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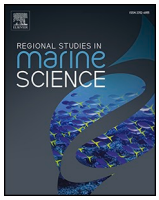

Reproductive traits of the African mud crab (*Panopeus africanus*) on the South Portuguese coast

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ABSTRACT

Species with high economic relevance are widely studied in contrast with low economic relevance species such as *Panopeus africanus*, a small crab with a wide distribution along the eastern Atlantic coast, which remains poorly studied in regarding to the biological and ecological issues. Knowledge of reproductive patterns is essential to understand populations dynamics and species biological life-cycle traits. The main objective of the present research was to study the reproductive patterns of *P. africanus*. The population size structure, maturation stages and gametogenic development aspect, size at maturation, reproductive periods and morphometric–fecundity relationships of *P. africanus* in the southern Portuguese coast (Ria Formosa) was analysed. Crabs were sampled monthly, from January 2019 to December 2021, with traps and hand collected. The crab's carapace width (CW) ranged between 7 to 52 mm, and it was observed that in smaller and larger CW size classes, the proportion of males was higher, however in intermediate CW size classes the sex ratio was near 1:1. The gonads development was characterized based on histological analysis. Males presented four maturity developmental stages where two stages were immature and two were mature. Females presented six maturity developmental stages, where two stages were immature, and four stages were mature. Regardless of sex, the mean maturity size was 20 mm CW. A high percentage of mature individuals was observed throughout the year however, in the colder months the percentage of mature individuals was lower. Additionally, ovigerous females were only observed in the warmer months, pointing to a short breeding period occurring in the warmer months. Fecundity ranged between 12368 and 84140 eggs/female, following a positive correlation between CW/egg number. These novel results allowed to shed light on reproductive patterns of *P. africanus* population inhabiting the southern Portuguese coast, contributing also to a better understanding of the basic reproductive biology of this crab populations.

1. Introduction

The African mud crab, *Panopeus africanus* (Milne-Edwards, 1867), is an endemic species of the eastern Atlantic, with a wide geographical distribution ranging from Angola to Portugal (Manning and Holthuis, 1981). It is an epibenthic species inhabiting shallow intertidal and subtidal habitats in coastal lagoons and estuaries, usually found under stones, burrowed in mud, or among oyster shells at a maximum depth of 4 m (Rodríguez et al., 1997). In crustacean species that have a high commercial or ecological relevance, the reproductive biology has been widely studied (Castiglioni and Negreiros-Fransozo, 2006). Despite the wide distribution of *P. africanus* along the east coast of the Atlantic, there is limited information on the reproductive biology of this species as these have no commercial value. This is also the case of the Algarve, south Portugal, where the species is frequently incidentally caught when harvesting for other crab species, such as the European green crab (*Carcinus maenas*).

Knowledge about reproductive patterns is essential to understand population reproduction biology, such as spawning season, reproductive potential, and size at first maturity (Costa and Soares-Gomes, 2009; Naderi et al., 2018). Relations between size and reproduction events, such as mating and physiological sexual maturity, are crucial to understanding stock dynamics (Bianchini and Ragonese, 2008) and management options regarding the preservation of populations (Hartnoll,

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1974).

The reproductive cycle of crustaceans includes a series of morphological and physiological events. In specimens completing the juvenile stages, these events include: 1) proliferation of gonadal cells; 2) differentiation, growth, and maturation of gametes; 3) reproductive behaviour associated with mating; 4) release of gametes; 5) ovulation; 6) spawning; and 7) incubation of embryos until hatching (Sastry et al., 1983). Hence, knowledge of the reproductive patterns (i.e., sexual maturation size, reproductive periods), assessed at micro or macroscopic level, are essential for understanding population reproduction dynamics and growth. For instance, fecundity is a measure of a species reproductive potential and as such, a key factor in the estimation of future stock levels. It allows the performance of a reproductive prognosis as well as the establishment of criteria for the exploitation and management of commercial species (Rizzo and Bazzoli, 2019). In most marine species fecundity is also directly related to biological characteristics of females such as body size, maturation age, reproductive effort, life spawn and environmental local conditions (Pinheiro and Fransozo, 1995; Llodra, 2002; Pinheiro et al., 2003; Monteiro et al., 2022).

Assessing reproductive parameters and fecundity allows for a better understanding of population fluctuations. The only scientific information published about the reproductive patterns of *P. africanus* is the work of Rodríguez et al. (1997) on the Cadiz Bay (Spain) where aspects of the reproductive cycle and larval development are studied. Thus, the present work aims to study reproductive patterns of *P. africanus,* namely: 1) macroscopic and microscopic (histological) maturation stages, 2) size at sexual maturation, 3) reproductive periods, 4) fecundity and 5) morphologic–fecundity relations in the southern Portuguese coast.

2. Materials and methods

2.1. Study Area and Sampling procedures

Samples were collected from the Ria Formosa, a shallow mesotidal coastal lagoon, a system of sand barrier islands that extends across 55 km along the southern coast of Portugal (Fig. 1). The average depth of the system is 3 m and 14% of its surface is permanently submerged (Falcao and Vale, 1990). Due to its shallowness, 50 to 75% of Ria Formosa water is exchanged with coastal Atlantic water during each tidal period and exchange rates vary according to the lunar calendar, which regulates tide intensity (Newton and Mudge, 2003). Salinity ranges from 36 to 38 PSU and the water temperature varies between 12.5 ºC in the winter to 25.5ºC in the summer (Barbosa, 2010).

Panopeus africanus were sampled monthly from January 2019 to December 2021 with fishing traps and hand collected. The fishing traps were deployed by professional fishermen and remained fishing between 12 to 24 h. The traps were settled at a mean depth of 2–3 m. A total of 3768 specimens were captured, where 2456 were males and 1312 females. In each field sampling the water temperature was measured with a hand-held meter (VWR Symphony SP90M5).

