

THE ROLE OF LIMB AUTOTOMY IN THE TERRITORIAL BEHAVIOR OF THE
FRESHWATER PRAWN, MACROBRACHIUM LAR (PALAEMONIDAE)

BY

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Title: The Role of Limb Autotomy in the Territorial Behavior of the Freshwater Prawn,
Macrobrachium lar (Palaemonidae)

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The role of limb autotomy in the territorial behavior of the freshwater prawn, *Macrobrachium lar*, was analyzed to determine whether or not prawns modified their defended territory size depending on the condition of cheliped autotomy. Territory size data were collected for sets consisting of four prawns interacting during 14-day measurement periods. Specific territory size measurements were obtained using Thiessen polygons demarcated by boundaries where agonistic encounters occurred and aggressive pressure was equal. Agonistic encounters were defined to include lunging, chasing, and fleeing. Measured territory sizes were then analyzed using a one-way Analysis of Variance (ANOVA) analysis with the Tukey-Kramer Multiple-Comparison test and the Kruskal-Wallis test employed where necessary.

No significant differences were found in the Control group, in which all 12 prawns defended a territory with a mean size of $5274.6 \pm 244.7 \text{ cm}^2$. Results for Experiment

Experiment Group 1, with one cheliped autotomized, showed that most prawns defended less territory compared to those prawns with both chelipeds intact. The results of Experiment Group 2 showed that most prawns with two chelipeds autotomized also defended less territory than most prawns with both chelipeds intact. However, all groups showed territory size variability consistent with previous findings that indicated variability in aggressiveness, a principal behavioral component of an organism's territorial defense capability. Chelipeds were found to regenerate rapidly, with available data showing single chelipeds regenerated in 20.4 ± 1.9 days and both chelipeds regenerated in 17.6 ± 1.7 days.

TO THE GRADUATE PROGRAMS

The members of the Committee approve the thesis of Richard Alan Seidel presented November 5, 2003.



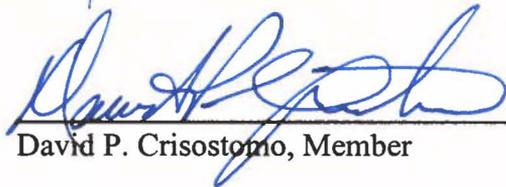
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INTRODUCTION

Territoriality is the prolonged occupancy of an area and its defense against intrusions by other individuals of the same species (Stimson 1973) or different species (Leiser 2003). Male individuals tend to be more territorial than females. For example, the male creosote-bush grasshopper *Boottettix argentatus* defends individual shrubs or shrub complexes against conspecifics, but females do not (Schowalter and Whitford 1979). Stimson (1973) has shown that certain invertebrates (specifically the limpet *Lottia gigantea*) exhibit territoriality as an adaptation for defending a food supply. In fact, one primary hypothesis explaining territoriality is the sufficient-resource hypothesis in which territories function to ensure an adequate supply of some resource (Verner 1977). Data from a study of another invertebrate, the desert spider *Agelenopsis aperta*, supported this hypothesis (Riechert 1981). Territory ownership in *A. aperta* has been shown to optimize food intake, thereby providing benefits to individual fitness (Riechert 1981).

A territory is a portion of the home range defended against conspecifics (Stamps 1999) and heterospecifics (Leiser 2003). Additionally, these territories do not overlap with the home ranges of neighboring conspecific residents (Stamps 1999). Boundaries between these territories are established and defended effectively with the use of aggressive pressure (Adams 1998). Stamps (1999) found that territorial animals are capable of spatial learning, specifically in the sense that rewarding experiences in an area increase the likelihood that an animal will return to or localize in that area. Lamanna and

Eason (2003) demonstrated that the blockhead cichlid, *Steatocranus casuarius* (Pisces, Cichlidae), successfully established territories in experimental trials using landmarks to set territory boundaries. Alternatively, aggressive interactions between conspecifics in a given area reduce the likelihood of individuals returning to that area (Stamps 1999).

In some species, there is considerable variation in fighting ability among interacting residents because of differences in size, health, weaponry, or experience (Adams 1998). Animals with greater fighting ability can exert greater pressure that tends to push boundaries away from stronger residents and towards their weaker neighbors (Adams 1998). In many agonistic exchanges, fights may not happen but instead a series of less damaging ritualized behaviors will occur. Stamps (1999) found that most interactions between neighboring individuals are symmetrical, with no winners or losers. Thus, the outcomes of these agonistic encounters between conspecifics can often be decided without injury to either party.

Should the defensive weaponry be damaged or lost completely during an agonistic exchange, the inability to display will result in losses in future encounters (Adams 1998). This inability to display because of injury could result in the territory holder being unable to defend its territory adequately resulting in the reduction or total loss of that individual's territory.

Territoriality is pronounced in male freshwater prawn, *Macrobrachium lar* (Crustacea, Palaemonidae). *Macrobrachium lar* is native to the freshwater streams and rivers of Guam, the Northern Mariana Islands, and elsewhere across the Western Pacific. *Macrobrachium* species also occur in freshwater streams throughout Southeast Asia (e.g.

M. rosenbergii in Malaysia, *M. carcinus* and *M. acanthurus* in the West Indies), North America (e.g. *M. ohionis* in Indiana, Ohio and Kentucky and *M. acanthurus* in Georgia and Florida), the Caribbean (e.g. *M. faustinum*, Puerto Rico) and Central and South America (e.g. *M. olfersii* from Mexico to Brasil) (Hedgpeth 1949, Holthuis 1952).

Macrobrachium lar is amphidromous, which means that individuals migrate regularly between freshwater and the sea but not for the purpose of breeding (McDowall 1992). *Macrobrachium lar* most often occurs in isolated freshwater streams and rivers with an abundant supply of freshwater algae (Holthuis 1952).

This prawn follows the basic anatomical plan for decapod crustaceans, with a hard chitinous exoskeleton and a protective carapace covering the thorax. On the underside of the carapace are five pairs of appendages: four pairs of pereopods and one pair of chelipeds. Posteriorly, the abdomen terminates at the uropod and on the underside contains five pairs of pleopods used for swimming (D'Abramo and Brunson 1996).

