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Morphometric analysis of swimming crabs *Callinectes danae* (Crustacea, Portunidae) from the Santa Cruz Canal, Pernambuco (Brazil)

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Abstract: A generalized linear model was used to analyze the relationships among the morphometric measures, sex and maturity of 547 specimens of the swimming crab, *Callinectes danae*, from the Santa Cruz Canal in Pernambuco, Brazil. Morphometric variables were width of the cephalothorax (WC), width of the fifth abdominal segment (W5), and the length of the largest chela (LC). The response variable was WC, while all the others were considered as explanatory variables. Mature males presented largest WC and longest LC. The lowest W5 values were recorded for juvenile males, and the highest values were recorded for mature females. Coefficients of main effects and of interactions between sex and maturity, maturity and LC, and W5 and LC, were all significantly different from zero. Overall correlations between WC and the covariates were positive, specially between WC and LC. Sexual dimorphism concerning reproductive development of *C. danae* includes differences in body size, abdomen shape, and the length of the chelae. Width of cephalothorax can be explained by a single equation integrating categorical (maturity and sex) and continuous (abdomen width and chela length) variables.

Key words: decapoda, dimorphism, maturity, morphometry, generalized linear model

Resumo: Caracterização morfométrica do siri *Callinectes danae* (Crustacea, Portunidae) capturado no Canal de Santa Cruz, Pernambuco (Brasil). Um modelo linear generalizado foi utilizado para analisar relações entre medidas morfométricas, sexo e maturidade de 547 espécimes de *Callinectes danae* coletados no Canal de Santa Cruz (Pernambuco-Brasil). As variáveis morfométricas foram largura do cefalotórax (WC), largura do quinto segmento abdominal (W5) e o comprimento da maior quela (LC). A variável resposta foi WC, e as demais foram consideradas como variáveis explicativas. Os machos maduros apresentaram os maiores valores de WC e de LC. Os menores valores de W5 foram registrados para machos juvenis, e o maiores valores para fêmeas maduras. Os coeficientes para os efeitos principais e para as interações entre sexo e maturidade, maturidade e LC, e W5 e LC, foram todos significativamente diferentes de zero. De maneira geral as correlações entre WC e as covariáveis foram positivas, especialmente entre WC e LC. O dimorfismo sexual relacionado ao desenvolvimento reprodutivo do *C. danae* inclui diferenças no tamanho, na forma do abdômen, e no comprimento da quela. A largura do cefalotórax pode ser explicada por uma equação única que integra variáveis categóricas (sexo e maturidade) e contínuas (largura do abdômen e comprimento da quela).

Palavras-chave: decapoda, dimorfismo, maturidade, morfometria, modelo linear generalizado.

Introduction

Crabs of the Portunidae family are characterized by their paddle-shaped fifth pair of legs adapted for swimming, a dorso ventrally flattened cephalothorax, and a series of tooth-like projections along the anterolateral margin of the carapace (Melo, 1996). In Brazil, the portunids of the genus *Callinectes* are well-known especially due to their importance to the artisanal fishing communities of coastal and estuarine regions (IBAMA, 2008). The swimming crab *Callinectes danae* Smith, 1869 distribution ranges from Florida (USA) to the southernmost state of Brazil, Rio Grande do Sul (Melo, 1996). It is one of the most common South American portunids (Nevis *et al.*, 2009; Teixeira & Sá, 1998, Severino-Rodrigues *et al.*, 2002, Branco & Freitas-Junior, 2010). However, there are no data on fishery stocks or the productive potential of the species.

Morphometric studies provide an important tool for the evaluation of fishery stocks and the understanding of population dynamics. Data on morphometric parameters may offer valuable insights into the wellbeing of an animal, as well as other aspects of its biology (Mantelatto & Fransozo, 1992). Morphometric variation may also be used for the description of "phenotypic stocks", which are defined as groups with similar rates of growth, mortality, and reproduction (Cadrin, 2000). These groups can be modeled and managed separately in order to guarantee the sustainable exploitation of fishery stocks (Cadrin & Silva, 2005).

