

## Size at sexual maturity in female *Euphylax robustus* (Brachyura: Portunidae) collected in coastal waters off Ecuador

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### ABSTRACT

Size at sexual morphometric and physiological maturity was estimated in females of the swimming crab, *Euphylax robustus*. The individuals were caught as bycatch in an artisanal trawl fishery targeting Titi shrimp (*Protrachypene precipua*) in coastal waters off Ecuador. A total of 73 females measuring 37 to 97 mm CW were analyzed. Size at morphometric sexual maturity was estimated using the following models: linear, broken stick, and two segments. The best model was selected by Akaike's information criteria bias corrected. Physiological maturity was established by identifying individuals as mature or immature by the color and width of their ovaries; subsequently, a logistic curve was fitted. Size at morphometric sexual maturity was 71.52 mm CW, and the physiological was 69.31 mm CW. Immature individuals showed ovaries with a cream color or that were semitransparent. The ovaries in mature individuals were light orange-yellow, and intense orange. This is the first report of sexual maturity in *E. robustus*. We assume that different crab species, with a similar mean size or modal like female *E. robustus*, could present size at sexual maturity between 67 to 72 mm CW.

### KEYWORDS

Kernel density estimator, morphometric maturity, ovigerous females, physiological maturity, swimming crabs



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## INTRODUCTION

*Euphyllax robustus* A. Milne-Edwards, 1874 is a swimming crab (Portunidae) distributed from Baja California-Mexico to Paita-Peru (Campos and López, 1998) that inhabits soft benthic habitats between 7 and 85 m depth, and the maximum sizes reported are 12.0 cm carapace width (CW) males and 10.1 cm CW females (Hendrickx, 1995; Moscoso, 2012). Information about *E. robustus* is limited to its distribution and corresponds to sporadic taxonomic and fishery records (Rathbun, 1930; Buitendijk, 1950; Lemaitre and Álvarez-León, 1992; Correa, 1993; Campos and López, 1998; Álvarez-León, 2015; Carbajal and Santamaría, 2017).

An increase in the abundance of *E. robustus* has been reported between March and June in the coastal waters off Ecuador in front of the provinces of Manabí and Esmeraldas (Jiménez and Martínez, 1982). This fact has been currently mentioned by fishermen, who are highlighting a high abundance after ENSO (El Niño-Southern Oscillation) events. *Euphyllax robustus* is part of the bycatch in an artisanal trawl fishery directing at the Titi shrimp (*Protrachypene precipua* Burkenroad, 1934) in coastal waters off Ecuador. *Euphyllax robustus* is a fishing species with economic importance for coastal communities and in this sense, the estimation of population parameters in fishery resources is necessary for their management and sustainable use.

Sexual maturity is a parameter used in fishery management, principally as the basis for establishing a minimum catch size. That measure assumes juvenile individuals are able to participate in at least one reproductive event prior to recruitment into the fishery, and this prevents overfishing of recruitment cohorts (King, 2007; Rodríguez-Félix et al., 2015).

Sexual maturity can be a biological indicator or reference point in fishery species considering the fishing effect on their populations (Jørgensen et al., 2007; Cotter et al., 2009). In crabs, the size at sexual maturity could be of three types, morphometric (i.e., allometric changes related to secondary sexual characters), physiological (i.e., gonadal development),