2.2. Laboratory procedures and data analysis

2.2.1. General procedures

In the field, crabs were placed on ice to reduce their physiological activity. After the transport to the laboratory, the crab's activity was reduced throughout a hypothermic shock by placing specimens in the freezer (−20 ºC) for five minutes for histological analysis (gonads tissues dissection) and during 48 h for the remaining analyses. The carapace width (CW, mm) of all specimens were measured with a vernier calliper (with an accuracy of 0.01 mm) and both total weight (TW, g) and gonad weight tissue (GW, g) were weighed with an analytical digital balance scale (0.0001 g precision) respectively.

2.2.2. Histological analysis

The gonads of 5 specimens of each macroscopically maturity stage (see Tables 1 and 2), were removed and fixed in a buffered 4% formaldehyde solution for 24–48 h. This process was performed for both sexes with a total of 40 specimens analysed, 15 males (3 maturity stages) and 25 females (5 maturity stages). The histological methodology was carried out according to Campinho et al. (2007). After fixation, the samples were placed in histological cassettes (n = 5/stage maturity) and transferred to 70% ethanol for at least two hours until further processing. For paraffin embedding, the samples were dehydrated through a graded ethanol series from 70% to 100% followed by xylene (100%) using an automated tissue processor (Leica, TP1020), and fixed in paraffin wax (Histosec, Merk, Germany). The blocks were stored at − 20 ºC until future use. The fixed samples were cut by cross-section into thin slices of 5 micrometers, using a rotary microtome (MICROM, HM340E), subsequently, the slices were mounted on glass slides coated

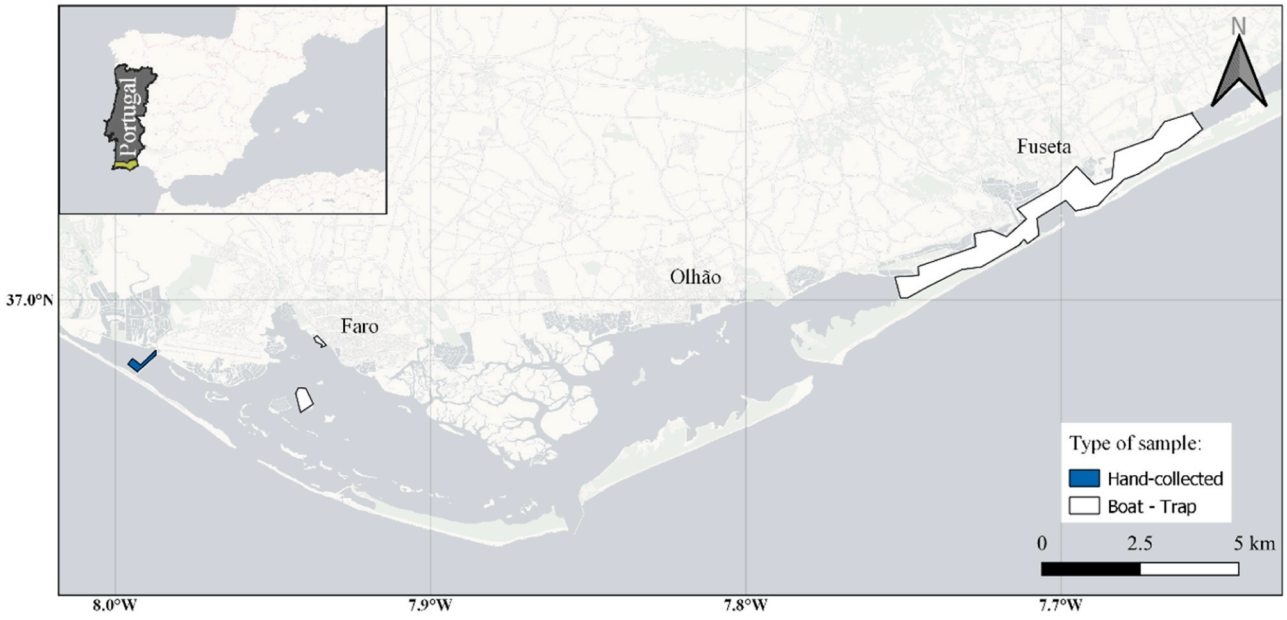
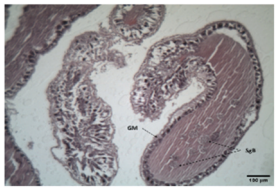
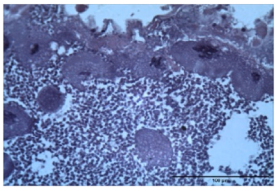
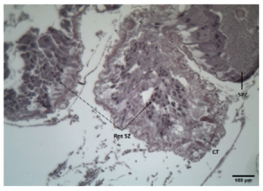


Fig. 1. Geographical location of the sampling areas, in blue the area where *Panopeus africanus* were collected by hand, and in white colour the areas where the specimens were collected with traps by boat.

Table 1
Reproductive stage key applied to classify the different gonadal maturity stages of *Panopeus africanus*, including illustrative photomicrographs and a brief description of each male macro and microscopic maturity stage. Scale bars 100 μm.