The life cycle and growth characteristics of *Macrobrachium* prawns have been well studied (Gunter 1937, Hughes and Richard 1973, Lindenfelser 1984, Covich et al. 1996, D'Abramo and Brunson 1996). Because freshwater prawns have a rigid exoskeleton, they must molt regularly in order for growth to occur. Increases in prawn weight and size occur soon after each molt (D'Abramo and Brunson 1996). The hormone ecdysone stimulates the molting of the chitinous exoskeleton, allowing for a growth period to occur before the new exoskeleton hardens. In other crustaceans, such as shrimps, individuals between molts are able to autotomize limbs in order to avoid predation by fish, octopuses, squid, or cuttlefish (Col 2000). Indeed, many crustaceans

can autotomize and regenerate their limbs throughout both juvenile and adult stages (Cooper 1998). The lost limbs of decapod crustaceans are subsequently regenerated within a period of several molts, as observed in the red swamp crayfish, *Procambarus clarkii* (Griffis et al. 2001).

A structure that is essential for immediate survival will not be a strong candidate for regeneration, because an individual losing the structure would probably die before the structure could properly regenerate (Reichman 1984). During my preliminary work in this study, I observed *M. lar* using chelipeds to enforce territorial perimeters. Display patterns of chelipeds have an important role in agonistic and aggressive interactions (Mariappan et al. 2000). Chelipeds are versatile organs of offense and defense which also make them vulnerable for autotomy (Mariappan et al. 2000). However, it has been noted that a lost structure, such as a cheliped, must be important enough to warrant its redevelopment (Reichman 1984). Regeneration of autotomized chelipeds imposes an additional energy demand, the regeneration load, on the incumbent, altering energy allocation for somatic and/or reproductive processes (Mariappan et al. 2000). Thus, it could be inferred that because lost chelipeds are regenerated, the prawn must require their presence for optimized defense beyond the expense of the nutritional resources required for limb regeneration.

Questions related to crustacean limb regeneration have stimulated much research. The grass shrimp *Palaemonetes pugio* has been used to study chemically regulated limb regeneration (Rao et al. 1978, and Rao and Conklin 1986). The crayfish *Procambarus clarkii* has been observed to regenerate its walking legs (Cooper 1998). Crayfish have

also been used to study muscle phenotypes and innervation patterns during limb regeneration (Griffis et al. 2001). However, no prior research on crustaceans has demonstrated a link between limb regeneration and territorial interactions.

In the present study, the underlying assumption is that a prawn which loses a cheliped (or chelipeds) will lose previously defended territory in part or in whole. The series of experiments in this study demonstrated that *M. lar* modifies its territorial behavior as a result of limb autotomy. With compromised mobility and defensive capabilities resulting from limb autotomy, it is advantageous for *M. lar* to regenerate the autotomized limb or limbs. The purpose of this study was to determine and describe the relationship between limb autotomy and territorial behavior in *M. lar* and test the following hypotheses. The first null hypothesis is that the freshwater prawn *M. lar* will retain defended territory despite cheliped autotomy. The first alternative hypothesis is that the freshwater prawn *M. lar* will lose defended territory (in part or whole) as a result of cheliped autotomy.

In preliminary experiments, the sufficient-resource hypothesis (Verner 1977) appeared to underpin the territorial behaviors exhibited by *M. lar*, and the modulation of food supply (addition/deprivation of food pellets) seemed to produce territorial interactions at the boundaries. Adams (1998) demonstrated that aggressive pressure occurs at these boundaries, thereby demarcating the territories being defended. If the first null hypothesis is accepted, *M. lar* will retain defended territory despite cheliped autotomy and it will decouple the connection between intact chelipeds and effective territory defense. If the first null hypothesis is rejected in favor of the first alternative hypothesis, *M. lar* will lose defended territory in part or whole. A measured loss in

territory size following cheliped autotomy will demonstrate that less weaponry means less territory.

The second null hypothesis is that the freshwater prawn *M. lar* will be unable to retake defended territory upon regeneration of autotomized chelipeds. The second alternative hypothesis is that the freshwater prawn *M. lar* will retake defended territory upon regeneration of autotomized chelipeds. If the first null hypothesis is rejected in favor of the first alternative hypothesis, then my second set of hypotheses will be relevant.

Because *M. lar* regenerate their autotomized chelipeds, I predict that once a prawn's chelipeds are regenerated the prawn will be able to retake previously defended territory.

- Dominance mediated through aggressive behavior (Lange and Leimar 2003) is a likely cause of successful prior residency, and I predict that regenerated chelipeds will reinstate the competitive advantage of the original territory holder. Studies on territorial male willow ptarmigan have shown that familiarity with neighbors and with a particular territory can reduce the costs of territory establishment (Eason and Hannon 1994). Territorial familiarity would likely increase the probability of territory reestablishment were it temporarily lost. If the second null hypothesis is accepted, it will mean that lost territory cannot be retaken due to a factor other than cheliped autotomy. If the second null hypothesis is rejected in favor of the second alternative hypothesis, it will mean that *M. lar* can retake defended territory when its autotomized chelipeds regenerate.

MATERIALS AND METHODS

Experiments were conducted in a freshwater tank measuring 41 cm deep x 118 cm x 185 cm, filled to a depth of 16 cm. A grid was marked on the base of a concrete tank. This grid divided the tank's surface area into 851 squares measuring 5 cm x 5 cm each. This provided stationary points of physical reference that were helpful in the quantitative description of established territories. Adams (1998) described the use of geometric procedures that divided habitats into cells called Thiessen, or Voronoi, polygons. While often irregularly shaped, these polygons facilitated the development of quantitative predictions of territory size and shape (Adams 1998).

Prawn lodges (5 cm diameter PVC pipe in 20 cm lengths) were placed symmetrically about each tank to provide shelter for each respective prawn. In addition to providing shelter and safety, these lodges were intended to encourage the localization of each prawn within each respective quadrant on the grid, as with the landmarks employed by Lamanna and Eason (2003).