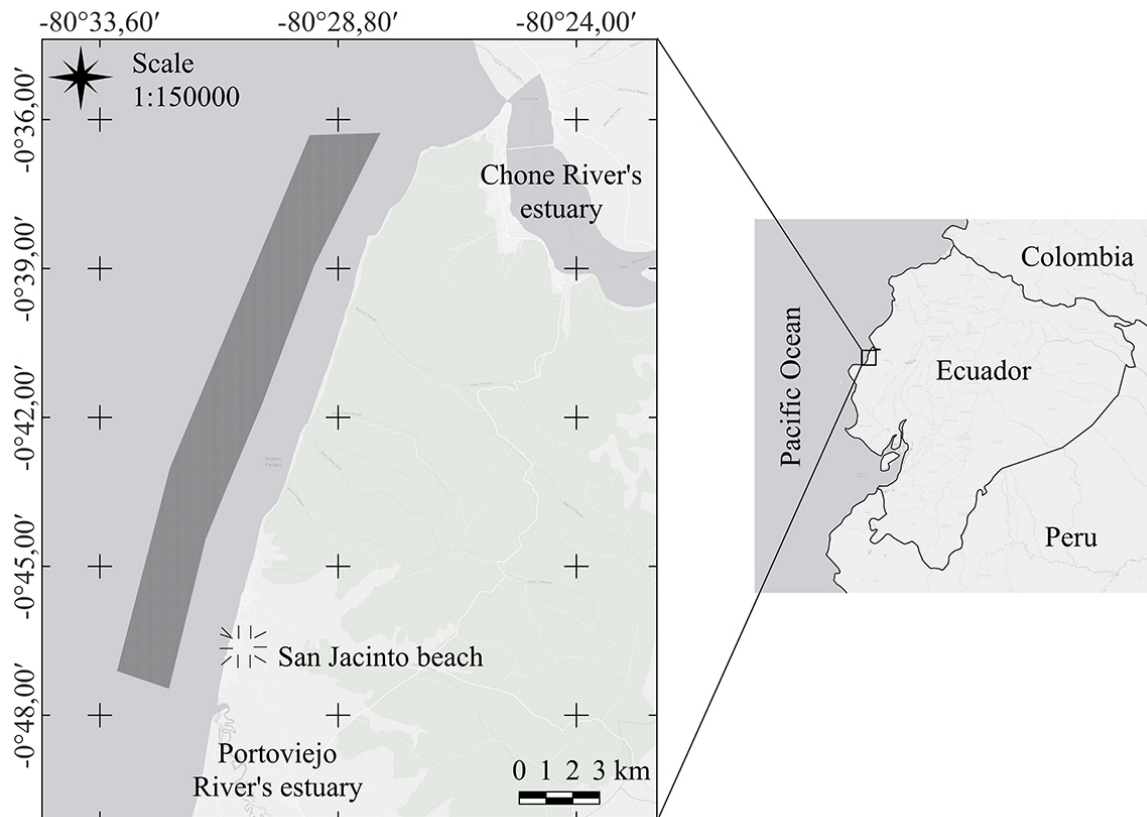
and functional (i.e., mating ability) (Waiho et al., 2017). For swimming crab species such as *Callinectes arcuatus* Ordway, 1863, *Callinectes danae* Smith, 1869, *Callinectes sapidus* Rathbun, 1896, *Portunus pelagicus* (Linnaeus, 1758), *Portunus segnis* (Forskål, 1775), and *Portunus sanguinolentus* (Herbst, 1783) the size at sexual maturity (physiological and morphometric) was previously estimated by logistic and linear models (Marochi et al., 2013; Sumeria et al., 2013; Zairion and Fahrudin, 2015; Ortega-Lizárraga et al., 2016; Wimalasiri and Dissanayake, 2016; Tureli and Yesilyurt, 2017).

The study aim is estimating the size at physiological and morphometric sexual maturity of female *E. robustus*. Even though it is a non-target commercial species, it is subject to fishing pressure, and it is necessary to generate biological indicators and reference points for the sustainable fishery of the species.

