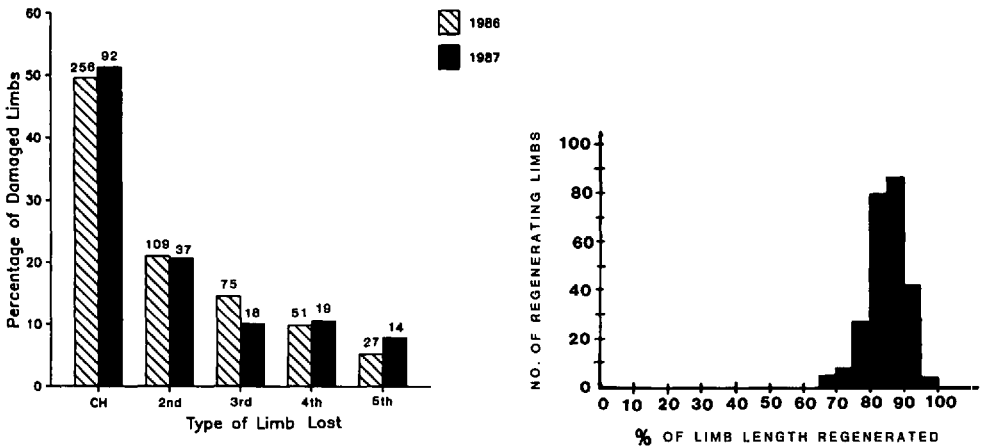


Figure 2 (left). The types of limbs missing or regenerating per crab expressed as a percentage of damaged limbs for 1986 and 1987. Damaged limbs = 518 in 1986 and 180 in 1987. Number of damaged limbs per category is given above each bar. Percentages of crabs autotomizing chelipeds (CH), second (2nd), third (3rd), fourth (4th), and fifth (5th) pereopods calculated from combined losses of both right and left limbs. Between-year differences among damaged limb types were not significant ($P > 0.1$).

Figure 3 (right). The percent limb length regenerated for all regenerating limbs in 1986 and 1987 Rhode River populations combined. Percent limb length regenerated = [(regenerating limb length/undamaged, contralateral limb length) \times 100]. Regenerating limbs included only appendages that had undergone at least one molt since autotomy.

right and left sides for either year (G -test, $P > 0.5$, $df = 4$). The relative frequencies of missing or regenerating limb types did not differ between years (G -test, $P > 0.1$, $df = 4$; Fig. 2).

Numerous combinations of limb loss and various stages of regeneration (e.g., scarred stumps, papillae, limb buds, regenerating limbs) were observed in both years. The four most common patterns, however, involved loss of a single cheliped, loss of a first walking leg, loss of both a cheliped and a first walking leg, and loss of a second walking leg (Table 1). Regenerating limbs typically were 10–20% shorter in length than contralateral, undamaged limbs (Fig. 3).

Approximately 80% of all crabs caught possessed dimorphic chelipeds with a crusher claw on the right side and a cutter claw on the left (Table 2). Double cutters were observed in roughly 10% of the crabs. Left crushers were relatively rare in both years (<5%; Table 2).

Table 1. Summary of the four most common patterns of limb loss in the Rhode River, Maryland in 1986 and 1987 expressed as a percentage of damaged crabs (i.e., those crabs missing or regenerating at least one limb) and as a percentage of total crabs (i.e., damaged and undamaged) caught. Percentages include both right and left limbs

Limb type	Percentages of damaged crabs and total crabs caught			
	1986		1987	
	Damaged (N = 348)	Total (N = 1,400)	Damaged (N = 117)	Total (N = 622)
Cheliped	44.5	11.1	46.2	8.7
2nd pereopod	10.0	2.5	5.1	1.0
Cheliped + 2nd pereopod	6.6	1.6	12.8	2.4
3rd pereopod	5.7	1.4	4.3	0.8

Table 2. Summary of crusher/cutter cheliped patterns in the Rhode River, Maryland expressed as a percentage of total crabs caught in 1986 (N = 1,400) and 1987 (N = 622)

Year	Percentage of morphological patterns of crab chelipeds				
	Right crusher left cutter	Left crusher right cutter	Double cutters	Double crushers	Right absent left cutter
1986	79.1	4.4	10.3	0.07	6.1
1987	82.5	2.9	10.9	0.00	3.7

Growth Experiments.—Variances for carapace width measurements typically were greater than those for carapace length because of broken spines and allometric differences between sexes at maturation (Gray and Newcombe, 1938); therefore, postmolt size and growth increment are discussed in terms of carapace lengths, unless noted otherwise. Many blue crab studies, however, report only carapace widths, so carapace width data are presented in this study also.

