

MORPHOTYPIC DIFFERENTIATION OF MALE MALAYSIAN GIANT PRAWNS, *MACROBRACHIUM ROSENBERGII*

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ABSTRACT

Mature male *Macrobrachium rosenbergii* differentiate into 3 distinct morphotypes differing in behavior and growth rate. We provide an operational definition of these morphotypes using relative growth techniques supplemented by observations on color and spination. Transition from the small male to the orange claw morphotype was gradual and a recognizable intermediate form, the weak orange claw, was identified and characterized. The shift from the orange claw to the blue claw morphotype occurred at a single metamorphic molt. Maturation from a juvenile male, lacking an appendix masculina, to the small male morphotype was not accompanied by an allometric change in the claw segments. Recognition of the morphotypes will be useful for genetic work using controlled matings between blue claw males and appropriate females and will facilitate experimentation on growth, behavior, and the evolutionary significance of morphotypic differentiation in *M. rosenbergii*. This analysis also implicates the androgenic gland in the control of growth rates of these prawns.

The Malaysian giant prawn *Macrobrachium rosenbergii* (de Man) has received considerable attention as a fresh-water aquaculture organism. Although growth rates are often quite high in these intensive culture systems, it has been observed that male growth rates are highly variable (Fujimura and Okamoto, 1972; Smith *et al.*, 1978; Brody *et al.*, 1980; Ra'anan, 1982; Malecha *et al.*, 1984). Recently, Ra'anan (1982) and Ra'anan and Cohen (1985) observed that differences in growth rate of adult prawns were associated with three morphologically distinguishable kinds of males. These morphotypes were partially characterized based on animals recovered from ponds at the end of the summer growth season. Ra'anan and Cohen (1985) and Ra'anan and Sagi (1985) demonstrated that all three morphotypes were sexually mature, but had markedly different growth rates. Some of the large males had very long second pereopods (claws) that were deep blue in color and were termed blue claw (BC) males (Ra'anan, 1982). These males were dominant and territorial (Ra'anan, 1982), sequestered postmolt adult female prawns prior to mating (Ra'anan, 1982; Sagi, 1984; Ra'anan and Sagi, 1985), and grew slowly (Ra'anan, 1982).

Sandifer and Smith (1977) noted the presence of orange-clawed males in ponds. These males were also large and had long claws (but shorter than BC males) that were usually orange in color and therefore were called orange claw (OC) males by Ra'anan (1982). These subordinate animals were not territorial, had poor mating success, and had very high growth rates (Ra'anan, 1982; Ra'anan and Sagi, 1985).

The remaining males were small and had short claws that were often relatively unpigmented and translucent. These were termed small (SM) males. These animals were subordinate, not territorial (Ra'anan, 1982), and mated with females using "sneak" reproductive behavior in the presence of BC males (Sagi, 1984; Telecky, 1984; Ra'anan and Sagi, 1985). The SM males grew slowly (Ra'anan, 1982).

Mature sperm can be demonstrated in the sperm ducts of all three types of adult males (Sagi, 1984). The three morphotypes of adult males can further be distinguished from undifferentiated juvenile males (UN), since the latter lack an appendix masculina on the endopod of the second pleopod.

Development of the adult male morphotypes is an environmental consequence,

strongly influenced by the social organization of prawn populations (Ra'anan and Cohen, 1985). Selective removal of the large BC and OC males permits SM males to grow rapidly and differentiate into the BC and OC males. Thus, differentiation of these forms of males does not appear to be primarily genetically determined.

Herein we characterize these male morphotypes allometrically and provide supplemental observations on claw color and surface sculpture. By following individually marked animals we have also described the transformation between morphotypes. This information will permit more precise experimental work on the physiological basis of morphotypic differentiation in *M. rosenbergii*.

MATERIALS AND METHODS

Animals were taken from ponds at Kalya, Afkim, and Ginnosar, Israel, 2 months after these ponds were stocked with hatchery-reared juvenile prawns of the same age. Additional measurements were made on breeding stock at the Genetics Experimental Farm of the Hebrew University in Jerusalem and a hatchery in Ein Hamifratz operated by Aquaculture Production Technology (Israel), Ltd. One additional set of measurements was taken from an animal captured in the Sakhneh, a geothermally heated stream containing prawns released from the Nir David nursery. This animal was at least 1.5 years old. Observations on color and spination were confirmed and extended using pond-reared animals from Amoriant, Inc., Hawaii, and the University of Hawaii, Prawn Aquaculture Program, Honolulu, Hawaii.

We used allometric growth techniques to distinguish the male morphotypes and to follow growth of animals from one form to another. Relative growth was described by the power function $y = \alpha x^\beta$, where x is the reference dimension, y is the dependent variable, and α and β are constants. This curvilinear relationship becomes linear when logarithmically transformed, so that $\log y = \beta \log x + \log \alpha$, where α represents the size of y at $x = 1$, and β is the slope of the transformed equation and describes the rate of growth of the dependent variable relative to the reference dimension. If $\beta > 1$, allometry is positive; if $\beta < 1$, allometry is negative, and if $\beta = 1$, growth is isometric (Teissier, 1960; Gould, 1966; Hartnoll, 1982). Growth will be considered isometric if $0.9 < \beta < 1.1$ (Kuris and Carlton, 1977).

Generally the reference dimension is selected as the best representative measurement of overall size. Three potential reference dimensions were considered. Wet weight was recorded to 0.01 g if the animal was < 10 g; if > 10 g, it was recorded to 0.1 g. Total length was measured with a ruler (to 1 mm) from the posterior margin of the orbit to the tip of the telson when the animal was extended. Carapace length was measured with a vernier caliper (to 0.1 mm) as the distance from the posterior margin of the right orbit to the posterior margin of the carapace at the midline.

All dependent variables were measured with a vernier caliper to 0.1 mm. All limb joints were measured on the second pereopods (chelipeds, claws). The dactyl was measured from the proximal dorsal midline to the distal tip. The propodus, with the joint flexed, was measured along the lateral face from the proximal lateral condyle to the distal tip. Propodus width was measured from the dorsal to the ventral midline of the propodus just proximal to its articulation with the dactyl. The carpus was measured when flexed from the end of the proximal lateral condyle to the distal margin along the lateral face. The merus and the ischium were each measured along the dorsal midline from the proximal to the distal margins. Our measurements of merus and ischium were similar to those of Nagamine and Knight (1980). All measurements represented the maximum dimensions that could be measured without damaging the living animals. Total claw length (Ra'anan, 1982) was measured along the extended cheliped with a ruler (to 1 mm), from the proximal base of the ischium to the distal tip of the propodus along the ventrolateral surface.

Regression statistics were computed with SAS (Statistical Analysis System; Ray, 1979) programs. Other statistics were calculated according to Sokal and Rohlf (1981).

RESULTS

Determination of Morphotypes

Color.—Color generally provides a reliable basis to separate OC from BC males. The color of all segments of the claw of living BC males is deep blue. In contrast, the propodus of OC males is usually orange. However, the distal tip of the propodus and its proximal dorsal surface may be suffused with blue in OC males. The medial face of the propodus of OC males is often white or pale blue. At ecdysis