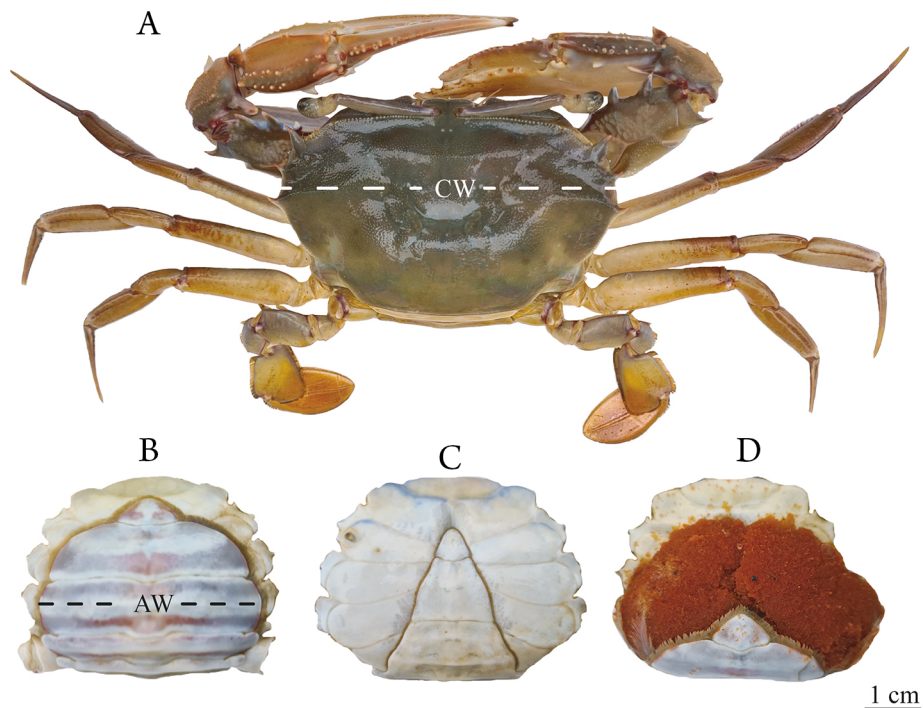
## MATERIAL AND METHODS

The samples were obtained from fishery landings of Titi shrimp (*P. precipua*) in front of San Jacinto Beach, between July and December 2022 (Fig. 1). The females were identified and selected according to the abdomen shape of the individuals sampled: ovoid in females and triangular in males. Morphometric variables, carapace width (CW), and abdomen width (AW) were recorded using a Vernier caliper. Ovigerous females were included in the sampling (Fig. 2). A database was uploaded into Mendeley Data (Cedeño and Zambrano, 2023).

Size frequency distribution (SFD) was built using a Kernel density estimator (KDE) with a Gaussian function (Rosenblatt, 1956). Bandwidth was estimated as the mean between the optimal (Silverman, 1986) and the oversmoothed (Scott, 1992); on the other hand, the origin of SFD was the mean of rounded points in 30 distributions (Zambrano et al., 2018). The analysis was run in Stata software by the commands *bandw* and *warpdenm* (Salgado-Ugarte et al., 2005; Salgado-Ugarte and Saito-Quezada, 2020).



**Figure 1.** Area of artisanal trawl fishery targeting *Protrachypene precipua* in coastal waters off Ecuador (gray polygon). San Jacinto beach is a landing site and where collection of *Euphylax robustus* as part of the bycatch of that fishery occurred.



**Figure 2.** Morphometric variables recorded for female *Euphylax robustus* collected in coastal waters off Ecuador, including an image of the male abdomen. **A**, Carapace width; **B**, female abdomen width (abdomen shape in females is ovoid); **C**, male abdomen (abdomen shape in males is triangular), **D**, ovigerous masses. CW = carapace width; AW = abdomen width.

*Size at morphometric sexual maturity*

The models: linear, broken stick, and two segments were fitted to the female data which was transformed

into a natural logarithm (Marochi et al., 2013). Those models have been used for allometric studies in related to identifying the size at sexual maturity (Hall et al., 2006). The equations were:

**Linear**

$$\ln AW = \ln a_1 + b_1 \times \ln CW$$

**Broken stick**

$$\ln AW = \ln a_1 + b_1 \times \ln CW \text{ if } \ln CW \leq B$$

$$\ln AW = \ln a_1 + (b_1 - b_2) \times \ln(B) + b_2 \times \ln CW \text{ if } \ln CW > B$$

