



Reproductive potential of *Ucides cordatus* (Linnaeus, 1763) (Decapoda: Brachyura: Ocypodidae) from two mangrove areas subject to different levels of contaminants

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ABSTRACT

Ocypodid crabs are common in mangrove areas, and the *uçá*, *Ucides cordatus* (Linnaeus, 1763), is a key species. It is abundant and economically relevant in these marine wetlands on the southeastern Atlantic coast of Brazil. Mangroves suffer from intense anthropogenic impact by pollutants affecting the reproductive biology of the animals inhabiting this ecosystem. We analyzed the reproductive potential of *U. cordatus*, comparing fecundity and fertility equations between two mangroves with distinct pollution levels in São Paulo state, Brazil: a pristine (Juréia-Itatins Ecological Station) and a contaminated (Cubatão) area. There was no difference in egg production (fecundity) between crabs from both areas, possibly due to tolerance mechanisms exhibited by the crabs. There was, however, a substantial difference in larval production, which was three times higher in crabs from the contaminated mangrove than in those from the pristine mangrove. These results are explained by different reproductive conditions of the ovigerous females arising from the fertility analysis: primiparous (first spawning from the contaminated area) and multiparous females (second or subsequent spawning from the pristine area). Multiple spawning in the same reproductive season had not been previously reported for this species. Results, however, do not explain the relationship between environmental contaminants and fertility, which should be investigated in the future.

Key Words: fecundity, fertility, reproductive biology, Ucidinae.

INTRODUCTION

Ucides cordatus (Linnaeus, 1763) is a semi-terrestrial brachyuran crab typical of mangrove areas. The species is widely distributed in the western Atlantic (Melo, 1996) and is considered an important fishery resource (Diele *et al.*, 2005) and a key species because crabs maintain energy and nutrients within mangrove forests by recycling senescent leaves (Nordhaus *et al.*, 2009). These crabs have a relevant role in environmental monitoring due to their high longevity (~10 years; Pinheiro *et al.*, 2005), reduced mobility (Nordhaus *et al.*, 2009), endemism, and easy capture (Pinheiro & Fiscarelli, 2001). They show seasonal reproduction (November to March) with showing two peaks (December and February), each with a different frequency of ovigerous females (Pinheiro & Fiscarelli, 2001; Sant'Anna *et al.*, 2014; Moraes *et al.*, 2015).

Reproductive strategies in brachyurans vary with season, spawning number, loss of eggs, and the spawning condition of females (Sainte-Marie, 1993; Diez & Lovrich, 2010; Verísimo *et al.*, 2011; Hartnoll, 2015; McLay & Becker, 2015). Reproduction can also be affected by exogenous factors such as salinity, temperature, photoperiod (Sastry, 1983; Bembe *et al.*, 2017), and environmental contamination (Depledge & Fossi, 1994; Penha-Lopes *et al.*, 2009; Almeida *et al.*, 2016). Species respond in different ways to contaminants, showing tolerance that change with exposure time and concentration of pollutants (Depledge & Fossi, 1994). Estuarine species can be affected by local contamination (Harris & Santos, 2000; Rotter *et al.*, 2011) but some crustaceans have strategies to survive when exposed to such contaminants (Ortega *et al.*, 2016). One of the strategies is the synthesis of metallothionein, a protein that binds metals (Bjerregaard *et al.*, 2005; Gao *et al.*, 2012).

Studies on reproductive strategies help in the management of fishery resources, and previous contributions on this topic have been reported for *U. cordatus* (Nascimento, 1993; Góes *et al.*, 2000; Sampaio, 2002; Pinheiro *et al.*, 2003; Pinheiro & Hattori, 2003; Hattori & Pinheiro, 2003; Dalabona *et al.*, 2005; Sant'anna *et al.*, 2007; Castilho-Westphal *et al.*, 2008, 2011, 2013; Wunderlich *et al.*, 2008; Sant'anna *et al.*, 2014). Fecundity and fertility of *Ucides cordatus* have been studied by Pinheiro *et al.* (2003) and Hattori & Pinheiro (2003), respectively. There are, nevertheless, studies showing differences between egg and larval production from contaminated and pristine mangroves areas, which are relevant to optimal management (Pinheiro *et al.*, 2003; Gonçalves & Reigada, 2012).