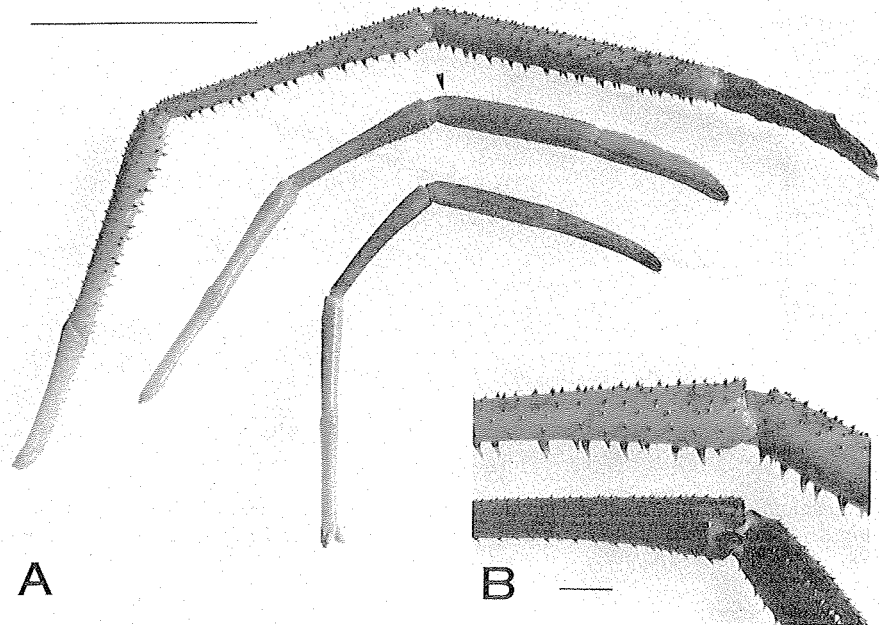


Fig. 1. A. Chelipeds of *Macrobrachium rosenbergii* from BC (top) and OC (middle and bottom) morphotypes. The OC claws are represented by the typical SOC form (middle) and the WOC form (bottom) that is transitional from the SM morphotype as discussed in the text. The chelipeds shown here were removed from prawns of similar size (carapace length 39–41 mm). The scale bar represents the mean carapace length of 40 mm. The arrowhead indicates the small blue area on the proximal part of the propodus of an OC prawn. B. The inset compares spination on the carpus of a BC male, carapace length = 39 mm (top) and an SOC male, carapace length = 44 mm. All prawns were obtained from Amorient ponds on Oahu, Hawaii.

the proximal blue area of the propodus is confined to that portion of the cuticle proximal to the ecdysial suture (Fig. 1) which splits to facilitate removal of the soft postmolt claw from the old exoskeleton of the propodus (Kuris, in preparation). In animals reared in laboratory aquaria or dark outdoor tanks the entire propodus of OC males is often blue or bluish green but even here the color does not match the intensity of the deep blue propodus of the BC males. Color of pond-reared animals from Hawaii (Anuenue stock) was indistinguishable from the color of the animals from Israel.

The claw color of SM males is quite variable and it is sometimes difficult to distinguish SM from OC males on the basis of color alone. One common color pattern of the SM males includes a blue fixed finger of the propodus, a red spot on the propodus at the articulation with the dactyl, and a red band on the distal end of the carpus. Most of the propodus is white or faintly blue in color. The intensity of color in the SM males also varies with environmental background color.

Spination.—Spination of the chelipeds is a reliable means to distinguish OC and

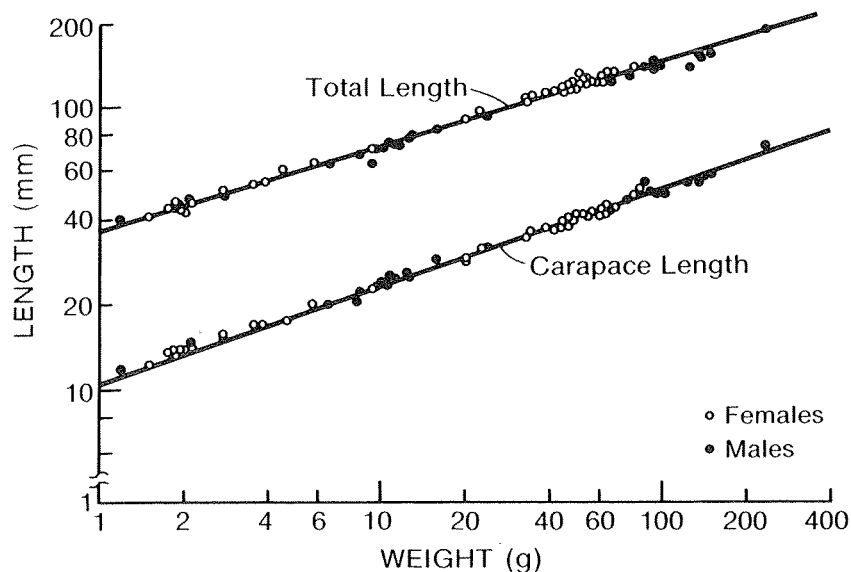


Fig. 2. Relationship between total length and carapace length compared with body weight for *Macrobrachium rosenbergii* after logarithmic transformation. See Table 1 for sample sizes and regression statistics.

BC males. In BC males the spines are long and robust. They may add 7% (range 2–11%, $N = 10$) to the width of the propodus. In life the spines are deep blue in color. After preservation in 70% ethanol, the basal 30–50% of the spines is opaque while the tips are transparent. In comparison, the spines of the OC males are small and fragile (readily crushed with a vernier caliper). In living animals they are orange in color with a black horny tip. When preserved the entire spine is transparent. The spines on the claws of SM males resemble those of OC males.

The orientation of the spines also differs between BC and OC morphotypes. The spines on the merus, carpus, and propodus of the BC males point distally at an angle of 60–75° ($N = 10$ spines on each of 6 animals, measured along the medial face of the carpus and propodus). In contrast, the spines on the chelipeds of OC males form a more acute angle of 30–45° ($N = 10$ spines on each of 10 animals). This difference in spine orientation is evident in Fig. 1. Spination of animals from Hawaii and Israel was indistinguishable.

Reference Dimension.—Weight, the usual measurement of overall growth in *M. rosenbergii*, does not provide the most useful reference dimension for a study of the relative growth of the claws because changes in claw size will also contribute to changes in weight. Ideally the reference dimension and the relative growth measure should be independent. However, since weight is so widely used as a measurement of growth in *M. rosenbergii*, it is desirable to determine the relationship between weight and the other potential reference dimensions, carapace length and total length.

The logarithmic relationships between weight and each of these other dimensions is linear and does not differ between sexes (Fig. 2). Large males tend to fall below the regression line because their large claws add disproportionately to their weight in relation to either total length or carapace length. Carapace length was selected over total length as the reference dimension because the former: (1) is

more precisely measured (abdominal flexure and extension affect estimation of total length); (2) may be measured as accurately on exuviae as on intact animals; and (3) has a higher slope when regressed on weight (Table 1) than does total length. This suggests that a change in carapace length more closely represents gain in weight than does an equivalent change in total length.

Relative Growth Analysis of Morphotypes

The existence of the three male morphotypes was first detected in pond-reared prawns; therefore, we used animals from such populations to establish morphometric criteria to distinguish these morphotypes. These animals were obtained from ponds at Kalya, Afikim, and Ginnosar, Israel. Animals from these localities were combined in the analyses, since no differences between localities were detected (Bartlett's test, ANCOVA). Propodus length, carpus length, and propodus width provided the most information and are treated in detail here.

Characterization of BC Males.—Prior to the relative growth analysis, all measured animals were classified into morphotypes on the basis of color, spination, and total claw length (this preliminary classification was done by AS and ZR who had considerable experience in recognition of morphotypes using these features). The relative growth analysis showed complete agreement with these other attributes for the recognition of BC males. The BC males are readily distinguished from other male morphotypes, having dramatically greater propodus and carpus lengths (Figs. 3, 4) in relation to carapace length. The BC measurements were disjunct from the OC measurements and no intermediate or indeterminate animals were observed in pond populations in the course of this study (see below for laboratory broodstock data). Carpus length is the best discriminator of BC from OC males (Fig. 4). The carpus of a BC male averages 61% longer than that of an OC male of similar size. By comparison, the difference between these males in terms of propodus length is only 47%. Within the BC morphotype, growth of the propodus is positively allometric, $\beta = 1.26$. However, growth of the carpus is more nearly isometric, $\beta = 1.09$.

The BC morphotype is also readily distinguished in the analysis of cheliped length in relation to total length (Fig. 5), the morphometric criterion previously used (Ra'anani, 1982; Ra'anani and Cohen, 1985) to determine morphotypes. The total length of the cheliped of BC males is unequivocally greater than for other males of similar body size. The relatively long propodus and carpus of the BC morphotype account for this increase in cheliped length. Accordingly, total cheliped length shows strong positive allometry, $\beta = 1.31$.

