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THE POPULATION DYNAMICS AND MORPHOLOGICAL TRAITS OF THE NEW SPECIES *Alpheus qatari* IN THE GULF OF CADIZ (SPAIN)

Thesis in: Modelling physical-biological processes

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Abstract

There are about more than 600 of *Alpheus* species have been identified under the 36 genera. *Alpheus* species are possible to be found in tropical, subtropical, and temperate seas and inhibiting a wide range of habitats, from tidal pool to deep sea areas, in both temperate and tropical areas. Some of them live in cool-temperate waters and few have colonized freshwater habitats, as well as mangroves and estuarine areas Major prominent feature of *Alpheus* species in enlarged or modified major chelea of the male organism. This group of species is highly abundant in spring and summer (warmer seasons) probably because the high nutrient availability and high primary production typical of those seasons help to maintain a high survival rate. *Alpheus qatari*, in the Persian Gulf, inhabiting the muddy substrate in the mangroves, in channels under rocks, and in the intertidal zone. The genetic analysis demonstrated that *Alpheus qatari* n. sp**.** is a new species, genetically different from the closest congeners *A. buckupi* and *A. lobidens. Widely distributed Alpheus species are morphologicalyy divers group.* The immense diversity of orbital hoods and chelipeds likely facilitated the diversification of snapping alpheids. Most of *Alpheus* species are burrowing organisms and In the alpheid group, one of the first pereiopods (chelipeds) bears a large claw with a snapping mechanism on the fingers, known as the snapping claw.

No studies to date have examined the relationship between food availability and distribution patterns in alpheid shrimp populations. Some species are detritus feeders. the amount and quality of food present in the sediments could influence where they decide to construct a burrow. Snapping shrimp have different feeding habits, however most of them are omnivores. Usually feeding is occurred during night time, while day time they are staying in the burrows. Epiphytes on sea grass leaves are also possible food for the shrimp. These algae to be a primary source of organic matter for higher top levels in tropical sea grass beds. Despite the high number of *Alpheus* species, there are few studies about their population biology, especially in comparison with the

extensive knowledge about the taxonomy and diversity of the family Alpheidae. There is a lack of information on the biological, ecological, and behavioral aspects of Alpheidae species. Information on population biology, such as the sex ratio, reproductive period, recruitment and the size at first maturity is essential to understand a species' life history.

Since *A. qatari* is a new exotic species in the Bay of Cadiz, we explore which factors (both biotic and abiotic) shape the distribution of this species. The main objective of this study is to analyse the *A. qatari* population structure on the San Pedro River (SW Atlantic Spanish coast) focusing on: (a) studying the population size distribution and sex-ratio during an annual period; (b) assessing whether a temperate region could shape the population dynamic of a tropical species. Bearing in mind that this is a new species, the morphological traits in males and females will also be analysed.
Individuals of the snapping shrimp *Alpheus* qatari were collected along the 275 m stretch of the San Pedro River right bank, Cadiz, Spain (36°32'03.4"N 6°12'50.8"W) from June 2021 to April 2022, and carried alive to the laboratory at the Institute of Marine Science of Andalusia (CSIC) for further analysis. The sampling periods covered all four seasons: Summer (June to September), Autumn (September to December), Winter (December to March), and Spring (March to May).

Total 383 organisms were recorded during sampling times July-2021 to May 2022, of which 174 were females (50 non-ovigerous and 124 ovigerous) and 188 were males. Ovigerous females were observed during the study period July-2021 to May 2022 .The average Sex ratio (Male:Female) was 1:0.92, i.e., its value did not differ significantly from the expected equal proportion (X^2 = 1.43, df =1, P >0.05). There is significant difference between male and females for the cephalothorax length (M–nn - Whitney = 15060, P < 0.05), while there was no significant difference for the cephalothorax length between non-ovigerous and ovigerous females (M–nn - Whitney = 3802, P > 0.05). The largest individuals were observed during the months of August, September, October, and March (male=14 -14.99mm CL). For the ovigerous females, the largest individuals were observed in April (ovigerous female = 15.66mm CL) and the largest non-ovigerous females were observed in August (–on - ovigerous female = 14.75 mm CL). the percentage of females increased from July -to December in 2021. The maximum percentage of females was recorded during December 2021. With regards to males, the percentage increased from July to October and gradually decreased up to December. In the case of Females with eggs, the percentage of individuals was higher in summer and in spring.

The size of females gradually decreased from July 2021 to November 2021 and increased from December 2021 to 2022 May. Ovigerous females were collected during the entire sampling period, with the smallest ones appearing mainly in summer (July) and the largest ones in spring (April-May). Two cohorts could be identified in males and females from July to October and, from November to May of the following year. The relationships between weight and lengths and the correlation coefficients were analyzed for all the demographic categories of *Alpheus qatari* during the 11-month study period at the San Pedro River. The significant correlation between the total weight and cephalothorax length was found to be a power function ($P<0.05$).

Results from the morphological analysis showed that there is a significant difference between the relative chela length of males and females ($W= 33473$, $p<0.05$).A Kruskal-wallis test was performed to determine significant difference of the relative chela length between the three demography categories ($H=155.58(2)$, $p<0.001$). Males showed a significantly larger chela than females. Female and ovigerous females did not show significant differences among their length.

The Fulton´s condition factor (K index) was calculated for the males, females and ovigerous females at each month. A PERMANOVA test was performed for the factors Sex, Month, and Position of Chela, and the covariate cephalotorax length. Among these factors, Sex, Month and cephalothorax length showed significant differences, and there was no evidence for a two and three-way significant interactions. The Hureau Index was calculated for the males, females and ovigerous females for each month. A PERMANOVA test was performed for the factors Sex, Month, and Position of Chela, and the covariate cephalothorax length. Among these factors, Sex and Month showed significant differences. Overall, the fullness index (FI) of the stomach showed the highest values in autumn and, among the different demographic categories, the highest values occurred in females.

The population traits of *A. qatari* in the San Pedro River are similar to other *Alpheus* populations. According to the results obtained, the sex ratio of the *Alpheus qatari* is not significantly different from the 1:1 ratio characteristic of other *Alpheus* species. In this study, most of the individuals collected were adults and only few of them had a small size, similar to other populations (Moraes et al, 2020). According to Costa-Zouza et al (2018), adults and juveniles are available during the whole annual period with a dominance of adults in most months. Thus, it could be assumed that adults are reproducing in another ground and migrate to the sampling area to grow. The significant correlation between the total weight and total length was found to be a power function in both sexes of *A. qatari* in the San Pedro River with a high correlation between both covariates. For instance, the values obtained from the length/weight relationship (LWR) of some shrimp species in the Iko River estuary showed that there was a high and significant correlation between both covariates. Our results revealed that *A. qatari* displayed a sexual dimorphism of the snapping claw with males having larger claws than females and showing a steeper allometric claw-body relation than females. The highest condition factor was recorded in summer, especially in August 2021. Males chela were heavier than the female ones at a similar size. These differences could explain that they are capable to reproduce or mate several times per year and protect females in their galleries. The fullness index value in this study also showed the fullness of gut of the organisms and, according to the results, the highest gut fullness value occurred in summer-autumn and the lowest one in winter, similar to other studies. During spring and summer, the seasonal food availability increases and enhances productivity (primary and secondary) within the coastal area, perhaps explaining the greater feeding intensity of the *Alpheus* species. This news *Alpheus* Qatari also follow some common feature of the family Alpheadea as sex ratio is not significantly apart from 1:1, males have significantly large chelea and Males are relatively larger than females.

Key words: *Alpheus qatari,* Sex ratio, Population Dynamics, Condition factor, Fullness index,

1. Introduction

1.1 General description

The family Alpheidae includes around 36 genera and about 600 species. It is an abundant and ecologically diverse group of decapod crustaceans (Anker et al, 2006). Some of them are commercially exploited in China, Japan, and Vietnam, but they account for a small proportion of fisheries (Holthuis, 1980). *Alpheus* species, also known as snapping shrimps, are possible to be found in tropical, subtropical, and temperate seas (Chace, 1988), inhabiting a wide range of habitats, from tidal pool to deep sea areas. Large snapping shrimps prefer to live in microhabitats among the intertidal area, under the rocks or creating holes in muddy and sandy sediments (Rodrigues et al., 2009, Mathews & Anker, 2009). Some of them live in cool-temperate waters and few have colonized freshwater habitats, as well as mangroves and estuarine areas (Anker et al, 2006). They are benthic organisms, and live in mutualistic or commensal association with other decapods, fishes, anemones, and sponges (Moraes et al., 2020). Many snapping shrimps live in permanent symbiosis with other organisms, such as sponges, cnidarians, mollusks, echinoderms, or other crustaceans (Anker et al.2001,2006). This group of species is highly abundant in spring and summer (warmer seasons) (Moraes et al, 2020), probably because the high nutrient availability and high primary production typical of those seasons help to maintain a high survival rate (Schettini et al., 1998; Sant'Anna Neto, 2005).

Giraldes et al (2021) recorded a new species, *Alpheus qatari*, in the Persian Gulf, inhabiting the muddy substrate in the mangroves, in channels under rocks, and in the intertidal zone. The genetic analysis demonstrated that *Alpheus qatari* **n. sp.** is a new species, genetically different from the closest congeners *A. buckupi* and *A. lobidens* (Giraldes et al, 2021).