Loss of a right cheliped in the single autotomy treatment resulted in the average removal of 8.9% of the total wet body weight, while the multiple autotomy of four limbs resulted in a 19.9% average reduction in total wet weight. A small papilla formed on the stump wound within 4 d after autotomy and a folded limb bud was present by the seventh day. No significant differences in the percent carapace length increment were observed among treatment groups in the pre-autotomy molt (i.e., when all crabs possessed intact appendages; one-way ANOVA, $P > 0.52$; Fig. 4A). Carapace lengths increased 23–25% at this molt (Fig. 4A), while wet weight increased 86–96% compared to previous intermolt weight (Fig. 5A). In the first molt after limb removal (see molt 2, Fig. 4A), growth increments did not differ between intact crabs and those suffering single autotomy (also Fig. 5A, Table 3). Crabs experiencing multiple autotomy, however, demonstrated a smaller carapace length increase at molt (17.6%) than intact crabs (22.8%; linear contrast, $P < 0.001$, $df = 1$; Fig. 4A). By the second molt after autotomy, no significant differences in size increase were observed among treatment groups (one-way ANOVA, $P > 0.42$; Fig. 4A, see also Fig. 5A, Table 3).

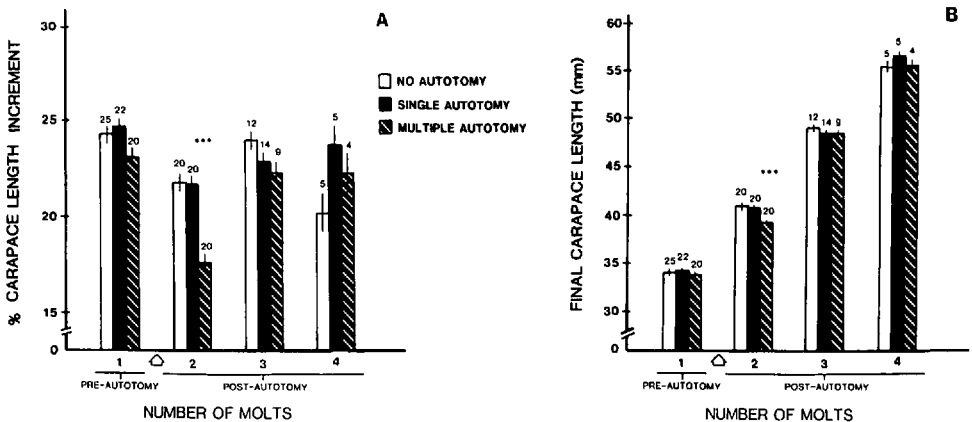


Figure 4. Comparisons of: A) the percent carapace length increment (± 1 SE) and B) the final mean carapace length (± 1 SE) among treatment groups (no, single, and multiple autotomy) for four molt cycles. In 4A, percent carapace length increment = $[(\text{postmolt} - \text{premolts length})/\text{premolts length} \times 100]$. In 4B, final carapace lengths (mm) are least-square means adjusted for premolt length. All crabs possessed intact appendages in pre-autotomy molt 1. The arrow along the horizontal axis denotes limb removal in single and multiple autotomy treatments. Molts 2, 3, and 4 were post-autotomy molts. Sample sizes are indicated above the bars. ***, $P < 0.001$.

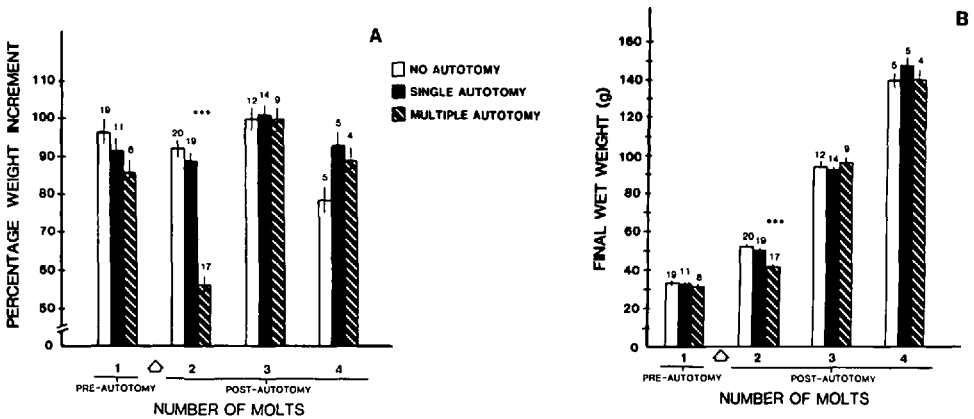


Figure 5. Comparisons of: A) the percent wet weight increment (± 1 SE) and B) the final mean wet weights (± 1 SE) among treatment groups (no, single, and multiple autotomy) for four molt cycles. In 5A, percent weight increment = [(postmolt - premolt weight)/premolts weight \times 100]. In 5B, final weights (g) are least-square means adjusted for premolt weight. All crabs possessed intact appendages in pre-autotomy molt 1. The arrow along the horizontal axis denotes limb removal in single and multiple autotomy treatments. Molts 2, 3, and 4 were post-autotomy molts. Sample sizes are indicated above the bars. ***, $P < 0.001$.

