Acta Zoologica
Published for The Royal Swedish Academy of Sciences and The Royal Danish Academy of Sciences and Letters

Acta Zoologica (Stockholm) doi: 10.1111/j.1463-6395.2010.00473.x

Phenotypical traits and gonadal development in mangrove land crab, *Ucides cordatus* (Decapoda: Ocypodidae)

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Keywords:
coloration, mangrove, reproduction

Accepted for publication:
29 July 2010

Abstract

Castilho, G.G., Ostrensky, A., Pie, M.R. and Boeger, W.A. 2010. Phenotypical traits and gonadal development in mangrove land crab, *Ucides cordatus* (Decapoda: Ocypodidae). —*Acta Zoologica* (Stockholm) **00**:1–5.

The association between phenotypical and reproductive traits has been the focus of considerable research in decapod crustaceans, particularly with the goal of facilitating the management and sustainable use of natural stocks of commercial interest. The goal of the present study is to evaluate the existence of a relationship between ovarian maturation and the coloration of the ovary and carapace of *Ucides cordatus* using histological methods and macroscopic observation. Monthly samples of adult females were obtained between October 2002 and March 2005 in mangroves of the Baía de Antonina, Southern Brazil (25°25′S; 48°42′W). A total of 367 specimens were collected throughout the study. Each specimen was classified subjectively into five ovarian colorations (whitish, yellow, orange, brown, and purple) and five carapace colorations (yellow, blue, dark gray, brown, and purple). Although substantial differences in ovarian development were found among females with different carapace and ovarian colorations, the variability within each class precluded their use as reliable indicators of ovarian development.

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Introduction

The mangrove land crab *Ucides cordatus* (Decapoda: Ocypodidae) plays a fundamental role in mangrove ecosystems of the western Atlantic, being responsible for the consumption and degradation of more than half of the leaf litter in these environments (Schories *et al.* 2003). This species also represents an essential fishery resource for local fishermen communities along the Brazilian coast, which often rely on its harvest as an important source of food and income (Glaser 2003). Recent concerns about the possibility of overexploitation as well as an emerging disease caused by pathogenic black yeast the Lethargic Crab Disease (Boeger *et al.* 2005) have raised concern regarding the long-term sustainability of this fishery.

The study of the population dynamics of the mangrove land crab requires an understanding of its reproductive biology (Diele 2000). Microscopic and macroscopic aspects of anatomy of the male and female reproductive systems of the mangrove land crab have already been described (Alves 1975; Nakamura 1979). However, little is known about how gonadal development of mangrove land crab females is correlated with their external morphological traits. The existence of such correspondence could represent a valuable tool in monitoring and managing natural populations, particularly by modulating their harvest through the female reproductive phenology.

External phenotypical modifications in adult decapod crustaceans might indicate underlying reproductive processes. Molting, for instance, causes morphological changes, such as in cheliped and abdomen size, which allow for the differentiation between adults and juveniles (Corgos and Freire 2006). Morphometric variation has been widely used to detect individuals in reproductive age (Armstrong 1988; Branco and Masunari 2000; Dalabona *et al.* 2005; Pinheiro *et al.* 2005). In addition, exoskeleton coloration of males of *Carcinus maenas* has been shown to correlate strongly with reproductive index, with red individuals showing higher indices than green individuals (Styrishave *et al.* 2004). Similar associations between external phenotypic traits and reproductive state have been shown in other crab species (Detto *et al.* 2004; Styrishave *et al.* 2004). In addition to exoskeleton traits, ovarian coloration might reflect the level of maturation of oocytes because of changes in the accumulation of reserve substances in oocytes and pigmentation patterns. For instance, the portunid crab *Ovalipes catharus* showed that transparent to