**Two segments**

$$\ln AW = \ln a_1 + b_1 \times \ln CW \text{ if } \ln CW < B$$

$$\ln AW = \ln a_2 + b_2 \times \ln CW \text{ if } \ln CW > B$$

Where the parameters are: intersects ( $a_1, a_2$ ), slopes ( $b_1, b_2$ ) and threshold ( $B$ ). Models were fitted using seed values, intersects = "0", slopes = "1". The  $B$ -value was determined by a threshold regression using Stata

software (Zambrano and Olivares, 2020). The seed values were re-parameterized by log-likelihood ( $LL$ ) according to equations presented by Haddon (2011):

$$LL = -\frac{n}{2} [\ln(2\pi) + 2\ln(\hat{\sigma}) + 1]$$

$$\hat{\sigma}^2 = \sum_{i=1}^n \frac{(\ln(x_i) - \ln(\hat{x}_i))^2}{n}$$

The best model was selected by Akaike information criterion bias corrected ( $AIC_c$ ) due to  $n/K < 40$ , i.e., the ratio between the data number ( $n$ ) and parameter ( $K$ ) was less than 40 (Akaike, 1973; Sugiyama, 1978; Hurvich and Tsai, 1989; Burnham and Anderson, 2002). Additionally, the plausibility of each model was established by Akaike weight ( $W_i AIC_c$ ), in percentages. The equations were obtained from Burnham and Anderson (2002). The fit of two lines to the data was validated statistically by the method of Draper and Smith (1966).

*Size at physiological sexual maturity*

The stage (ST) of gonadal development in females was identify using the Morpho Chromatic scale generated for *Callinectes bellicosus* (Stimpson, 1859). The scale presented five gonadic stages with different

colors: ST1, small and translucent; ST2, white to yellow; ST3, yellow intense; ST4, orange and purple; ST5, light yellow and presence of ovigerous mass (Castañeda-Fernández de Lara et al., 2015).

The Morpho Chromatic scale, and that of other authors, consider the color as characteristic of maturation level in crab species (Stewart et al., 2007; Brown, 2009; Castañeda-Fernández de Lara et al., 2015). The ovaries were dissected for recording the ovary width (OW) using a digital Vernier caliper. The mean and standard deviation (ST) of OW were estimated. Mature individuals were considered in stages ST3, ST4, and DT5.

The physiological sexual maturity of female *E. robustus* was estimated using a logistic model fitted by minimizing the negative log-likelihood ( $-LL$ ) (Brouwer and Griffiths, 2005):



$$P_i = \frac{1}{1 + \exp^{-(L_i - L_{50})/a}}$$

$$-LL = -\sum_{i=1}^n \left[ m_i \times \ln\left(\frac{p_i}{1-p_i}\right) + n_i \times \ln(1-p_i) + \ln\left(\frac{n_i}{mi}\right) \right]$$

where:  $P_i$  = proportion of mature females in size class  $i$ ;  $L_i$  = size of class  $i$ ;  $L_{50}$  = size at physiological sexual maturity in 50% of the females;  $a$  = width of the curve resulting from the proportion of mature fish at a given size;  $n_i$  = number of females in class  $i$ ;  $mi$  = mature females in class  $i$ .

The confidence interval of physiological sexual maturity of female *E. robustus* was estimated using log-likelihood profiles (Venzon and Moolgavkar, 1988). The method was taken from Haddon (2011), and the equations were:

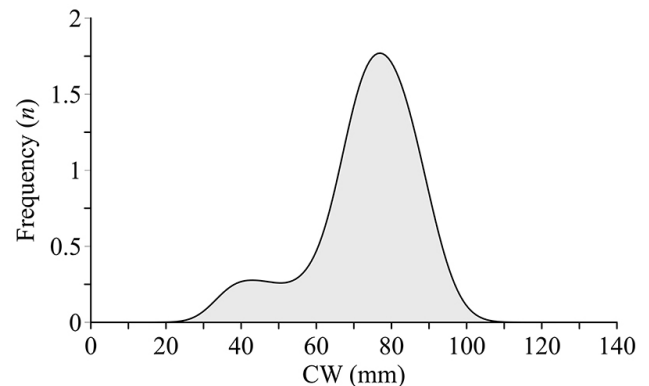
$$2 \times [LL(\theta)_{Max} - LL(\theta)] \leq X^2_{1,1-\alpha}$$