Santa Cruz Canal is used for intensive fishing by local residents from the towns along the northern coast of Pernambuco state. Official government figures (*e.g.* IBAMA, 2008) indicate that the swimming crab is an important resource harvested by the artisanal fishermen of Pernambuco. More than one third of all swimming crabs caught in Brazil (2,415 t in 2006) (FAO, 2014) comes from the Santa Cruz Canal (944.5 t in 2006) (IBAMA, 2008). Swimming crabs are exploited commercially only in Brazil, hence the Santa Cruz Canal is the main fishing ground of swimming crabs in the Atlantic Ocean.

A number of morphometric studies have been based on bivariate allometry (Branco & Masunari, 1992, Pinheiro & Fransozo, 1993, Masunari & Swiech-Ayou, 2003, Shinozaki-Mendes *et al.*, 2012). Bivariate analyses are useful but make difficult to study interactions between multiple relationships when there are more than two variables. However, other approaches are available, such as multivariate analysis, geometric morphometry, or even linear models, which provide an integrated framework analysis of the set of variables. Such analyses could provide more information on the relationship between the biometrics measurements, and also on differences in growth according to sex and period of the life cycle.

Relationships among severa morphometric measurements of *C. danae* collected in the Santa Cruz Canal, Pernambuco, Brazil were analyzed in this paper byusing generalized linear models. The results contribute to the understanding of the local *C. danae* phenotypes and the variation in sexual dimorphism during the maturation process. Also, the estimations of the parameters of the models in this paper can be compared to estimations calculated for other fishing grounds, in an attempt to assess if there are different phenotypic stocks. Finally, the paper also contributes to show the potential application of generalized linear models in biological studies with multiple variables.

Materials and Methods

The specimens of *C. danae* were collected in the Santa Cruz Canal (approximately 08º47'30" S, 034º53'20" W) (Figure 1), during monthly excursions between March 2009 and February 2010. The crabs were captured using two different techniques – beach seine measuring 15 m x 1.85 m with a 12 mm mesh, and line with bait of low commercial value fish (e.g. engraulids, gerreids, and carangids). Specimens were collected twice a month, once with each technique, always at low tide.

The live specimens were taken to the laboratory, where they were cryo-anesthetized for biometric analysis, and identified using the brachyuran identification key proposed by Melo (1996). The sex and maturity of the specimens were determined based on the shape and attachment of the abdomen (Van Engel, 1990). Immature females have a triangular abdomen which is attached to the thoracic sternites, while in the mature females, it is semicircular and not attached to the sternites. In the males, the abdomen is shaped like an inverted T, and is attached to the sternites in the immature specimens and unattached in the mature individuals.

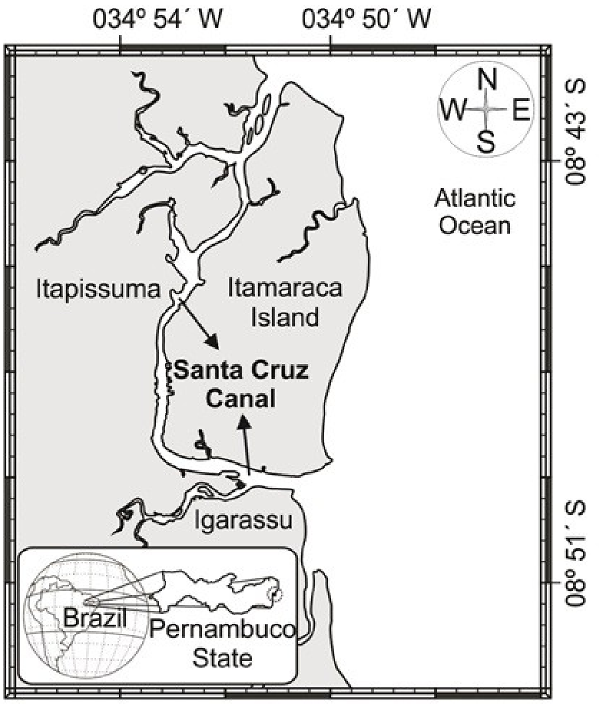

Figure 1. Location of the Santa Cruz Canal on the coast of the Brazilian state of Pernambuco, where the *Callinectes danae* specimens were collected for analysis in the present study.