Mangroves forests have been affected worldwide by anthropogenic impact (e.g., Alongi, 2002; Duke *et al.*, 2007; Abreu-Mota *et al.*, 2014). Such human pressure has been affecting mangroves along the central coast of São Paulo state, Brazil since European colonization starting in the 1500's (Pinheiro *et al.*, 2010). The port of Santos and the Cubatão industrial complex are the main sources of pollution on this coastal region (Luiz-Silva *et al.*, 2006; Nascimento *et al.*, 2006; Zündt, 2006); stilt houses and untreated sewage are also important (Pinheiro *et al.*, 2012). Studies have looked at benthic macroinvertebrates such as *U. cordatus* as indicator of pollution aiming to obtain information about local anthropogenic conditions (Eisler, 2010; Duarte *et al.*, 2017; Pinheiro *et al.*, 2017). Recognizing the mechanisms that allow the survival of these species in highly stressful environments is essential for studies of populations impacted by different levels of pollution (Ortega *et al.*, 2016). We evaluated the reproductive strategies of *U. cordatus*, the uçá crab, by comparing fecundity (number of eggs), fertility (number of hatched larvae), and hatching rate in a pristine (the Juréia-Itatins Ecological Station) and a polluted mangrove forest in the municipality of Cubatão.

MATERIALS AND METHODS

Study area

This study was conducted in two mangrove areas in São Paulo state, Brazil (Fig. 1) impacted by different levels of pollution (Duarte *et al.*, 2016, 2017): Cubatão, defined as a probably high impacted (PHI) area and the Juréia-Itatins Ecological Station, Peruibe, defined as a probably non-impacted (PNI) area.

Sampling

Ovigerous *Ucides cordatus* during the same reproductive season were collected in Cubatão (December 2015) and Juréia (February 2016). Ovigerous individuals were manually captured by directly inserting the arm of a catcher into the burrow (Pinheiro & Fiscarelli, 2001), and kept individually in plastic bags to avoid the loss of appendages or eggs.

Specimens were identified in the laboratory following the diagnostic characters of Melo (1996) and measured (CW, carapace width) with precision calipers (0.01 mm). Eggs (~5) were removed from each individual and observed under a microscope for identification of the embryonic stage, according to stage characterization already reported for *U. cordatus* by Pinheiro & Hattori (2003). Individuals with eggs in the first stages (second to fourth) were used for fecundity analysis, whereas those in the last stages (seventh and eighth) were placed in plastic containers until the larvae hatched and later used for fertility analysis.

Fecundity

Fecundity is the number of eggs produced by a female in a single egg batch or during a given period of its life cycle (Sastrý, 1983), and is related to the size of females. Ovigerous females from the fecundity analysis were treated to the procedures of Pinheiro *et al.* (2003). Crabs were chilled at 4 °C prior to sacrifice. Egg masses

were removed with scissors and tweezers and transferred to 70% ethyl alcohol for 48 h and subsequently to absolute alcohol for 72 h. Each egg mass was dried (60 °C) until weight was stabilized (~72 h). Pleopod fragments and setae of each dry egg mass were removed and discarded. Total dry weight of the egg mass was obtained using an analytical balance (to 0.0001 g precision). Fecundity was determined by counting the number of eggs in three subsamples (0.3 mg each) under a stereomicroscope. Calculation of the total number of eggs was performed by the rule of proportions, together with mean number of eggs (NE) for each female. The coefficient of variation (CV) between three subsamples of each female was calculated to avoid analytical error, with significant deviations substituted by another counting when CV was larger than 15%.

A regression analysis of NE *versus* CW was performed using NE as a dependent variable and CW as an independent variable; the empiric point was fitted by a power function ($NE = aCW^b$). The fecundity equation was evaluated for each mangrove area according to the determination coefficient (R^2), considering $R^2 > 0.70$ as a good fit. Log-transformed data were compared by t-test for each mangrove area to evaluate possible differences between fitted curves. A significance level of 5% was used.