1.2 Morphology of Alpheid species

Shrimps from the family Alpheidae are morphologically divers and widely distributed. Their eyes constitute one of the proper characteristics to identify this group of species among the decapod crustaceans, because they are dorsally covered by orbital hoods (Anker et al., 2006). The immense diversity of orbital hoods and chelipeds likely facilitated the diversification of snapping alpheids (Banner and Banner 1982). The genus *Alpheus* are characterized by a laterally compressed, sub-cylindrical body. In the most diverse alpheid group, one of the first pereiopods (chelipeds) bears a large claw with a snapping mechanism on the fingers, known as the snapping claw (Anker et al.,2006). The major chela of this pair of appendages is well developed, with fixed and mobile fingers and this chela produces an audible, snapping sound for prey capture and in agonistic interactions (Anker et al. 2006, Moraes et al., 2020).

1.3 Feeding behaviour

No studies to date have examined the relationship between food availability and distribution patterns in alpheid shrimp populations (Palomar et al.,2004). Previous studies on coral reef dwelling alpheids report that they are primarily detritus feeders (Karplus,1987). Thus, the amount and quality of food (in the form of particulate organic matter) present in the sediments could influence where they decide to construct a burrow (Karplus et al., 1974). According to Palomar et al. (2004), the gut content analysis provided evidence that the major source of food for the *Alpheus macellarius* is sea grass among 8 food materials under laboratory conditions. However, Palomar et al. (2004) classified *A. macellarius* as an omnivore species according to their laboratory study. Hazlett (1962) also found that a species of *Alpheus* collected in Bermuda was omnivorous. Burrows were created to stay during day time, except during feeding time, which usually occurred at night. The structure of *A. macellarius* burrows further supports a deposit-feeding habit. Alpheids have been observed to harvest fresh sea grass and leaf litter and bring these into their burrows (Stapel & Erftemeijer, 1997.); Griffis & Suchanek (1991) explained this behavior as 'biological gardening'. This is the accumulation of plant material in the burrow for cultivation of micro- organisms inside the burrow. Funnel shaped openings, expanded chambers and deep burrows are all indicative of sediment processing during feeding and the importance of scavenged material in the shrimp's nutrition (Nickell & Atkinson,1995; Bird & Poore,1999).

Epiphytes on sea grass leaves are also a possible food for the shrimp. This may explain the observation of shrimp directly feeding on sea grass. The leaves have little nutritional content compared with their epiphytic algae. The importance of epiphytic algae is further supported by the study of Moncreiff & Sullivan (2001) using stable isotope techniques, which found these algae to be a key source of organic matter for top level trophic levels in tropical sea grass beds.

Postlarval and juvenile *A. heterochaelis* feed avidly on Artemia nauplii, grasping large numbers of nauplii with the slender second chelae. Studies on their feeding habits have shown that they are primarily detritus feeders. However, in some species like *Alpheus macellarius*, sediment organic matter and sea grass were found to be important food sources. Thus, it has been postulated that substratum preference may be based on diet, as many burrowing shrimp feed on organic matter in the sediment, epifauna or infauna. If food was a resource in short supply, a significant negative relationship between condition and shrimp density would be observed (Corfield and Alexander,1995).

Hazlett (1962) found that a species of *Alpheus* collected in Bermuda was omnivorous.. Observations on *SynAlpheus fritzmuelleri* have shown that this species also has varied dietary habits. During several days of observations, this shrimp fed on algae such as Dictyota sp., filamentous ectoprocts and a eunicid polychaete.

It has been proposed that substratum preference may be based on diet, as many burrowing shrimp feed on organic matter in the sediment, epifauna or infauna. However, according to Corfield Andalexander (1995), differences in substratum preference between *A. edwardsii* and *A. lobidens* seem to be unrelated to diet, as the diets of the two species are same.

Fulton's condition factor is one method to describe the condition of the organisms which is widely use in aquatic science or fisheries science studies. The ratio between length and weight is used to measure the condition of the organisms. The ratio varied due to reproductive state and the general "conditions of nutrition" brought about by seasonality or "other circumstances" in the environment (Nash et al., 2006, Ouakka et al , 2017). Feeding habit of the organisms determine the nutrient condition of the organisms. When organisms have good nutrient foods, they can achieve their maximum growth. The fullness index has several applications, in the field ecology and fisheries science. Mainly it provides information on stomach fullness of the organisms. Fullness index indirectly provide information on food availability and feeding intensity of the organisms (Ouakka et al , 2017).The food supply changes during the year and the climate season.

Generally feeding intensity was lower in winter and moderate in spring and autumn (Nikolsky 1963). Maturity stage of the organisms also determine the fullness index and feeding intensity. The immature and maturing organisms fed more intensively than spawning organisms. This can be attributed that the ovigerous organisms spent the entire accrued energy for reproductive activity and the immature or maturing organisms spent the energy used for feeding activity for growth and other physiological activities (Ouakka et al, 2017).

1.4 Population dynamic of *Alpheus* species

Despite the high number of *Alpheus* species, there are few studies about their population biology, especially in comparison with the extensive knowledge about the taxonomy and diversity of the family Alpheidae (Anker et al., 2006). There is a lack of information on the biological, ecological, and behavioral aspects of Alpheidae species (Pescinelli, et Al.,2016). Information on population biology, such as the sex ratio, reproductive period, recruitment and the size at first maturity is essential to understand a species' life history (Stearns 2000). Many aspects of organismal ecology may be explained by population processes and structure such as size-frequency distribution, sexual maturity, reproductive period, recruitment, longevity, mortality, and sex ratio(; De Grave & Fransen, 2011).

Populations are affected by abiotic factors that change with geographical location (Bauer 1989, 1992; Cobo and Fransozo 2003; Castilho et al. 2007; Lardies et al. 2008). According to the Mossolin et al. (2006) studies on snapping shrimp are limited and they include mainly information of reproductive cycle and secondary sexual characters.

Social monogamy is defined as the close association of one male and one female and involves cooperation with respect to breeding activities (Wickler & Seibt, 1981). Social monogamy has been demonstrated for several species (Wickler & Seibt, 1981; Mathews, 2002a). Monogamy is the most common mating system among Alpheidae (Correa & Thiel, 2003).

Sex ratio is an important aspect that might represent the behavior of species (e.g., sexual behavior or mating system), as well as the sexual system. Mossolin et al (2006), conducted a study on the population structure of *Alpheus armillatus* in Sao Sebastiao and Ilhabela, southeastern Brazil, and according to the study, sex ratio has not changed from 1:1 ratio similar to the species *Alpheus dentipes* (Fernandes Munoz and Garcia-Raso, 1987), *A. angulatus* McClure, 1995 (Mathews, 2002) and *A. brasileiro*

(Costa-Souza, 2017). This is probably related to the monogamous behavior, hence producing a sex ratio not apart from 1:1 (Correa and Thiel 2003). Same as Mossolin et al. (2006), who commonly observed pairs of *Alpheus armillatus* individuals' underneath rocks or boulders. Normally, females were larger than males (Mossolin et al., 2006). Ovigerous females were observed all over the year, presenting three main spawning periods, both during warm and cool months: April-May, July-August, and March. Continuous reproduction and multiple recruitments per year were also confirmed in *A. normanni* in the seagrass meadows of Puerto Rico (Bauer, 1989) especially in summer. The recruitment period and intensity vary among Alpheid population. For instance, *SynAlpheus longicarpus* and *A.dentipus* present a continuous recruitment (Erdman and Black,1987, Fernández-Muñoz and Garcia-Raso, 1987) and *SynAlpheus fritzmuelleri* Coutière, 1909 has seasonal recruitment (Felder 1982). The intensity of recruitment is related with several factors, including the constancy of embryo production and the temporal variation in the mortality of first life stages. (Bauer 1989). High temperatures allow a rapid gonadal development providing a constant input (renewal) of individuals to the population (Sastry 1983; Bauer 1992; Cobo and Fransozo 2003; Litulo 2005). Costa- Souza et al. (2017) recorded a continuous reproductive period for the population of *A. estuariensis*, which keep same population size throughout the years. The presence of juveniles along the entire year indicates a continuous recruitment in the study area. Many subtropical and tropical crustacean species, including typical mudflat species, show continuous recruitment (Bauer 1992; Litulo 2005; Bezerra and Mathews-Cascon 2006, 2007). However, in *A. estuariensis*, the abundance in the first two size classes was extremely low and was only observed in 4 months. This might indicate a higher natural mortality of juveniles in the first few life stages.

Food availability, otherwise nutrition availability, is also an important factor for the development of embryos and the releasing of planktonic larvae into the environment, which determines the population structure through the survival of individuals (Sastry,1983). Species who are living in the lagoons get benefits because they have high level of nutrients available throughout the year (Levinton, 1995). In the estuarine environments, they have a constant supply of resources, due to a high input of organic matter (Little 2000; Silva et al. 2016). According to Moraes et al. (2020), shrimps are more abundant in spring and summer (warmer seasons) than in all other seasons, because of the higher nutrient availability and higher primary production (Schettini *et al.,* 1998; Sant'Anna Neto, 2005).

The choice of substrate to settle influenced by several factors such as surface texture and sediment types, adult-related cues, salinity, estuarine water, vegetation, and presence of shells for hermit crabs (Strasser and Felder 1998, 1999; Anger 2006). The type of sediment might affect the burrow structure, density, and distribution of burrowing crustaceans, which might also influence the distribution of burrow associated fauna (Dworschak 1983; Yanagisawa 1984; Hall-Spencer and Atkinson 1999; Palomar et al. 2005). Fine sediments are characterized by the low space between particles, hence exhibit poor drainage, lower oxygen concentration, higher organic matter concentration, and cohesive properties. Such cohesiveness is important for burrowing organisms and allows the construction and maintenance of their shelters in the sediments (Little 2000). *A. estuariensis* selects substrates with fine sediments due to their particular properties. Higher cohesive properties might help burrowing due to the sediment's poor drainage capacity, which prevents drying during the low tide. The high concentration of organic matter content in fine sediments declines the shrimps' exposure time during foraging. In summary, the population dynamic could be altered by these other factors.