Biometric measurements were taken with a pair of calipers (0.01 cm precision). The following measurements were taken – width of the cephalothorax (WC), measured between the external extremities of the lateral spines, width of the fifth abdominal segment (W5) measured in the median portion of the segment, and the length of the largest chela (LC), measured from the extremity of the fixed dactyle to the longitudinally opposite extreme (Figure 2).

For the analysis of possible differences of the mean WC, LC, and W5 values recorded for males and females, juveniles and adults, the data were first tested for normality (Shapiro-Wilk) and homoscedasticity of the variances (Bartlett's test), which are prerequisites for the application of the Analysis of Variance (ANOVA). When the data did not satisfy these prerequisites, the nonparametric Kruskal-Wallis test was applied (Zar, 1984).

In the generalized linear models (GLMs), the WC was considered as the dependent variable, while LC and W_5, sex (male/female), and morphometric maturity (immature/mature) were the independent variables. Other variables, such as the length of the cephalothorax and body weight, are known to be related to WC (Baptista Metri *et al.*, 2005, Furia *et al.*, 2008, Shinozaki-Mendes *et al.*, 2012), and could have been included in the models. However, the present study focused on elements more closely-related to sexual and age-related differences, such as the abdomen (represented by W_5) and the chela (LC).

The stepwise approach, based on Akaike's Information Criterion, or AIC (Akaike, 1974), was used to select the explanatory variables and the model. That information criterion can be used to select a parsimony model with good balance between bias and variance of the etimations (Burnham & Anderson, 2002). Stepwise approach was initiated using a complete model (including all the explanatory variables and the first-order interactions). Interactions were then excluded each at a time, and the results of AIC calculations were compared to the AIC of the complete model. Whenever there was an improvement in the performance of the model following the exclusion of one interaction, it was eliminated and the reduced form of the model was considered to be the new complete model. This procedure was repeated until no further interactions could be eliminated. Procedure to select main effects was similar to that applied to select interactions. However, exclusion of main effects were only considered if they were not in selected interactions. All analyses were run in the R program (R Core Team, 2012).

Results

A total of 547 *Callinectes danae* specimens were collected, of which, 292 were male (151 immature and 141 morphologically mature) and 255 were females (171 immature and 84 mature). The

WC, LC, and W_5 values for the four crossing sex-age classes (immature/mature females and immature/mature males) did not present homoscedasticity of variance in any case, with $p < 2.2x10^{-16}$ being recorded in Bartlett's test for WC and W_5, and $p = 6.2x10^{-15}$ for LC. Similarly, the data were not normally distributed (Shapiro-Wilks, $p < 0.05$) in most cases, with the exception of WC for the mature females ($p = 0.1091$), W_5 for immature males ($p = 0.3449$), and LC for mature females ($p = 0.6329$). Given these violations of the prerequisites required for the parametric ANOVA, the data were analyzed using the nonparametric Kruskal-Wallis test.