Stage	0 Immature	I Development	II Mature	III Spent
Macroscopic maturity stage	No gonad tissue could be visually identified	Thread-like testis, difficult to distinguish from, the hepatopancreas. Translucent in colour	Testis increased in diameter. More discernable from the hepatopancreas. Creamy/ White in colour	Testis reduced in size. Creamy/White in colour
Gonad external image				
Microscopic maturity stage	No gonad tissue could be visually identified	When spermatogonia B (SgB) are present germ lineage has started. In this stage also spermatocytes (Sc), spermatids (St) are present.	Spermatozoa (Sz) are present throughout the testis, either in the sperm duct, cysts or lobules. Spermatogonia (Sg) and spermatids are present.	Residual spermatozoa (Res Sz) present in the sperm duct. Degeneration and resorption of sperm cells, in all development stages, in the testis.
Gonad internal image				

with 3-Aminopropyltriethoxysilane (Sigma-Aldrich) (a solution that contains an additive which keeps the section attached to the slide) and then, samples were dried overnight in an oven at 37 °C. The slides were stained with hematoxylin/eosin as described in Campinho et al. (2007). Finally, the sections were mounted for definitive preparations in DPX (Sigma) and photomicrographs of histological sections of *P. africanus* gonad tissue were taken using a ZEISS Axiolab drb KT optical microscope coupled to an OPTICA 3 digital camera. Image analysis was carried out using ImageJ (http://rsb.info.nih.gov/ij/).

2.2.3. Maturity and Reproductive analysis

Sex and gonad maturity stage of all specimens were assessed macroscopically, using a modified maturity scale defined by Ovelheiro et al. (2023) (Tables 1 and 2). The gonad maturity stages of males were divided into four groups: Immature (0), Developing (I), Spawning (II), and Spent (III), with stages 0 and I classified as immature and II and III as mature; The gonad maturity stages of females were divided into six groups: Immature (0), Early development (I), Late development (II), Mature (III), Spawning (IV), Spent (V), being the stage 0 and I classified as immature and II, III, IV, and V as mature. The reproductive period of *P. africanus* was analysed monthly by classifying the crabs as mature or immature and according to the different stages of maturation and sex.

Carapace width structure for each sex was analysed. The specimens were grouped in CW size intervals (2 mm), and the Kolmogorov-Smirnov test was performed to analyse differences between sexes in the CW distribution. Moreover, the overall sex ratio (males:females), sex ratio by size intervals, and sex ratio by month were examined. Differences in the sex ratio proportion were assessed using the chi-square test (χ^2), with the application of the Bonferroni correction to mitigate the potential risk of type I errors arising from multiple monthly comparisons. Assuming that the sex ratio was 0.5, the null hypothesis was rejected if the χ^2 estimated value was greater than expected value ($p < 0.05$). The Pearson correlation test was computed to assess the relationship between the percentage of mature individuals and the water temperature in each month. Statistical analysis was performed with the IBM SPSS statistic software (error level of significance $\alpha = 0.05$).

The size at first maturation was determined for each sex by estimating the proportion of mature individuals in every carapace width class (1 mm), being only the specimens collected exclusively during the spawning season considered. The carapace width at which 50% (L_{50}) of individuals were mature was calculated following (Roa et al., 1999) by fitting to a logistics regression model (Eq. 1). Where P is the proportion of mature crabs, CW the carapace width in mm, α the intercept parameter, and β the slope parameter. The standard errors of L_{50} were calculated with the delta method by using the package msm (vers. 1.6.9; Jackson, 2011) in R, and a Wald-type confidence interval was also calculated.

$$P(CW) = \frac{1}{1 + e^{\alpha+\beta CW}} \quad (1)$$

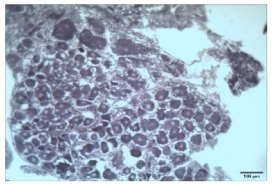
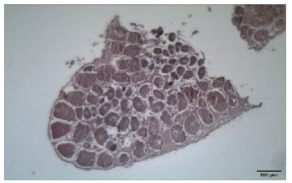
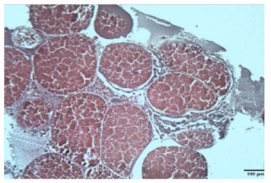
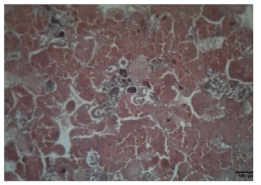
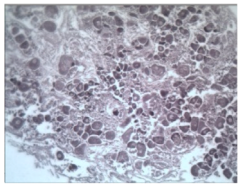
2.2.4. Fecundity analysis

To analyse the fecundity patterns of *P. africanus*, 51 egg-bearing females were captured between May and July 2020 (egg mass black colouration). The specimens were collected in order to include different ovigerous female's carapace width range. After the body measures of the egg-bearing females, three sub-samples of the egg mass were removed from each specimen (Rodríguez-Félix et al., 2018). Each sub-sample was weighed (*Wr*) and stored in a 4% formaldehyde solution. The remaining egg mass was carefully removed from the pleopods and the females' body weight (g) was registered again, this time without eggs. This allowed the calculation of the total egg mass weight (*Wegg*). Each sub-sample was placed in a Bogorov counting chamber and the number of eggs (*n*) was counted under a Leica Stereoscope. The fecundity (*F*) for each sub-sample was estimated by the gravimetry method (Eq. 2), where *Wegg* is the weight of all the eggs; *Wr* is the weight of the sub-sample, and *n* is the number of eggs in the subsample.

$$F = \frac{n \cdot w_{egg}}{w_r} \quad (2)$$

The mean fecundity and respective standard deviation of each egg-

Table 2
Reproductive stage key applied to classify the different gonadal maturity stages of *Panopeus africanus*, including illustrative photomicrographs and a brief description of each female macro and microscopic maturity stage. Scale bars 100 μm.