With the exception of the multiple limb loss treatment group in the first post-autotomy molt, mean increases in carapace length for all treatments did not differ significantly between molts one and four (Kruskal-Wallis, $P > 0.07$, $df = 3$; Fig. 4A). In contrast, mean carapace width increments increased significantly between molt one (26.6%) and molt four (33.3%) (Kruskal-Wallis, $P < 0.001$, $df = 3$; Table 3). Mean carapace length and width increments differed significantly for each molt (t -tests, $P < 0.01$, $df = 3$), indicating allometric growth.

Slight decreases in carapace length ($< 2\%$) and wet weight ($< 4\%$) increments (but not carapace width increments) were observed for all treatments between the first and second molts. These decreases in growth may have been due to higher metabolic rates caused by temperature increases from June to July or the result of a delayed adjustment to a new diet. Since all treatments were affected equally, the outcome of the experiment was unchanged.

A comparison of final (i.e., postmolt) mean carapace lengths adjusted for initial (i.e., premolt) size variations showed no differences among treatment groups after the pre-autotomy molt (one-way ANCOVA, $P > 0.44$; see molt 1, Fig. 4B). In

Table 3. Results of one-way ANOVA models comparing mean % carapace width increments among no, single, and multiple autotomy treatments for four molts. All crabs possessed intact appendages in pre-autotomy molt 1. Molts 2, 3, and 4 were post-autotomy treatment molts. Mean values per treatment, sample sizes (in parentheses), mean square errors (MSE), and significance levels of the ANOVAs are presented. Results of linear contrasts comparing treatment means in molt 2 presented at bottom

Molt number	Mean % carapace width increments			MSE	Significance level
	No autotomy	Single autotomy	Multiple autotomy		
1 (pre)	27.2 (25)	26.6 (22)	25.8 (20)	0.67	$P > 0.72$
2 (post)	29.6 (20)	27.6 (20)	20.1 (20)	0.75	$P < 0.0001^*$
3 (post)	32.8 (12)	31.6 (14)	30.6 (9)	0.97	$P > 0.67$
4 (post)	30.6 (5)	36.4 (5)	32.9 (4)	1.2	$P > 0.10$

* Multiple vs. no autotomy, $P < 0.0001$, $df = 1$. Single vs. no autotomy, $P > 0.24$, $df = 1$.

Table 4. Results of one-way ANCOVA models comparing final least-square mean carapace widths among no, single, and multiple autotomy treatment groups for four molts. All crabs possessed intact appendages in pre-autotomy molt 1. Molts 2, 3, and 4 are post-autotomy treatment molts. Mean values per treatment (mm), 1 standard error (in parentheses), and significance levels for treatments in ANCOVA models are presented. Results of linear contrasts comparing treatments in molt 2 presented at bottom

Molt number	Final mean carapace width			Significance level
	No autotomy	Single autotomy	Multiple autotomy	
1 (pre)	71.0 (0.58)	70.7 (0.63)	70.4 (0.67)	$P > 0.82$
2 (post)	89.2 (0.90)	87.8 (0.92)	82.5 (0.90)	$P < 0.0001^*$
3 (post)	112.4 (1.3)	111.0 (1.2)	111.7 (1.6)	$P > 0.74$
4 (post)	132.4 (2.0)	137.6 (1.8)	133.7 (2.2)	$P > 0.19$

* Multiple vs. no autotomy, $P < 0.0001$, $df = 1$. Single vs. no autotomy, $P > 0.28$, $df = 1$.

the first molt following limb removal (molt 2, Fig. 4B), final carapace lengths did not differ between intact crabs and those missing one limb (linear contrast, $P > 0.76$, $df = 1$). Crabs missing four limbs, however, were significantly smaller in size when compared to intact crabs (linear contrast, $P < 0.001$, $df = 1$, see molt 2, Fig. 4B; also Fig. 5B, Table 4). Carapace lengths among the three treatment groups did not differ significantly in the second or third molts following autotomy (molt 3, one-way ANOVA, $P > 0.44$ and molt 4, one-way ANOVA, $P > 0.28$; Fig. 4B). Similar patterns were observed in postmolt measurements for carapace width (Table 4) and wet weight (Fig. 5B). Crabs in all treatments increased significantly in carapace lengths and widths (Kruskal-Wallis, $P < 0.001$; Fig. 4B, Table 4) and wet weights (Kruskal-Wallis, $P < 0.01$; Fig. 5B) over four molts.