Fertility

Fertility is defined as the number of larvae hatched in a single batch, and is related to female's size (Sastrý, 1983). Fertility was analyzed as discussed by Hattori & Pinheiro (2003). Each ovigerous female in final embryonic stages was maintained in plastic recipients (3 l), with controlled salinity (15 ± 1 psu), temperature (26 ± 1 °C) and photoperiod (12:12 h), until hatching. Larvae were transferred after hatching to a glass recipient and preserved in alcohol 70% until analysis. Each set of larvae released by a female was placed in a larger plastic recipient with water (5 l) in constant aeration for the proper dispersion of larvae in the water column. Fertility was determined by quantifying the number of larvae in ten subsamples (2 ml) using a Stempel pipette and calculating the mean by total volume extrapolation (5 l). The total number of larvae (NL) was determined per female by the rule of proportion, with sample elimination when $CV > 15\%$.

Empirical points of NL *versus* CW relationship were submitted to regression analysis, with NL the dependent variable and CW the independent variable. These data were fitted by a power function ($NL = aCW^b$) and confirmed by a determination coefficient (R^2). The same procedure was applied to fecundity equations, with log-transformed data used to compare mangrove fertility equations by t-test at a significance level of 5%.

Hatching rate

Hatching rate (HR) was calculated comparing fecundity and fertility equations obtained from each mangrove area. The percentage of hatching was organized by size class (5 mm CW) in both sampled areas, and expressed by the equation $HR = (NL/NE) \times 100$ (Hattori & Pinheiro, 2003) where HR is the mean of hatching rate, NL the mean number of larvae, and NE the mean number of eggs. HR means were tested for normality (Shapiro-Wilk test) and homoscedasticity (Levene's test). Data were compared by t-test or by non-parametric tests (Mann-Whitney test), according to the previously obtained results.

RESULTS

A total of 108 ovigerous females of *U. cordatus* were captured in the mangroves of Cubatão (December 2015) and Juréia (February 2016). The composition of females with eggs in initial embryonic stage and pre-hatching stage varied between areas (initial embryonic, 45.3% and pre-hatching, 54.7% in Cubatão; initial embryonic, 58.2%, pre-hatching, 41.2% in Juréia). Fifty-six females (Cubatão, $N = 24$; Juréia, $N = 32$) were used for fecundity analyses, and 52 females (Cubatão, $N = 29$; Juréia, $N = 23$) for fertility analyses.