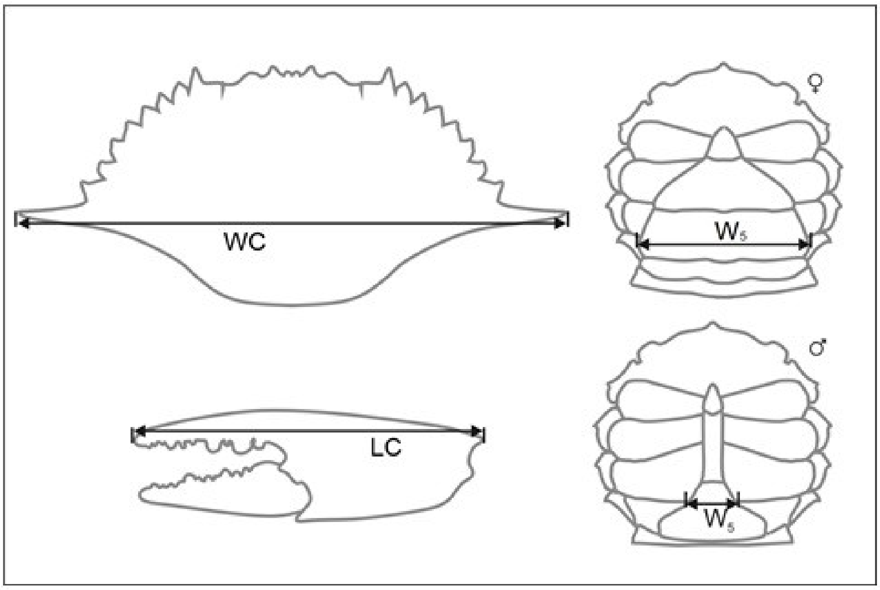

Figure 2. Morphometric measurements of *Callinectes danae* collected during the present study: WC = Width of the Cephalothorax, LC = Length of the chela, and W_5 = width of the fifth segment of the abdomen in males and females.

The WC values ranged from 2.35 cm to 12.09 cm. The difference of values for the juveniles of the two sexes was not significant (overall median, x = 6.25 cm for both sexes combined). However WC values of juveniles were significantly lower than those recorded for the adult females (x= 8.56 cm). Values recorded for the adult males (x= 10.50 cm) were significantly higher than those recorded for juveniles and adult females (Figure 3).

The fifth abdominal segment width (W_5) ranged from 0.21 cm to 3.95 cm (Fig. 3). Median W_5 of the juvenile females was indistinguishable from that of the adult males (x = 0.96 cm for the sexes combined). Juvenile males had significantly narrower abdomens (x = 0.60 cm), while those of the adult females were significantly wider (x = 2.60 cm).

Chela lengths (LC) ranged from 1.05 cm to 7.42 cm, and followed a similar pattern to the WC values (Figure 3), with those recorded for the immature specimens being indistinguishable by sex (x = 2.98 cm for the two sexes combined), while the adult females LC values were intermediate (x = 4.17 cm), and the adult males values were significantly longer (x = 6.20 cm) ($p < 0.0001$).

The coefficients for the generalized linear model selected are shown in Table I. Coefficients significantly different from zero are associated with the main effects of sex, morphometric maturity, width of the fifth abdominal segment and the length of the chela. The interactions between sex and maturity, maturity and chela length, and between W5 and chela length were also significantly different from zero.

The design matrix was constructed with the immature females as the base level, with the effects for sex (male) and maturity (mature) being expressed as coefficients related to baseline values. Estimations of main effects indicate that WCs of males were 0.143 cm larger, on average, than WC of females whenever other explanatory variables are fixed. Similarly, WCs of mature individuals were 0.494 cm larger, on average, than WC of juveniles, whenever other explanatory variables are fixed. With regard to the covariables, the steepest slope (1.722) was observed for the exclusive linear relationship between WC and LC. The slope for the principal effect of the W5 was slightly lower.

All the coefficients estimated for the principal effects were positive (Table I). A reduction in the value for the slope was found only for the interactions between maturity and chela length, and between the abdominal width and chela length. However, these coefficients were low in comparison with the principal effects.