Stage	0 Immature	I Early Development	II Late Development	III Mature	IV Spawning	V Spent
Macroscopic maturity stage	No gonad tissue could be visually identified	Thread-like ovary, difficult to distinguish from the hepatopancreas. Yellowish in color. Oocytes are not visible.	Ovary has increased in size. More discernable from the hepatopancreas. Dark-brown in color. Oocytes are not visible	Ovary increased in size. Occupies the majority of the body cavity. Dark-brown in color. Oocytes visible	Ovary reduced in size, with a filamentous aspect and with a light-brown color. Residual oocytes are visible	Ovary reduced in size, with a filamentous aspect and with a light-brown color. Residual oocytes are visible
Gonad external image						
Microscopic maturity stage	No gonad tissue could be visually identified	Primary growth (PG), cortical alveoli (CA) and early vitellogenic (VT1) oocytes are present. Absence of postovulatory follicles (POFs).	PG, CA, and/or VT1 and/or mid vitellogenic (VT2) oocytes are present. Some atretic oocytes (AT) can be present. Absence of POF's.	Late vitellogenic (VT3) oocytes, migratory nucleus (MIG) and/or hydrated (HYD) are mainly present. PG together with CA can also be observed.	POFs present in dominance, some PG, CA can be present. Thick ovarian wall. Remaining hydrated oocytes (HYD), or HYD at atretic state may also be present.	PG oocytes dominate. Massive AT and POFs present some CA and/or, VT1, VT2 can be present.
Gonad internal image						

bearing female were calculated using the results of three sub-samples. Linear relationships between fecundity and morphometric variables were tested: (i) egg count regressed against carapace width; (ii) egg wet weight regressed against egg count; and (iii) egg wet weight regressed against body wet weight. The linear regressions were performed with IBM SPSS statistic software (a level of significance of $\alpha = 0.05$ was used).

3. Results

P. africanus specimens exhibited a range of carapace width (CW) from 7.05 to 51.74 mm (mean ± SD: 34.47 ± 7.89 mm) (Fig. 2) and their total body weight varied between 0.14 and 47.75 g (mean ± SD: 14.90 ± 8.32 g). For males, the carapace widths ranged from 7.05 to 51.74 mm (mean ± SD: 36.48 ± 7.48 mm), and for females the CW ranged from 7.81 to 50.35 mm (mean ± SD: 30.71 ± 7.23 mm). The total weight of males varied between 0.14 to 47.75 g (mean ± SD: 17.51 ± 8.28 g) and for females between 0.22 to 44.04 g (mean ± SD:10.01 ± 5.80 g). No statistically significant differences between male and female's CW distribution were recorded (Kolmogorov– Smirnov test, $p > 0.05$), although almost all specimens with CW> 45 mm were male.

3.1. Histology

The reproductive maturity of *Panopeus africanus* was characterized based on histological analysis, males presented four developmental stages and females presented six developmental stages of reproductive maturity. These stages were assigned according to the presence/absence of gametogenic cells, maturation, and the shape and size of these cells.

3.1.1. Male gametogenic development

Male reproductive maturity was categorized into four developmental stages, where stages 0 and I were considered immature, and stages II and III were considered mature (Table 1).

Macroscopically, the gonads in stage I (development) were difficult to distinguish from the hepatopancreas, as the testes were thread-like and translucent in colour. In stage II (sexual maturity or spawning), the testes were large in diameter and cream/white in colour and were more distinguishable from the hepatopancreas. In stage III (spent), testes were smaller than stage II and cream/white in colour. In the first reproductive cycle, males are considered immature in stages 0 and 1, and mature in stages 2 and 3. After completing the first reproductive cycle (stage 3 – spent), males are considered mature until the end of their lifespan and return to stage 2 to start a new reproductive cycle. See supplementary information for detailed images of stages I, II, III and clarity about the reproductive cycle (FigS1, S3, S5 and S17, respectively).

Microscopically, the gonads in stages 0 (immature) and stage I (development) only presented spermatogonia A and B (Sg A and Sg B). Stage II (spawning) presented mature testis, where spermatozoa (Sz) were present. In stage III (spent), residual spermatozoa (Res Sz) were identified, and the process of cell resorption dominated. See supplementary information for detailed images of stages I, II, III (Fig. S2, S4 and S6, respectively).

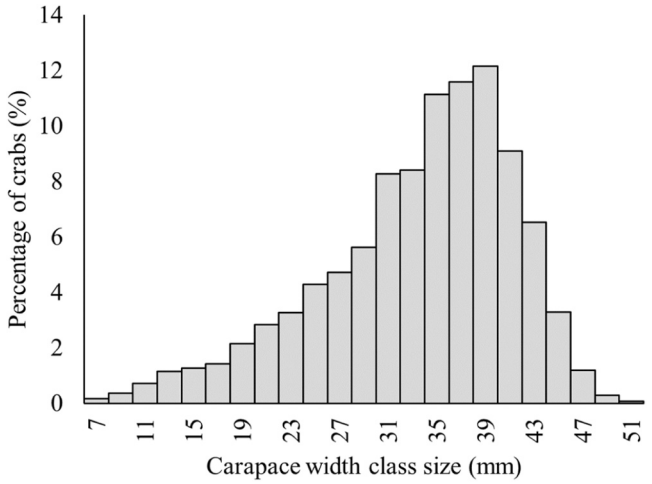


Fig. 2. - Carapace width class size-frequency of *Panopeus africanus* population.

3.1.2. Female gametogenic development

Female reproductive maturity was categorized into six developmental stages, where stages 0 and I were considered immature and stages II, III, IV, and V were considered mature (Table 2).