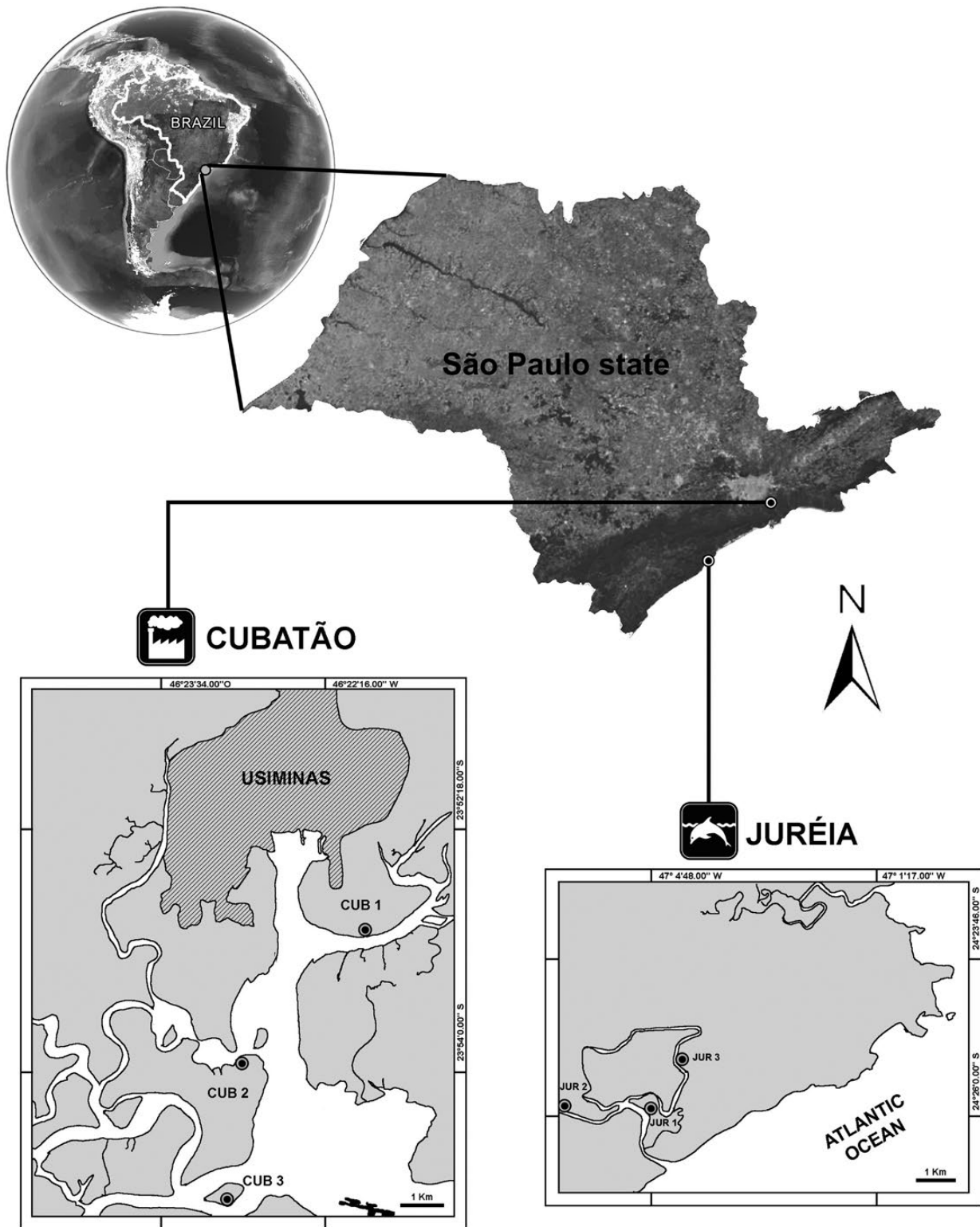


Figure 1. Mangrove areas in São Paulo state, southeastern Brazil: Juréia (JUR) on the south coast and Cubatão (CUB) on central coast, each comprising three subareas. From Google Earth® satellite images by G. Pinheiro).

Fecundity

The size of the ovigerous females was significantly higher in Cubatão ($F = 2.137$; $P < 0.0001$) (mean \pm standard deviation 65.09 ± 5.32 mm CW) than in Juréia (57.73 ± 6.01 mm CW). A positive correlation was confirmed between NE and CW, regardless of location (Cubatão $r = 0.79$, $P = 0.00024$; Juréia

$r = 0.88$, $P = 2.06 \cdot 10^{-9}$), with empiric points better fitted to a power function ($R^2 > 60\%$, Table 1).

The number of recently extruded eggs (NE) in Juréia ranged 52,334–176,654 eggs ($129,503 \pm 21,134$ eggs) and was significantly lower than in Cubatão (76,545–212,801 eggs; $152,368 \pm 31,148$ eggs) ($F = 12.82$, $P < 0.001$), corroborating the differential fecundity and positive correlation between this reproductive variable

Table 1. Summary of biometric variables and fecundity equations obtained for *Ucides cordatus* in a pristine (Juréia) and contaminated (Cubatão) mangrove based on the regression of the dependent variable (NE, number of eggs) versus independent variable (CW, carapace width). Both equations were significant ($P < 0.05$). \bar{x} , mean; s, standard deviation.

Mangrove areas	N	NE			CW (mm)			NE × CW ($Y = aX^b$)	R^2 (%)
		Minimum	Maximum	$\bar{x} \pm s$	Minimum	Maximum	$\bar{x} \pm s$		
Juréia	32	52,334	170,654	129,503 ± 21,134	43.0	66.4	60.05 ± 4.4	NE = 6.75CW ^{2.41}	77.8
Cubatão	24	76,545	212,801	152,368 ± 31,148	51.3	78.3	65.03 ± 5.9	NE = 49.8CW ^{1.92}	61.8
Total	56	52,334	212,801	134,869 ± 33,845	43.0	78.3	60.5 ± 6.8	NE = 19.6CW^{2.15}	76.2

and female size (Fig. 2). There were no significant differences between fecundity equations (Cubatão versus Juréia) when intercept (a) and slope (b) constants were compared (a: $t = 0.901$, $P = 0.37$; b: $t = 1.25$, $P = 0.29$). This suggests a single general fecundity equation (NE = 19.6CW^{2.15}; $R^2 = 76.2\%$) despite the distinct areas.