1.5 Size Structure

Alpheus sp. populations from temperate regions might have larger individuals. They have late sexual maturity and short and seasonal reproductive period & recruitment (Bauer 1992; Litulo 2005). On the opposing, subtropical and tropical populations have smaller individuals and early sexual maturity. They have continuous reproductive period and recruitment (Bauer 1992; Litulo 2005; Almeida et al. 2012, Hirose et al. 2012). The snapping shrimp *A. brasileiro* did not show sexual dimorphism in body size, which can be explained by its social and reproductive behavior that may include social monogamy (Pescinelli, Davanso et al. 2017).

2 Scope of the research

Since *A. qatari* is a new exotic species in the Bay of Cadiz, we explore which factors (both biotic and abiotic) shape the distribution of this species. The main objective of this study is to analyse the *A. qatari* population structure on the San Pedro River (SW Atlantic Spanish coast) focusing on: (a) studying the population size distribution and sex-ratio during an annual period; (b) assessing whether a temperate region could shape the population dynamic of a tropical species. Bearing in mind that this is a new species, the morphological traits in males and females will also be analysed.

Our hypotheses are that a) this exotic species will be present in the non-native area of the San Pedro River during the full study period; b) despite of being a tropical species, its density and the density of the different demographic categories will vary as if it was a native species typical of a temperate area; c) the sex ratio will follow a pattern similar to its congener species of other *Alpheus* species, and hence the overall sex ratio will not be biased to any demographic category; d) being an exotic and invasive species, it will show a good performance throughout the year irrespective of the food abundance and, hence, the fullness and condition indexes will present good values in all the seasons.

3 Method

3.1 Sampling and sampling site

Individuals of the snapping shrimp *Alpheus qatari* were collected along the 275 m stretch of the San Pedro River right bank, Cadiz, Spain (36°32’03.4”N 6°12’50.8”W) (Figure 1). The San Pedro River is shallow, and its dynamics is tidally dominated, with the tidal regime being mesotidal and semidiurnal. The river bottom presents different sediment types, including mud and sand. The vegetation is mostly halophyte and is adapted to the tidally driven salinity changes.

The samples were picked by hand during daytime from June 2021 to May 2022. They were always collected when the low tide was at its lowest amplitude and were carried alive to the laboratory at the Institute of Marine Science of Andalusia (CSIC) for further analysis. The sampling periods covered all four seasons: Summer (June to September), Autumn (September to December), Winter (December to March), and Spring (March to May).

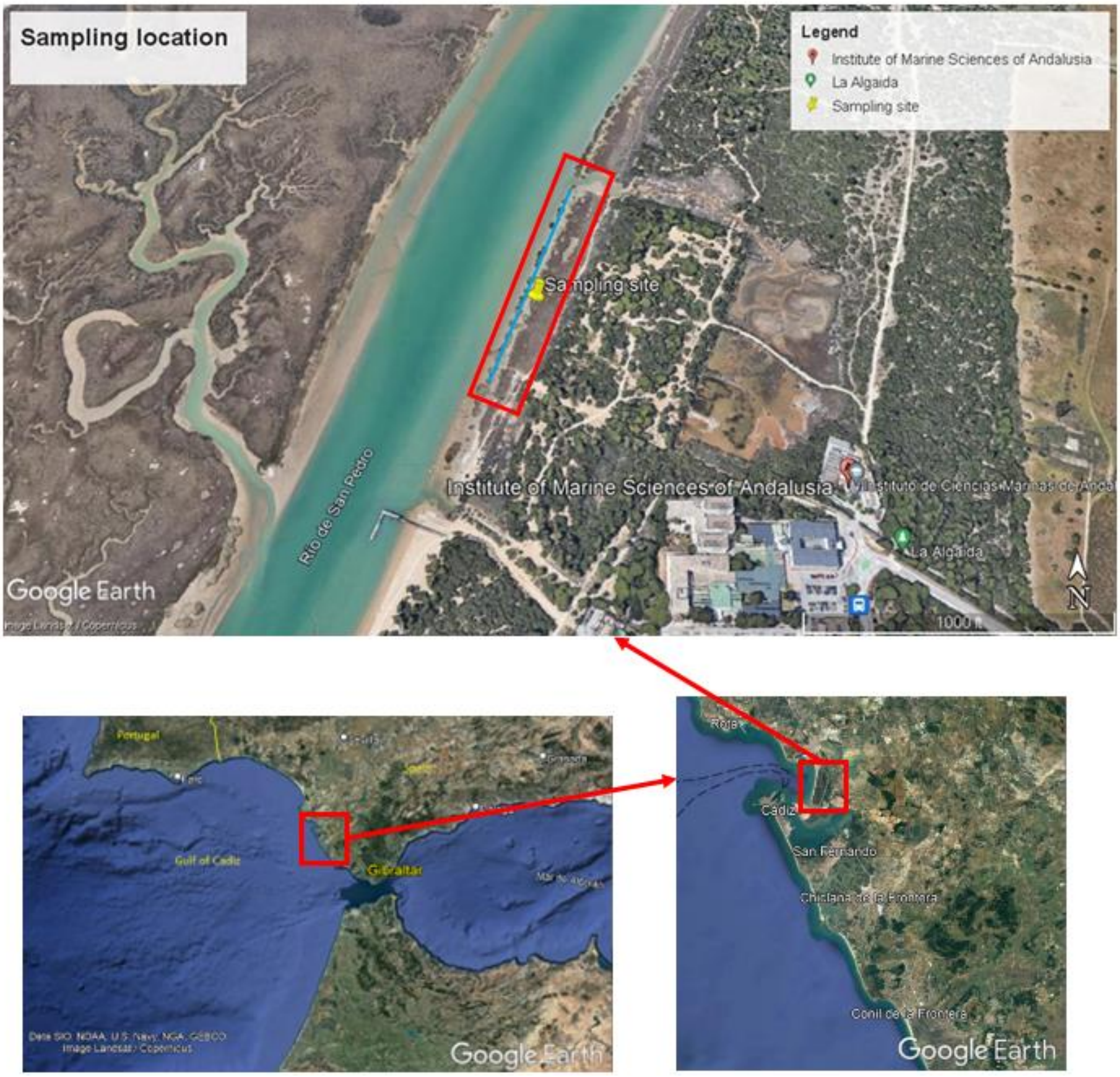

Figure 1: Sampling location

3.2 Laboratory analysis

Shrimps were sexed and sized with a vernier calliper (carapace width, CW) to the nearest 0.1 mm. The total length (TL) of the body (distance from the tip of the rostrum to the posterior margin of the telson, excluding the setae), the Cephalothorax length/carapace length (CL) of the body, the Length and width of the main chelae organisms, and its position (on right or left side) were measured and recorded for each shrimp (Figure 2). The period when ovigerous females were observed was considered the reproductive period of the species. Monthly temperatures were consulted on the site: "Red de Estaciones Meteorológicas Portuarias (REMPOR)", the network of "Puertos del Estado", Spain (see http://www.puertos.es).

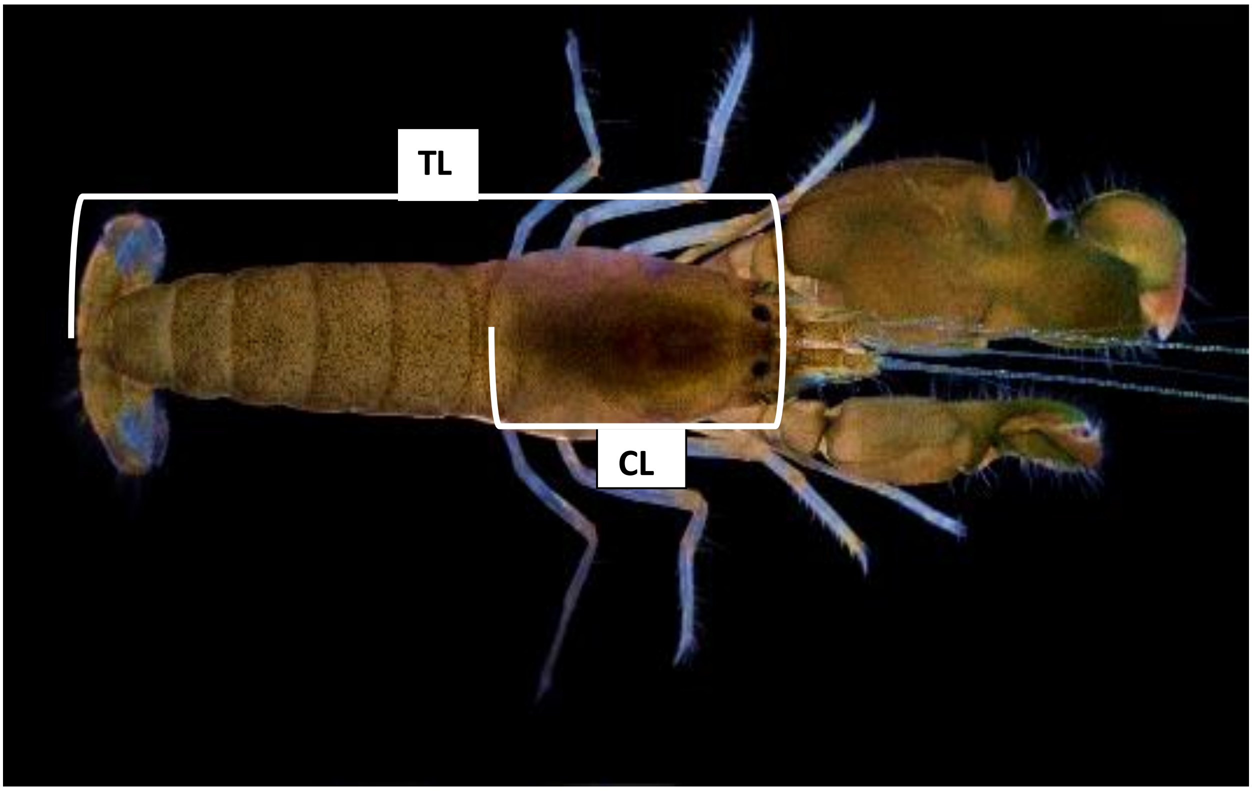

Figure 2: Morphometric characters of the organisms: Alpheus qatari (Male). TL stands for Total body Length, and CL stands for Cephalothorax length/carapace length.