Tank water was filtered using a three stage freshwater filter system, which facilitated mechanical (via sponge), chemical (via activated charcoal), and biological (via invaginated plastic spheres supporting nitrifying microbes) filtration. The water was delivered to the top of the filter via a Rio 600 water pump and returned to the tank via a 2 cm plastic input hose. Water aeration was accomplished using a 30 cm air wand.

The tank was thermally regulated using a shade screen constructed from ½ inch dowels, ½ inch PVC elbows and tees, and black (60% shade factor) shade cloth. In addition to the cover provided by this shade screen, a 40 cm styrene lip was constructed to deter specimens from escaping. The water level of the tank was lowered to 16 cm following the introduction of *M. lar* specimens into the tank. Reduced water level confined the territorial interactions of *M. lar* to the two-dimensional grid marked on the tank substrate.

All *M. lar* were collected March through October, 2003, from the Pigua River, in Merizo, Guam. Small hand nets were used to capture *M. lar* from the series of eight shallow pools (Priest's Pools) eroded by the Pigua River in the basalt substrata. Only adult male *M. lar* in the size class 9.0 ± 1.5 cm were retained, all others were released. Most prawns were introduced immediately into the experiment tanks at the University of Guam Marine Laboratory. Extra prawns were stored temporarily in 8.0 L basins with aeration. All prawns were fed Wardley Pond Ten pellet food, made of crude protein, crude fat, and crude fiber. Shade cloth (60% shade factor) was used to cover each basin.

The boundaries between territories develop where the aggressive tendencies of residents match those of adjacent neighbors (Adams 1998). Numerous experiments have shown that neighbors restrict one another's territory areas by fighting and display (e.g., Watson 1967, Krebs 1971, Welsh 1975, Nursall 1977, Norman and Jones 1984, Gordon 1992). This was confirmed by preliminary observations of *M. lar*. Prior to this study, there has been little effort to predict the geometric consequences of these boundary conflicts (Adams 1998).

I attempted to predict the positions of territorial boundaries formed by these neighborhood interactions and focused specifically on the role of limb autotomy that modulated these territorial boundaries (i.e. the size of the boundaries). Food resource competition (via the addition of food pellets) was used to induce territorial interactions at the interface zones between adjacent territories. Boundaries were identified and used to calculate territorial areas (in square centimeters) defended by each respective prawn. To quantify the territory sizes, I summed the areas of the 5 x 5 squares contained within each Thiessen polygon (defined by afore mentioned territory boundaries) where agonistic exchanges occurred. This permitted good estimation of the overall territory sizes. An analysis of variance (ANOVA) was preferable because it is used to test for significant differences among several means without increasing the Type I error rate.

A simple experiment was conducted prior to the replication trials to demonstrate that the agonistic exchange frequency increased when food supply stimulated competition. Six *M. lar* individuals were placed in the same tank for ten minute intervals, observed, and their territorial behaviors quantified. During this first 10-minute observation period, no food pellets were added to the tank. The system remained undisturbed for a period of 5 minutes before the second 10-minute observation period. During the second 10-minute observation period, food pellets were added (4 pellets per prawn) and again the agonistic encounters were quantified. The mean number of agonistic exchanges when pellets were absent (A) was 6.7 ± 0.9 per 10-minute interval. The mean number of agonistic exchanges when pellets were present (P) was 11.3 ± 0.9 per 10-minute interval. The one-way ANOVA analysis indicated a statistically

significant difference ($\alpha = 0.05$) between the means for the pellets absent (A) group and the pellets present (P) group. The Tukey-Kramer multiple-comparison test indicated a significant difference between groups A and P, which demonstrated that the degree of agonistic exchange could be experimentally manipulated.

During induced encounters, I observed the following territorial behaviors: lunging, chasing, and fleeing. All behaviors that involved an advance of less than 5 cm were scored as lunging. All behaviors that involved an advance of more than 5 cm were scored as chasing. And, all behaviors that involved a retreat of more than 5 cm were scored as fleeing. One point was recorded every time one or several of these behaviors were displayed in a single dyadic encounter.

As with other decapod crustaceans, *M. lar* autotomized limbs along anatomically defined autotomy planes. In Experimental Groups 1 and 2, prawns were induced to autotomize chelipeds by the method of Skinner and Graham (1970). This study followed the guidelines for the treatment of animals in behavioral research set forth by the Animal Behavior Society (available: www.animalbehavior.org). I also observed closely all provisions of the Animal Welfare Act (AWA), part 7 USC 2143 (a)(7)(A) of the United States of America.

The Control group for this phase of the experiment used four adult male *M. lar* specimens (per replication set) with both chelipeds intact. Prawns in all groups rotated positions during the 14-day observation periods, but still defended measurable territory sizes. A box plot was used to visually display data on the sizes of territories (in cm²) defended by the 12 individuals in the Control group. Defended territory sizes were

monitored visually, quantified, and recorded for each *M. lar* for 14 days. Individual means for territory size were calculated for each Control group individual. Finally, the Tukey-Kramer Multiple-Comparison Test was used to analyze for significant differences between the groups.

Experiment 1 involved single cheliped removal in two of four prawns (per replication set), again with territories monitored visually and data recorded for 14 days. A \log_{10} transformation was required to normalize Experiment 1 territory data for parametric ANOVA analysis. A box plot was used to visually display the data on transformed territory sizes (in $\log_{10} \text{ cm}^2$) defended by the 12 individuals in Experiment 1. Defended territory sizes were recorded for each *M. lar* for 14 days. The treatment differed in Experiment 1 compared to the Control group, in that six of 12 individuals had one cheliped autotomized. Individual means for territory size were calculated for each Experiment 1 individual. The Tukey-Kramer Multiple-Comparison Test was used to analyze for significant differences between the groups.