Fertility

Ovigerous females were significantly smaller in Juréia ($t = 2.02$; $P = 7.28 \times 10^{-6}$) (58.2 ± 4.4 mm CW) than in Cubatão (66.1 ± 5.2 mm CW), following the same pattern observed in the fecundity analysis.

Fertility data were submitted to residue analysis, excluding 24 ordination pairs (Cubatão $N = 15$; Juréia $N = 9$). The variables NL and CW were positively correlated, without an effect of mangrove area (Cubatão $r = 0.81$, $P = 0.00057$; Juréia $r = 0.84$, $P = 0.0016$). Empiric points of NL versus CW relationship were fitted through a power function ($R^2 > 65\%$, Table 2).

The fertility of *U. cordatus* (155,813 ± 27,264 larvae) was four times greater in Cubatão than in Juréia (38,589 ± 9,463 larvae) ($t = -14.18$, $P < 0.0001$). The result was confirmed by comparing the same size class between mangrove areas. Fertility equations between areas differed in relation to intercept constant (a: $t = -18.47$, $P < 0.0001$) but not the slope (b: $t = 1.59$, $P = 1.42$) (Fig. 3). Line equations did not coincide, confirming different fertility conditions of crabs of different mangrove areas.

Hatching rate

Hatching rate (HR) in Juréia ranged from 25.6 to 24% (30.8 ± 3.8%). HR was higher (82.4 to 100%; 94.3 ± 7.2%) in Cubatão, representing a higher larval release rate. Hatching rate data from Juréia ($N = 4$) and Cubatão ($N = 5$) showed normal distribution ($0.845 \leq W \leq 0.899$; $0.180 \leq P \leq 0.426$) and homoscedastic variance ($L = 0.876$; $P = 0.380$). Means were different ($t = -15.79$, $P = 0.1 \cdot 10^{-5}$); HR was three times higher in Cubatão than in Juréia (Fig. 4).

DISCUSSION

Fecundity and fertility in decapod crustaceans are generally represented by curvilinear equations (Pinheiro & Terceiro, 2000; Hamasaki et al., 2006; Rameshbabu et al., 2006; Rasheed & Mustaqim, 2010). These equations relate the cubic relationship of the number of eggs (NE) and larvae (NL) with the linear variable that represents the width of the carapace (CW). A positive correlation between egg production and size of ovigerous females in *U. cordatus* was confirmed as a function of the limited cephalothoracic space that varies with the shape and volume of the cephalothorax occupied by the branchial chambers (Hines, 1982; Hartnoll & Gould, 1988).

Egg production in *U. cordatus* was not significantly different in mangroves with different levels of contamination. These results confirm those of Ortega et al. (2016) regarding contaminant

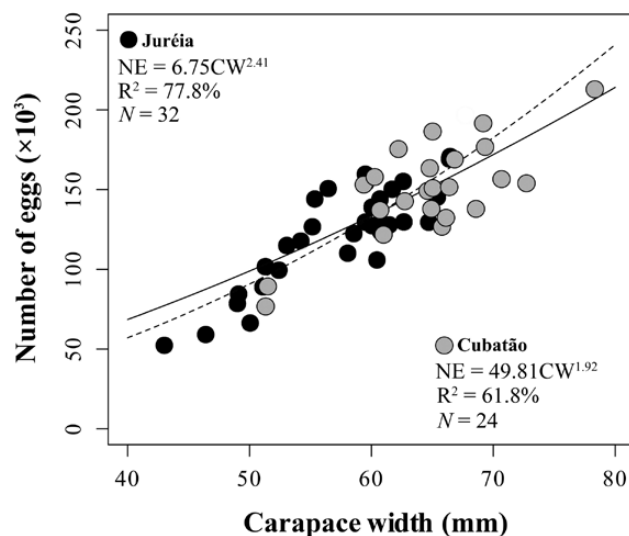


Figure 2. Fecundity (NE versus CW) of *Ucides cordatus* based on empiric points established on each mangrove area (Cubatão, gray points and continuous line; Juréia, black points and dashed line) fitted by a power function. NE, number of eggs; CW carapace width.