Figure 3: Alpheus qatari (Female)

3.3 Morphology

Considering that this is a new species and that the study by Giraldes et al.,(2021) did not describe the morphology of *A. qatari* in detail, we have included here a comparison of morphological description between males (Figure 02) and females (Figure 03) (Cuesta et al., unpublished data). As mentioned before, the individuals were sexed and their morphological traits were analyzed to differentiate between male and female, according to the appendix interna of the second pleopod or using the sexual dimorphism trait of the snapping claw (Figure 4). As observed in Figures 02 and 03, male organisms have a larger and more robust snapping claw than female organisms.

Relative chela length (RCL)

The chela length was measured and the relative chela length for the male, female and ovigerous female was estimated with the cephalothorax length as following:

Relative chela length = $\boldsymbol{Chela\ length/Cephalothorax\ length}$

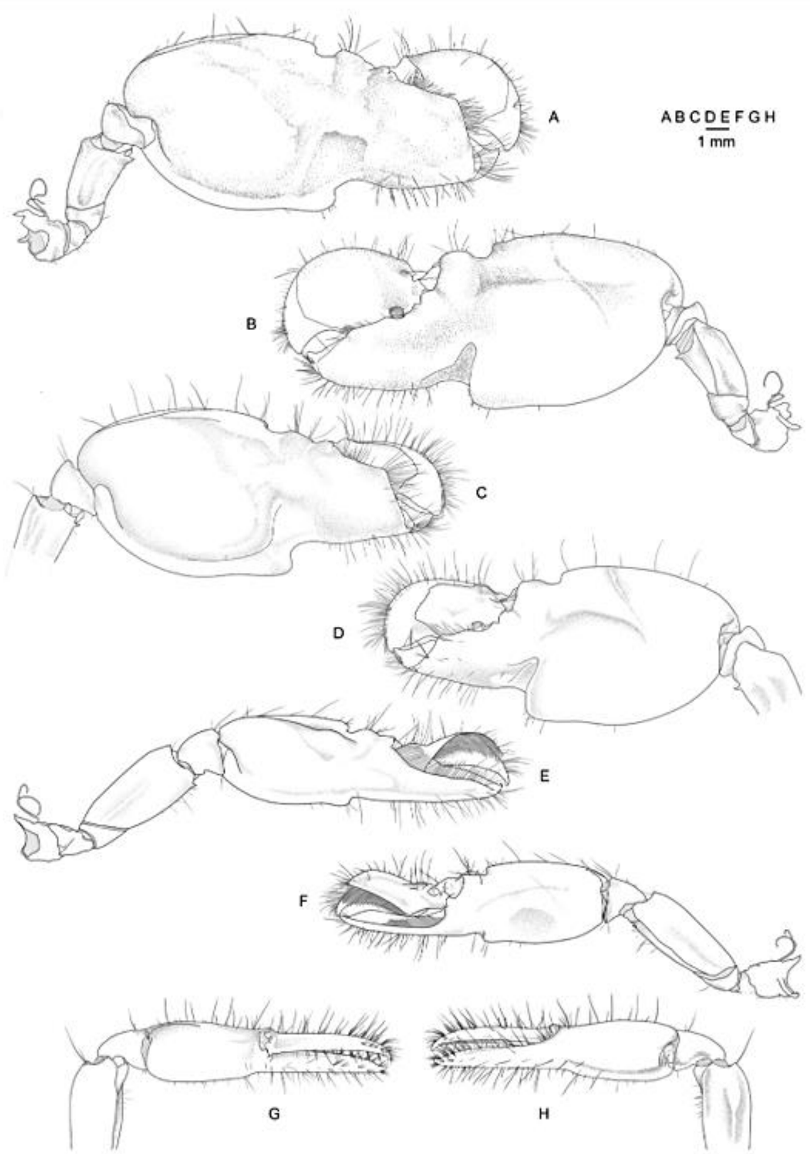

Figure 4: Alpheus qatari, male

(IEOCD-ICMAN/2964) [A-B; E-F] and female (IEOCD-ICMAN/2963) [C-D; G-H] from the intertidal zone of the San Pedro River inlet, Puerto Real (Cádiz); A) major cheliped, mesial view; B) major cheliped, lateral view; C) major cheliped, mesial view; D) major cheliped, lateral view; E) minor cheliped, mesial view; F) minor cheliped, lateral view; G) minor cheliped, lateral view; H) minor cheliped, mesial view. Scale bars: A, B, C, D, E, F, G, H = 1 mm.

3.4 Fulton´s condition factor (K)

Fulton’s condition factor (K) expresses the condition of the organisms and it is calculated with the following equation Fulton, 1911):

$$K = (W/L^3) \times 100$$

Where *K* is theFulton’s condition factor, *W* is the animal’s weight, and L is the length (Total length).

The value of the condition factor is used to measure the condition or wellbeing of a species.

The collected snapping shrimps were measured (total length in mm) and weighted (in grams) immediately after they were transferred to the laboratory.

3.5 Hureau Index (Fullness index)

The fullness index (FI) is defined as the ratio between the food ingested by an organism and weight of the organism. Its values were calculated for all the collected organisms during the study period.

Concerning the measurement of the fullness index, the entire stomach was detached from the alimentary system by removing the posterior part of the esophagus and intestine and then it was weighted with the contents (TSW). In addition, stomach contents (FW) and the empty stomach (ES) were dissected and weighted separately. The difference of the two weights gives the weight of food (FW) (Kagwade 1964):

WF (g) = TSW (g) - ES (g).

The measurement of ingested food weight (FW) is expressed as a percentage of the total organism weight (W) according to the formula defined by Hureau (1969):

FI = FW / W x 100,

where FI is the index of fullness (fullness index).

3.6 Statistical analysis

The normality of the data was checked using the Shapiro-Wilk test, the Mann-Whitney test was used to test differences in size between the males and females (–on - ovigerous and ovigerous), and a t-test was carried out to explore the differences between ovigerous and non-ovigerous females. The chi-square (X^2) test was used to analyze whether the overall sex ratio as well as the adult sex ratio deviate from the theoretical proportion. The sex ratio was calculated as the number of adult males divided by the total number of adult individuals (males and females) observed during each monthly campaign. The Wilcoxan-man-whitten test (W) was performed to determine whether there was any significant difference between male and female for the relative chela length.

PERMANOVA test (version 6, PRIMER -E Ltd., Plymouth, UK) for fullness and K indexes were used to test differences in the factors sex, months, main chela position, and the covariate size (cephalothorax length). The position of the main chela (the snapping chela) could be found at the right or left first pereiopod. This position (right or left) was used as a factor in the analysis.

All the statistical analyses were done using Microsoft Excel, R studio and PRIMER.

4 Results

4.1 Population structure and sex ratio

A total of 383 individuals were recorded between July 2021 and May 2022 , of which 174 were females (50 non-ovigerous and 124 ovigerous) and 188 were males (Table 1). Ovigerous females were observed during the study period July-2021 to May 2022 (Figure 1).The average Sex ratio (Male:Female) was 1:0.92, i.e., its value did not differ significantly from the expected equal proportion (X^2 = 1.43, df =1, P >0.05).

Table 1: Descriptive statistics of *Alpheus* qatari recorded at the San Pedro River (SW Spain)

All measurements are in mm (CL). NO females indicate non-ovigerous females; O females, ovigerous females; SD, standard deviation.

Category	Range	Mean ± SD	N
Males	3.73-19.97mm	11.90±1.84	188
Females			
NO females	7.75-14.75mm	11.97±1.45	50
O females	5.89-15.66mm	11.94±1.75	124

The size distribution of the different demographic categories in the 11-month sampling period is shown in Figure 5. There is significant difference between male and females for the cephalothorax length (M–nn - Whitney = 15060, P < 0.05), while there was no significant difference for the cephalothorax length between non-ovigerous and ovigerous females (M–nn - Whitney = 3802, P > 0.05). Males were more abundant in the sizes 12 – 12.99 mm CL. Ovigerous and non-ovigerous females were more abundant in larger sizes 11-12.9 mm CL. The largest individuals were observed during the months of August, September, October, and March (male=14 -14.99mm CL). For the ovigerous females, the largest individuals were observed in April (ovigerous female = 15.66mm CL) and the largest non-ovigerous females were observed in August (–on - ovigerous female = 14.75 mm CL).