The lengths of the intermolt periods adjusted statistically for premolt size differences did not differ significantly among intact animals and those missing one or four limbs within any molt (one-way ANCOVAs, $P > 0.11$). The average

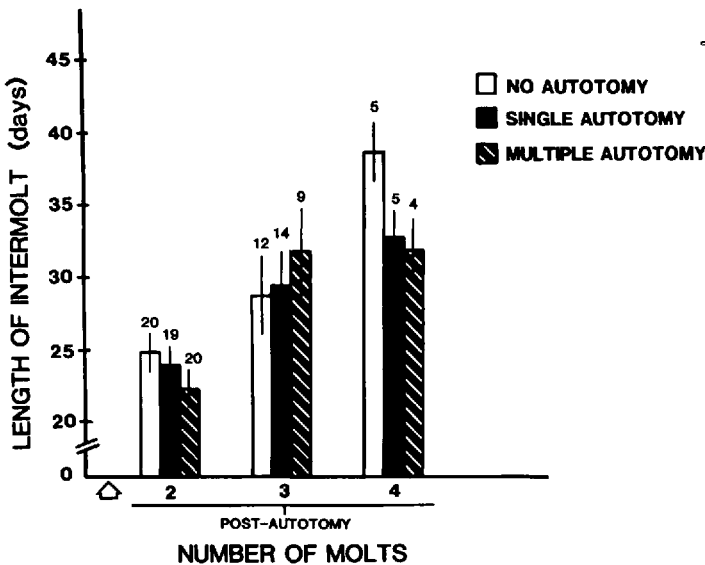


Figure 6. Comparison of intermolt length (d) among no, single, and multiple autotomy treatment groups for post-autotomy molts. The arrow along the horizontal axis denotes limb removal in single and multiple autotomy treatments. Differences in intermolt length among treatment means were not significant within any molt ($P > 0.11$).

Table 5. Percent limb length regenerated (± 1 SD) for the right cheliped in the single autotomy treatment and the right cheliped, second (2nd) and fifth (5th) pereopods in the multiple autotomy treatment groups for post-autotomy molts 2, 3, and 4. Percent limb length regenerated = [(regenerating limb length/full limb length) \times 100]. Sample sizes are given for each limb type and molt number

Molt number	Single autotomy	Multiple autotomy		
	Cheliped	Cheliped	2nd	5th
2	88.3 (3.75) N = 17	84.7 (5.25) N = 10	84.4 (3.58) N = 12	86.5 (6.83) N = 13
3	97.2 (2.47) N = 12	91.9 (6.58) N = 7	92.2 (2.36) N = 7	94.3 (3.82) N = 8
4	98.6 (2.10) N = 5	93.9 (5.41) N = 3	97.4 (4.54) N = 3	98.5 (6.40) N = 4

intermolt length between molts, however, increased significantly from 23.6 days to 34.4 days (Kruskal-Wallis, $P < 0.001$, $df = 2$; Fig. 6). These increases probably were a function of decreasing temperature and increasing size of the crabs (Leffler, 1972). Mature females were excluded from calculations of intermolt duration, since they are thought to undergo a terminal ecdysis upon reaching maturity.

The mean percent limb length regenerated (=regenerating limb length/full limb length \times 100) of right chelipeds did not differ significantly between single and multiple autotomy treatment groups for any molts (t -tests, $P > 0.5$), although regeneration values were slightly lower in animals suffering multiple limb loss (Table 5). Because multiply-autotomized crabs lacked a normally-sized, contra-lateral cheliped, "normal" cheliped lengths were estimated by regressing carapace width against cheliped length for intact crabs (carapace width = $4.02 + 1.04$ cheliped length; $R^2 = 0.94$). Mean regeneration percentages for right cheliped lengths in the first post-autotomy molt were 84.7% and 88.3% in the multiple and single autotomy treatment groups, respectively (Table 5). By the second molt after autotomy, nearly 100% of the cheliped length had been regenerated in animals subjected to single limb loss (Table 5). The percent limb length regenerated for the three limb types (i.e., chelipeds, first walking legs, swimming legs) did not differ significantly for any post-autotomy molts (one-way ANOVAs, $P > 0.47$).

Crabs in both single and multiple autotomy treatment groups lacked a distinct crusher claw even after three post-autotomy molts. Instead, all crabs appeared to possess two cutter claws. Qualitative observations showed slightly larger chelar teeth and formation of an incipient molar on left claws (i.e., the original cutter) in 11 of 13 (84.6%) animals in which only the right crusher had been removed. The remaining two animals possessed chelar teeth of equal size. Molar formation apparently resulted from the fusion of the two most proximal teeth on the cutter dactyl. In animals missing both chelipeds, larger chelar teeth and signs of molar formation were observed on regenerated right claws (i.e., the original crusher) in 6 of 11 animals (54.5%). In the remaining animals, one crab possessed slightly larger teeth on the left cheliped; another showed early signs of molar formation on both chelipeds; and three possessed small, equally-sized teeth.

