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Sexual dimorphism in *Litopenaeus vannamei* (Decapoda) identified by geometric morphometrics

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Abstract. Sexual dimorphism is a dominant condition between species of crustaceans. Although sometimes the morphological discrimination may be shown conspicuous, in others it can only be accessed through statistics analyses based on comparative morphometric techniques. In particular, the geometric morphometrics (GM) have incremented the power of discrimination between the sexes, by identifying minimal morphological differences in body structures unnoticed by traditional morphometrics. With objective to identify the level of sexual dimorphism between males and females breeding of *Litopenaeus vannamei*, GM analyzes were performed. The females showed greater robustness in the cephalothorax, besides a greater width in the second abdominal segment compared to males. The sexual dimorphism here observed is probably linked to functional aspects of reproduction of this species and contributes to new morphometric information of *L. vannamei* individuals in adult phase.

Key words: sex differentiation, aquaculture, Penaeidae, sex discrimination, geometric morphometrics

Resumo. Dimorfismo sexual em Litopenaeus vannamei (Decapoda) identificado por morfometria geométrica. Dimorfismo sexual é uma condição dominante entre espécies de crustáceos. Embora algumas vezes a discriminação morfológica possa se mostrar conspícua, em outras somente pode ser acessada através de estatísticas baseadas em técnicas morfométricas comparativas. Em particular, a morfometria geométrica (GM) tem incrementado o poder de discriminação entre os sexos, pela identificação de diferenças morfológicas mínimas, em estruturas corporais, não percebidas através de morfometria tradicional. Objetivando identificar o nível de dimorfismo sexual entre machos e fêmeas reprodutores da espécie *Litopenaeus vannamei* foram empregadas análises através de MG (morfometria geométrica). As fêmeas demonstraram uma maior robustez no cefalotórax, além de uma maior largura no segundo segmento abdominal em comparação com os machos. O dimorfismo sexual observado revela-se atrelado a aspectos funcionais da reprodução da espécie e contribui com novas informações morfométricas da sua fase adulta.

Palavras chave: diferenciação sexual, aquicultura, Penaeidae, discriminação sexual, morfometria geométrica

Introduction

Sexual dimorphism, morphological differences between the sexes mediated by the action of sex hormones is a dominant condition among crustacean species, group in which females often grow larger and reach larger sizes than males (Gopal *et al*. 2010). Although sometimes morphological discrimination can be conspicuous (Bildstein *et al*. 1989), in other sexual differentiation is quantitative and qualitatively accessed only through statistics analysis based on comparative morphometric techniques (Bertin *et al*. 2002). Morphometry includes analysis of body shape or particular morphological characteristics (Begg & Waldman

1999). Resolutive analysis of shape through geometric morphometric methods has been successfully applied in several aquatic species (Valentin 2000, Cadrin & Silva 2005) with the purpose of population analysis and biogeography (Hopkins & Thurman 2010, Giri & Loy 2008), phylogeny (Astrop 2011), breakdown of morpho-stocks, and phenotypic plasticity (Silva *et al*., 2010) studies. In particular, the functional morphology has been used in a number of studies to natant decapod to compare geographically isolated populations (Tzeng *et al*. 2001, Tzeng 2004), identify intraspecific variation (De Grave 1999, Kapiris & Thessalou-Legaki 2001), interspecific differences, sexual dimorphism (Barría *et al.* 2011) and establish bodily morphological differences in relation to habitat (Maynou & Sarda 1997).

In some crustaceans studies of sexual dimorphism, using geometric morphometrics (GM) are particularly suitable due to the accurate identification of homologous landmarks on the hard exoskeleton (Abelló *et al.* 1990, Rufino *et al.* 2004. Indeed, studies of MG have increased the discrimination power between the sexes, by identifying minimal morphological differences in body structures unnoticed by traditional morphometrics (Barría *et al.* 2011).

Much biological information is obtained through analysis of sexual dimorphism and particularly useful in taxonomic, ecological, behavioral and evolutionary studies. Although, the existence of sexual dimorphism in *L. vannamei* is popularly known, some of its morphological structures were not compared by accurate morphometric techniques, especially involving breeders. In this way, here is showed for the first time qualitative evidences of sexual dimorphism in *L. vannamei* based in GM approach.