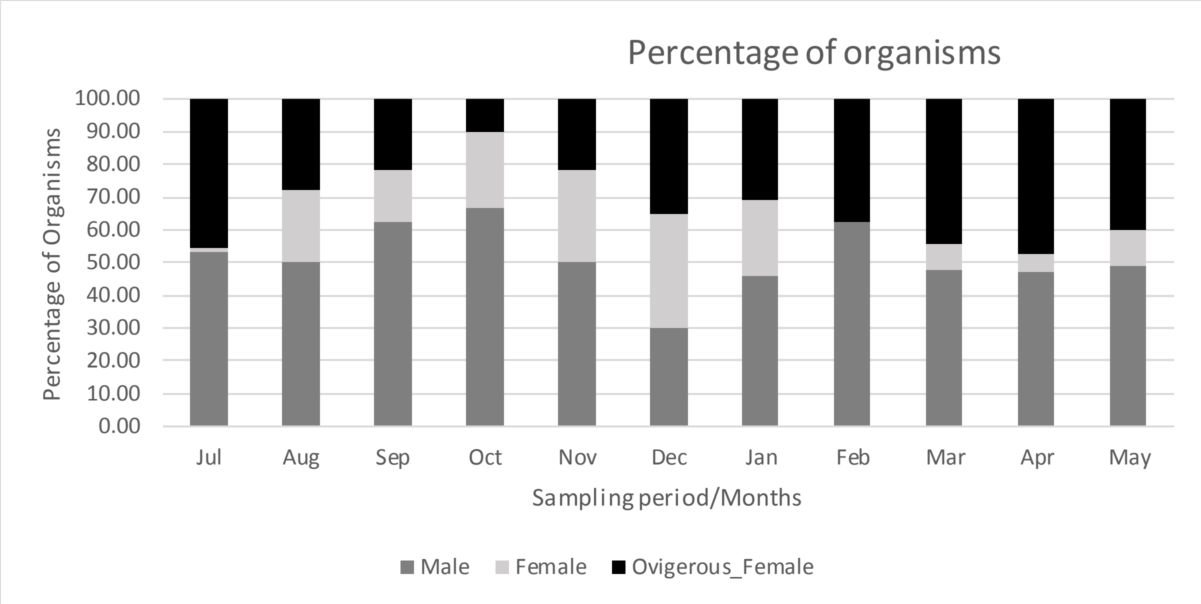

Figure 5: Percentage of different demographic categories of Alpheus qatari at the San Pedro River (SW Spain) during the 11 months of study (July 2021 – May 2022). OF, Ovigerous females; F, Non-ovigerous females; M, Males

According to Figure 5, the percentage of females increased from July -to December in 2021. The maximum percentage of females was recorded during December 2021. With regards to males, the percentage increased from July to October and gradually decreased up to December. In the case of Females with eggs, the percentage of individuals was higher in summer and in spring.

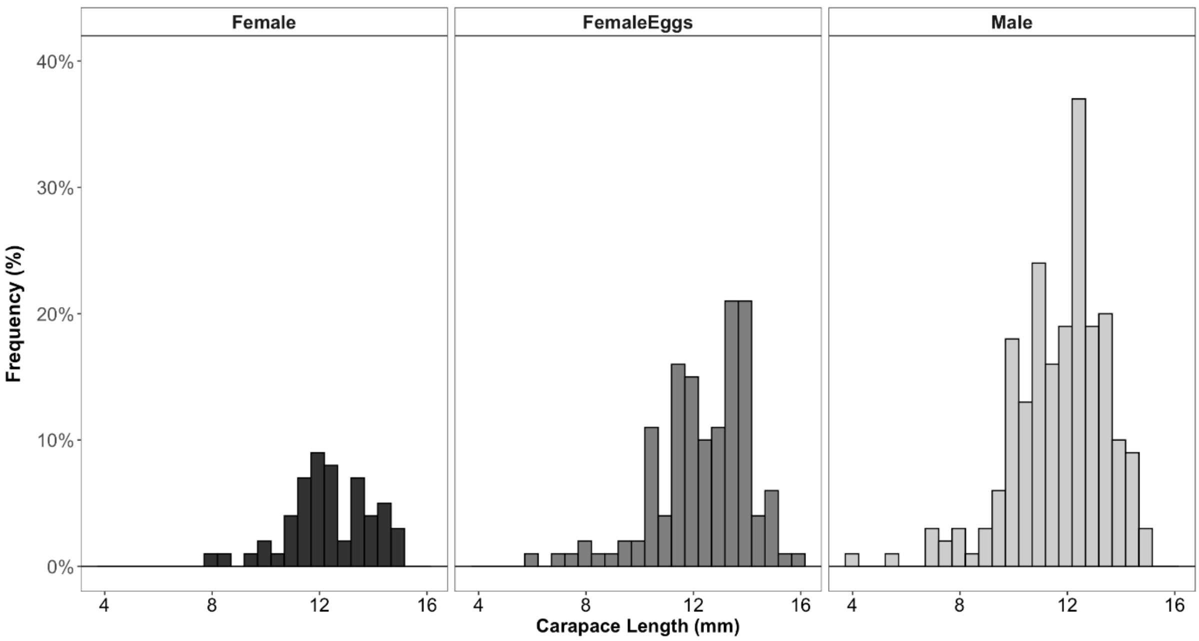

Figure 6: Size - frequency distribution of demographic categories of Alpheus qatari during the 11-month study period at the San Pedro River (SW Spain).

The carapace length ranged between 3 mm to 16 mm for males, between 7 mm and 15 mm for females, and between 5 mm to 15 mm (the highest size) for ovigerous females(Figure 6).

4.2 Temporal variation of carapace length

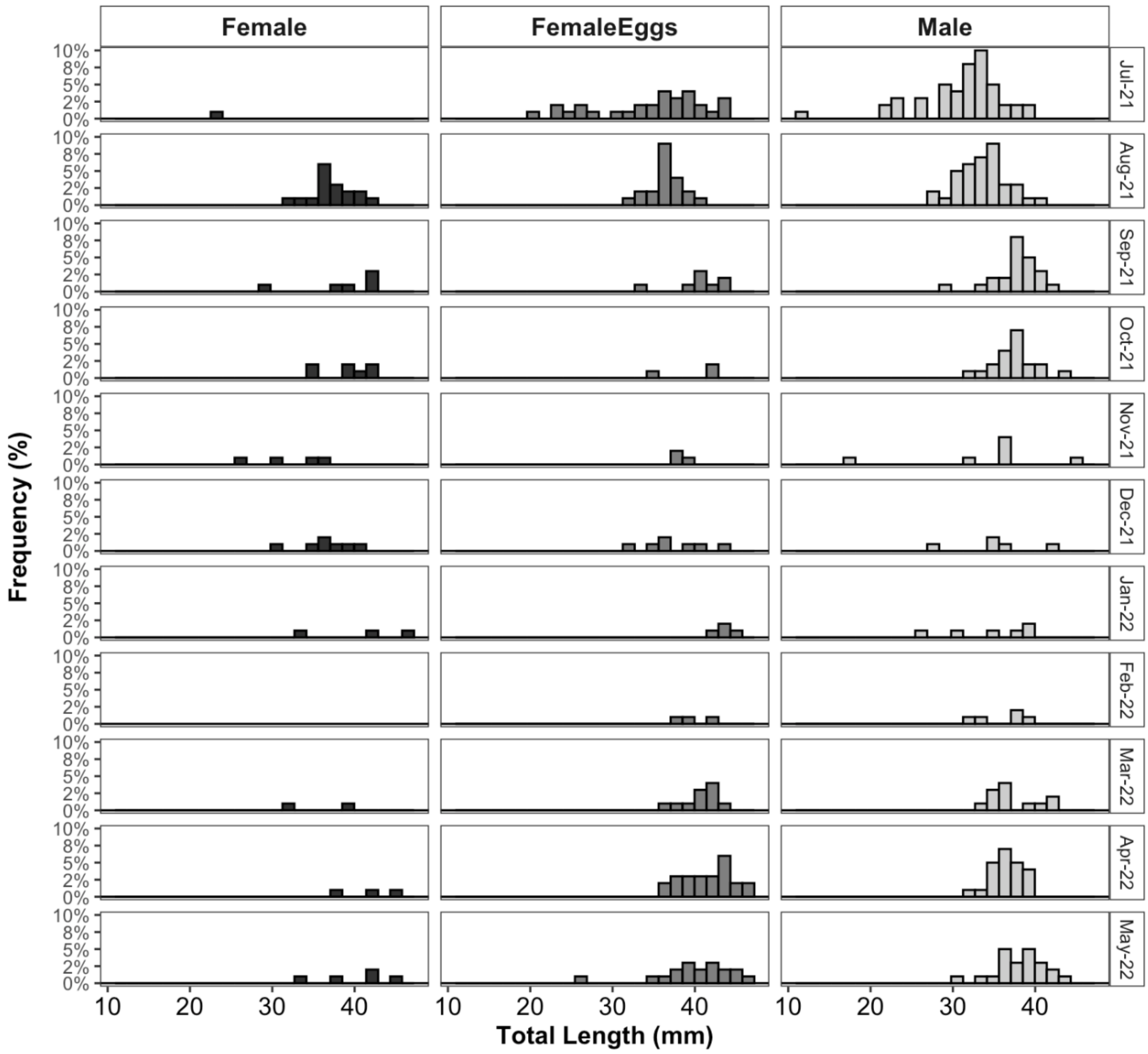

Figure 7: Temporal variation of carapace length for females, ovigerous females, and males

The carapace length changed during the study period for males, females and ovigerous females (Figures 7 to 11). The size of females gradually decreased from July 2021 to November 2021 and increased from December 2021 to 2022 May (Figure 7). Ovigerous females were collected during the entire sampling period, with the smallest ones appearing mainly in summer (July) and the largest ones in spring (April-

May). Two cohorts could be identified in males and females from July to October and, from November to May of the following year (Figure 7).

The average cephalothorax length in males was 11.94±1.78 mm, and ranged between 10.6 mm and 12.4 mm from July 2021 to May 2022, i.e., during the 11 months of the study period (Figure 8). The smallest individuals were recorded in July-2021 and the largest ones in March-2022. The lowest values in the samplings 1 and 5, refer to the new cohorts of the population, thus decreasing the low average size of the population of those samplings in summer (sampling 1, July) and autumn (sampling 5, November).