DISCUSSION

A substantial percentage of the blue crab population in the Rhode River sub-estuary was either missing or regenerating at least one limb in both 1986 (24.8%) and 1987 (18.8%). The degree to which such limb loss might handicap an animal should vary with the type and number of missing or regenerating limbs and with the length of time the animal must function without its services. In blue crabs

(Fig. 2) and a number of other decapod crustaceans (e.g., *Carcinus maenas*, Needham, 1953; McVean and Findlay, 1979; *Cancer magister*, Durkin et al., 1984; Shirley and Shirley, 1988), the anterior-most limbs typically suffer the highest frequency of damage. Crabs often spread their chelipeds and rise up on their walking legs in a threatening posture during agonistic encounters or when confronted by predators (Schone, 1968; Robinson et al., 1970; Jachowski, 1974; Hazlett, 1977; Sinclair, 1977; Hyatt and Salmon, 1978; Vannini, 1980). Such postures would make the anterior limbs particularly prone to injury and might explain the consistency of autotomy patterns observed in the Rhode River over 2 years. The loss of chelipeds or raptorial appendages in crustaceans can be costly; their absence has been shown to reduce competitive ability for refuge in alpheid shrimp (Conover and Miller, 1978), lobsters (O'Neill and Cobb, 1979), and stomatopods (Berzins and Caldwell, 1983), and may adversely affect mate acquisition in *Carcinus maenas* (Sekkelsten, 1988) and *C. sapidus* (Smith, unpubl. data).

Disadvantages arising from limb loss could be lessened if regeneration of the missing appendage(s) is rapid. Following the loss of a single cheliped, *C. sapidus* were notable in their ability to regenerate almost 90% of the normal limb length in the first post-autotomy molt and nearly 100% of the length by the second molt (Table 5). Small- to medium-sized blue crabs molted every three to four weeks in laboratory experiments (Fig. 6), so even severely handicapped blue crabs should be able to replace limbs completely in a single season (ca. May to November in the Rhode River). In contrast, other crab species may replace missing limbs more slowly. For example, Skinner and Graham (1972) recorded that regenerating limbs were only two-thirds the size of normal limbs in land crabs, *Gecarcinus lateralis*, forced to autotomize one to six pereopods. Small increases in regenerating limb size coupled with long intermolt periods (e.g., up to one year in *G. lateralis*, Skinner, 1985 and *Cancer magister*, Warner, 1987) means that some individuals must endure long periods with missing or smaller limbs. Edwards (1972) estimated that complete regeneration of appendage length in king crabs, *Paralithodes camtschatica* could take 4–7 years. Even in blue crabs, limb loss occurring at the end of the growing season, in large crabs, or in mature females could be costly since molting frequency may be either slowed by falling temperatures or increasing age or halted because of a terminal molt.

The benefits of limb regeneration may be countered to some extent if body size is reduced in subsequent post-autotomy molts. The removal of two chelipeds and two pereopods in this experiment resulted in a 32% decrease in carapace width increment but only a 7.5% decrease in actual carapace width compared to intact controls (Tables 3, 4). Small disparities in size, however, can affect chances of surviving encounters with intra- and interspecific predators (Reaka, 1987; Smith, unpubl. data) or competing successfully with conspecifics (Caldwell and Dingle, 1979). In the Rhode River, crabs less than 91 mm in carapace width were significantly more vulnerable to injury or death from predators than were larger conspecifics (Smith, unpubl. data). Quick recovery of body size following multiple autotomy, therefore, could be critical to survival. In laboratory experiments, size differences resulting from multiple limb loss were confined to the first post-autotomy molt. By the second molt following autotomy, postmolt carapace measurements and wet weights were statistically indistinguishable from those of undamaged animals.

The relatively rapid recovery of size deficits in damaged *C. sapidus* did not involve shortening subsequent intermolt periods. Results of this study and those of Ary et al. (1987) showed that limb removal in early stages of anecdyosis (C) in *C. sapidus* did not significantly affect the duration of the intermolt compared to

intact controls. Ary et al. (1987) postulated that crabs lacked sufficient physiological preparation to begin a precocious ecdysis when limbs were lost early in the intermolt. It is possible that an insufficient number of limbs were removed in this study or Ary et al.'s (1987) experiments to reach a threshold necessary to alter the intermolt length. Skinner and Graham (1972) indicated that removal of two chelipeds and four pereopods was sufficient to stimulate a precocious molt in *C. sapidus*. Since times to ecdysis for their control animals were not reported, the degree to which limb removal shortened the molt cycles in their treatment group is unclear. It is possible that a threshold number greater than four limbs is needed to stimulate a precocious molt in blue crabs; however, such severe instances of limb loss rarely were encountered in the Rhode River population (<0.1%).