Comparison of propodus width with propodus length disclosed that here too BC males were readily distinguished from the other morphotypes (Fig. 6). Even though the propodus width measurement of the BC males included the prominent cuticular spines, the propodus of BC males was relatively narrower than the propodus of other male morphotypes. However, occasional BC males exhibited a relatively wide propodus (Fig. 6).

Redefinition of Morphotypes on the Basis of Propodus Length.—Surprisingly, after removal of the readily identified BC morphotype from our analysis, three groups of males still remained. The correlation for log propodus length on log carapace length of the non-BC males was very highly significant ($r = 0.989$). Despite this, examination of the residuals for this regression revealed that this relationship was nonlinear (Fig. 7). A saddle-shaped distribution of residuals indicated that the relationship was either curvilinear or it was composed of at least two overlapping

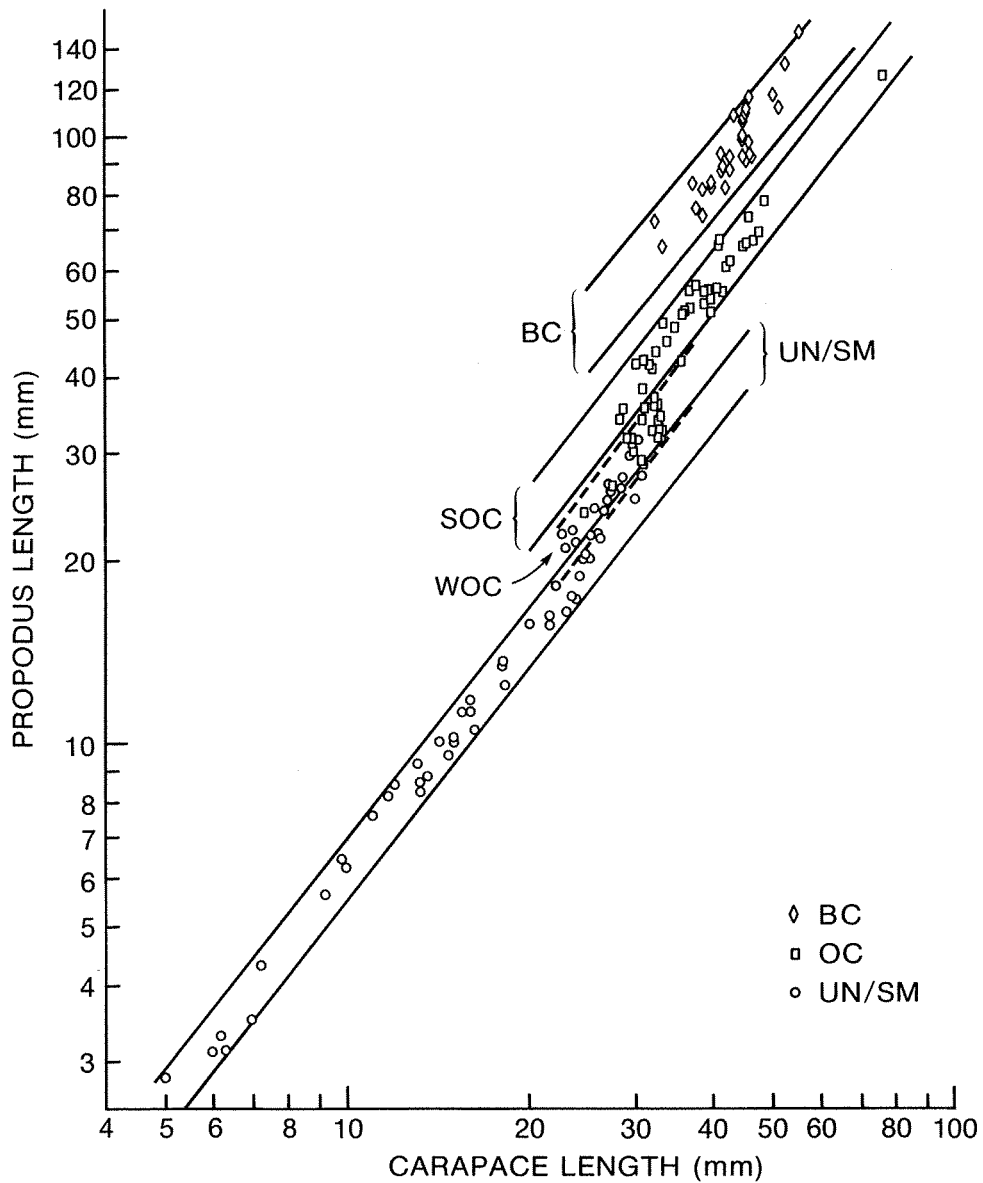


Fig. 3. Relationship between log carapace length and log propodus length for male *Macrobrachium rosenbergii*. Morphotype labels for individual points are based on field determinations by ZR and AS. Solid lines are the upper and lower 90% predicted confidence limits for the BC, SOC, and SM/UN morphotypes (from top to bottom). The confidence intervals shown here for SOC and SM/UN animals are, respectively, those extended from the calculations based on OC animals ≥ 34.0 -mm carapace width and < 22.0 -mm carapace width as discussed in the text. The dashed lines represent the 90% confidence interval for WOC males excluded from the SOC and UN/SM categories as described in the text.

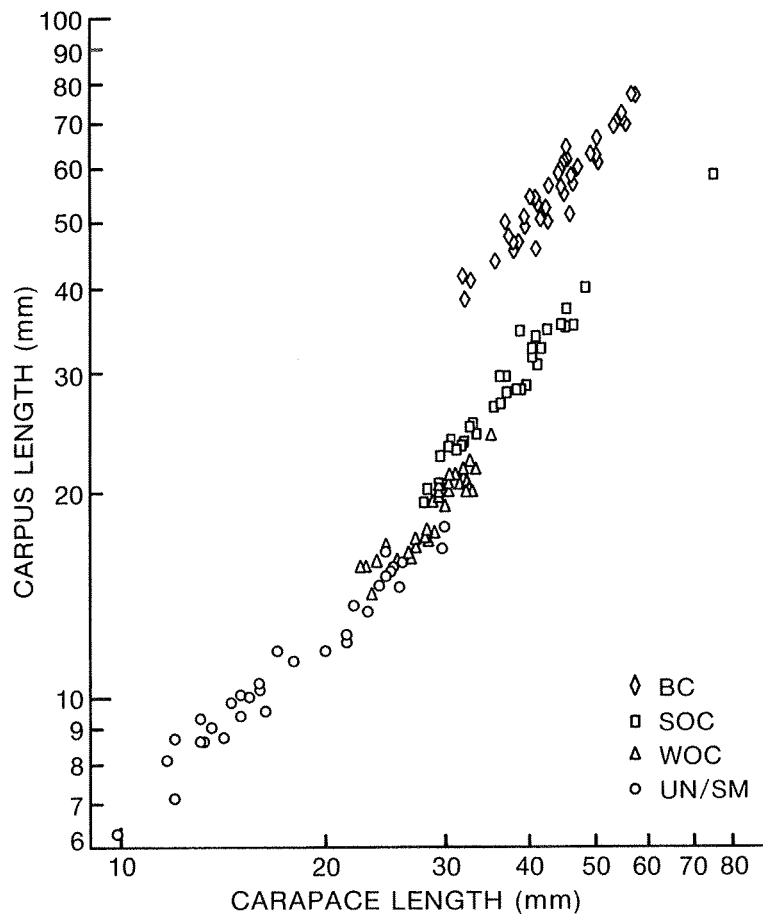


Fig. 4. Relationship between log carapace length and log carpus length for male *Macrobrachium rosenbergii*. Morphotype labels are based on field identification for BC males. For the separation of UN/SM, WOC, and SOC males, morphotype labels are based on the analysis of linearity and 90% confidence intervals of the propodus length-carapace length relationship as described in the text.

linear clusters. Inspection of Fig. 3 suggested that each end of this distribution was linear. Hence, regression statistics were obtained for all males ≥ 34.0 mm carapace length and for those < 22.0 -mm carapace length (Table 1). Since all the males ≥ 34.0 -mm carapace length were classed as OC by AS and ZR in the field, this group tentatively represented the OC males. The 32 males < 22.0 -mm carapace length had all been termed SM or UN males upon capture. Most important, the residuals for each of these groups were evenly distributed about the regression line confirming that each was linear.