tolerance in this species. Some studies nevertheless argue that pollutants can stimulate or inhibit reproduction in decapod crustaceans. Martin-Diaz et al. (2004, 2005) stated that some trace metals (Cd, Cu, and Zn) can stimulate vitellogenesis in the brachyuran crab *Carcinus maenas* (Linnaeus, 1758), whereas the opposite was observed in the crayfish *Procambarus clarkii* (Girard, 1852), where ovary maturation was inhibited after exposure to Cd and Hg (Reddy et al., 1997). Few studies present an in-depth analysis of the effects of metal contamination in the reproductive potential of brachyurans. Fecundity can be reduced by premature loss of eggs when exposed to contaminants (DeCoursey & Vernberg, 1972), whereas an increase of egg production in environments with high concentrations of organic matter and metals (Penha-Lopes et al., 2009). Bergey & Weis (2008) found that egg production in *Minuca pugnax* (Smith, 1870) was not significantly different in mangrove areas with distinct contamination levels of organic pollutants and metals. *Minuca pugnax*, like *U. cordatus*, is a scavenger ocy podid from mangroves areas and promotes sediment bioturbation.

In a review of biomarkers, Depledge & Fossi (1994) indicated that chronic exposure to chemical pollutants could trigger regulatory mechanisms and generate tolerant populations. The same fecundity rate of *U. cordatus* in the two studied areas confirms this hypothesis, given the long-term contamination in Cubatão (Pinheiro et al., 2008; Banci et al., 2017) in contrast to the relative pristine conditions in Juréia (Pinheiro et al., 2013; Duarte et al., 2016, 2017). The reduction in both fertility and hatching rate, however, occurred only in the pristine mangrove area (Juréia) and raised important questions. Besides the observed differences, the fertility rate in Juréia was lower to that reported by Hattori & Pinheiro (2003) for the same species. These authors conducted their study

Table 2. Summary of biometric variables and fertility equations obtained for *Ucides cordatus* in a pristine (Juréia) and contaminated (Cubatão) mangrove based on the regression of the dependent variable (NL, number of larvae) versus independent variable (CW, carapace width). Both equations were significant ($P < 0.05$). \bar{x} , mean; s, standard deviation.

Mangrove Areas	N	NE			CW (mm)			NE × CW ($Y = aX^b$)	R^2 (%)
		Minimum	Maximum	$\bar{x} \pm s$	Minimum	Maximum	$\bar{x} \pm s$		
Juréia	14	24,500	55,000	38,589 ± 9,463	50.2	66.2	58.2 ± 4.4	NL = 0.565CW ^{2.73}	70.1
Cubatão	14	102,000	199,000	155,813 ± 27,264	56.7	76.05	66.1 ± 5.2	NL = 102.9CW ^{1.75}	66.7

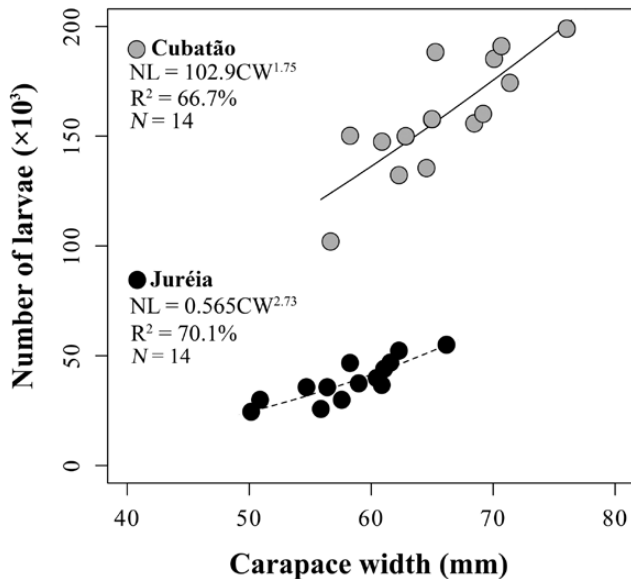


Figure 3. Fertility (NL versus CW) based on empiric points established on each mangrove area (Cubatão, gray point and continuous line; Juréia, black points and dashed line) fitted by a power function. NL, number of larvae; CW, carapace width.