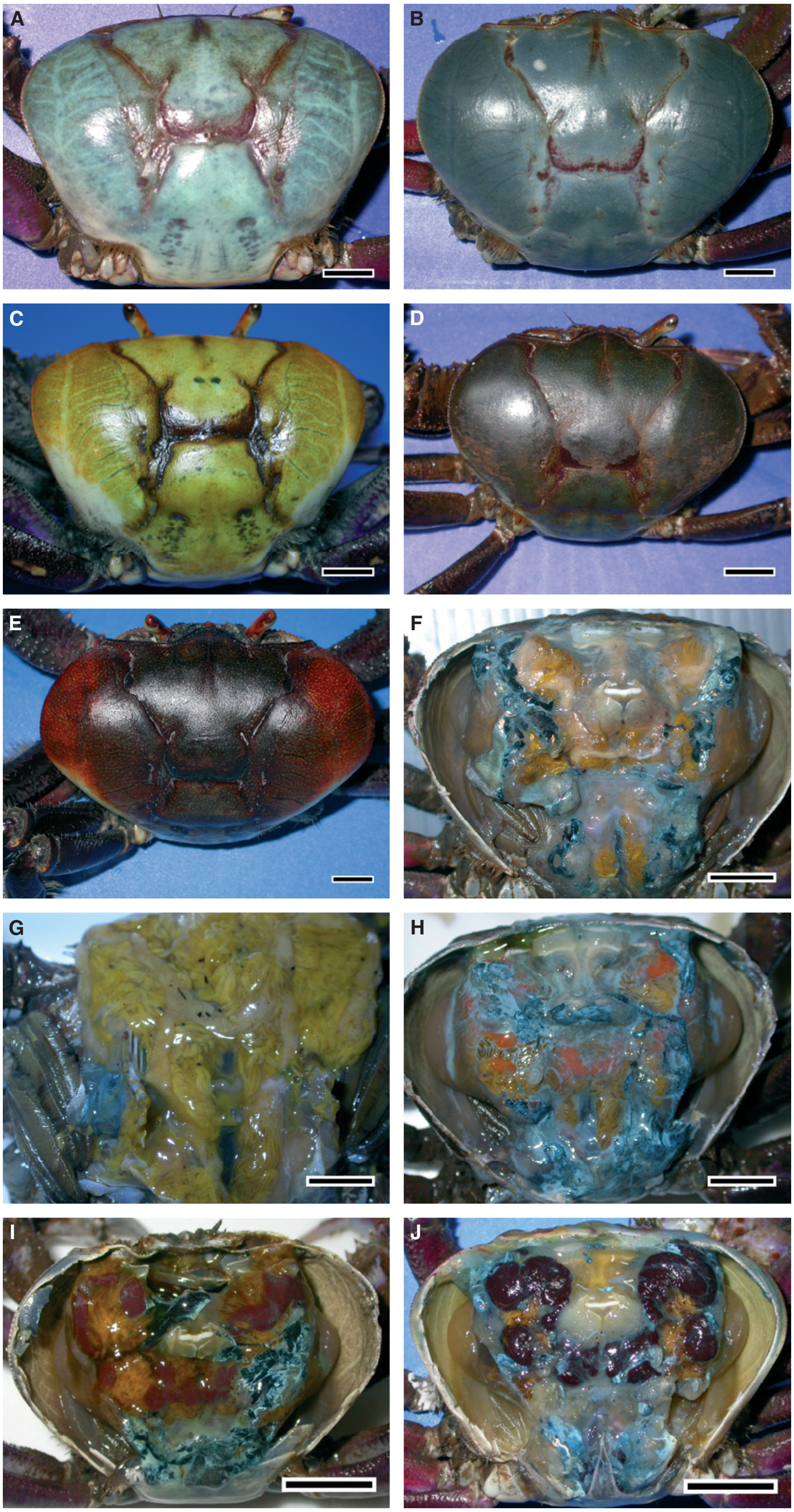

Fig. 1—Carapace and ovarian colorations of *Ucides cordatus* observed in the present study. Carapace colorations (scale = 2 cm): (A) blue, (B) dark gray, (C) yellow, (D) purple, (E) brown. Ovarian colorations (scale = 1.5 cm): (F) whitish, (G) yellow, (H) orange, (I) brown, (J) purple.

light-brown ovaries was associated with the presence of vitellogenesis (Armstrong 1988). Similar results were obtained in *Portunus pelagicus*, where gonadal stages were determined based on the coloration of the ovaries, which ranged from white to orange. The goal of the present study is to test whether macroscopic features such as ovarian coloration and carapace coloration could be used as proxies to describe gonadal development in *U. cordatus*.

Materials and Methods

The animals used in this study were collected monthly in mangroves of the Baía de Antonina (25° 25′ S; 48° 42′ W), Southern Brazil, between October 2002 and March 2005. A total of 367 females (average carapace width = 6.6 cm, range of 3.5–8.0 cm) were captured directly in their burrows and transported alive to the laboratory. Carapace coloration of each individual was subjectively scored as yellow, blue, dark gray, brown, or purple (Fig. 1). Gonadal coloration was assessed during necropsy and scored as whitish, yellow, orange, brown, or purple (Fig. 1).