The overall effect of autotomy on growth in the blue crab population in the Rhode River subestuary appears minor when autotomy patterns from the field are combined with findings from laboratory experiments. In both 1986 and 1987, approximately 90% of the damaged crabs had lost only one or two limbs. Loss of a single cheliped in *C. sapidus* in laboratory experiments, however, had no significant effect on the molt increment or molt interval compared to intact animals. Since cheliped removal represented the most extreme form of single limb loss in terms of biomass, the loss of one or two walking or swimming appendages should have similarly little effect on the growth increment. Unless foraging efficiency is hindered greatly by the absence of certain limbs, growth rates for the majority of damaged crabs in the Rhode River should not differ significantly from those of undamaged animals. Multiple autotomy, in contrast, did reduce growth increments in laboratory experiments; however, the relative rarity of such severely damaged animals in the Rhode River suggests that multiple autotomy also has little impact on overall growth in the population. For example, 9.4% to 13.7% of the blue crabs in the subestuary were missing three or more limbs in 1987 and 1986, respectively (Fig. 1B). Assuming a 32% reduction in carapace width in the first molt following autotomy for these individuals, overall growth in the Rhode River crab population still would be reduced by only 3–4.4%. Similarly, Bennett (1973) estimated that autotomy was responsible for only a 3% reduction in growth in *Cancer pagurus* populations off the English coast.

Lastly, the phenomenon of cheliped asymmetry reversal following removal of a major claw has been well-documented in certain decapod crustaceans (e.g., alpheid shrimp, Wilson, 1903; Mellon and Stephens, 1978 and *Menippe mercenaria*, Simonson, 1985) but only circumstantially so in blue crabs. In alpheid shrimp, the loss of a major claw results in transformation of the existing minor claw into a major claw. The autotomized limb subsequently is replaced by a minor claw. Hamilton et al. (1976) postulated that all blue crabs begin with right crushers and left cutters and that the appearance of double cutters or left crushers was evidence of past crusher autotomy and subsequent partial or complete reversal of asymmetry. If their basic postulate were correct, instances of unambiguous left crushers in blue crabs from the Rhode River would indicate that complete reversal occurred, but was rare. Results of the present growth experiments suggest that crabs greater than 35 mm carapace width may be incapable of reproducing a distinct crusher claw, since a large crushing molar was absent on all regenerating chelae even after three post-autotomy molts. The common occurrence of double cutters in Rhode River crabs (ca. 10% of all crabs caught) supports this contention. Regeneration of true crushers may occur only if these claws are lost very early in the crab's life. The lack of a crusher could lower foraging efficiency in juvenile crabs, since Blundon and Kennedy (1982) showed that crushers in *C. sapidus* had

greater mechanical advantage and strength than cutter claws. Complete regeneration of a crusher may be less critical for larger animals, because forces generated by cutters in large crabs appeared sufficient to crush most sizes of bivalves found in the Chesapeake Bay (Blundon and Kennedy, 1982).

In conclusion, limb loss was common in blue crabs in the Rhode River, but the effect of autotomy on overall growth in the population appeared slight when autotomy patterns from the field were combined with findings from laboratory growth experiments. Loss of one or two limbs, while frequent in damaged crabs, had no significant effect on growth. Severe multiple autotomy was observed rarely in natural populations, and costs in terms of reduced growth were minimized by the rapid recovery of limbs, size, and biomass in the second post-autotomy molt. Except for certain situations (e.g., autotomy in mature females or in animals at the end of the growing season), missing limbs can be replaced in the course of a season.

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LITERATURE CITED

- Ary, R. D., Jr., C. K. Bartell and M. A. Poirrier. 1987. The effects of chelotomy on molting in the blue crab, *Callinectes sapidus*. J. Shellfish Res. 6: 103-108.
- Bennett, D. B. 1973. The effect of limb loss and regeneration on the growth of the edible crab, *Cancer pagurus* L. J. Exp. Mar. Biol. Ecol. 13: 45-53.
- Berzins, I. K. and R. L. Caldwell. 1983. The effect of injury on the agonistic behavior of the stomatopod, *Gonodactylus bredini* (Manning). Mar. Behav. Physiol. 10: 83-96.
- Bliss, D. E. 1960. Autotomy and regeneration. Pages 561-589 in T. H. Waterman, ed. The physiology of Crustacea: metabolism and growth, Vol. 1. Academic Press, New York.
- Blundon, J. A. and V. S. Kennedy. 1982. Mechanical and behavioral aspects of blue crab, *Callinectes sapidus* (Rathbun), predation on Chesapeake Bay bivalves. J. Exp. Mar. Biol. Ecol. 65: 47-65.
- Botsford, L. W. and D. E. Wickham. 1978. Behavior of age-specific, density-dependent models and the northern California dungeness crab (*Cancer magister*) fishery. J. Fish. Res. Board Can. 35: 833-843.
- Bowmer, T. and B. F. Keegan. 1983. Field survey of the occurrence and significance of regeneration in *Amphiura filiformis* (Echinodermata: Ophiuroidea) from Galway Bay, west coast of Ireland. Mar. Biol. 74: 65-71.
- Caldwell, R. L. and J. Dingle. 1979. The influence of size differential on agonistic encounters in the mantis shrimp, *Gonodactylus viridis*. Behaviour 69: 255-264.
- Congdon, J. D., L. J. Vitt and W. W. King. 1974. Geckos: adaptive significance and energetics of tail autotomy. Science 184: 1379-1380.
- Conover, M. R. and D. E. Miller. 1978. The importance of the large chela in the territorial and pairing behaviour of the snapping shrimp, *Alpheus heterochaelis*. Mar. Behav. Physiol. 5: 185-192.
- Durkin, J. T., K. D. Buchanan and T. H. Blahm. 1984. Dungeness crab leg loss in the Columbia River estuary. Mar. Fish. Rev. 46: 22-24.
- Edwards, J. S. 1972. Limb loss and regeneration in two crabs: the king crab *Paralithodes camtschatica* and the tanner crab *Chionoecetes bairdi*. Acta Zool. 53: 105-112.
- Fingerman, M. and S. W. Fingerman. 1974. The effects of limb removal on the rates of ecdysis of eyed and eyestalkless fiddler crabs, *Uca pugilator*. Zool. Jb. Physiol. 78: 301-309.