Materials and Methods

A total of 64 specimens of *Litopenaeus vannamei* (Boone 1931), 33 males and 31 females, weighing between 35 and 40 grams (approximately eleven months old) was used for analyzes of sexual dimorphism in the species. The specimens were sampled in a shrimp farm from Rio Grande do Norte State, Northeastern Brazil. The sexing was established by visual identification of gonads and the presence of secondary sexual characteristics, such as petasma.

For analyzes by geometric morphometrics were used specimens free of injuries and damage point. The digitalized images were obtained from the left lateral face, using digital capture of image with a resolution of 8.1 megapixels (Sony H10) under standardized distance and position.

After the digital record of each individual, the tpsUtil software was used to order the images of specimens in a single file with TPS format. The tpsDig2 software (Rohlf 2006) was used to locate landmarks on two rigid body structures of both sexes, the cephalothorax - CT (seven landmarks) and the second abdominal segment – SA (six landmarks).

The information in the form of the coordinates of landmarks by analysis of Procrustes superposition (Dryden and Mardya, 1998) were obtained by MorphoJ 1.02b ® software (Klingenberg 2011). The numerical data were analyzed by canonical variable analysis (CVA), from which the MANOVA, the Mahalanobis distance (D2), the discriminant function analysis (DFA) and comparative deformation grids were obtained from the first canonical variable. In addition, the coordinates to landmarks coordinates were generated warped outlines to demonstrate, more clearly the grids vector variations of deformation of each sex.

Results

Significative differences in the shape and disposition of the centroid in relation to *landmarks* defined in regions CT and SA were identified between males and females. For the two body regions analyzed the first canonical variable was responsible for 100% of the total variation of the sample. The discriminant function correctly analyzed the samples on its sex, with greater divergence between the sexes for the region of CT (Mahalanobis $D^2 = 3.67$) and lower for the region of SA (Mahalanobis $D^2 = 1.57$) (Table 1).

Compared to males, females showed cephalothorax more robust at its base, angular modification of facial thorn and slight posterior position of the ocular insertion and of antennas. Variations in the shape of SA show greater width and lower height for females while the males have thinner segments (Figure 1).

Discussion

Morphological distinction between males and females of *L. vannamei* adults to the structures here analyzed was evident. The detection of differences in size at maturity from changes in the allometry of sexual characteristics is a common example of interspecific heterochrony identification. Many decapod crustaceans exhibit accentuated changes until sexual maturity as a result of discontinuous growth (Cadrin 2000).

Table 1. Statistical analysis of shape in males and females adults of *Litopenaeus vannamei*.

Structure	MANOVA					CVA					DFA		
	SS	*df*	*F*	*p*	*Pillai´s trace*	*Eigenvalue*	Var (%)	Cum (%)	*p***	D^2	T^2	T^2 *p*	*p*
CT	0.0101	10	5.05	<0.01	0.78	3.47	100	100	<0.01	3,67	215.28	<0.01	<0.01
SA	0.0102	8	8.12	<0.01	0.39	0.63	100	100	<0.01	1,57	39.44	<0.01	<0.01

MANOVA - Multivariate analysis of variance; CVA - Canonical variance analysis; DFA - Discriminant function analysis, SS - Sum square, df - degrees of freedom; D² - Mahalanobis distance, T2 - Hotteling's test, CT - cephalothorax; SA - Second abdominal segment.

In many species of crustacean have been observed ontogenetic sexual dimorphism in which females generally grow faster and reach a size larger than males of the same age (Villegas & Barquero 2000, Diaz *et al.* 2001, Otoshi *et al.* 2003, Perez-Rostro & Ibarra 2003, Gitterle *et al.* 2005).

Differential morphological characteristics present in the cephalothorax are shown conspicuously differentiated between the sexes of *L. vannamei*. Such changes have been identified in several crustaceans (Kapiris & Thessalou-Legaki 2001, Tzeng *et al.* 2001, Tzeng 2004, Anastasiadou *et al*. 2004, Anastasiadou & Leonardos 2008). One of dimorphic of the characters observed in females is the presence of large abdomens, selectively important condition for species that do not show parental care (Thiel 2003). Analysis by multivariate morphometry identified a conspicuous sexual dimorphism expressed mainly in body height and in the second abdominal segment in *Palaemonetes antennarius* (Anastasiadou *et al.* 2009). In fact, the sexual dimorphism in crustaceans in great part is tied to reproduction or reproductive strategy (Anastasiadou *et al.* 2009).