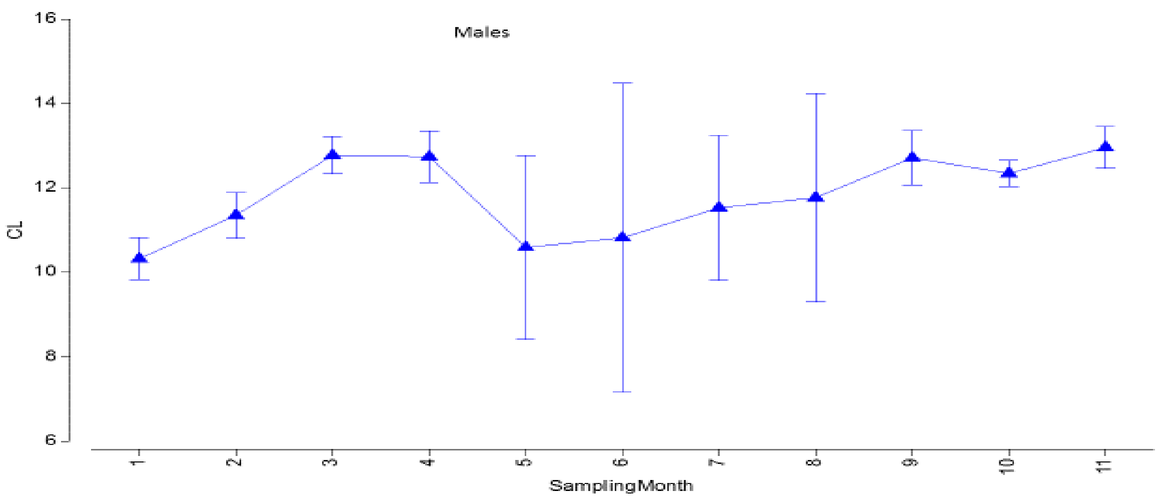

Figure 8: Average carapace length variation of Males during the sampling period

(1:July 2021, :2:August 2021, 3:September 2021, 4: October 2021, 5: November 2021, 6: December 2021, 7:-January 2022, 8: February 2022, 9: March 2022,10: April 2022, 11: May 2022).

With regards to ovigerous females, the average cephalothorax length was 12.06 ± 1.76 mm, and the size variation was low, with the exception of autumn (October – November) due to the low number of individuals in this demographic category (Figure 9).

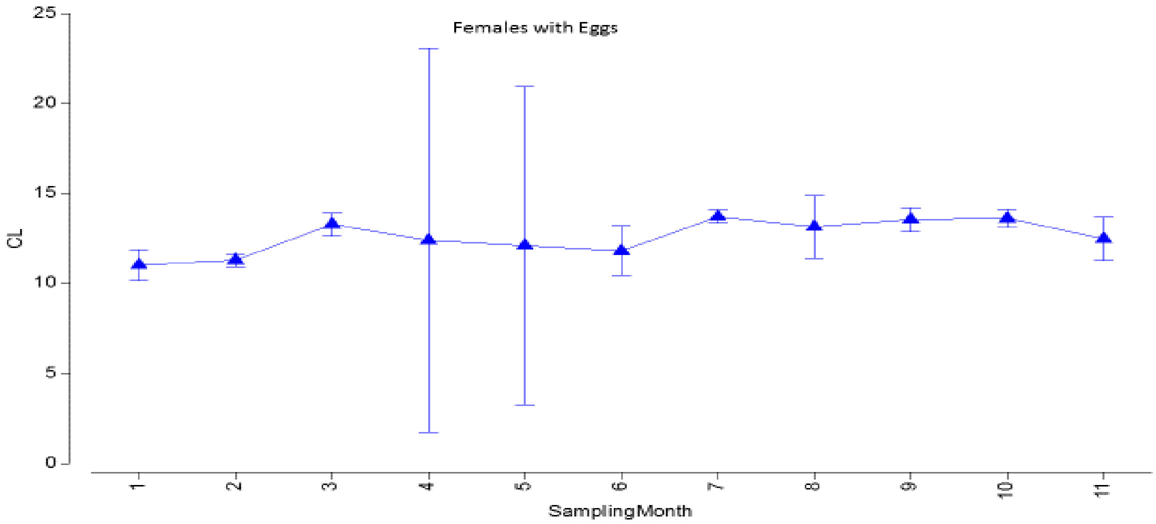

Figure 9: Average carapace length variation of ovigerous females during sampling period

(1:July 2021, :2:August 2021, 3:September 2021, 4: October 2021, 5: November 2021, 6: December 2021, 7:-January 2022, 8: February 2022, 9: March 2022,10: April 2022, 11: May 2022).

With regards to the cephalothorax length of females *A.qatari*, the average carapace length was 12.15±1.68 mm with a size range between 7.75 mm and 12.71 mm during the study period (Figure 10). Similar to the males, the lowest values in the samplings 1 and 5 refer to the new cohorts of the population, the low average size of the population of those samplings decreasing in summer (sampling 1,July) and autumn (sampling 5, November).

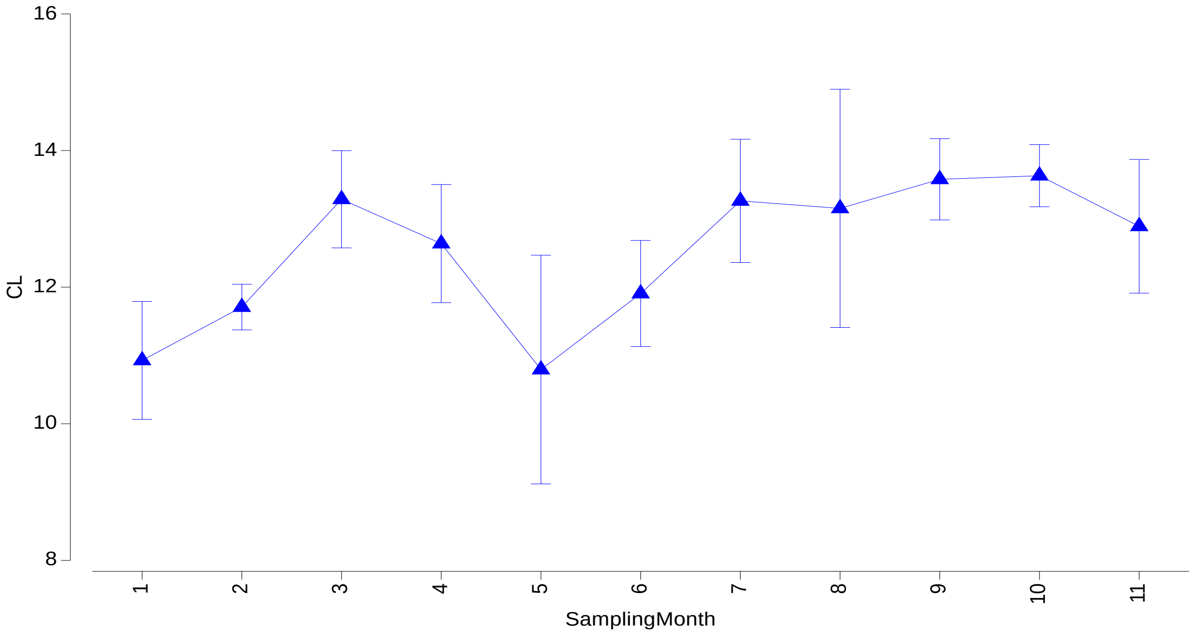

Figure 10:Average carapace length variation of all females (Non-ovigerous and ovigerous females) during the sampling period

(1:July 2021, :2:August 2021, 3:September 2021, 4: October 2021, 5: November 2021, 6: December 2021, 7:-January 2022, 8: February 2022, 9: March 2022,10: April 2022, 11: May 2022).

4.3 Length-weight relationship

The relationships between weight and lengths and the correlation coefficients were analyzed for all the demographic categories of *Alpheus qatari* during the 11-month study period at the San Pedro River.

All data – Length Weight Relationship

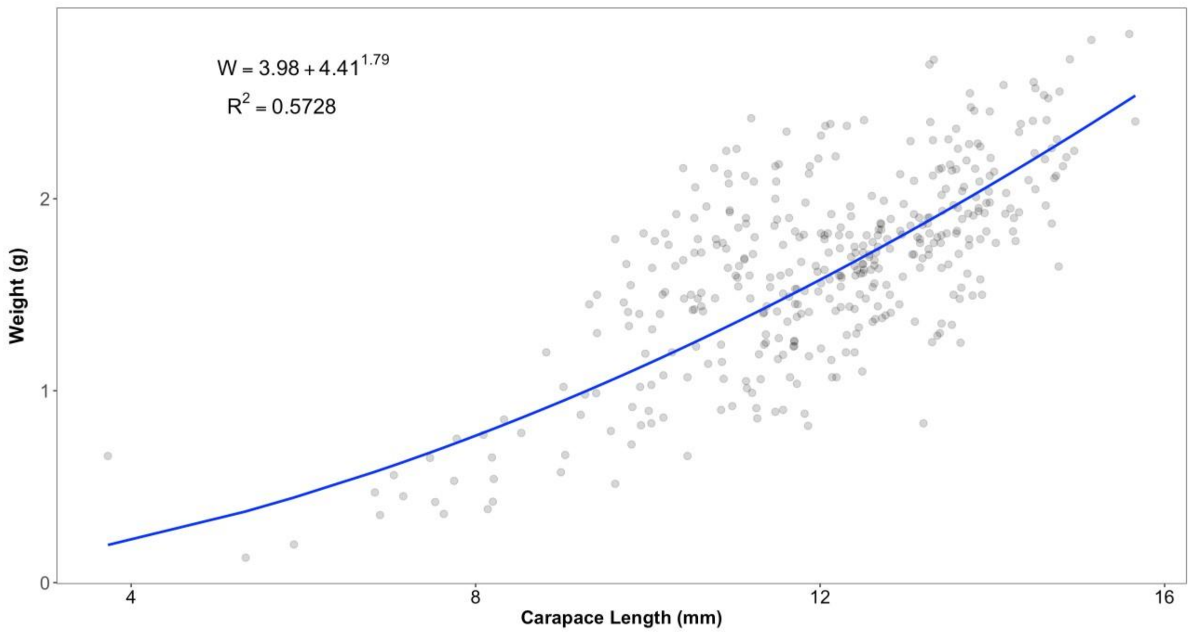

Figure 11:Length-weight relationship curves for the San Pedro River population.