Macroscopically, in stage I, the ovary was thread-like, difficult to distinguish from the hepatopancreas, and had a yellowish colour. In stage II, the ovary was more discernible from the hepatopancreas and had a dark-brown colour. In stage III, the ovary occupied most of the body cavity and had a dark-brown colour. In stage 4, the eggs were carried externally in the pleopods, and the ovary had filamentous aspects with a light-brown colour. In stage V, the ovary retained the filamentous aspect with a light-brown colour; however, the eggs were already released by the female. In the first reproductive cycle, females are considered immature in stages 0 and 1, and mature in stages 2 to 5. After completing the first reproductive cycle (stage 5 – spent), females are considered mature until the end of their lifespan and return to stage 2 to start a new reproductive cycle. See supplementary information for detailed images of stages I, II, III, IV, V and clarity about the reproductive cycle (Fig. S7, S9, S11, S13, S15, and S17 respectively).

Microscopically, it was possible to observe primary oocytes in all the gonadal development stages (I to V), varying only in number within each maturation stage. In Stage I, only non-vitellated oocytes (primary oocytes) were present, with small sizes. In Stage II, the beginning of vitellogenesis was verified, evidenced by the presence of partially vitellated oocytes. Akin to stage I, non-vitellated oocytes were present, and some vitellated, although with a diminutive presence. The difference between these two stages referred to the oocyte diameter mean, which was higher in stage II (Table 3). Stage III (mature) presented three types of oocytes: non-vitellated, at the beginning of vitellogenesis (partially vitellated), and vitellated, where the latter appeared in large numbers and were larger in diameter. Stage IV (spawning) presented the most oocyte types, including hydrated, atretic oocytes, and post-ovulatory follicles (POFs), although they were present in small numbers, corresponding to the final stage of oocyte maturation and

Table 3
Panopeus africanus gonadal development data in Ria Formosa lagoon (South Portugal). MS, microscopic maturity stage; OT, oocytes type; N, number of oocytes; M±SD (μm), mean and standard deviation; OD Min-Max (μm), minimum and maximum oocyte diameter. Remark: (*) Due to dehydration, the oocyte is difficult to measure, the measurements were only made for the largest diameter.

MS	OT	N	M ± SD (μm)	OD Min - Max (μm)
0	-	-	-	-
1	Non - vitellated	108	51.74 ± 22.27	23 – 87
	Partially vitellated	46	53.90 ± 14.32	28 – 121
2	Non - vitellated	64	49.82 ± 27.49	30 – 90
	Partially vitellated	108	84.95 ± 37.01	43 – 182
3	Non - vitellated	38	44.18 ± 52.17	31 – 72
	Partially vitellated	46	77.22 ± 56.90	52 – 115
	Vitellated	80	188.87 ± 58.08	114 – 301
4	Non - vitellated	38	43.44 ± 39.31	36 – 55
	Partially vitellated	34	115.29 ± 46.61	59 – 157
	Vitellated	88	146.84 ± 24.80	86 – 220
5	Non - vitellated	38	37.86 ± 21.94	25 – 57
	Partially vitellated	76	72.82 ± 28.64	43 - 130
	Vitellated	42	114.14 ± 18.44	84 - 188
	Hydrated	2	87.42 ± 23.52 *	87 - 87 *
	Atretic	2	44.22 ± 23.72	44 - 44

release. Stage V (Spent) had many partially vitellated oocytes which corresponded to the beginning of a new reproductive cycle. The presence of atretic oocytes increased towards the end of the laying period. It was possible to observe incidence in stage II and IV (Table 2), which demonstrated that the reproductive cycle was continuous. See supplementary information for detailed images of stages I, II, III, IV, and V (Fig. S8, S10, S12, S14, and S16, respectively).

3.2. Carapace width structure, Sex ratio, Reproductive periods and Size at maturity

3.2.1. Sex ratio

The sex ratio (m/f) of *P. africanus* in this study was m/f = 1.81, but the sex ratio varied according to the size classes ($\chi^2 = 615.5, p < 0.001$). The proportion of *P. africanus* males in CW size-classes between 7 and 13 mm comprised 80% of the total sample measured (Fig. 3A). The proportion of females surpassed that of males in the size class of 15 mm and remained higher in the size classes up to 31 mm CW. The mean proportion of males in these intervals was 40%. After the size class of 31 mm, the proportion of males in the population increased and remained higher than females until the maximum size class (51 mm) where the proportion of males in the population was 80%. For size-classes between 45 to 51 mm most of the population were males, with the proportion of males in this interval reaching 95%. The sex ratio exhibited monthly variation ($\chi^2 = 59.3$, $p < 0.001$) (Fig. 3B), with a prevalence of males throughout the year. However, in January ($\chi^2 = 0.117$, $p = 0.732$), March ($\chi^2 = 0.610$, p = 0.435), June ($\chi^2 = 1.973$, $p = 0.160$), and December ($\chi^2 = 2.189$, $p = 0.139$), the sex ratio did not exhibit statistically significant differences and remained close to the expected 1:1 ratio.

3.2.2. Reproductive periods

The proportion of mature individuals in the population (Fig. 3B) was always higher than 50%. The months with a lower percentage of mature individuals were the winter months (December to March). After this period most of the population (>80%) was comprised by mature individuals. A positive correlation was found between the percentage of mature individuals and the water temperature (Pearson correlation: R= 0.74, $p = 0.002$).

Immature *P. africanus* specimens were observed from the end of autumn (November) until the beginning of spring (April), with the maximum portion of immatures being observed in December (Figs. 3C and 3D). Mature individuals appeared year around but were most frequent during the spring/summer months (April to August). The highest percentage of mature females was observed in May (99%), while mature males prevail in June (100%). Spawning individuals occurred between April and August in females, and during the whole year for males, with most frequency between February and June. Spent period occurred between May and January, most during August to October in females and October and January in males.