Ovarian fragments were kept in Davidson's fixative for 24 h, followed by routine histological processing and staining with Harris hematoxylin and eosin, Mallory trichromic, and by a histochemical reaction with Periodic Acid – Schiff, according to Behmer *et al.* (1976).

Ovarian maturation stages were determined histologically based on the prevalence of oogonia and oocytes in different developmental stages (oocyte I – reduced basophile cytoplasm; oocyte II – previtellogenic oocyte; III – oocyte in vitellogenesis, with slightly eosinophilic cytoplasm; oocyte IV –oocyte in vitellogenesis, with eosinophilic cytoplasm and weakly condensed nucleus; and oocyte V – mature oocyte). The following classification was used, agreeing Castilho (2006): E1 (immature ovary), when only oogonia and oocytes in the I and II stages are present, with the latter occupying a large portion of the ovary; E2 (ovary in initial maturation), when oogonia and oocytes in the I, II, and III stages are present, with a higher prevalence of the latter; E3 (ovary in final maturation), when oocytes in the I, II, III, and IV stages are present, with a prevalence of oocytes in the IV stage; E4 (mature ovary), when oocytes in the I, II, and V stages are present, with a prevalence of oocytes in the V stage, which occupy a large gonadal area; and E5 (regenerating ovary), when oocytes in the V stage are being reabsorbed and considerable gonadal restructuring takes place, with a high number of oogonia and oocytes in the I and II stage.

The existence of a non-random association between gonadal or carapace coloration and ovarian development was assessed using a chi-square test, where the frequency of individuals in each color class was scored as either reproductive (stage IV in gonadal maturation) or non-reproductive (all other maturation stages together). This analysis was followed by a graphical representation of the frequency distribution of gonadal maturation stages in each color class.

Results

The distribution of ovarian developmental stages in each color class was significantly non-random with respect to both ovarian ($\chi^2 = 51.6$; df = 4; $P < 0.001$) and carapace coloration ($\chi^2 = 51.0$; df = 5; $P < 0.001$) (Table 1). That is, the frequency of reproductive females was not evenly distributed among different age classes. However, such correspondence was far from being precise. The proportion of reproductive females in each class of carapace coloration varied from 9 to 67% (Table 1). In the case of gonadal coloration, the frequency of reproductive females was even more evenly distributed, from 4 to 18% (Table 1). In other words, if a decision is made to prevent harvesting some coloration class, any such decision would not only preserve a considerable proportion of non-reproductive females but also would fail to protect a large portion of the reproductive females in the population.

The above-mentioned conclusion is corroborated by an inspection of the frequency of different levels of gonadal development in each carapace and ovarian coloration classes (Figs 2 and 3). There is considerable variability within different carapace and ovarian coloration classes in their level of gonadal development. This result indicates that the studied macroscopic traits cannot be used as reliable indicators of the underlying stage of vitellogenesis of a given female.

Discussion

The possibility of using external phenotypical traits such as gonadal or carapace coloration to infer ovarian maturation

Table 1 Number of studied individuals of *Ucides cordatus* classified as either reproductive (stage IV in ovarian maturation) or non-reproductive (all other stages)

Carapace coloration	Reproductive	Non-reproductive	Ovarian coloration	Reproductive	Non-reproductive
Yellow	8	4	Orange	15	92
Blue	24	30	Yellow	2	48
Gray	15	159	Whitish	3	32
Brown	13	54	Transparent	3	14
Purple	10	23	Brown	1	24
			Purple	43	56
Total	70	270	Total	67	266