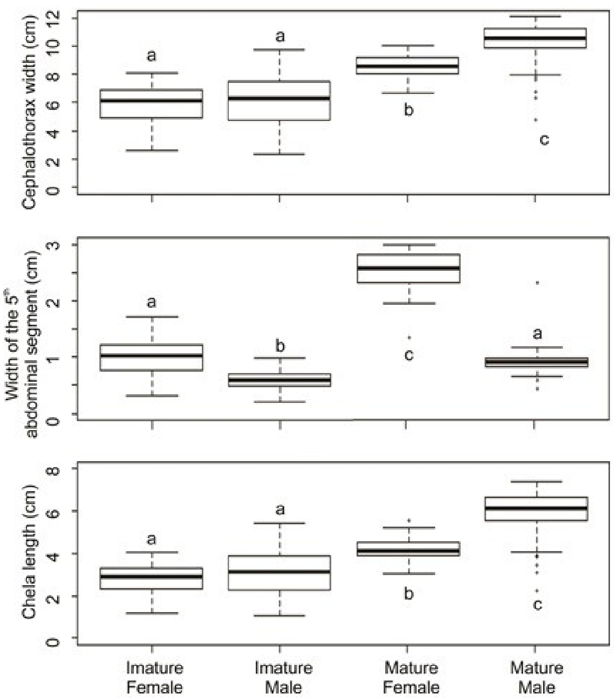

Figure 3. Morphometric parameters for the population of *Callinectes danae* from the Santa Cruz Canal (Pernambuco, Brazil) – median width of the cephalothorax and the fifth segment of the abdomen, and the length of the chela. The box plots show maximum, 75% quartile, median, 25% quartile, and minimum. The isolated points represent the outliers. Different letters indicate significant differences between medians, according to the results of the Kruskal-Wallis test.

The first-order interaction between sex and maturity indicated that the WCs of adult males were 0.261 cm, on average, than WCs of immature males whenever the other explanatory variables (LC and W5) are fixed. Another way to interpret the coefficient estimated for interaction between sex and maturity is that WCs of adult males were on average larger than WCs of adult females whenever the other explanatory variables are fixed. In general, then, mature males are characterized by a wider cephalothorax.

The relationship between maturity (factor) and LC (covariable) is negative. This reflects the fact that the slope for the regression between WC and LC is slightly lower in mature individuals in comparison with juveniles. This means that, during the molt which marks the transition to maturity, the development of the chela is disproportionately greater in relation to the WC in comparison with the preceding molts.

The effect of the interaction between LC and W_5 is significant. This means that the slope of continuous variable LC on the response variable (WC) changes as the values of W_5 change, and vice-versa. The effect is negative (-0.206), hence the regression slope of LC on WC when W_5 is long, is lower than the slope when W_5 is short. This means that in mature specimens (more well-developed W5) the growth of LC with respect to the growth of WC is higher than in immature specimens.

Table I. Estimations of coefficients of the Generalized Linear Model fitted to dataset of the *Callinectes danae* specimens collected in the Santa Cruz Canal in Itapissuma (Pernambuco, Brazil). Cephalothorax width is the response variable. The explanatory variables were the factors sex (female and male) and maturity (immature and mature), and the covariables length of the chela (LC) and the width of the fifth abdominal segment (W_5). The reference levels are female and immature.

Coefficient	Estimate	Standard Error	t	p (>| t |)
Intercept	0.270	0.087	3.103	2.00×10^{-3}
LC	1.722	0.022	79.900	5.120×10^{-301}
W5	1.469	0.150	9.825	4.55×10^{-21}
Maturity (mature)	0.494	0.178	2.781	5.61×10^{-3}
Sex (male)	0.143	0.044	3.249	1.23×10^{-3}
LC:W5	-0.206	0.030	-6.938	1.14×10^{-11}
LC:maturity(mature)	-0.238	0.039	-6.174	1.31×10^{-9}
Maturity(mature):Sex(male)	0.261	0.101	2.583	1.01×10^{-3}

The results of the analysis of deviance are shown in Table II, in which each line represents the result of the inclusion of the term shown in the first column. For example, the inclusion of LC as an explanatory variable results in proportional deviance reduction of 96.37%. This emphasizes the importance of LC, of all the variables analyzed, for the explanation of the variability of WC. The inclusion of additional terms other than LC results in a much smaller reduction in deviance. In any case, the model selected (using all the terms and interactions) provides relatively accurate predictions for the WC values, emphasized by the reduction of more than 98% in total deviance. Standard residual diagnostics plots are shown in Figure 4. Overall the model is not biased, residuals were homoscedastic and the distributions is approximately normal.

Table II. Results of the analysis of deviance. Cephalothorax width is the response variable. The factors are sex (female and male) and maturity (immature and mature) and the covariables are the length of the chela (LC) and the width of the fifth abdominal segment (W_5). The reference levels are female and immature. DF ─ Degrees of Freedom; SS ─ Sum of the Squares; F ─ statistic; Δ Res. (%) ─ proportion of the deviance eliminated when the term is included in the model.