Experiment 2 involved double cheliped removal in two of four prawns (per replication set), again with territories monitored visually and data recorded for 14 days. Because Experiment 2 data did not meet the assumptions of a parametric ANOVA, a nonparametric ANOVA, the Kruskal-Wallis test, was employed. A box plot was used to visually display data on the sizes of territories (in cm^2) defended by the 12 individuals in the Experiment 2. Defended territory sizes were recorded for each *M. lar* for 14 days. Individual means for territory size were calculated for Experiment 2. The treatment differed in Experiment 2 in that six of 12 individuals had two chelipeds autotomized.

The Kruskal-Wallis one-way ANOVA was used to assign median ranks and to determine corresponding decisions on hypotheses. Finally, the Tukey-Kramer Multiple-Comparison Test was used to test for significant differences between groups in Experiment 2.

The second null hypothesis was that *M. lar* retook defended territory upon regeneration of autotomized chelipeds. The second alternative hypothesis was that *M. lar* did not retake defended territory upon regeneration of autotomized chelipeds. This phase of the experiment was intended to follow the corresponding set in the first phase.

Preliminary observations showed that adult male *M. lar* regenerated lost pereopods in 25 ± 5 days, and I estimated cheliped regeneration time to be about 20% longer.

Territory sizes in Experiments 1 and 2 were compared with those of the Control group. The purpose in testing acquired territory data was to demonstrate that territory sizes undergo a statistically significant change in size as a result of cheliped autotomy. In Experiment 1 and Experiment 2, each set of four prawns was run with three replications (total of $n = 12$) to increase the power of statistical analyses. Territory data was analyzed with parametric one-way ANOVA analysis. These tests helped determine the relationship between limb autotomy and territoriality in *M. lar*.

RESULTS

Individual Control group prawns defended territories that varied in size from $4862.9 \pm 347.6 \text{ cm}^2$ to $5927.0 \pm 146.0 \text{ cm}^2$ (Table 1); there was also variability in each individual's territory individual mean (Figure 1). The defended territory size grand mean for the Control was $5274.6 \pm 244.7 \text{ cm}^2$. There were no significant differences between each individual mean in the Control group (one-way ANOVA: $\alpha = 0.05$, F-Ratio = 2.07, $P = 0.025$, Power = 90.4%) (Table 1).

In Experiment 1, prawns with one cheliped autotomized defended smaller territories than prawns with both chelipeds intact. Territory sizes for each prawn were variable (Figure 2). Mean territory sizes for individual intact prawns ranged from $5364.6 \pm 192.1 \text{ cm}^2$ to $6716.9 \pm 182.1 \text{ cm}^2$. Mean territory sizes for individual treatment prawns ranged from $3647.2 \pm 186.4 \text{ cm}^2$ to $4650.2 \pm 234.9 \text{ cm}^2$. Experiment 1 territory size data were not normally distributed, so they were transformed logarithmically to permit parametric analysis with a one-way ANOVA test (Table 2). Retesting these data following logarithmic transformation confirmed that they were parametric. There were significant differences detected between individual means in Experiment 1 (one-way ANOVA: $\alpha = 0.05$, F-Ratio = 26.03, $P = 0.000$, Power = 100%) (Table 2).

Macrobrachium lar was variable in aggressiveness and territorial defense, and differences in territory size of some Experiment 1 prawns in the same treatment were just

barely significant (e.g. prawn *7 and *8, prawn *11 and *8). Also, no significant differences were found between several intact prawns and treatment prawns (e.g. prawn 10 and *3, prawn 10 and *8). Because most Experiment 1 prawns in a different treatment differed significantly from each other, however, the conclusion was that single cheliped autotomy resulted in significantly decreased territory size. This allowed the rejection of the first null hypothesis in favor of first alternative hypothesis which meant that the freshwater prawn *M. lar* lost defended territory (in part of whole) as a result of cheliped autotomy.

In Experiment 2, prawns with two chelipeds autotomized defended smaller territory sizes than prawns with both chelipeds intact. Variability in mean sizes of territories was observed for each prawn (Figure 3). Mean territory sizes for intact prawns ranged from $5258.2 \pm 203.7 \text{ cm}^2$ to $7522.2 \pm 207.5 \text{ cm}^2$. Mean territory sizes for treatment prawns ranged from $2917.8 \pm 143.6 \text{ cm}^2$ to $4057.4 \pm 257.3 \text{ cm}^2$ (Table 3). There were significant differences detected also between group territory size means in Experiment 2 (one-way ANOVA: $\alpha = 0.05$, F-Ratio = 47.61, P = 0.000, Power = 100%). Most territory size medians for Experiment 2 prawns in a different treatment differed significantly from each other (Table 4).

Mean territory sizes in Experiment 2 had statistically significant differences, and these were important because differences between groups were predicted based upon induced differences in cheliped autotomy. Territory sizes of most prawns with both chelipeds autotomized were significantly different than those of most prawns with both chelipeds intact. *Macrobrachium lar* was variable in aggressiveness and territorial

defense, differences in territory size of some Experiment 2 groups in the same treatment were significant (e.g. prawn *3 and *12, prawn 10 and 5, prawn 10 and 1, prawn 1 and 2, prawn 1 and 9, and prawn 1 and 6). Prawn 1 from Experiment 2 was an outlier; its larger territory size was significantly different from prawns of the equivalent treatment. Again, the results allowed for the rejection of the null hypothesis in favor of alternative hypothesis. Thus, *M. lar* lost defended territory (in part of whole) as a result of double cheliped autotomy.

Because *M. lar* molted about every three weeks under laboratory conditions, any newly molted prawns left in a tank with aggressive intermolt conspecifics were invariably damaged or killed. *Macrobrachium lar* was observed feeding regularly on the molted exoskeletons of same-tank conspecifics, presumably to exploit the available chitinous material needed for its own exoskeleton, as well as protein for growth and maintenance. Newly molted prawns also fell prey while in their vulnerable, soft-shelled condition. Because prawns were extremely vulnerable immediately following ecdysis, lost territory was not regained.