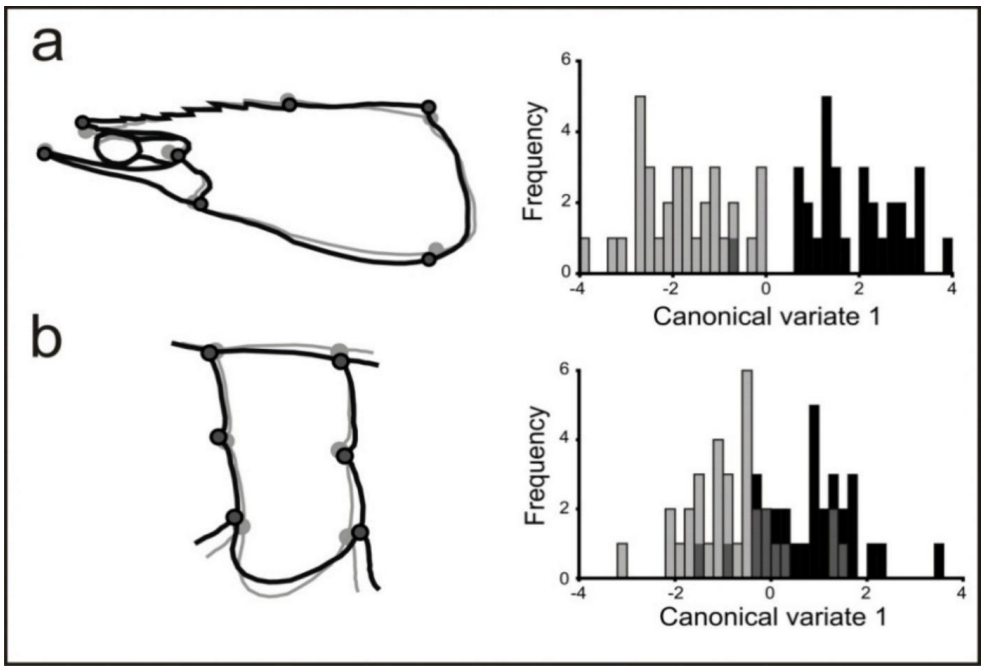

Figure 1. Comparison of body shapes of females and males of *L. vannamei*, generated from warped outlines and frequencies obtained from the first canonical variable. a. Cephalothorax; b. Second abdominal segment. Lines and bars in gray indicate structures and frequency of Eigenvalue in males and in black in females.

The same pattern of sexual dimorphism occurs for a number of species of commercially important penaeid shrimps (Choe 1971, Hartnoll 1985, Bray & Lawrence 1992, Primavera *et al.* 1998, Hoang *et al.* 2003). It has been suggested that in *L. vannamei* the shape of the abdomen represents a key factor in the competition for food (Hansford & Hewitt 1994). Sexually dimorphic females

demonstrate a greater food conversion efficiency and apparent digestibility (Daborn 1973, Strong & Daborn 1979, Moss & Moss 2006).

Sexual dimorphism identified by geometric morphometrics in *L. vannamei* is expressed in females for greater ruggedness in the cephalothorax greatest width in the second abdominal segment compared to males. The establishment of morphological differences between the sexes of *L. vannamei*, at different ontogeny stages, are of great interest for shrimp farming, having in view handling and using potentials, as early separation of males and females (monosex cultivation) and for ensuring a uniform size of shrimp stocks cultivated, since that part of the size variation among individuals may be related to sexual dimorphism.

Conclusions

In many animal groups, early sex identification has enormous biotechnological value and is related with management practices that increase production. Moreover, dimorphic characters are useful in the taxonomic and ecological aspects. This work presented for the first time subtle but significant variations between *L. vannamei* sexes based on GM analyses. The sexual dimorphism is associated with functional structures related to adaptation in this species. These results may represent an important tool for the appropriate management of *L. vannamei* stocks. Subsequent researches based on these data will enable to estimate sexual differentiation during early ontogenetic phases subsidizing protocols to obtain monosex stocks with significant impact on commercial production.

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