To assign the animals in the 22.0–33.9-mm interval of carapace length to either the OC or UN/SM groups, the predicted 90% confidence limits were calculated for males ≥ 34.0 mm. The width of the 90% confidence interval at the lowest observed value of x , 35.7-mm carapace width, was similar to the confidence interval at the grand mean, 40.8 mm, exceeding the latter by less than 5%. The error in the confidence interval at the grand mean reflects only the error in the estimate of the intercept and not the error in the slope. Therefore, the estimate

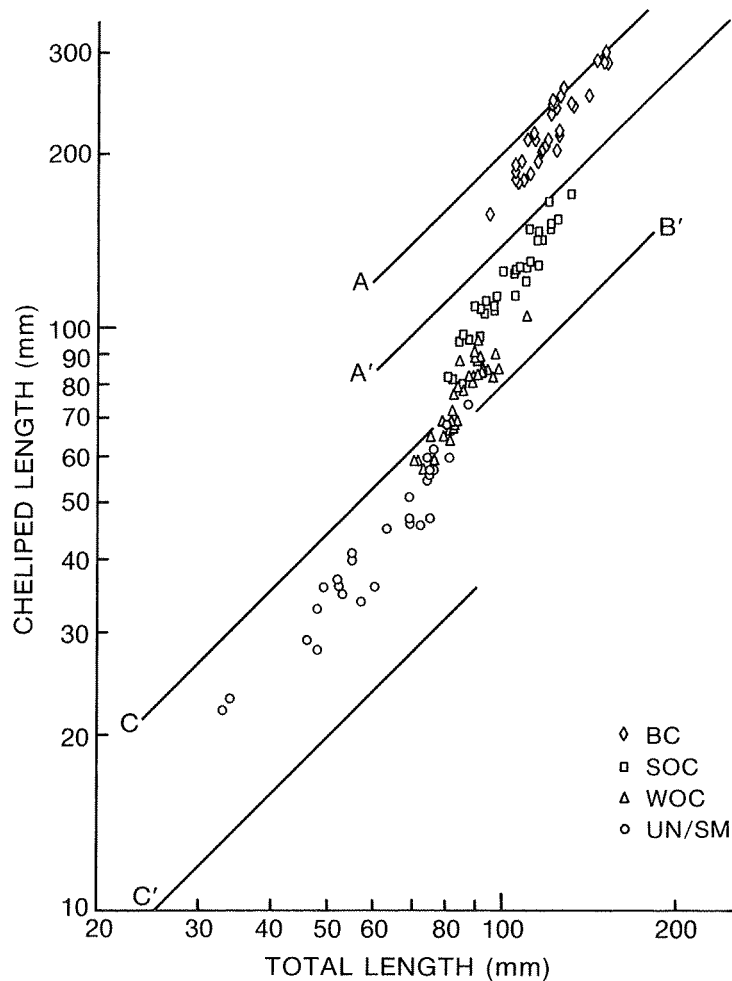


Fig. 5. Relationship between log total length and log cheliped length for male *Macrobrachium rosenbergii*. Morphotype labels as in Fig. 4. The diagonal lines represent the upper and lower proportional limits of the morphotypes as previously defined by Ra'anan (1982). Lines A, A' include the BC males, A', B' include OC males, and C, C' include SM males.

of the slope was very precise, the variance at limiting values was low, and the confidence interval scarcely expanded as it departed from the grand mean. Thus, extrapolation of the confidence interval beyond the observed range of x would be a suitable method to classify observations below a carapace length of 34.0 mm. Hence, the confidence limits were extended below 34-mm carapace length and observations that fell above the lower confidence limit were now included in the OC classification. Residuals for these 13 observations were evenly distributed about the regression line calculated for animals ≥ 34 -mm carapace length. Addition of these observations to the OC group increased the slope and the correlation coefficient (Table 1, compare OC ≥ 34 mm with SOC). These additional animals had also been classified as OC in the field.

Similarly, the confidence intervals for the SM/UN group were extended above 22.0-mm carapace length. Eleven observations below the extended upper confi-

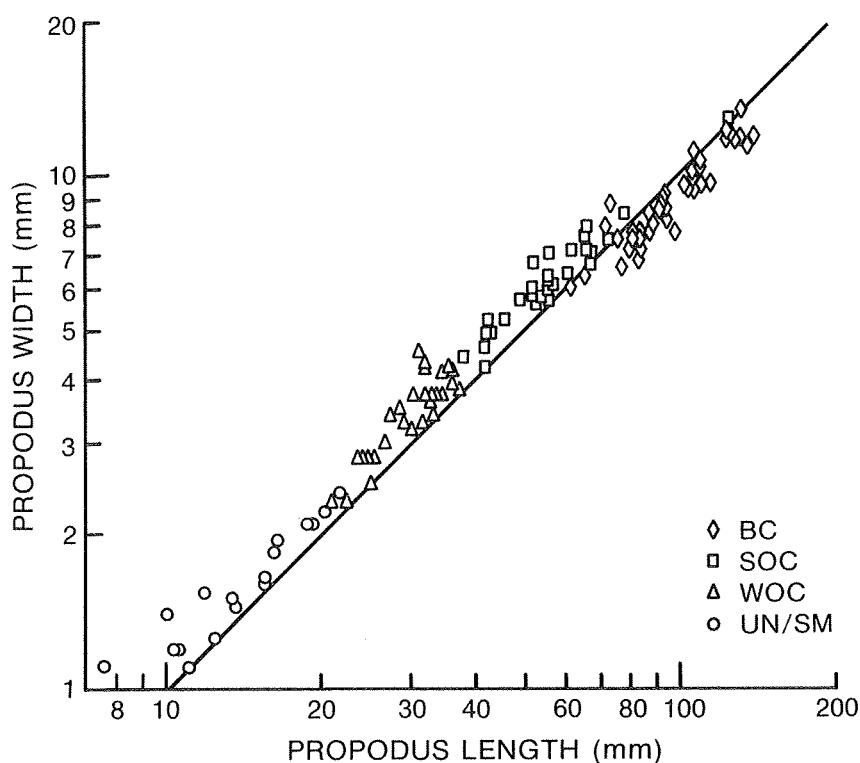


Fig. 6. Relationship between log propodus length and log propodus width for male *Macrobrachium rosenbergii*. Morphotype labels as in Fig. 4. The line represents the isometric relationship represented by the ratio of 10:1 for propodus width : propodus length.

dence limit were included in the SM/UN classification. Inclusion of these observations had a negligible effect on the regression and correlation coefficients (Table 1, compare UN, SM <22 mm with all UN, SM). The 90% confidence intervals, extended beyond the observed data as described above, are shown in Fig. 3.

The important result of this reclassification process was the emergence of a set of observations that belonged to neither the OC nor the UN/SM groups as defined above. Most of these animals were classified as OC in the field (21 of 33), the remainder being termed SM. For this group, field identifications were often tentative or the animals were described as "becoming OC." Since most of them had been previously regarded as OC and since their growth performance in population studies was similar to larger OC males, they are termed weak orange claw (WOC) males. The 90% confidence intervals for log propodus length on log carapace length were calculated for the WOC males (Fig. 3). The morphotypic significance of this group will be treated in detail below. In contrast, OC males that fell within the confidence limits of the regression for OC males ≥ 34.0 mm will be termed strong orange claw (SOC) males.

Examination of Fig. 3 shows that WOC and SOC males are progressively larger in terms of body size (carapace length) than are UN/SM males. The size ranges of SOC and BC males overlap substantially. The slope of the log propodus length on log carapace length regressions increases progressively for the UN/SM, WOC, and SOC males, all being positively allometric (Table 1).