- Fox, L. R. 1975. Cannibalism in natural populations. *Ann. Rev. Ecol. Syst.* 6: 87-106.
- Gray, E. H. and C. L. Newcombe. 1938. The relative growth of parts in the blue crab *Callinectes sapidus* Rathbun. *Growth* 2: 235-246.
- Hamilton, P. V., R. T. Nishimoto and J. G. Halusky. 1976. Cheliped laterality in *Callinectes sapidus* (Crustacea: Portunidae). *Biol. Bull. (Woods Hole)* 150: 393-401.
- Hartnoll, R. A. 1982. Growth. Pages 111-196 in D. E. Bliss, ed. *The biology of Crustacea*, Vol. 2. Academic Press, Inc., New York.
- Hazlett, B. A. 1977. Responses to agonistic postures by the spider crab *Microphrys bicornutus*. *Mar. Behav. Physiol.* 1: 85-92.
- Hines, A. H. 1982. Allometric constraints and variables of reproductive effort in brachyuran crabs. *Mar. Biol.* 69: 309-320.
- , R. N. Lipcius and A. M. Haddon. 1987a. Population dynamics and habitat partitioning by size, sex and molt stage of blue crabs (*Callinectes sapidus*) in a subestuary of central Chesapeake Bay. *Mar. Ecol. Prog. Ser.* 36: 55-64.
- , P. J. Haddon, J. J. Miklas, L. A. Wiechert and A. M. Haddon. 1987b. Estuarine invertebrates and fish: sampling design and constraints for long-term measurements of population dynamics. Pages 140-164 in T. P. Boyle, ed. *New approaches to monitoring aquatic ecosystems*, ASTM STP 940. American Society for Testing and Materials, Philadelphia.
- Hopkins, P. M. 1982. Growth and regeneration patterns in the fiddler crab, *Uca pugilator*. *Biol. Bull. (Woods Hole)*: 301-319.
- Hyatt, G. W. and M. Salmon. 1978. Combat in the fiddler crabs *Uca pugilator* and *U. pugnax*: a quantitative analysis. *Behaviour* 65: 182-211.
- Jachowski, R. L. 1974. Agonistic behavior of the blue crab, *Callinectes sapidus* Rathbun. *Behaviour* 50: 232-253.
- Johnson, P. T. 1980. Histology of the blue crab, *Callinectes sapidus*: a model for the Decapoda. Praeger Publ., New York. 440 pp.
- Kurihara, Y. and K. Okamoto. 1987. Cannibalism in a grapsid crab, *Hemigrapsus penicillatus*. *Mar. Ecol. Prog. Ser.* 41: 123-127.
- Kuris, A. M. and M. Mager. 1975. Effect of limb regeneration on size increase at molt of the shore crabs *Hemigrapsus oregonensis* and *Pachygrapsus crassipes*. *J. Exp. Zool.* 193: 353-360.
- Leffler, C. W. 1972. Some effects of temperature on the growth and metabolic rate of juvenile blue crabs, *Callinectes sapidus*, in the laboratory. *Mar. Biol.* 14: 104-110.
- McConaughy, J. R. and J. D. Costlow. 1980. Regeneration in larvae and juveniles of the mud crab *Rithropanopeus harrisi*. *J. Exp. Zool.* 213: 247-256.
- McVean, A. 1976. The incidence of autotomy in *Carcinus maenas* (L.). *J. Exp. Mar. Biol.* 24: 177-187.
- . 1982. Autotomy. Pages 107-132 in D. E. Bliss, ed. *The biology of Crustacea*, Vol. 4. Academic Press, Inc., New York.
- and I. Findlay. 1979. The incidence of autotomy in an estuarine population of the crab *Carcinus maenas*. *J. Mar. Biol. Assoc. U.K.* 59: 341-354.
- Mellon, DeF., Jr. and P. J. Stephens. 1978. Limb morphology and function are transformed by contralateral nerve section in snapping shrimps. *Nature* 272: 246-248.
- Needham, A. E. 1953. The incidence and adaptive value of autotomy and of regeneration in Crustacea. *Proc. Zool. Soc. Lond.* 123: 111-122.
- O'Neill, D. J. and J. S. Cobb. 1979. Some factors influencing the outcome of shelter competition in lobsters (*Homarus americanus*). *Mar. Behav. Physiol.* 6: 33-45.
- Polis, G. A. 1981. The evolution and dynamics of intraspecific predation. *Ann. Rev. Ecol. Syst.* 12: 225-251.
- Reaka, M. L. 1979. The evolutionary ecology of life history patterns in stomatopod Crustacea. Pages 235-260 in S. E. Stancyk, ed. *Reproductive ecology of marine invertebrates*. Belle W. Baruch Library in Mar. Sci. No. 9, Univ. of S. Carolina Press, Columbia, South Carolina.
- . 1987. Adult-juvenile interactions in benthic reef crustaceans. *Bull. Mar. Sci.* 41: 108-134.
- Robinson, M. H., L. G. Abele and B. Robinson. 1970. Attack autotomy: a defense against predators. *Science* 169: 300-301.
- SAS Institute. 1981. SAS for linear models. SAS Institute Inc., Cary, North Carolina. 231 pp.
- Schone, H. 1968. Agonistic and sexual display in aquatic and semi-terrestrial brachyuran crabs. *Amer. Zool.* 8: 641-654.
- Sekkelsten, G. I. 1988. Effect of handicap on mating success in male shore crabs, *Carcinus maenas*. *Oikos* 51: 131-134.
- Sheppard, L. and A. d'A. Bellairs. 1972. The mechanism of autotomy in *Lacerta*. *Br. J. Herp.* 30: 182-183.
- Shirley, S. M. and T. C. Shirley. 1988. Appendage injury in dungeness crabs, *Cancer magister*, in southeastern Alaska. *Fish. Bull.* 86: 156-160.