3.2.3. Size at maturity

P. africanus specimens collected during peak spawning (February - October) were used for calculating size at maturity. The smallest sexually matured specimen was 17.4 mm of CW for both sexes. The carapace width at which 50% (L_{50}) of the *P. africanus* population reach sexual maturity was 19.7 ± 0.5 mm ($\alpha = -7.17$; $\beta = 0.37$) for females and 19.8 mm ± 0.6 mm ($\alpha = -8.57$; $\beta = 0.43$) for males (Fig. 4).

3.3. Fecundity

CW of the egg-bearing females varied between 22.82 and 39.41 mm with mean (± SD) of 31.26 ± 4.51 mm. The fecundity varied between 12368 and 84140 eggs/female with a mean (± SD) around 43371 ± 17523 eggs/female.

The relationship between CW-EC yielded a statistically significant slope ($p < 0.001$). The linear relationship between egg counting (EC) and egg mass weight (EW) was also statistically significant ($p < 0.001$), and the coefficient of correlation ($r^2 = 0.76$) denoted a high correlation between variables. Lastly, the correlation between body wet weight (BWW) and egg mass weight (EW), was statistically significant ($p < 0.001$) (Fig. 5).

4. Discussion

Due to the low number of scientific studies on *P. africanus* regarding its biological and reproductive aspects comparative analyses of our results with previous studies are scarce. Still, our results point to a new

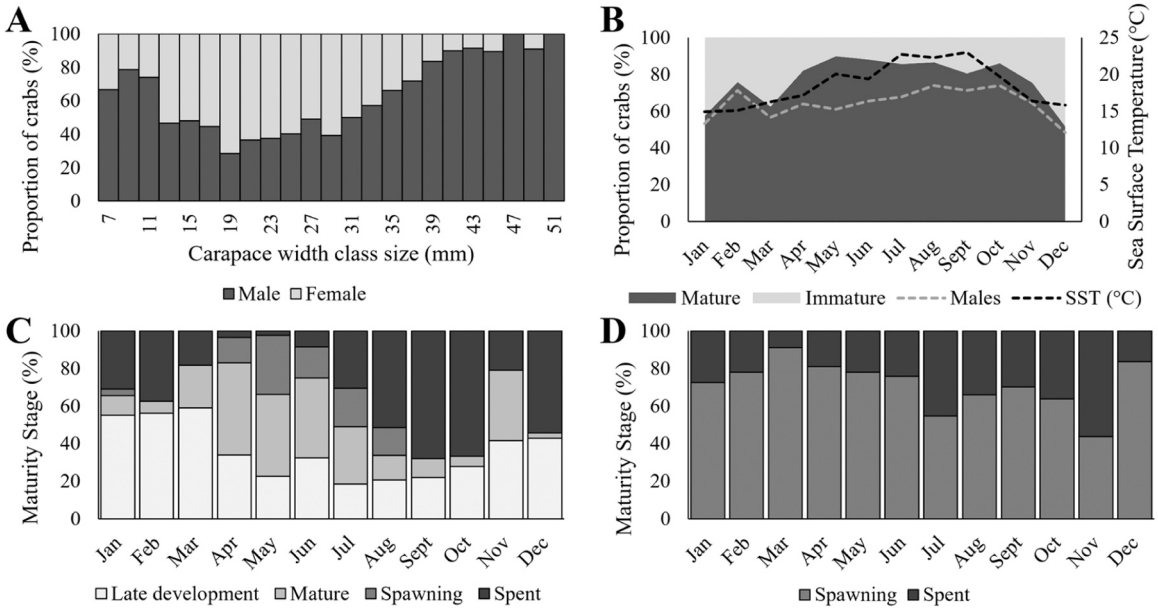


Fig. 3. - Sex ratio, and spawning season of *Panopeus africanus* on the South Portuguese coast. (A) Variation of sex ratio value with the size of *P. africanus* specimens; (B) Mean monthly proportion of mature individuals, mean monthly proportion of *P. africanus* males and Sea surface temperature (°C); (C) Monthly variations of mature female gonad maturity stages of *P. africanus*; (D) Monthly variations of mature male gonad maturity stages of *P. africanus*.

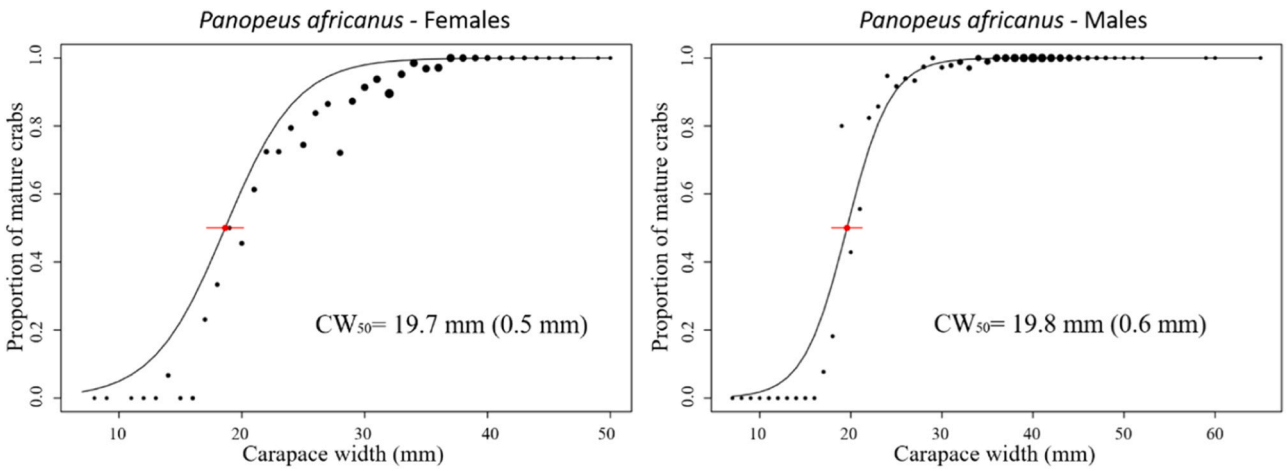


Fig. 4. - Carapace width (mm) maturity size for females (left) and males (right) specimens of *Panopeus africanus* on the Ria Formosa lagoon (South Portuguese coast). The red bar is the L_{50} with standard derivation.