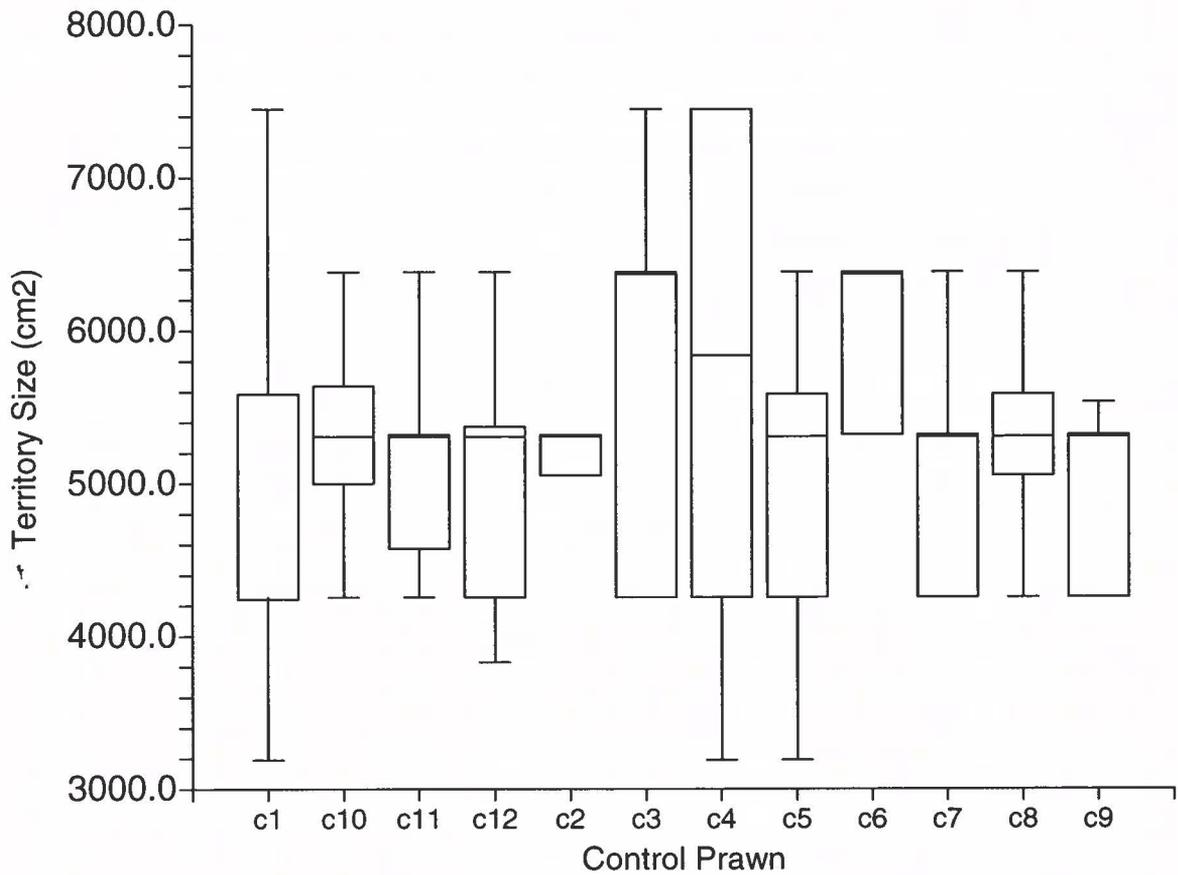


Figure 1. A box plot showing territory sizes defended by *M. lar* in the Control group; means are marked with horizontal lines, and error bars as shown.

Table 1. Mean territory sizes (cm²) of Control group *M. lar* individuals with corresponding standard errors. At right are results for the Tukey-Kramer Multiple-Comparison Tests with multiple comparisons analyzing for all pairwise differences between the Control group means ($\alpha = 0.05$, $df = 156$, $MSE = 838446.6$, Critical Value = 4.69). NS indicates that no significant differences existed between individual prawns.

Prawn	n	Mean	Standard Error	Different From Prawns
All	168	5274.6	244.7	
c1	14	4862.9	347.6	NS
c9	14	4954.2	147.0	NS
c12	14	4999.9	190.3	NS
c7	14	5015.0	206.5	NS
c5	14	5091.0	253.8	NS
c11	14	5091.1	160.0	NS
c2	14	5167.0	219.1	NS
c10	14	5319.0	172.8	NS
c8	14	5394.9	235.6	NS
c4	14	5698.7	395.9	NS
c3	14	5774.9	309.7	NS
c6	14	5927.0	146.0	NS