Variables			Residual		F	p value	Δ Res. (%)
	DF	SS	DF	SS			
			546	2962.91			
LC	1	2855.34	545	107.57	53905.45	0.00	96.37
W5	1	53.02	544	54.55	1001.05	5.64×10^{-125}	1.79
Maturity	1	9.43	543	45.12	178.06	2.67×10^{-35}	0.32
Sex	1	0.32	542	44.80	6.03	1.44×10^{-2}	0.01
LC:W_5	1	14.17	541	30.63	267.47	4.09×10^{-49}	0.48
LC:Maturity	1	1.725	540	28.904	32.566	1.90×10^{-8}	0.058
Maturity:Sex	1	0.353	539	28.550	6.671	1.01×10^{-2}	0.012

Discussion

C. danae is sexually dimorphic in the width of the cephalothorax (WC) and chela length (LC), which are both greater in the mature males than in females. By contrast, enlargement of the width of the abdomen at maturity is a characteristic of the females. These characteristics have different functionalities, but all are related to reproduction. Hartnoll (1982) concluded that the pubertal molt is easily identified by the marked morphological changes (e.g. chela length, width of abdomen) which indeed indicate that the individual has reached reproductive maturity.

Juvenile males and females have chelae of similar size, whereas adult males have significantly larger chelae in comparison with those of mature females. In this paper the importance of the size of the chelae for the males is emphasized by the interaction between chela length and maturity in the linear model in which the width of the cephalothorax is the dependent variable. The results of the present study indicate that the pubertal molt is characterized by a major growth spurt in the size of the cephalothorax, but an even greater spurt in the growth of the chelae, especially in the males.

Large size of the mature males is a typical characteristic of the portunid crabs. The size differentiation of males and females is related to the reproductive behavior. The pair engage in a premating embrace, when male wraps his legs and craws around the female. The male might prove through the hug, because he might be able to remain clasped to the female during a number of days prior to the pubertal molt, which is when copulation occurs (Christy, 1987). The male might be able to defend the female from other males and to obtain food for both individuals (Vogt, 2012). In this sense, a larger chela may have a number of advantages, when it may contribute to success during confrontations with competing males (Hartnoll, 1974), the defense and retention of the females during the pubertal molt (Guerrero-Ocampo *et al.*, 1998), as well as the harvesting of food. Hence the reproductive success of a male strongly depends on how large he is. In opposition, the probability of successful mating of a large female is reduced by the difficulty of finding a compatible partner, thought large females carries larger number of eggs.

Overall sexual differences in growth and size are related to the differentiated mode of energy use of females and males (Hartnoll, 1974). Males invest more energy in growth, given the importance of their size for the success of the mating embrace. By contrast, females invest a considerable portion of their energy in the development of ovaries, in the production of large number of eggs, and in the spawning migration. The change in the shape of the abdomen of the female, which goes from triangular to semicircular during the pubertal molt, results in a significant increase of W_5 values. The larger the abdominal area in the female, the larger the number of eggs she can hold. All brachyurans studied to date present change in the shape of the abdomen of the female at maturity, though the modification is less pronounced in some species (e.g. *Cardisoma guanhumi* – Shinozaki-Mendes *et al.*, 2013), than in other typical portunids.