in moderately impacted mangrove areas (Iguape, 24°42'8.0"S, 47°28'55.5"W), whereas Cubatão is a highly impacted mangrove area with subtle damage recorded for *U. cordatus* individuals (Duarte *et al.*, 2016). The mean fertility of *U. cordatus* in Iguape was given by Hattori & Pinheiro as 147,169 ± 32,070 larvae ($N = 58$; 41.7–76.8 mm CW) and therefore did not differ from the mean fertility in Cubatão (155,813 ± 27,264 larvae; $t = 1.29$; $P = 0.19$) we report. Furthermore, there was no difference between the a and b constants of the equations obtained of both locations (a: $t = 0.63$, $P = 0.53$; b: $t = 0.91$, $P = 0.36$), so the data could be represented by the equation $NL = 255.3CW^{1.53}$, $R^2 = 77.4\%$ (Fig. 5). The fertility was similar between locations that presented distinct levels of impacts by pollutants (see Duarte *et al.*, 2016). Hence, the contamination status of these mangroves does not explain the differences in the reproductive potential found here.

The second ovigerous peak (February) during the reproductive season of *U. cordatus* on the south coast of São Paulo state could be explained as a second spawning of females (Sant'anna *et al.*, 2014) using the remaining spermatophores from the copulation in October (two months before, according to Pinheiro & Fiscarelli, 2001) or from a second copulation in December. Most females spawn once a year and only a few of them spawn again in the same reproductive period, as indicated by a reduced ovigerous frequency in this second spawning peak (Diele, 2000). The fertility rate we reported for the Cubatão populations and those recorded for Iguape (Hattori & Pinheiro, 2003) were similar and based on ovigerous females captured in December (first peak). The fertility rate in these two mangrove areas was four times higher than

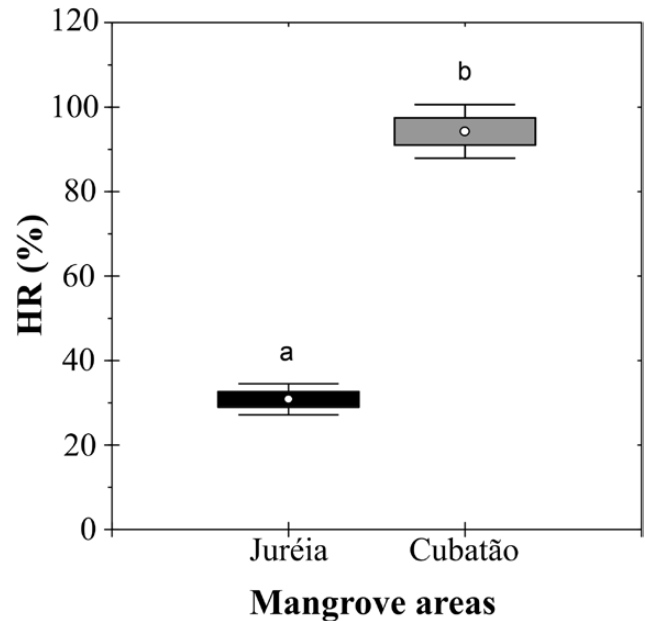


Figure 4. Hatching rate (HR) of ovigerous individuals of *Ucides cordatus* in mangroves: Cubatão (gray boxplot) and Juréia (black boxplot). White dot, mean; box, mean ± standard error; whisker, confidence interval of mean; means with different letters are statistically different ($P < 0.05$).