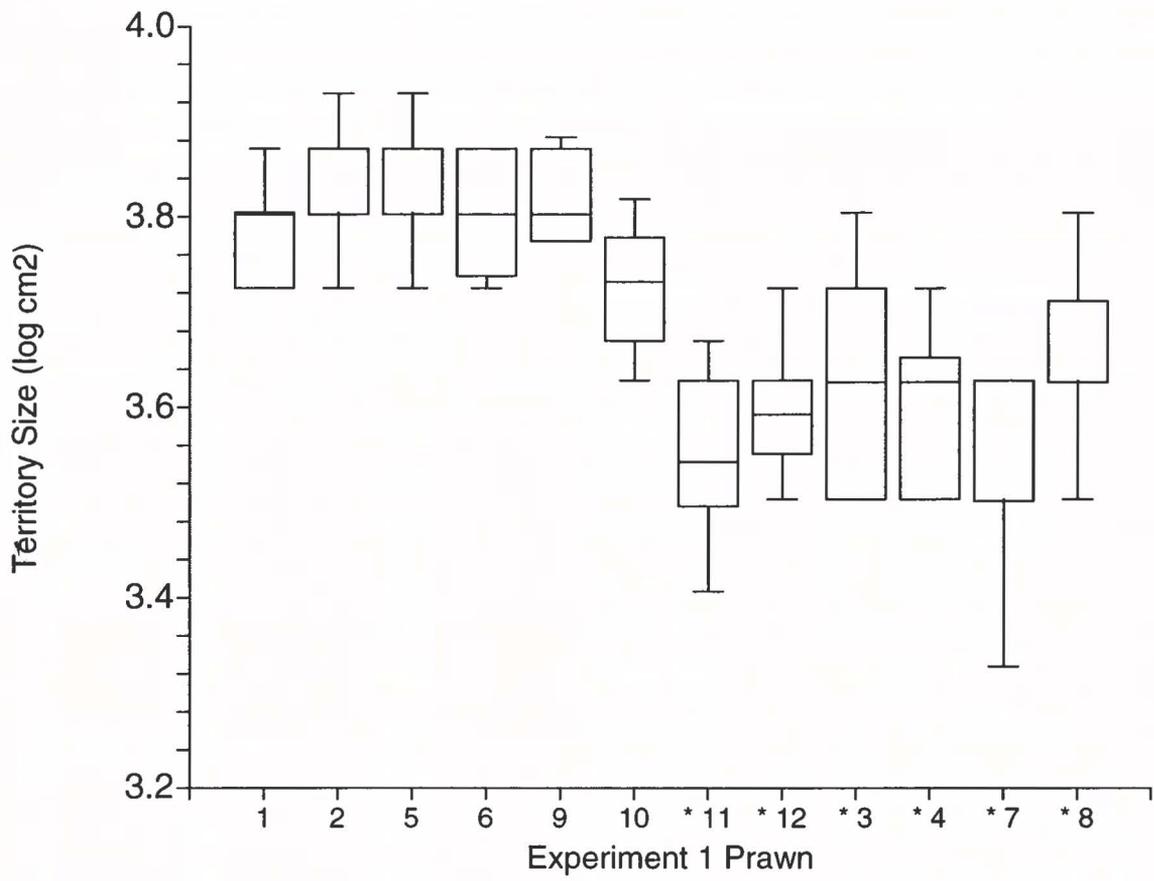


Figure 2. A box plot showing territory sizes defended by *M. lar* in Experiment 1, with prawns having one cheliped autotomized indicated with asterisks; means are shown as horizontal lines, with corresponding error bars.

Table 2. Mean territory sizes (\log_{10} transformed cm^2) of Experiment 1 *M. lar* individuals with corresponding standard errors. At right are results for the Tukey-Kramer Multiple-Comparison Tests with multiple comparisons analyzing for all pairwise differences between the Experiment 1 means ($\alpha = 0.05$, $df = 156$, $MSE = 6.35 \times 10^{-3}$, Critical Value = 4.69). Experiment 1 prawns with one cheliped autotomized are indicated with asterisks.

Prawn	n	Mean	Standard Error	Different From Prawns
All	168	3.69	2.13×10^{-2}	
* 7	14	3.55	3.64×10^{-2}	* 8, 10, 1, 6, 2, 5, 9
* 11	14	3.55	2.27×10^{-2}	* 8, 10, 1, 6, 2, 5, 9
* 12	14	3.60	1.61×10^{-2}	10, 1, 6, 2, 5, 9
* 4	14	3.61	2.19×10^{-2}	10, 1, 6, 2, 5, 9
* 3	14	3.63	2.68×10^{-2}	1, 6, 2, 5, 9
* 8	14	3.66	2.11×10^{-2}	* 7, * 11, 1, 6, 2, 5, 9
10	14	3.73	1.58×10^{-2}	* 7, * 11, * 12, * 4
1	14	3.78	1.24×10^{-2}	* 7, * 11, * 12, * 4, * 3, * 8
6	14	3.79	2.64×10^{-2}	* 7, * 11, * 12, * 4, * 3, * 8
2	14	3.82	1.52×10^{-2}	* 7, * 11, * 12, * 4, * 3, * 8
5	14	3.82	1.52×10^{-2}	* 7, * 11, * 12, * 4, * 3, * 8
9	14	3.83	1.17×10^{-2}	* 7, * 11, * 12, * 4, * 3, * 8