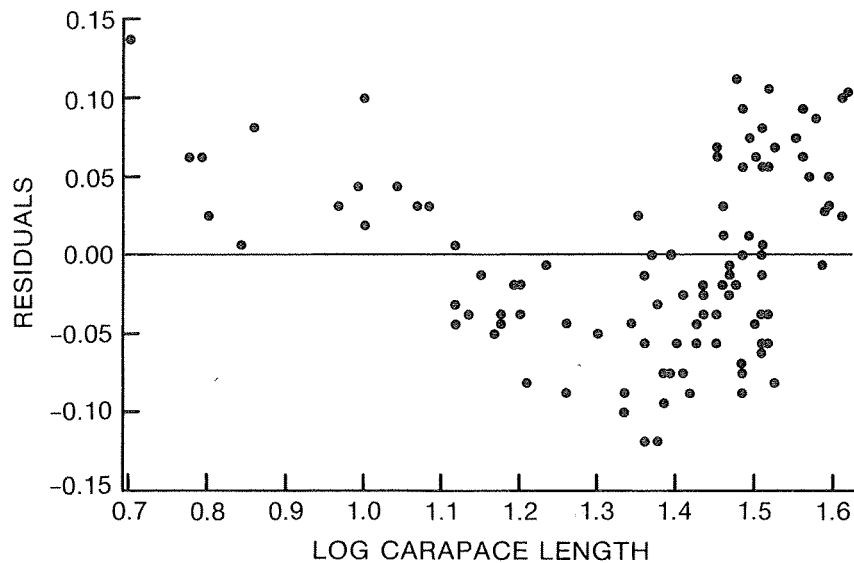


Fig. 7. The relationship in *Macrobrachium rosenbergii* between log carapace length and residuals of log propodus length following linear regression of the logarithmically transformed data.

Further Description of the UN/SM, WOC, and SOC Males.—Having operationally defined UN/SM, WOC, and SOC males on the basis of the propodus length-carapace length relationship, the efficacy of this classification procedure can be tested by comparing these three types of males using other morphometric relationships. For the logarithmically transformed carpus length on carapace length relationship all three groups exhibited linear and highly correlated associations (Table 1, Fig. 4). Slope for SM/UN males was negatively allometric, became isometric for WOC males, and positively allometric for SOC males. Unlike the propodus, growth of the carpus was isometric for BC males. Figure 4 shows considerable overlap for the carpus-carapace length relationship of WOC and UN/SM males.

When length of the extended cheliped was compared with total length of the body, the same general relationship emerged. All three groups of males showed a positively allometric slope with the WOC males having the highest value. Again, the data for the SM/UN and WOC groups overlapped.

The propodus width-propodus length relationship was constant over the range of UN, SM, WOC, and SOC males. Growth was isometric and always exceeded a ratio of 10:1 for propodus width : propodus length (Fig. 6). For BC males growth was also isometric, but the ratio of propodus width to propodus length usually did not exceed 10:1.

The only male captured from the seminatural escaped population in the geothermal warm spring, the Sakhneh at Kibbutz Nir David, proved to be the largest animal in the study (body weight = 230 g). It was an SOC on the basis of spination and relative dimensions, although the color of its propodus was bluish green. Its relative dimensions are plotted in Figs. 2–4. These measurements were not used to calculate regression and correlation statistics (Table 1), since, as an outlier, this male had a disproportionate statistical influence.

Maturation of Juvenile to Adult Males.—The SM males could not be distinguished

Table 1. Regression constants and correlation coefficients for relative growth data of *Macrobrachium rosenbergii*. All data were logarithmically transformed. W = weight, TL = total length, CCL = carapace length, CUL = carpus length, CHL = cheliped length, PL = propodus length, N = sample size, β = slope, α = intercept, r = correlation coefficient.

Morphotype, sex, size range (carapace length)	Independent variable	Dependent variable	N	β	α	r
Both sexes, all morphotypes	W	TL	90	0.298	36.99	0.995
Both sexes, all morphotypes	W	CCL	93	0.340	10.91	0.998
BC males	CCL	PL	42	1.265	0.80	0.936
UN, SM, and OC males	CCL	PL	108	1.552	0.17	0.989
OC males ≥ 34.0 mm	CCL	PL	19	1.242	0.60	0.880
All SOC males	CCL	PL	32	1.403	0.33	0.960
WOC males	CCL	PL	33	1.400	0.26	0.933
UN, SM males < 22.0 mm	CCL	PL	32	1.277	0.33	0.991
All UN, SM males	CCL	PL	43	1.282	0.32	0.994
Females	CCL	PL	67	1.359	0.29	0.993
BC males	CCL	CUL	42	1.094	0.89	0.956
SOC males	CCL	CUL	32	1.211	0.36	0.974
WOC males	CCL	CUL	33	1.025	0.60	0.913
UN, SM males	CCL	CUL	31	0.852	0.96	0.961
Females	CCL	CUL	63	0.986	0.71	0.985
BC males	TL	CHL	32	1.312	0.41	0.919
SOC males	TL	CHL	32	1.375	0.20	0.935
WOC males	TL	CHL	35	1.543	0.08	0.891
UN, SM males	TL	CHL	29	1.174	0.35	0.964
Females	TL	CHL	15	1.230	0.31	0.988

from undifferentiated juvenile (UN) males on the basis of the available relative growth data. Undifferentiated juveniles could readily be distinguished by the presence of a gonopore with flaps at the base of the fifth pereopod. An appendix masculina on the endopod of the second pleopod characterizes SM and other adult males. The UN males also lack an appendix masculina. All animals < 10 -mm carapace length were UN males. The UN males exhibited the same allometric relationship for propodus length on carapace length as did the SM males (Fig. 3).

Hatchery Broodstock, Aquarium, and Tank-reared Males.—Analysis of measurements from hatchery broodstock and other animals confined to aquaria and tanks showed these morphotypes to be readily recognized on the basis of the relative propodus and carpus dimensions, even though color of the propodus of OC males was often relatively bluish green or blue. Spination was also always a reliable character to separate BC from SOC males. Animals reared in aquaria frequently exhibited morphometric relationships characteristic of WOC and SOC males at carapace lengths smaller than typically seen in ponds. This suggested that environmental factors such as crowding may influence the size threshold at which transformations between morphotypes may occur.

Broodstock aquaria at the Genetics Experimental Farm and Ein Hamifratz typically contained six large males. One (at most two) of these males showed all the characteristic features of BC males as described above. The remaining animals showed SOC morphology, although one or both chelipeds were frequently missing, regenerated, or deformed. Four males, however, had the spination of a BC male but the morphometrics of the SOC morphotype. The frequency of such males among the 46 large males observed in broodstock populations suggests that this

Table 2. Change in the relative growth of propodus length as measured by the slopes of logarithmically transformed data for the propodus length-carapace length relationship following individual molts of *Macrobrachium rosenbergii* in the laboratory. Slopes of the regression lines for each of the morphotypes from the field data are included for comparison. Student's *t*-statistic and the probability that the mean slope of the observed molts were drawn from a population having the mean of the regression slopes are also provided. NS = not significant.

Morphotype before and after molt	<i>N</i>	Slope of observed molts	Slope of regression line for morphotype	<i>t</i>
UN/SM → UN/SM	28	1.33 ± 0.52	1.28	0.59, NS
SM → WOC	3	2.32 ± 0.91	—	—
WOC → WOC	7	1.70 ± 0.59	1.40	1.59, NS
WOC → SOC	9	4.69 ± 1.94	—	—
SOC → SOC	10	2.08 ± 0.60	1.40	3.06, 0.01 < <i>P</i> < 0.05
SOC → BC	6	14.88 ± 5.51	—	—
BC → BC	0	—	1.26	—

is a regular occurrence under these conditions. A similar male was photographed by Nagamine *et al.*, (1980: fig. 2), and termed a "normal mature male." The significance of such males is unclear. No such animals were ever recognized among the many pond-reared males examined.

Morphotypic Transformation

To study the transformation of males from one morphotype to another, male prawns were measured and their growth followed over one or more molts. Some animals were obtained from grow-out ponds, measured, held until they molted in laboratory aquaria, and measured again 1–2 days after ecdysis. Since size of the postmolt cuticle is determined in early premolt (Kuris and Mager, 1975), this approach provides measurements of growth under field conditions. When these measurements were compared with those of animals held in the laboratory for several successive molts, no differences were noted. Therefore both groups were combined in the following analysis.