maximum size for *P. africanus* at 51.74 mm, overtaking the previous public record of 51.5 mm (Fischer et al., 1981). The larger specimens collected in the southern Portuguese population (northern limit distribution) are likely related to the lower water temperatures that this population is subjected to, when compared to the other populations previously analysed. This is in line with temperature-size relationship described for crustacean crabs where lower temperatures lead to higher maximum CW (Atkinson, 1994). Moreover, the CW range in the present study is the widest reported, considering all *P. africanus* populations. The wide CW range can be also related to the sampling collection method because specimens were gathered not only by traps but complementary harvested during low tide. The use of both fishing methods was essential to allow the sampling of all size classes. The larger specimens were mostly caught by traps, while specimens with CW measuring < 30 mm were not retained by traps and were instead caught by hand inside their burrows and under rocks. The only class size that was not sampled corresponds to specimens where CW measures < 7 mm (juveniles).

Sex ratio analysis for different CW size classes revealed that the proportion of males was higher in the smaller and higher size classes while the proportion of females was higher in the intermediate size classes. In crustaceans, sex ratios are often biased due to several ecological and evolutionary processes (Ewers-Saucedo, 2019). The large percentage of juvenile males may be attributed to behavioural differences between males and females since in most crab's species males are more active and competitive (Styrishave et al., 2004), leading to a higher mortality rate of males in smaller size classes and thus more females than males reach the intermediate CW size classes. The increase in male percentages in the larger size classes can be explained by the maximum CW attained by each sex, which tends to be larger in males than in females and is likely driven by male competition in a bid to gain access to females and food resources (Klassen and Locke, 2007). Hartnoll (2006) suggests that in several crab species, females don't make intraspecific competition effectively resulting in more energy diverted into reproduction instead of growth.

We were able to record monthly changes in sex ratio, namely a decrease in the proportion of males in the winter months, which coincided with a lower proportion of mature crabs. This finding shows that the lower proportion of males was connected to the increase in the activity of females, that in these months spend more energy on growth rather than reproduction.

The analysis of the gametogenic cycle allowed us to ascertain the spawning season of *P. africanus* on the Southern Portuguese coast. The reproductive period had previously been analysed in Cádiz (Rodríguez et al., 1997), which is located at approximately the same latitude as Ria Formosa, with both areas representing the northern limit of this species. However, in Rodríguez et al. (1997) only the reproductive period of females was studied, whereas in the present study, we analysed both sexes.

The reproductive period of females belonging to the populations of Ria Formosa was similar to that reported by Rodríguez et al. (1997). In both populations, ovigerous females were observed during the warmer months (April to August), when the water temperature is warmer (>20ºC). *P. africanus* larvae are also more abundant in these months, and higher larvae survival rates are related to the higher water temperatures (20 to 25ºC) (Rodríguez et al., 1997). The periods during which females are mature were similar between the two studies populations, occurring between January and July. Finally, the major difference between the two studies is that in the present study, females in the resorption stage were considered mature, whereas in Rodríguez et al. (1997) females at this stage of development were not included. In the present research, females in resorption are found from August (after the ovigerous stage) until February when gonadal development restarts, in a similar process to that described for *Carcinus maenas* in Ovelheiro et al. (2023).

On the other hand, the reproductive periods of males are longer, with mature males being found throughout the year. Still in the colder months, from December to February, a decline in the percentage of mature males was observed. The reproductive periods of males had not yet been analysed or reported but from the present study, reproductive periods in *P. africanus* are linked to the reproductive periods of females and with the seasonal temperature. The breeding periods appear to be short and occur in the warmest months being observed a correlation between the breeding periods and the temperature. In comparison with the population of the west coast of Africa, the spawning season in the northern limit of their latitudinal range is shorter, since in African populations, ovigerous females are observed in January, March, April, July, and November (Manning and Holthuis, 1981).

Using the data obtained in the three years of the study it was possible to determine the percentage where 50% of the individuals were mature. Thus, the size of the first maturity of *P. africanus* was determined for the first time for the species. Interestingly, that CW maturity size was similar between the two sexes (approximately 20 mm CW). Our results are in line with previous studies in Cadiz Bay and West Africa (Manning and Holthuis, 1981; Rodríguez et al., 1997), where size of sexual maturation was not analysed, that reported that smaller ovigerous females around 17 mm were captured. Results are similar to those observed in other species of the genus *Panopeus*, where the carapace width at the stage which individuals reach sexual maturity varied between 15 mm and 20 mm (Carvalho-Batista et al., 2015; Brousseau and McSweeney, 2016; Santos et al., 2018). However, the size at maturity here calculated for *P. africanus* was the largest carapace width for the *Panopeus* genus. Still,