The significant correlation between the total weight and cephalothorax length was found to be a power function ($P<0.05$); the formulae are given in Figures 11-14. Based on the value of the correlation coefficient (R^2), the relationships in general had high R^2 values. Note that the R^2 for the regressions of the females was slightly higher than of males. Based on the t-test against the value of growth coefficient (b) at the 95% confidence interval, we could infer that the growth pattern of the organisms was negatively allometric ($b<3$, t-test, $P<0.05$), meaning that the increase in length of this species was accompanied by a smaller increase in weight.

Female - LWR

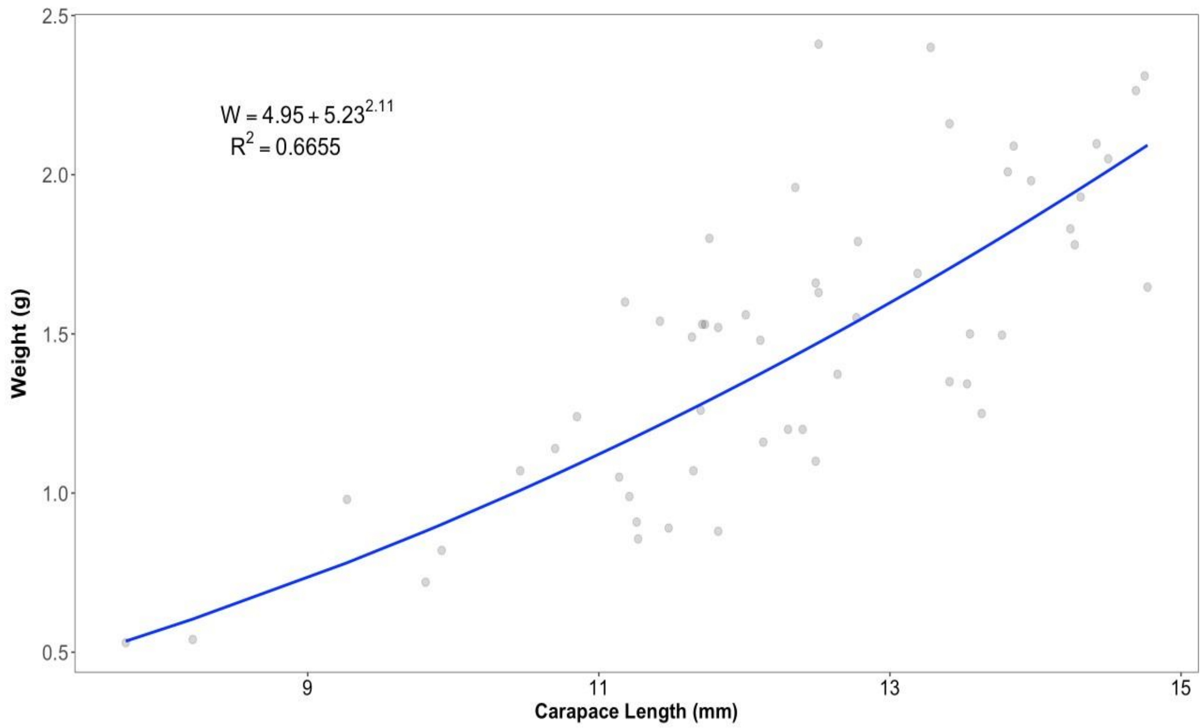

Figure 12: Length-weight relationship curve of females from the San Pedro River.

FemaleEggs - LWR

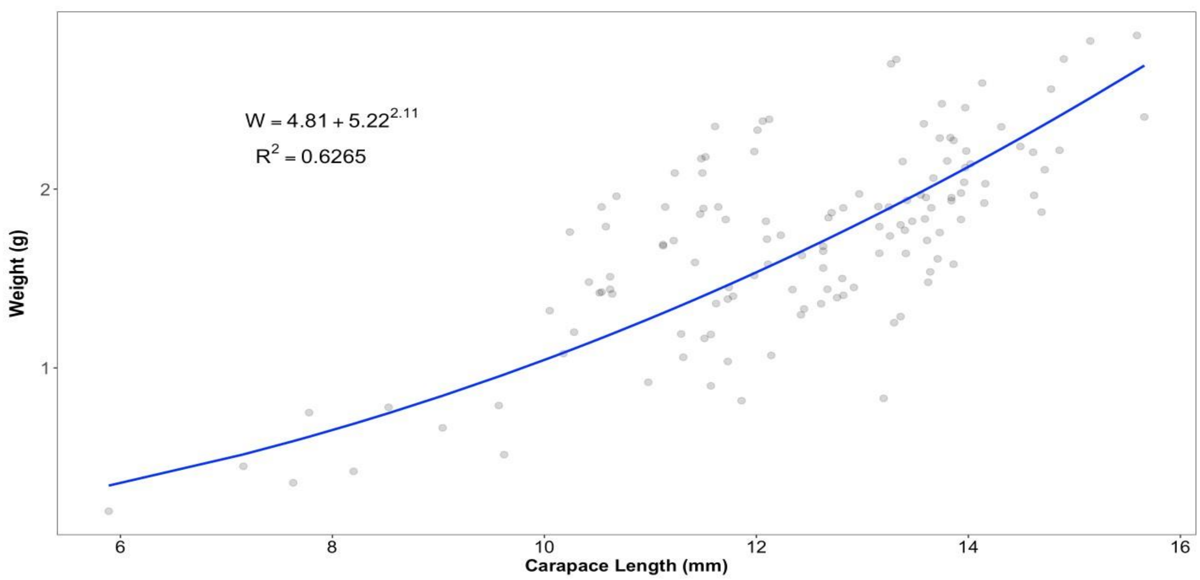

Figure 13:Length-weight relationship curve of ovigerous females from the San Pedro River.

Male - LWR

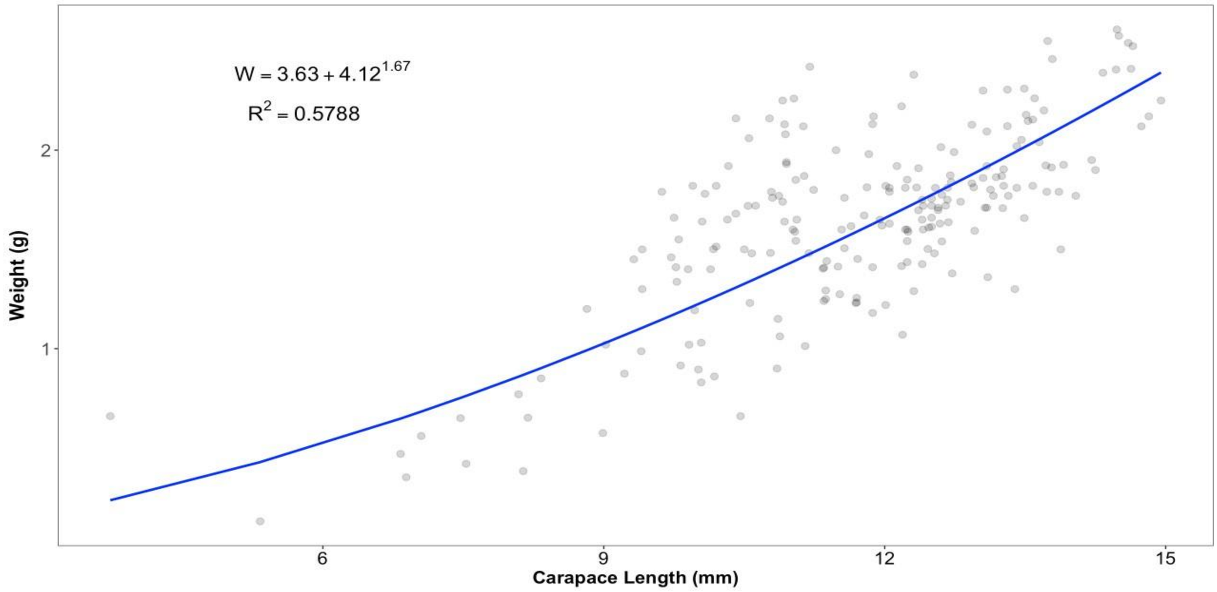

Figure 14: Length-weight relationship curve for males from the San Pedro River.

4.4 Morphological traits: Relative chela length

Results from the morphological analysis showed that there is a significant difference between the relative chela length of males and females (W= 33473, $p<0.05$).A Kruskal-wallis test was performed to determine significant difference of the relative chela length between the three demography categories (H=155.58(2), $p<0.001$). Males showed a significantly larger chela than females. Female and ovigerous females did not show significant differences among their length (Figure 15).