In Fig. 8 the growth of individuals is plotted in terms of the propodus to carapace length relationship and compared with the morphotypic boundaries as defined by the 90% confidence intervals described above. The slopes of propodus length on carapace length were calculated for all individual molts. If animals molt and do not change morphotypes, then for the propodus length-carapace length relationship, the mean slope of these individual molts will approximate the slope of the regression for the allometric relationship. The mean values of these observations were compared (*t*-test, Table 2) with the regression slopes (from Table 1) being used as the parametric value. For the molts within morphotype, the UN/SM and WOC observations were not significantly different from the parametric value, while the SOC observations had a marginally significant higher slope. If the slope of an individual molt crossed the 90% confidence limit of a morphotype, it was defined as a transformation to the next morphotype. Molts involving a change in morphotype had a higher slope (Fig. 8, Table 2) than did the within-morphotype molts.

Defining a transformation between morphotypes by the criterion of whether the slope of an individual molt crosses a line (the 90% confidence limit) and then comparing the slopes of those that cross the line (transform) with those that do not cross (retain the same morphotype) are informative procedures. They both

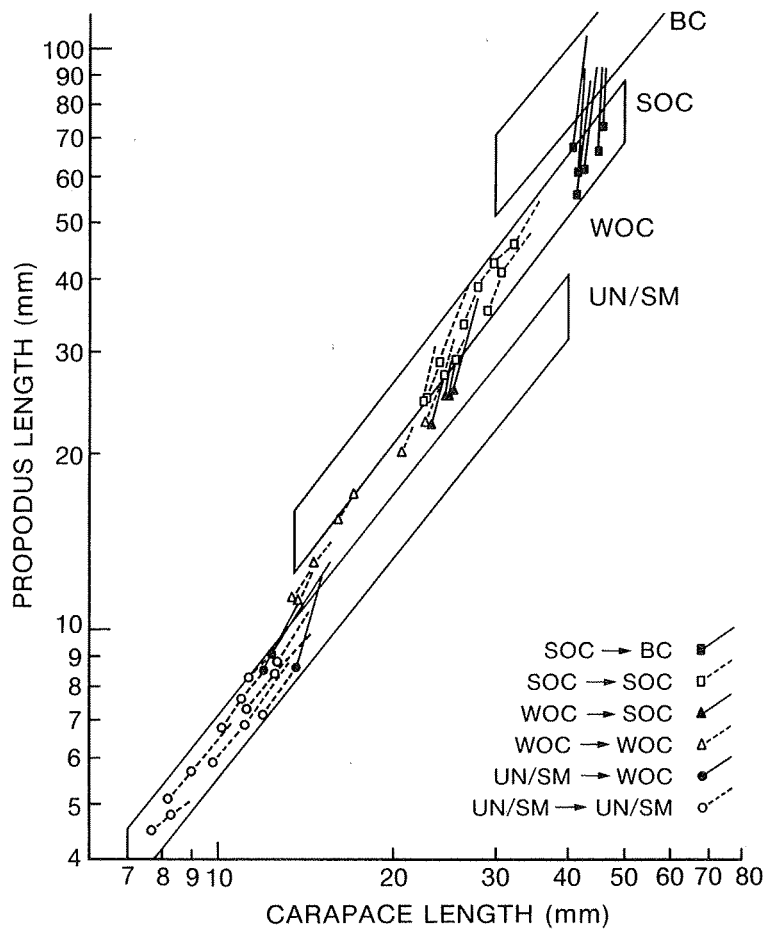


Fig. 8. Relative growth of log propodus length versus log carapace length for individual male *Macrobrachium rosenbergii* before and after one or more molts. Areas labeled UN/SM, SOC, and BC represent the 90% confidence interval of the respective morphotypes. The locations of individual animals before and after a molt were used to identify their morphotypes and determine whether a molt had involved a transition from one morphotype to another. For visual clarity, 15 UN/SM to UN/SM, 1 WOC to SOC, and 2 SOC to SOC molting observations were eliminated from the figure.

corroborate the hypothesis that growth involves morphotypic transformation and also show whether we have established the approximate boundary of the morphotypes using the 90% confidence limits. Consider the following cases. (1) If morphotypic transformation (a high slope at a transformation molt) does not occur, then individual growth slope measurements that cross a line (any line) will, on the average, have somewhat higher slopes than those that do not cross such a line. However, some molts initiated above such an arbitrary line would also cross the line and thereby show that such a line did not, in fact, define a morphotypic boundary (alternatively such molts could be considered a reversion to the earlier morphotype). (2) If morphotypic transformation does occur but the boundaries have been incorrectly determined, then the set of slopes crossing such a boundary line will perform include "false positives": nontransforming molts having a low slope. Similarly, the set of slopes that do not cross the line will include "false

negatives," transforming molts having a high slope. The more incorrectly such boundary lines are placed the less likely there will be a significant difference between the predicted high slopes of transformation molts and the low slopes predicted for the within-morphotype molts on either side of the boundary. Further, when tested against the regression slopes as parametric values for each morphotype (as above), the individual slopes will tend to be significantly greater than such a parametric mean. (3) If morphometric transformation did occur and the boundaries are correctly placed, then the set of slopes crossing the line will be significantly higher than those that do not. Further, the latter molts (within morphotype) will not have significantly greater slopes than the parametric mean. Thus, this test is conservative. Errors in the data on individual slopes or the location of the 90% confidence limits will tend to support the null hypothesis that transformation molts do not have a higher slope than within morphotype molts.

The first case is rejected, since no individual molts ($N = 63$) "reverted" to an earlier morphotype. Inspection of Table 2 shows that the morphotypic boundaries appear to be correctly placed for the SM to WOC boundaries, because the slopes of the individual molts within the SM and WOC morphotypes were not significantly different from the regression slopes for those morphotypes. Also, the slopes of the transforming molts (SM to WOC, WOC to SOC) were significantly higher than the molts within SM and within WOC morphotypes. However, the slopes of the individual molts within the SOC morphotype were significantly greater than the regression slope for the SOC animals.

Individual growth records for animals originally in the WOC morphotype were highly variable. Some molts were not accompanied by a change in morphotypic status. However, most growth records for animals initially classified as WOC were accompanied by a large increase in the relative length of the propodus (Table 2). In most instances this change was sufficient to be recognized as a transformation to SOC. Sometimes two molts appeared necessary to complete this change, as further suggested by the high slopes following the first SOC instar (alternatively these animals may have been misclassified). Thus, the longitudinal growth data support the relative growth analysis. The WOC to SOC transformation is relatively gradual. Our preliminary observations indicate that it usually occurs over one molt but sometimes requires two. These observations suggest that the change from SM to WOC is also recognizable. However, the variation in the slope of the individual molts of the UN/SM group overlaps the range of the SM to WOC transformation molts. In aquaria the UN/SM animals transformed to WOC status at smaller sizes (10-mm carapace length) than animals reared in ponds (minimum 22-mm carapace length) (compare Figs. 3 and 8).

DISCUSSION

Our observations support and extend earlier work (Sandifer and Smith, 1977; Ra'anana, 1982; Cohen and Ra'anana, 1983; Sagi, 1984; Ra'anana and Cohen, 1985; Ra'anana and Sagi, 1985) defining male morphotypes on the basis of color, growth characteristics, and behavior. Four types of males can be identified using the allometric relationship between propodus length and carapace length. Figures 4-6 show that animals classed according to the propodus length on carapace length relationship fall into the same recognizable groups when considered on the basis of relative growth of carapace length, cheliped length, or (for the SOC-BC comparison) propodus width. For all four sets of observations the SOC-BC distinction is always unequivocal, WOC and SOC groups are readily distinguished and the SM and WOC groups overlap but may be statistically recognized.

Since a BC male appears to traverse all the other forms during the course of its development (Ra'anani, 1982; Ra'anani and Cohen, 1985), we will discuss the growth and differentiation of the morphotypes in sequence from juveniles to the BC morphotype. Maturation of juvenile (UN) to adult male morphotypes is accompanied by the development of the appendix masculina on the pleopod (Nagamine and Knight, 1980). Among hatchery-nursed postlarvae, sexual maturation occurs at about 10-mm carapace length. Interestingly, no change in claw shape appears to accompany sexual maturation to the SM morphotype.