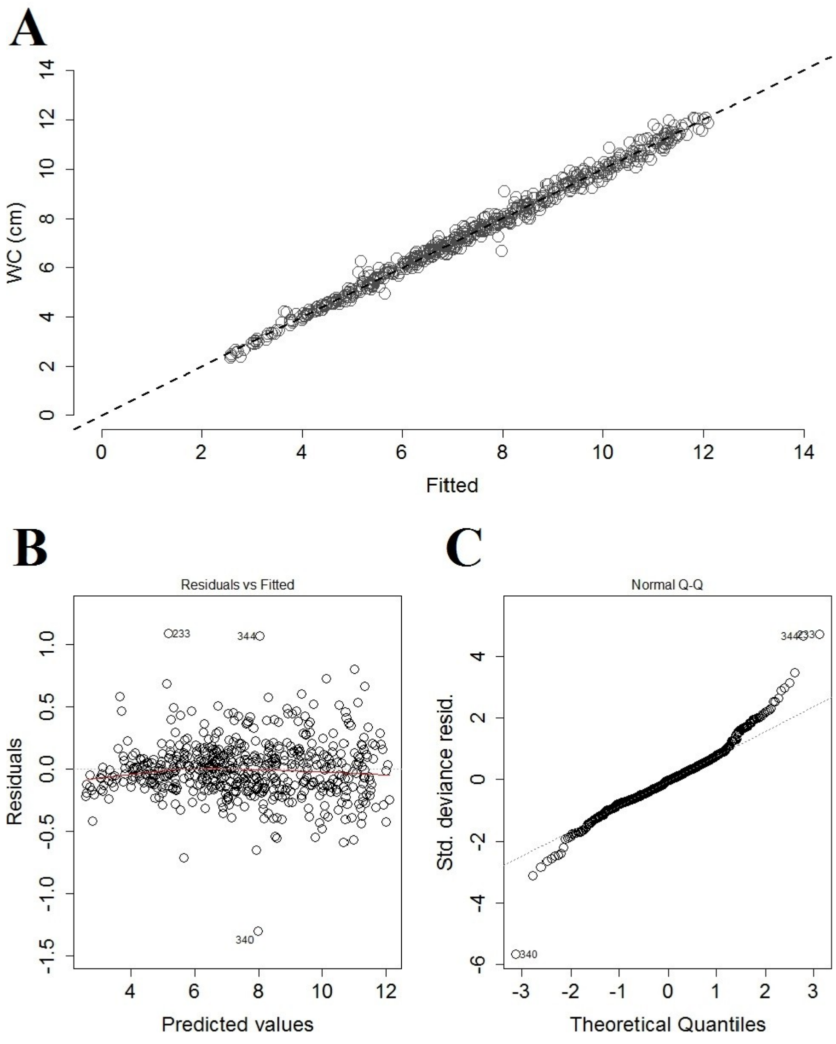

Figure 4. Relationship between fitted and observed values of the width of the cephalothorax (WC) (A), between fitted and residuals (B), and normal quantile plot of residuals (C).

The variation in the size of the chela for *C. danae* is relatively discreet in comparison with other brachyuran species, such as *Cardisoma guanhumi* (Shinozaki-Mendes *et al.*, 2013), *Ucides cordatus* (Dalabona *et al.*, 2005), and *Uca mordax* (Masunari & Dissenha, 2005). This difference may be at least partly related to habitat, given that aquatic species not only require good hydrodynamics for efficient swimming, but also an ability to bury themselves in the sediment to escape predators, whereas the semi-terrestrial species normally construct burrows to hide from predators. In support of this conclusion, the aquatic crab *Chaceon affinis* (Fernández-Vergaz *et al.*, 2000) presents a similarly reduced pattern of growth of the chela like observed in *C. danae*.

Calculations in this paper indicate that length of chela (LC) is the main variable accounting for the width of the cephalothorax, while width of abdomen (W_5) is also important, albeit to a lesser extent. The set of coefficients produced by this analysis represent numerical characteristics that define the "phenotypic stock" exploited by fishermen in Santa Cruz Canal on the Pernambuco State coast. Replication of the analysis for crabs caught in other places maybe of major interest. Comparisons of coefficients estimations as calculated for different fishing grounds along the coast would be useful to assess if there are different stocks or groups, and if they should be considered separated for practical purposes management.

The application of a GLM for the quantitative analysis proved to be an important analytical tool. The additive structure of the model allows integrating multiple variables, categorical or continuous, into a single framework. In this case, it is not necessary to run separated smaller models, which represent the phenomenon in a fragmented manner. However, it is important to note, that the selection of model structure depends on the objective of the researcher. Partial bidimensional analysis of the relationship among WC, W_5, and LC, in different crossing levels sex and maturity factors (e.g. Shinozaki-Mendes *et al.*, 2012), may also result useful to better understand morphometric relationships.

Acknowledgments

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