$$LL(\theta) = LL(\theta)_{Max} - \frac{X^2_{1,1-\alpha}}{2}$$

## RESULTS

A total of 73 female *E. robustus* were analyzed. The range of sizes was 37-97 mm CW while the modal size was 77 mm CW (SD = 13.66 mm). Bandwidth was 0.71 mm (Fig. 3). The best model was two segments, but in all cases the intercepts showed negative values while the slopes were higher than the unit (Tab. 1). The fitting of two lines to the female data was validated statistically ( $F_{(3,12)} = 37.31$ ).

Immature individuals of female *E. robustus* showed ovaries with a cream color or semitransparent, the mean of ovary width (OW) was 1.3 mm (SD  $\pm$  0.33 mm). They were difficult to appreciate visually and to manipulate. Mature ovaries were light orange-yellow, intense orange or purple, and the value mean was 3.6 mm OW (SD  $\pm$  1.66 mm). Individuals with these mature ovary colors were used to estimate the size at physiological sexual maturity (Fig. 4).

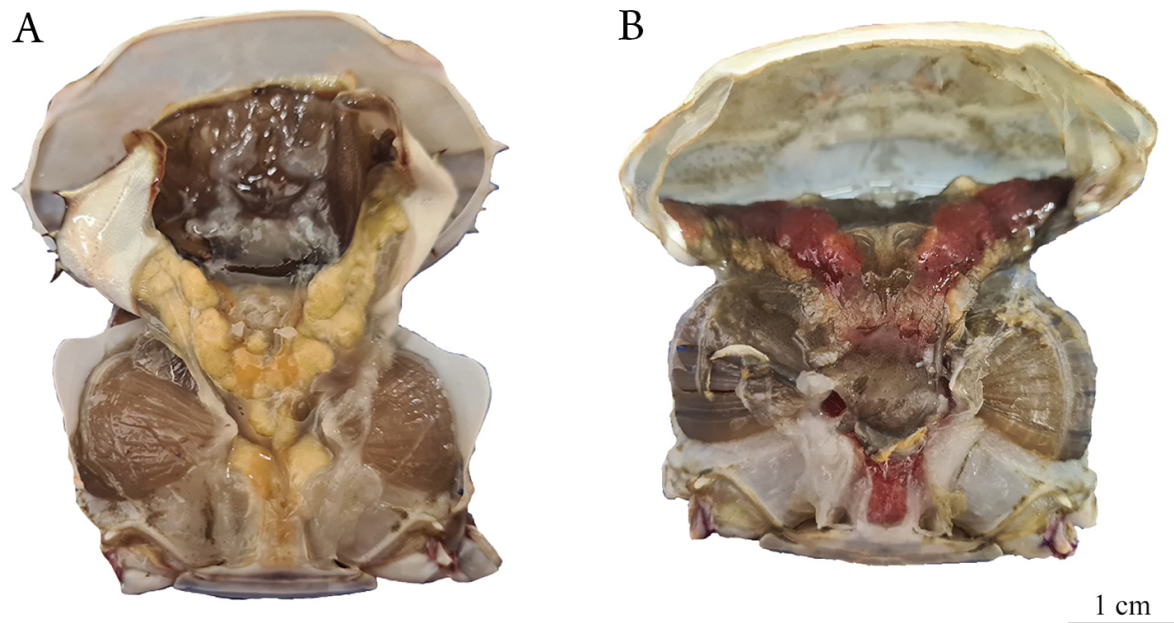


**Figure 3.** Size frequency distribution for female *Euphyllax robustus* collected in coastal waters off Ecuador. CW = carapace width.