After release of UN and SM males into ponds, the rapidly growing larger animals begin to differentiate into other morphotypes. While the SM and WOC groups were statistically recognizable, individuals could not always be assigned to these groups with certainty. Hence, we suggest that WOC males are a previously unrecognized intermediate form between the SM and SOC morphotypes. Since the size of WOC animals was relatively large (10–25 g) early in the growing season, the growth rate of the animals classed as WOC appears to correspond to the rapidly growing (OC) fraction of the population in grow-out pond studies (Cohen and Ra'anani, 1983; Ra'anani and Cohen, 1985). As the slope of the log propodus length on log carapace length relationship of WOC males is higher than for the SM morphotype, differentiation of WOC from SM males increases. The WOC form appears to be a transitory transitional phase from the SM to SOC morphotypes. A molt from WOC to SOC was accompanied by a marked increase in propodus length (Table 2). However, animals intermediate between WOC and SOC are to be expected occasionally. The term "OC" is still useful with reference to both WOC and SOC males.

As seen in Fig. 8 and as may be surmised from Fig. 3, animals of the SOC morphotype may molt several times as SOC males. The molt to the BC morphotype entailed a marked change in length of both the propodus and carpus. This was not accompanied by a proportionate increase in the width of the propodus. With the evident changes in coloration and spination this molt effectively represented a metamorphosis.

Preliminary analyses showed that allometric growth was most positive and proportional variation greatest for the propodus and carpus and least for the merus and ischium. Thus, the former segments contain more information. Indeed, growth of the ischium is nearly isometric to carapace length and was used as the reference dimension by Nagamine and Knight (1980) and Nagamine *et al.* (1980). Further problems with the ischium as a reference dimension include its limited size range which increases the proportional error, and measurement of the ischium which risks autotomy of the limb. Thus, variation in merus length, when compared to ischium length, permitted these workers to detect only a single inflection. Further information on body size and male maturation provided in their study suggests that this inflection probably represented the SM to WOC transformation. Merus and ischium measurements were obtained for 24 specimens assigned to morphotypes on the basis of relative propodus length. The BC males could be distinguished from OC males, since the former had relatively large merus : ischium proportions (Kuris, unpublished observations).

Tazelaar (1930) provided an early allometric analysis of the cheliped of *M. rosenbergii* (= *Palaemon carcinus*). Tazelaar noted a bimodality in claw size of large male prawns collected from a natural habitat in India. Comparison of the size range of her large specimens (40–70-mm carapace length) with Figs. 3 and 5 indicated that her samples contained both SOC and BC morphotypes. This suggests that these morphotypes occur in natural populations as well as in ponds, tanks, and aquaria. Tazelaar also noted a positively allometric inflection in claw

length at a carapace width that corresponds to the SM to WOC transition of the present study. She attributed this change to sexual maturation. Although this inference is commonly made from allometric data for male Crustacea (e.g., Somerton and MacIntosh, 1983), it is inappropriate for male *M. rosenbergii*. Small males are sexually mature (Ra'anan, 1982; Sagi, 1984; Telecky, 1982, 1984; Ra'anan and Sagi, 1985). Yet relative growth of the claw does not permit these adult males to be distinguished from juveniles lacking an appendix masculina (Fig. 2).

Variation in male morphology and behavior has been reported for other crustaceans. Crayfish regularly exhibit two distinctive adult male morphologies. Form I males are capable of breeding. These animals revert seasonally to the nonbreeding form II males (e.g., Stein, 1976; Boyd and Page, 1978). Most of the breeding size males undergo these transitions (Payne, 1972). This seasonal variation is correlated with enlargement of the androgenic gland in the form I males (Carpenter and de Roos, 1970). Thus, behavioral interactions between these types of males are reduced by their seasonal separation. Thampy and John (1973) have claimed a seasonal cycle in male dimorphism for *Macrobrachium idae* and have associated this variability with hypertrophy of the androgenic gland.

Recognition of the morphotypes will have an impact on several areas of research.

(1) The development and characterization of genetic stocks and hybrids for domestication (Malecha, 1980) may benefit by using only BC males for mating experiments. Presently some mating experiments fail because the males attack rather than mate with valuable females of known pedigree (D. Lund, personal communication). This is a behavior associated with OC males (Ra'anan, personal observation).

(2) *Macrobrachium* is one of the genera of carideans with the most species (Holthuis, 1950, 1952). Relative proportions and ornamentation of the second pereiopods of the males provide the diagnostic features of many if not most of the species. Holthuis (1950, 1952) has repeatedly observed that limb proportions of species of *Macrobrachium* change greatly during growth and that some large males have limbs shaped like females or juveniles. Long ago, Coutière (1901) recognized that these animals were sexually mature. For *M. rosenbergii*, at least, these males have now been shown to be a regular, socially mediated component of pond populations (Cohen and Ra'anan, 1983; Ra'anan and Cohen, 1985).

Morphotypic differentiation may be present in other species of *Macrobrachium*. Henderson and Matthai (1910), Holthuis (1950), Thampy and John (1973), and Koshy (1973) remark on the existence of "dimorphic males," "hypertrophied male secondary sex characters," "older male stages," or "mixtures of juvenile and adult males" in other species of *Macrobrachium* (e.g., *M. dayanum*, *M. idae*, *M. malcolmsonii*, and *M. scabriculum*). Reexamination and characterization of possible morphotypes in such species will substantially assist clarification of systematic relationships and species diagnoses in the genus *Macrobrachium*. At this point, as Koshy (1973) noted, use of relative proportions of all or part of the chelipeds in species diagnoses should be avoided. Strong positive allometry in the growth of this appendage (Table 1) results in large differences in the relative proportion of limb segments over the wide size range of adult males.

(3) The evolutionary significance of these behaviorally distinct morphotypes of male *M. rosenbergii* remains to be elucidated and may provide a valuable clue to the more successful manipulation of this complex system in aquaculture. Among crustaceans, such a morphologically based, complex social system is at present unique to *M. rosenbergii*. Parallel phenomena are known for many species of fishes (e.g., Warner, 1984). The suitability of *M. rosenbergii* for experimental

studies may facilitate the quantitative analysis of fitness in such complex life histories.

(4) Although the social aspects of the differentiation of morphotypes in male *M. rosenbergii* have been addressed, the underlying physiological mechanisms of these transformations remain unknown. However, a study of the endocrinological control of sexual maturation in male *M. rosenbergii* (Nagamine *et al.*, 1980) has provided an interesting starting point. Working before the morphotypes were recognized, Nagamine *et al.* (1980) noted two types of males with appendices masculinae present. These males differed in the relative length of the chelipeds. Comparison of sizes of these males with sizes in the present study indicated that these two types of males were SM and OC morphotypes. Andrectomy experiments showed that the androgenic gland was essential for this morphotypic transformation. If this work is confirmed using the more precise assays of morphotypic status developed herein, then some unexpected implications arise. Does the androgenic gland control growth rate of the morphotypes? This role seems possible since growth rate is closely associated with morphotypic status (Ra'anan, 1982; Ra'anan and Cohen, 1985). Heretofore, the androgenic gland has not been implicated in studies of crustacean growth and molting. Studies of the interactions of this gland with the better understood ecdysone-molt-inhibiting hormonal system would be intriguing. Experimental analysis of the role of the androgenic gland in growth of *M. rosenbergii* will be challenging, because such experiments must be conducted in a social context rather than upon isolated individuals. The operational definition of the morphotypes, and the nature of the transitions described herein, will facilitate such analyses.

ACKNOWLEDGEMENTS

We thank the hatchery and pond management staffs of Kibbutzim Nir David, Ein Hamifratz, Kalya, Almog, Afikim, and Ginnosar for their cooperation and generous assistance with this project. We also thank Jeffrey Shields for his able assistance with data collation and statistical computation, Dottie McLaren for the illustrations, and Becky Boehrs for manuscript preparation. John Born, Jeffrey Shields, Keith Nelson, Peter Connors, Carolyn Ashbaugh, James Roberts, Ehud Spanier, Sam Sweet, Ilan Karplus, Gidi Hulata, Robert Warner, and Dennis Lund provided very helpful discussions or commented on the manuscript. We thank Aquaculture Production Technology, Ltd., Israel, and the University of California for financial and logistic support, and the Prawn Aquaculture Program of the University of Hawaii which provided additional specimens for confirmation of the observations in Israel. AMK also gratefully acknowledges Professor Dan Cohen and the Department of Genetics of the Hebrew University of Jerusalem for facilities at the Genetics Experimental Farm and support on sabbatical leave in Israel.