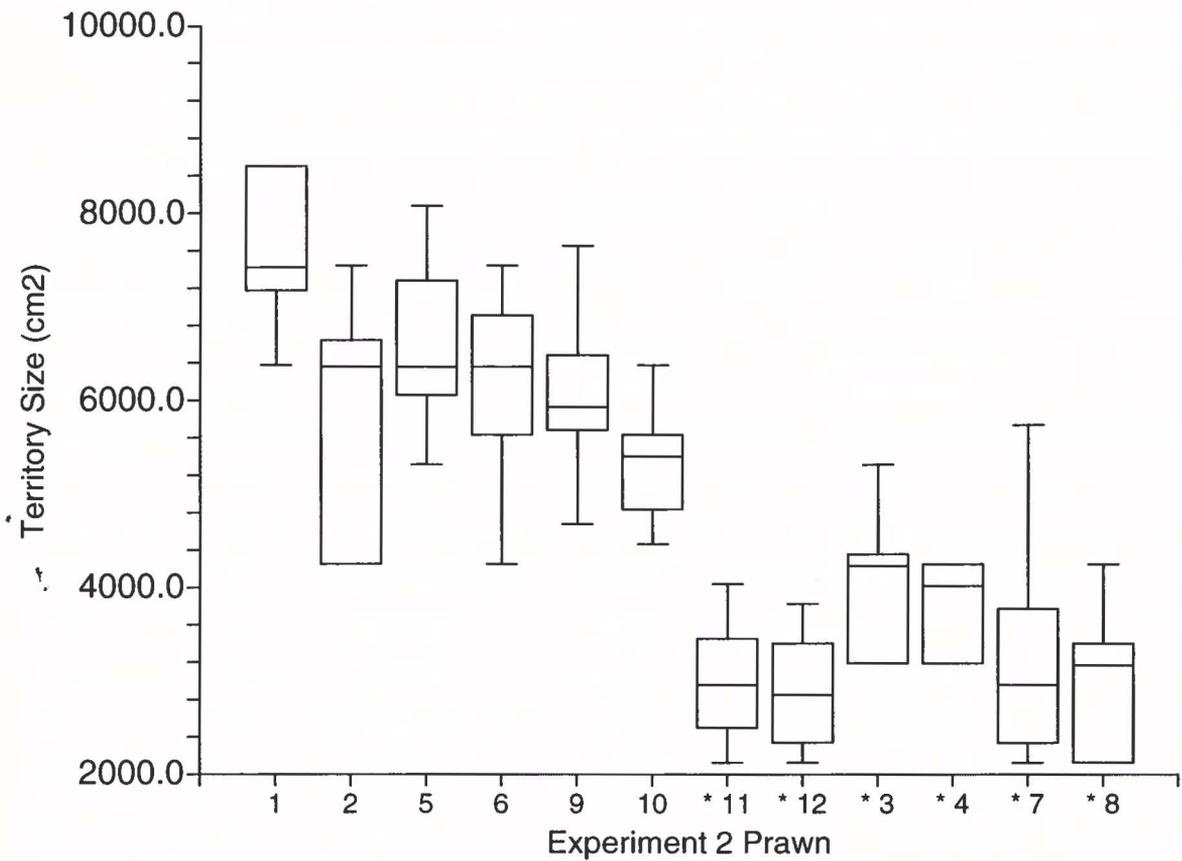


Figure 3. A box plot showing territory sizes defended by *M. lar* in Experiment 2, with prawns having two chelipeds autotomized indicated with asterisks; means are shown as horizontal lines, with corresponding error bars.

Table 3. Mean territory sizes (cm²) of Experiment 2 *M. lar* individuals with corresponding standard errors. At right are results for the Tukey-Kramer Multiple-Comparison Tests with multiple comparisons analyzing for all pairwise differences between the Experiment 2 means ($\alpha = 0.05$, $df = 156$, $MSE = 781375.6$, Critical Value = 4.69). Experiment 2 prawns with two chelipeds autotomized are indicated with asterisks.

Prawn	n	Mean	Standard Error	Different From Prawns
All	168	4817.4	236.2	
* 12	14	2917.8	143.6	* 3, 10, 2, 9, 6, 5, 1
* 8	14	2963.3	184.5	10, 2, 9, 6, 5, 1
* 11	14	3160.9	256.1	10, 2, 9, 6, 5, 1
* 7	14	3312.9	352.4	10, 2, 9, 6, 5, 1
* 4	14	3768.6	141.8	10, 2, 9, 6, 5, 1
* 3	14	4057.4	257.3	* 12, 10, 2, 9, 6, 5, 1
10	14	5258.2	203.7	* 12, * 8, * 11, * 7, * 4, * 3, 5, 1
2	14	5926.8	329.2	* 12, * 8, * 11, * 7, * 4, * 3, 1
9	14	6154.8	200.0	* 12, * 8, * 11, * 7, * 4, * 3, 1
6	14	6200.2	243.1	* 12, * 8, * 11, * 7, * 4, * 3, 1
5	14	6565.1	216.1	* 12, * 8, * 11, * 7, * 4, * 3, 10
1	14	7522.2	207.5	* 12, * 8, * 11, * 7, * 4, * 3, 10, 2, 9, 6

Table 4. A Kruskal-Wallis one-way ANOVA on ranks of Experiment 2 data, with indicated decisions on hypotheses (H_0 : All medians are equal, and H_a : At least two medians are significantly different).

Method	DF	Chi-Square (H)	Prob Level	Decision(0.05)
Not Corrected for Ties	11	125.5	0.00	Reject H_0
Corrected for Ties	11	126.4	0.00	Reject H_0
Number Sets of Ties	23			
Multiplicity Factor	33186			

Prawn	n	Ranks	Sum of Rank	Mean Z-Value	Median	Different From Prawns
1	14	2143.0	153.1	5.5093	7446.0	2, 10, *11, *12, *3, *4, *7, *8
2	14	1638.0	117.0	2.6112	6383.0	*3, *4, *7, *8, *11, *12
5	14	1841.0	131.5	3.7761	6383.0	*11, *12, *3, *4, *7, *8
6	14	1717.5	122.7	3.0674	6383.0	*11, *12, *3, *4, *7, *8
9	14	1693.5	121.0	2.9297	5957.0	*11, *12, *3, *4, *7, *8
10	14	1362.0	97.3	1.0272	5425.5	1, *11, *12, *4, *7, *8
* 11	14	513.0	36.6	-3.8450	2979.0	1, 2, 5, 6, 9, 10
* 12	14	453.5	32.4	-4.1865	2872.5	1, 2, 5, 6, 9, 10
* 3	14	935.0	66.8	-1.4232	4255.0	1, 2, 5, 6, 9
* 4	14	837.0	59.8	-1.9856	4042.5	1, 2, 5, 6, 9, 10
* 7	14	590.0	42.1	-3.4031	2978.5	1, 2, 5, 6, 9, 10
* 8	14	472.5	33.8	-4.0774	3191.0	1, 2, 5, 6, 9, 10

DISCUSSION

Generally, animals have been shown to differ enormously in their territorial systems in that some species defend only small areas surrounded by undefended space while others defend large contiguous territories (Both and Visser 2003). Optimal territory size for an individual organism has been shown to depend on the relative abundance of certain divisible resources, such as food (Both and Visser 2003).

Food resource competition increased the frequency of agonistic exchanges in *M. lar*. These exchanges were necessary in order to delineate territorial boundaries between individuals. Aggressive pressure between conspecifics defined these territorial boundaries (Adams 1998) and the areas contained within the territory areas. According to the sufficient-resource hypothesis (Verner 1977), conspecifics interact agonistically to secure individual portions of available food resources. In the present study, food pellets were added to tanks and prawns competed for this resource. All *M. lar* individuals began feeding within several minutes after the introduction of food pellets. Similar behavior was described in the Antarctic krill *Euphausia superba* that fed in response to chemical cues even before contact with the source particles (Hamner et al. 1983). My results, then, supported the sufficient-resource hypothesis and allowed for the use of the methods employed here to stage territorial encounters.