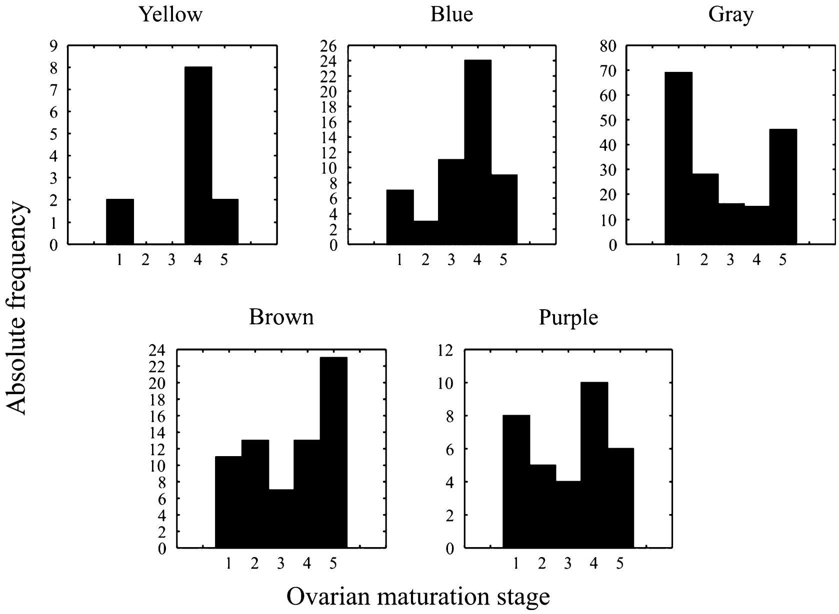

Fig. 2—Relative frequency of different levels of gonadal development of *Ucides cordatus* among different carapace coloration classes.

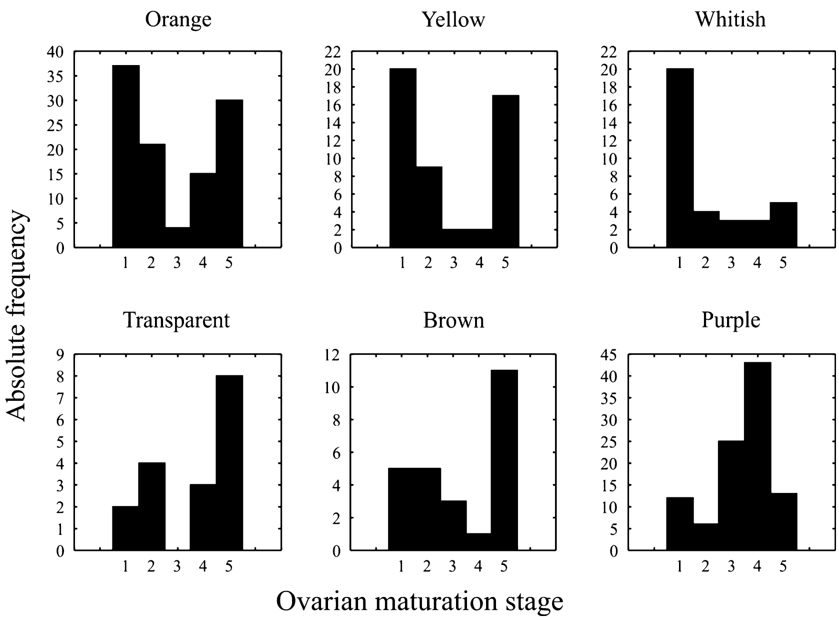

Fig. 3—Relative frequency of different levels of gonadal development of *Ucides cordatus* among different ovary coloration classes.

levels would represent a valuable tool to monitor natural populations and thus inform management initiatives. For instance, brown carapaces in *U. cordatus* have been suggested as indicatives of the molt that precedes copulation, whereas blue carapaces would be most frequent in the mating season (Pinheiro and Fiscarelli 2001). However, the results of the present study indicate that carapace coloration is a poor predictor of ovarian maturation stage. In particular, females with brown or blue carapaces did not show any distinctive level of gonadal development in relation to other carapace color classes (Fig. 2). In addition, although blue carapaces were indeed most common between November and February (the reproductive season), females with brown carapaces were present all year long in the study location.

Several authors have studied the variation in the ovarian coloration of *U. cordatus* (Alves 1975; Nakamura 1979; Góes *et al.* 2000; Dalabona 2001), which might reflect modifications because of differential accumulation of reserve substances in oocytes (Tsukimura 2001). However, there was little agreement among those authors with respect to the association between coloration and developmental stage, as well as the number of developmental stages. The results of the present study indicate that ovarian coloration might not reflect the underlying level of gonadal development. For instance, an E5 stage, which is characterized by the regeneration of the ovary and the onset of oocyte production for the following spawning in the same reproductive period, was observed in whitish, yellow, orange, and brown ovaries (Fig. 3).

In conclusion, the results of the present study indicate that there is little correspondence between macroscopic phenotypical traits such as gonadal or carapace coloration and the degree of ovarian maturation at the cellular level. Therefore, any management policies based these traits is not advised.

Acknowledgements

Financial support for the present study was provided by the SETI/UGF of the Government of the State of Paraná. We thank P. V. Costa, R. Ventura, and U. T. Silva for assistance in obtaining the crabs in the field.

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