LITERATURE CITED

- Boyd, J. A., and L. M. Page. 1978. The life history of the crayfish *Orconectes kentuckiensis* in Big Creek, Illinois.—*American Midland Naturalist* 99: 398–414.
- Brody, T., D. Cohen, A. Barnes, and A. Spector. 1980. Yield characteristics of the prawn *Macrobrachium rosenbergii* in temperate zone aquaculture.—*Aquaculture* 21: 375–385.
- Carpenter, M., and R. de Roos. 1970. Seasonal morphology and histology of the androgenic gland of the crayfish, *Orconectes nais*.—*General and Comparative Endocrinology* 15: 143–157.
- Cohen, D., and Z. Ra'anan. 1983. The production of the freshwater prawn *Macrobrachium rosenbergii* in Israel. III. Density effect of all-male *Tilapia* hybrids on prawn yield characters in polyculture.—*Aquaculture* 35: 57–71.
- Coutière, H. 1901. Les Palaemonidae des eaux douces de Madagascar.—*Annales des Sciences Naturelles, Zoologie, Série 8*, 12: 249–342.
- Fujimura, T., and H. Okamoto. 1972. Notes on progress in developing a mass culturing technique for *Macrobrachium rosenbergii* in Hawaii.—*In*: T. V. R. Pillay, ed., *Coastal aquaculture in the Indo-Pacific region*. Pp. 313–327. Fishing News (Books) Ltd., Surrey, England.

- Gould, S. J. 1966. Allometry and size in ontogeny and phylogeny.—*Biological Reviews* 41: 587–640.
- Hartnoll, R. G. 1982. Growth.—*In*: L. G. Abele, ed., *The biology of Crustacea*. Volume 2. Embryology, morphology, and genetics. Pp. 111–196. Academic Press, New York.
- Henderson, J. R., and G. Matthai. 1910. On certain species of *Palaemon* from South India.—*Records of the Indian Museum* 5: 277–306.
- Holthuis, L. B. 1950. The Palaemonidae collected by the Siboga and Snellius expeditions with remarks on other species. I. Subfamily Palaemoninae. The Decapoda of the Siboga Expedition, Part X.—*Siboga Expeditie* 39a9: 1–268.
- . 1952. A general revision of the Palaemonidae (Crustacea Decapoda Natantia) of the Americas. II. The subfamily Palaemoninae.—*Occasional Papers of the Allan Hancock Foundation* 12: 1–396.
- Koshy, M. 1973. Studies on the sexual dimorphism in the freshwater prawn *Macrobrachium dayanum* (Henderson, 1893) (Decapoda, Caridea), II.—*Crustaceana* 24: 110–118.
- Kuris, A. M., and J. T. Carlton. 1977. Description of a new species, *Crangon handi*, and new genus, *Lissocrangon*, of crangonid shrimps (Crustacea: Caridea) from the California coast, with notes on adaptation in body shape and coloration.—*Biological Bulletin* 153: 540–559.
- , and M. Mager. 1975. Effect of limb regeneration on size increase at molt of the shore crabs *Hemigrapsus oregonensis* and *Pachygrapsus crassipes*.—*Journal of Experimental Zoology* 193: 353–360.
- Malecha, S. R. 1980. Development and general characterization of genetic stocks of *Macrobrachium rosenbergii* and their hybrids for domestication.—*University of Hawaii Sea Grant Quarterly* 2(4): 1–6.
- , S. Masuno, and D. Onizuka. 1984. The feasibility of measuring the heritability of growth pattern variation in juvenile freshwater prawns, *Macrobrachium rosenbergii* (de Man).—*Aquaculture* 38: 347–363.
- Nagamine, C., and A. W. Knight. 1980. Development, maturation, and function of some sexually dimorphic structures of the Malaysian prawn, *Macrobrachium rosenbergii* (de Man) (Decapoda, Palaemonidae).—*Crustaceana* 39: 141–152.
- , ——, A. Maggenti, and G. Paxman. 1980. Effects of androgenic gland ablation on male primary and secondary sexual characteristics in the Malaysian prawn *Macrobrachium rosenbergii* (de Man) (Decapoda Palaemonidae), with first evidence of induced feminization in a nonhermaphroditic decapod.—*General and Comparative Endocrinology* 41: 423–441.
- Payne, J. F. 1972. The life history of *Procambarus hayi*.—*American Midland Naturalist* 87: 25–35.
- Ra'anan, Z. 1982. The ontogeny of social structure in the freshwater prawn *Macrobrachium rosenbergii*.—Ph.D. Thesis, Hebrew University of Jerusalem. Pp. 1–101.
- , and D. Cohen. 1985. The ontogeny of social structure and population dynamics in the freshwater prawn *Macrobrachium rosenbergii* (de Man).—*In*: F. M. Schram and A. Wenner, eds., *Crustacean issues II: Crustacean growth*. Pp. 277–311. A. A. Balkema, Rotterdam.
- , and A. Sagi. 1985. Alternative mating strategies in males of the freshwater prawn *Macrobrachium rosenbergii* (de Man).—*Biological Bulletin* 169: 592–601.
- Ray, A. A. 1979. *Statistical analysis system user's guide*.—SAS Institute, Cary, North Carolina. Pp. 1–494.
- Sagi, A. 1984. Alternative reproduction strategies in male populations of the freshwater prawn *Macrobrachium rosenbergii*.—M.Sc. Thesis, Hebrew University of Jerusalem. Pp. 1–50.
- Sandifer, P. A., and T. I. J. Smith. 1977. Preliminary observations on a short-claw growth form of the Malaysian prawn, *Macrobrachium rosenbergii* (de Man).—*Proceedings of the National Shellfisheries Association* 67: 123–124.
- Smith, T. I. J., P. A. Sandifer, and M. H. Smith. 1978. Population structure of Malaysian prawns, *Macrobrachium rosenbergii* (de Man), reared in earthen ponds in South Carolina, 1974–1976.—*Proceedings of the World Mariculture Society* 9: 21–38.
- Sokal, R. R., and F. J. Rohlf. 1981. *Biometry*, 2nd Edition.—W. H. Freeman and Co., San Francisco. Pp. i–xviii, 1–859.
- Somerton, D. A., and R. A. MacIntosh. 1983. The size at sexual maturity of the blue king crab, *Paralithodes platypus*, in Alaska.—*Fishery Bulletin, United States* 81: 621–628.
- Stein, R. A. 1976. Sexual dimorphism in crayfish chelae: functional significance linked to reproductive activities.—*Canadian Journal of Zoology* 54: 220–227.
- Tazelaar, M. A. 1930. The relative growth of parts in *Palaemon carcinus*.—*Journal of Experimental Biology* 7: 165–174.
- Teissier, G. 1960. Relative growth.—*In*: T. H. Waterman, ed., *The physiology of Crustacea*, Volume I. Pp. 537–560. Academic Press, New York.
- Telecky, T. M. 1982. The proportion of runt-fertilized females in two captive populations of *Macro-*

- brachium rosenbergii* and the behavior of runts associated with a courting bull and female.—M.A. Thesis, University of Nevada, Reno, Nevada. Pp. 1–53.
- . 1984. Alternate male reproductive strategies in the giant Malaysian prawn, *Macrobrachium rosenbergii*.—*Pacific Science* 38: 372–373.
- Thampy, D. M., and P. A. John. 1973. Observations on variations in the male sex characters and their relation to the androgenic gland in the shrimp *Macrobrachium idae* (Heller).—*Acta Zoologica* 54: 193–200.
- Warner, R. R. 1984. Deferred reproduction as a response to sexual selection in a coral reef fish: a test of the life historical consequences.—*Evolution* 38: 148–162.

RECEIVED: 28 April 1986.

ACCEPTED: 13 August 1986.

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