- Simonson, J. L. 1985. Reversal of handedness, growth, and claw stridulatory patterns in the stone crab *Menippe mercenaria* (Say) (Crustacea: Xanthidae). *J. Crust. Biol.* 5: 281-293.
- Sinclair, M. E. 1977. Agonistic behaviour of the stone crab, *Menippe mercenaria* (Say). *Anim. Behav.* 25: 193-207.
- Skinner, D. M. 1985. Molting and regeneration. Pages 43-146 in D. E. Bliss and L. H. Mantel, eds. *The biology of Crustacea*, Vol. 9. Academic Press, Inc., New York.
- and D. E. Graham. 1970. Molting in land crabs: stimulation by leg removal. *Science* 169: 383-385.
- and ———. 1972. Loss of limbs as a stimulus to ecdysis in Brachyura (true crabs). *Biol. Bull. (Woods Hole)* 143: 222-233.
- Sokal, R. R. and F. J. Rohlf. 1981. *Biometry*. W. H. Freeman, New York. 859 pp.
- Steel, R. G. D. and J. H. Torrie. 1980. *Principles and procedures in statistics*. McGraw-Hill, New York. 633 pp.
- Stein, R. A., S. T. Threlkeld, C. D. Sandgren, W. G. Sprules, L. Persson, E. E. Werner, W. E. Neill and S. I. Dodson. 1988. Size structured interactions in lake communities. Pages 161-179 in S. R. Carpenter, ed. *Complex interactions in lake communities*. Springer-Verlag, New York.
- Van Engel, W. A. 1958. The blue crab and its fishery in Chesapeake Bay. Part 1—Reproduction, early development, growth, and migration. *Commer. Fish. Rev.* 20: 6-17.
- Vannini, M. 1980. Notes on the behaviour of *Ocypode ryderi* Kingsley (Crustacea, Brachyura). *Mar. Behav. Physiol.* 7: 171-183.
- Warner, R. W. 1987. Age and growth of male dungeness crabs, *Cancer magister*, in northern California. *Calif. Fish. Game* 73: 4-20.
- Werner, E. E. and J. F. Gilliam. 1984. The ontogenetic niche and species interactions in size-structured populations. *Ann. Rev. Ecol. Syst.* 15: 393-425.
- Wilkie, I. C. 1978. Functional morphology of the autotomy plane of the brittle star *Ophiocoma nigra* (Abildgaard) (Ophiuroidea, Echinodermata). *Zoomorph.* 91: 289-305.
- Wilson, E. B. 1903. Notes on the reversal of asymmetry in the regeneration of the chelae in *Alpheus heterochelis*. *Biol. Bull. (Woods Hole)* 4: 197-210.
- Wood, F. D. and H. E. Wood, II. 1932. Autotomy in decapod Crustacea. *J. Exp. Zool.* 62: 1-55.

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