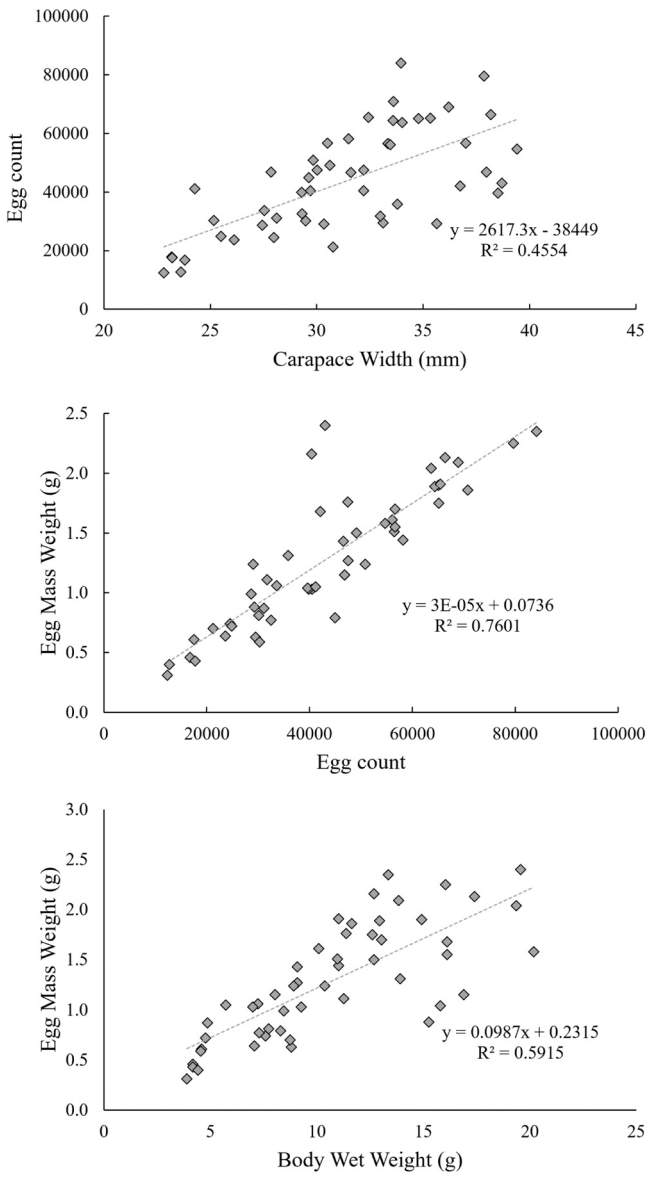


Fig. 5. - *Panopeus africanus* fecundity linear regression analysis. On the top, *P. africanus* egg count regressed against carapace width; In the middle, *P. afticanus* egg weight regressed against egg count; On the bottom, *P. africanus* egg weight regressed against body wet weight.

it should be noted that the population analysed in this study is at the limit of its geographical reported distribution and the water temperatures are lower relatively to other estuaries where the species inhabit. In the present study, the relative size at maturation (L_{50}/CWmax), defined as the ratio between the carapace width at maturation (L50) and the maximal carapace width (CWmax) of *P. africanus*, was close to 40%. This proportion was similar to what was observed in other crabs habiting the Ria Formosa, such as *Carcinus maenas* and *Afruca tangeri*, where the relative size at maturation was also around 40% (Personal data). The relative size at maturation for other species within the *Panopeus* genus was also close to 40%. In Santos et al. (2018), the relative size at maturation of *Panopeus occidentalis* was 40% for males and 47% for females, and in Brousseau and McSweeney (2016), the relative size at maturation of *Panopeus herbstii* was 30%. Moreover, in the previous study, the relative size at maturation for *Hemigrapsus sanguineus* species was also determined to be close to 30%, being observed that both species analysed in Brousseau's study had a similar relative size at maturation. Such differences between latter studies can be explained by the habitat's environmental conditions, which affect the reproductive patterns of crab species (Hartnoll, 2006; Groner et al., 2018).

Beyond the environmental conditions, the morphological characteristics also influence reproductive patterns, such as the fecundity (Hartnoll, 2006; Monteiro et al., 2022). The positive correlation between morphological parameters and fecundity of *P. africanus* observed in this study presents evidence that fecundity is proportional to body size. The relation is explained by the abdomen size/carapace width: the larger abdomen, the higher number of eggs a female can bear (higher fecundity) (Rodríguez et al., 1997; Rodríguez-Félix et al., 2018; Monteiro et al., 2022). The relation between fecundity and female size had been previously analysed in the Cadiz population (Rodríguez et al., 1997) and the results were quite similar. However, Rodríguez et al. (1997) used carapace length (CL) instead of carapace width (CW), while in the present research, the CW was used. We also observed the relation between egg weight against the egg number and the weight of females, two other well-defined morphometric relationships in crab species (Baklouti et al., 2013; Rodríguez-Félix et al., 2018; Monteiro et al., 2022), supporting also that morphological characteristics influence fecundity.

5. Conclusions

This study contributes to a better understanding of the basic reproductive biology of *P. africanus* with regards to its spawning seasons, sexual maturation size and population sex ratio. A positive correlation between the water temperature and the reproductive periods, and between the fecundity and the morphological parameters were also observed. Future studies about growth rates and feeding ecology should be done to complement the knowledge of this species' biological and ecological characteristics.

Ethics approval

This study did not require ethics approval.

CRediT authorship contribution statement

João Nuno Monteiro: Research design, Investigation, Data analysis, Discussion of results and methodology, Writing and editing the original draft. **Andreia Ovelheiro:** Investigation, Review of the original draft. **Miguel Pinto:** Investigation, and review of the original draft. **Maria Alexandra Teodósio:** Discussion of results and methodology, Review of the original draft, Funding. acquisition. **Francisco Leitão:** Research design, Data analysis, Discussion of results and methodology, Writing and review of the original draft, Funding acquisition.

Declaration of Competing Interest

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

Data will be made available on request.

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Research data policy and data availability

The datasets generated during and/or analysed during the current study are not publicly available due to the possible use of the data for further analyses/studies but are available from the corresponding author on request.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.rsma.2023.103339.

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