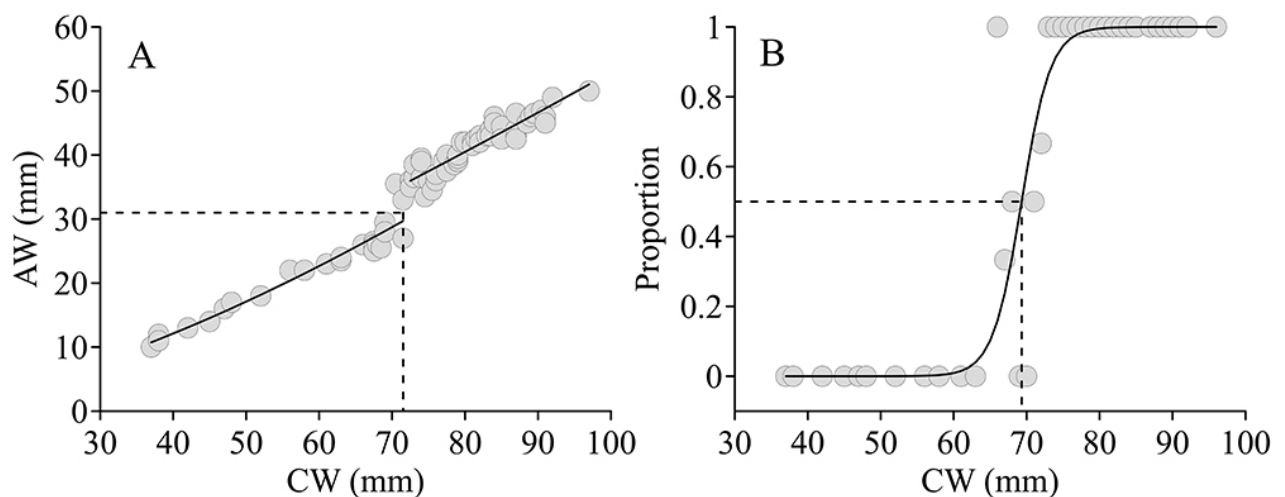
The size at morphometric sexual maturity of female *E. robustus* corresponds to 71.52 mm CW, based on the  $B$ -value from the two segments model. The physiological maturity was 69.31 mm CW, and the confidence interval estimated was 68-71 mm CW (Fig. 5).

**Table 1.** Values of model parameters, Akaike information criterion bias corrected ( $AIC_c$ ) and Akaike weight ( $Wi AIC_c$ ) estimated on females of *Euphyllax robustus* collected in coastal water off Ecuador.

Models/ Parameters	$a$	$b$	$a_2$	$b_2$	$B$	$AIC_c$	$Wi AIC_c$
Linear	-3.93	1.74				-176.33	0.00
Broken stick	-2.92	1.46		1.77	3.81	-172.81	0.00
Two segments	-3.19	1.54	-1.56	1.20	4.27	-223.12	100.00



**Figure 4.** Coloration of mature ovaries in female *Euphylax robustus* collected from coastal waters off Ecuador. **A**, Light orange-yellow ovary; **B**, purple ovary.



**Figure 5.** Size at sexual maturity (segmented line) for female *Euphylax robustus* collected from coastal waters off Ecuador. **A**, morphometric maturity; **B**, physiological maturity. CW = carapace width; AW = abdomen width.

## DISCUSSION

Size at physiological sexual maturity was reached before morphometric sexual maturity in female *E. robustus*. That order is present in other swimming crab species such as *C. danae*, *C. ornatus*, *Charybdis* (*Charybdis*) *hellerii* (A. Milne-Edwards, 1867), and *Necora puber* (Linnaeus, 1767) (González-Gurriarán and Freire, 1994; Carvalho et al., 2011; Marochi et al., 2013; Abdul et al., 2021; Marcio et al., 2022).

However, in other crab species, the order is reversed, for example, in *C. sapidus*, *Po. pelagicus*, *Po. segnis*, *Arenaeus cribrarius* (Lamarck, 1818), *Chaceon notialis* Manning & Holthuis, 1989, *Anamathia rissoana* (Roux, 1828), *Menippe nodifrons* Stimpson, 1859, *Cancer pagurus* Linnaeus, 1758, and *Dilocarcinus pagei* Stimpson, 1861 (Pinheiro and Fransozo, 1998; Delgado and Defeo, 2004; Mura et al., 2005; Bertini et al., 2007; Sumeria et al., 2013; Zairion and Fahrudin,

2015; Davanzo et al., 2016; Tureli and Yesilyurt, 2017; Moore et al., 2022).