Macrobrachium lar has been shown to exhibit highly variable acts of individual aggressiveness and submissiveness within groups (Donaldson 1981); this individuality also contributed to variability in defended territory sizes of males measured in this study. Because crustaceans have been shown to be less aggressive when their exoskeletons are

soft (Tamm and Cobb 1978), it would be undesirable, but more likely, for ecdysis to occur during a longer measurement period. In the present study, a 14-day period was long enough to achieve good territory size measurements but not so long as to risk an ecdysis event and conspecific predation. While some attention has been given to the subtle variations in crustacean molt state (Reaka 1975, Cromarty et al. 2000), all *M. lar* individuals with hardened exoskeletons were assumed to be in equivalent molt state (mid-intermolt) in this study. This was important to insure that no ecdysis occurred during the 14-day measurement intervals in any group for the reasons described previously.

Macrobrachium spp. employ a defense strategy whereby cheliped displays regulate aggressive interactions (Mariappan et al. 2000) and determine territorial dominance hierarchies. The Control group prawns (both chelipeds intact) in this study were shown to exhibit variable territory size within and among groups. Despite some variability in territories defended by Control group prawns, there were no statistically-significant differences at the $\alpha = 0.05$ level.

Prawns subject to single cheliped autotomy were shown to defend significantly decreased territory sizes compared with intact prawns. Prawns subject to double cheliped autotomy were shown to defend significantly decreased territory sizes compared with intact prawns, as well. Because chelipeds are versatile organs of offense and defense (Mariappan et al. 2000) and are important in determining the outcome of agonistic encounters (Donaldson 1981), single or double cheliped autotomy resulted in less successful territory defense. Single or double cheliped autotomy also resulted in compromised signaling ability, and that contributed also to less successful territory defense.

Most defended territories of Experiment 1 treatment prawns (one cheliped

autotomized) were significantly different from most defended territories of intact. So, the first null hypothesis, in which *M. lar* would retain defended territory despite cheliped autotomy, was rejected. The Kruskal-Wallis test favored the alternative hypothesis because most territory size medians for Experiment 2 prawns in a different treatment differed significantly from each other. This was consistent with the results from the Tukey-Kramer Multiple Comparison test that showed statistically significant differences between most territories defended by prawns of different treatment.

Conclusions from Experiment 1 and Experiment 2 were consistent with Adams' (1998) findings that animals with greater fighting ability exerted greater pressure that tends to push territorial boundaries towards weaker conspecific neighbors. In *M. lar*, greater fighting ability was possible because of the use of intact chelipeds.

Because exoskeletons are soft after ecdysis, wild prawns probably take refuge in naturally irregular substrata during and following ecdysis to avoid predation. The tanks used for this study were artificial and lacked natural refuges. Because of these circumstances, the second set of hypotheses may not have been addressed effectively. While no prawn was able to retake lost territory in the artificial habitat, wild prawns probably are able to defend territory again once their chelipeds regenerate and their exoskeletons harden. Soft exoskeletons following ecdysis were a serious impediment to territory defense, primarily because of increased vulnerability.

One emergent discovery was the rapid regeneration time of replacement chelipeds in *M. lar*. Limb regeneration rates were important and therefore tracked in this study for their relevance to territory defense. There was a significant inverse relationship shown between effective territory defense and limb autotomy. Skinner and Graham (1970) found that when the Bermuda land crab *Gecarcinus lateralis* loses numerous pereopods or both

chelipeds it undergoes almost immediate preparations for molting with attendant limb regeneration. However, the adult intermolt period for *G. lateralis* was found to be 180 to 300 days after acclimation to laboratory conditions, and in all groups missing limbs, *G. lateralis* molted after a mean elapsed time of 53 days (Skinner and Graham 1970).

As discussed previously, the *M. lar* individuals in the present study molted about every 21 days. *Macrobrachium lar* individuals regenerated a single lost cheliped in 20.4 ± 1.9 days ($n = 7$). *Macrobrachium lar* individuals regenerated also both lost chelipeds in 17.6 ± 1.7 days ($n = 9$). Additional data are required to verify limb regeneration rates in *M. lar*, but these preliminary results support Skinner and Graham's (1970) observations.

Another aspect of *M. lar* cheliped regeneration was the mechanism by which the task was accomplished. Taylor and Kier (2003) have shown that the soft water-inflated body of newly molted blue crabs may rely on a hydrostatic skeleton, similar to that of worms and polyps. Taylor and Kier (2003) described how these blue crabs take in water to both cast off the old exoskeleton and move legs and claws until the new exoskeleton hardens. It is probable that *M. lar* used water in a similar way to inflate hydraulically newly regenerated chelipeds, thus facilitating the observed length increase of up to 400%. After 48 hours elapsed, *M. lar* achieved a hardened exoskeleton and had the ability to grip effectively with the new chelipeds if not attacked by conspecifics in the tank. Limb regeneration in *M. lar* is critical to effective territory defense, in that the presence of the intact cheliped facilitated the maintenance of or a complete loss of a territory.

When fully intact *M. lar* individuals encountered one another, they defended equivalent territory sizes. When fully intact individuals encountered conspecifics with one or both chelipeds autotomized, the fully intact conspecifics defended significantly larger territories. The conclusion is that *M. lar* requires intact chelipeds to defend maximum

territory size.

This study identified several items that warrant further investigation. The rapid limb regeneration in *M. lar* has only been described incompletely in mechanistic terms. While the hydraulic inflation mechanism suggested in this work was plausible, a more detailed mechanism description would be desirable. This mechanism would need to incorporate models for muscle attachment and innervation. Also, *M. lar* was observed to employ forward gliding motility and this could be investigated for its behavioral and ecological significance. Another undescribed behavior observed in *M. lar* was an asymmetric charging posture. This behavior could be investigated for possible behavioral and ecological significance because it might facilitate some selective advantage.

Finally, captive *M. lar* developed progressively lighter coloration while in captivity. Wild prawns were dark brown in color, but individuals in captivity became more grey with each molt. Possible explanations might include different diet in captivity, less exposure to direct sunlight in captivity, or possibly even adaptation to artificial substrata lighter in color compared with freshwater stream beds. So, further investigation could uncover the physiological mechanism responsible for this observed color change and its significance.

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