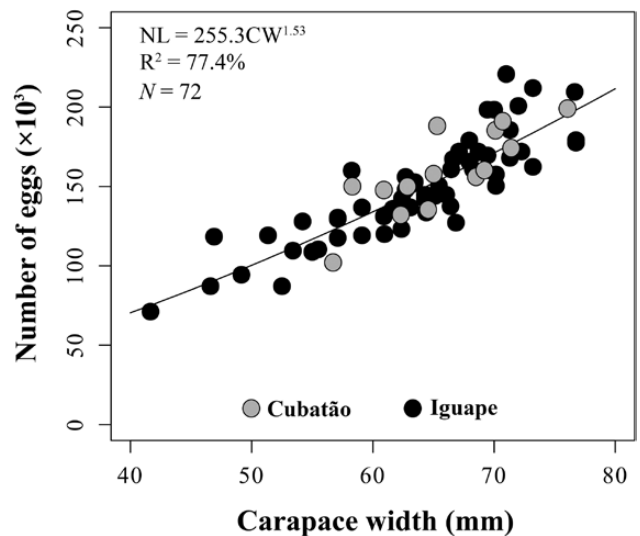


Figure 5. Fertility data and equations (NL versus CW), obtained from female *Ucides cordatus* captured in December in two mangrove areas: Cubatão (present study; sampled in 2015, gray points) and Iguape (Hattori & Pinheiro, 2003; sampled between 1998 and 2003, black points). NL, number of larvae; CW, carapace width.

those obtained from Juréia, which were captured in February (second peak). This information indicates a change in fertility rate along the reproductive period of *U. cordatus* as previously reported by Pinheiro *et al.* (2003). According to Some females can produce embryos even if deprived of a new copulation by using the spermatophores stocked from previous copulations (Castilho-Westphal *et al.*, 2013). A single copulation can produce more than one spawning (K. Diele, personal communication). The number of larvae produced varies over time and these differences can be associated with multiple spawning (multiparity) in this species. This reproductive strategy, which seems to occur in *U. cordatus*, was previously reported in other brachyuran families such as Oregoniidae (Sainte-Maire, 1993) and Majidae (Verisimo *et al.*, 2011).

The number of spawnings per year (or in the same life cycle) has a direct effect on reproductive potential in brachyurans (González-Pisani & Greco, 2014). Females of aquatic crabs copulate immediately after molting (e.g., *Callinectes sapidus* Rathbun, 1896), with no difference in reproductive potential among consecutive spawnings (Graham *et al.*, 2012). This is possibly due to the greater size of their seminal receptacles and a higher number of spermatozoa that allow consecutive multiple spawnings. Females of some aquatic crabs, on the other hand, copulate during intermolt, as reported for *Chionoecetes opilio* (Fabricius, 1788) and *C. bairdi* Rathbun, 1924, showing a reduction of 70–80% in reproductive potential in multiparous species (Somerton & Meyers, 1983 and Sainte-Marie, 1993, respectively). Copulation in *U. cordatus* also occurs during intermolt in both sexes; spermatozoa are stored as spermatophores in sufficient amounts to fertilize the ova (Sant'anna *et al.*, 2007), assuring multiple spawning, as previously confirmed for crabs in captivity by M. Pinheiro (personal communication). This second reproductive peak suggests that females were spawning for a second time, while the first peak was correlated to primiparous spawners (first spawning), thus explaining a higher quantity of larvae produced. Histological evaluation of the seminal receptacles of *U. cordatus* by Sant'anna *et al.* (2007) confirmed the presence of spermatophores throughout the year, showing that the remaining spermatophores can be used in subsequent spawnings and thus reducing the number of larvae produced.

The semi-terrestrial crabs such as *U. cordatus* are the most derived group due to its hard-shell mating (Hartnoll, 1969; McLay & López-Greco, 2011). The occurrence of two spawnings in the same reproductive period is thus a novel biological information for *U. cordatus*, although it might be more frequent than previously suspected. Our results could fill a gap in the life cycle of this species, encouraging future studies on multiparity.

Conservation measures prevent the capture of ovigerous *U. cordatus* throughout the year in Brazil (IBAMA, 2003a, b). Change in these regulations should consider the differences in larval production over the reproductive period, especially in December, when a massive spawning occurs in primiparous females.

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