The order in which sexual maturity appears is a biological characteristic of each species, and it must be analyzed uniquely (Fernández-Vergaz et al., 2000; Corgos and Freire, 2006; Williner et al., 2014). This order is probably dictated by reproductive convenience. Species that mature physiologically first, such as *E. robustus*, can generate offspring prematurely, at the expense of maximizing their egg-carrying capacity. On the other hand, species that mature morphometrically first increase their capacity to carry eggs after fertilization but take longer to generate offspring. Finally, the order in which the types of maturity are reached in each species has been adapted to increase their capacity to generate offspring and for the maintenance of their populations.

Size at morphometric sexual maturity estimated for *E. robustus* was like that reported for *C. danae* (67.87 mm CW) in Brazil, *Cardisoma crassum* Smith, 1870 (66.02 mm CW) in Ecuador, and *Ch. notialis* (70.20 mm CW) in Uruguay (Delgado and Defeo, 2004; Marochi et al., 2013; Zambrano and Olivares, 2020). On the other hand, physiological sexual maturity of *E. robustus* was like that of *C. danae* (67.00 mm CW) and *Ch. notialis* (71.70 mm CW) (Delgado and Defeo, 2004; Marochi et al., 2013). We assume that different crab species, from neritic, pelagic or terrestrial environments in tropical latitudes, with a mean size or modal like *E. robustus* could present size at sexual maturity between 67 to 72 mm CW.

The two segments method was the best model for estimating the morphometric maturity in *E. robustus*. That procedure has been used in allometry studies in other crab species such as *C. arcuatus*, *Ca. crassum* and *Menippe frontalis* A. Milne-Edwards, 1879 (Ortega-Lizárraga, et al., 2016; Zambrano and Olivares, 2020; Zambrano and Ramos, 2020). Estimating morphometric sexual maturity requires identifying two subsets of data, related to both juvenile and adult individuals. The inflection or angular point between the identified groups indicates the size at sexual maturity. However, the procedure can only be conducted when the morphometric variables represent differentiation of secondary sexual characters, such as the abdomen width in female crabs (Conan et al., 2001).

The coloration of mature ovaries in *E. robustus* is like that reported for *Scylla olivacea* (Herbst, 1796), *S. serrata* (Forskål, 1775), *C. danae*, *C. sapidus*, *Portunus trituberculatus* (Miers, 1876), *Po. sanguinolentus*, *Po. pelagicus*, and *Danielethus crenulatus* (A. Milne-Edwards, 1879) (Stewart et al., 2007; Brown, 2009; Zara et al., 2013; Liu et al., 2014; Wimalasiri and Dissanayake, 2016; Farias et al., 2017; Che et al., 2018; Amin-Safwan et al., 2019). In this sense, we can assume, that females of swimming crab species are mature when their ovaries have a coloration of intense orange, light orange, red-orange, or yellow-orange.

This is the first record of sexual maturity for *E. robustus* along its distribution. The results presented can be used to assess the fishing impact on the species as well as to establish management measures. We recommend establishing 75 mm CW as the minimum fishing size considering the precatory principle and adaptative management (Walters and Hilborn, 1976; Walters, 1986; FAO, 1995).

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## ADDITIONAL INFORMATION AND DECLARATIONS

### Author Contributions

Conceptualization and Design: JC, RZ. Performed research: JC, RZ. Acquisition of data: JC, RZ. Analysis and interpretation of data: JC, RZ. Preparation of figures/tables/maps: JC, RZ. Writing - original draft: JC, RZ. Writing - critical review & editing: JC, RZ.

### Consent for publication

All authors declare that they have reviewed the content of the manuscript and gave their consent to submit the document.

### Competing interests

The authors declare that they have no known competing

financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Data availability

The data are available in Mendeley Data: <https://data.mendeley.com/datasets/6krwd4ppk4/2>

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