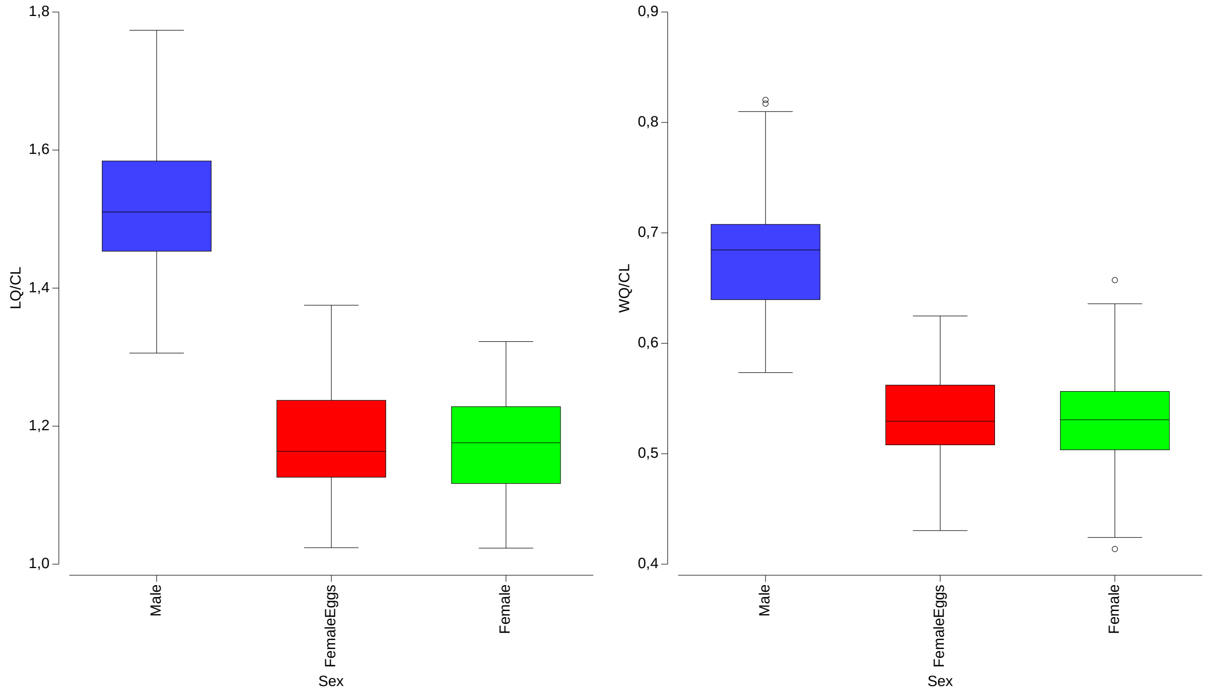

Figure 15: Relative chela length (left) and chela width (right) of the different demography categories (Male, Female and ovigerous females).

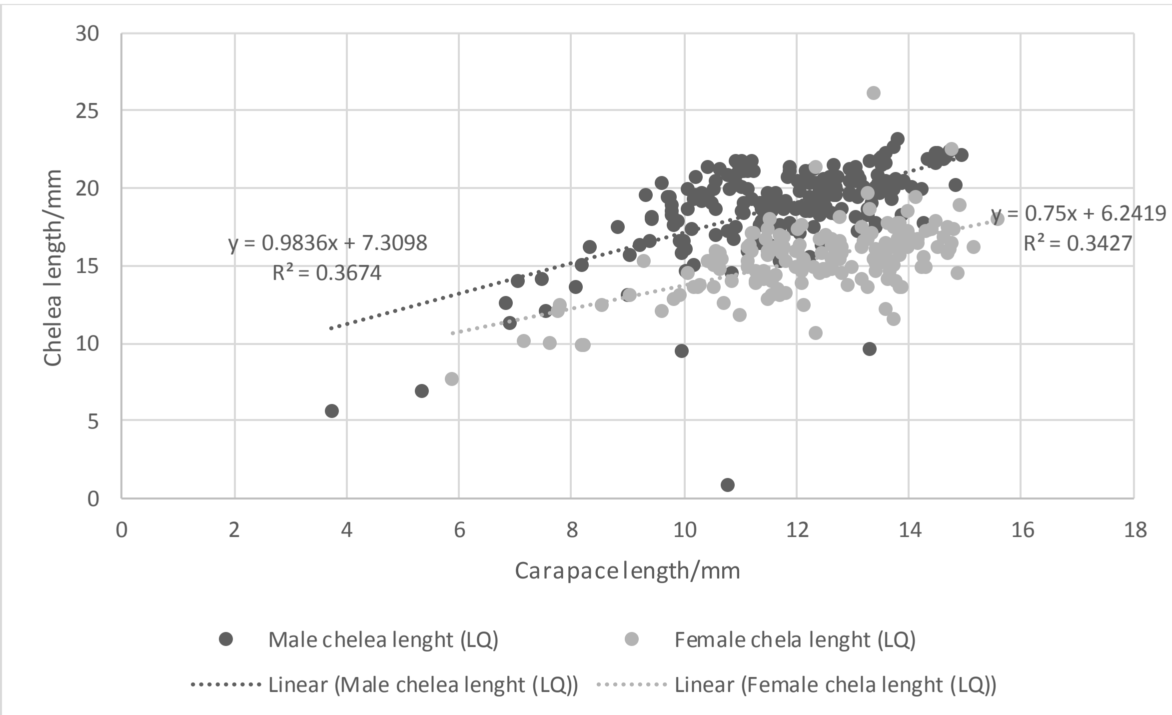

Figure 16: Comparison of the relationship of the length of the males and females with the carapace length between males and females of Alpheus qatari

The relationship between chelae and CL was steeper in males than in females, indicating that males have a larger major chela than females at a given carapace length (Figure 16).

4.5 Fulton´s condition factor (K)

The Fulton´s condition factor (K index) was calculated for the males, females and ovigerous females at each month. A PERMANOVA test was performed for the factors Sex, Month, and Position of Chela, and the covariate cephalotorax length (Table 2). Among these factors, Sex, Month and cephalothorax length showed significant differences, and there was no evidence for a two and three-way significant interactions.

Table 2. PERMANOVA table of results. CL = cephalothorax length, Mo = months, Se = demography categories (female, male and ovigerous females), Ma = Position of the main chela (right or left).

Source	df	SS	MS	Pseudo-F	P(MC)
CL	1	4,7397E-05	4,7397E-05	175,25	0,0001
Mo	10	7,8709E-05	7,8709E-06	29,102	0,0001
Se	2	5,138E-05	2,569E-05	94,988	0,0001
Ma	1	2,9517E-07	2,9517E-07	1,0914	0,2911
Res	262	7,0859E-05	2,7045E-07		
Total	320	0,00026087			

The highest Fulton's condition factor (K index) was recorded for the summer months July and August 2021, both in males and in females (Figure 17).

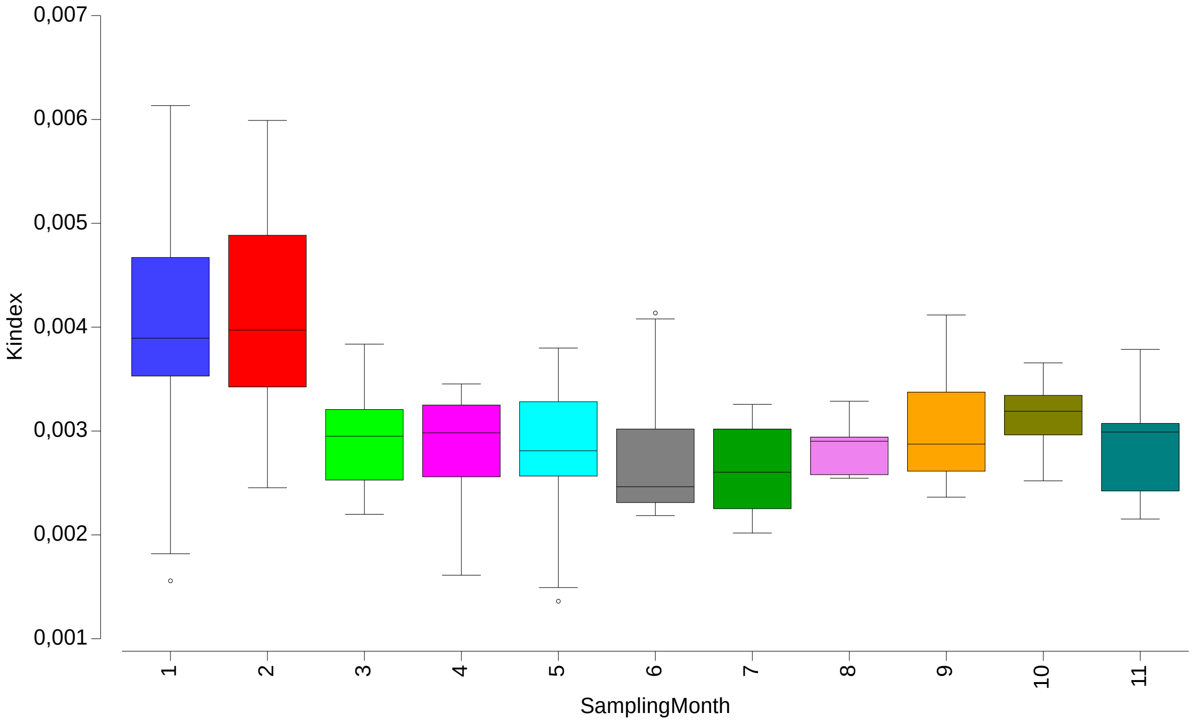

Figure 17: Distribution of condition factor (K index) during the sampling period

(1:July 2021, :2:August 2021, 3:September 2021, 4: October 2021, 5: November 2021, 6: December 2021, 7:-January 2022, 8: February 2022, 9: March 2022,10: April 2022, 11: May 2022).

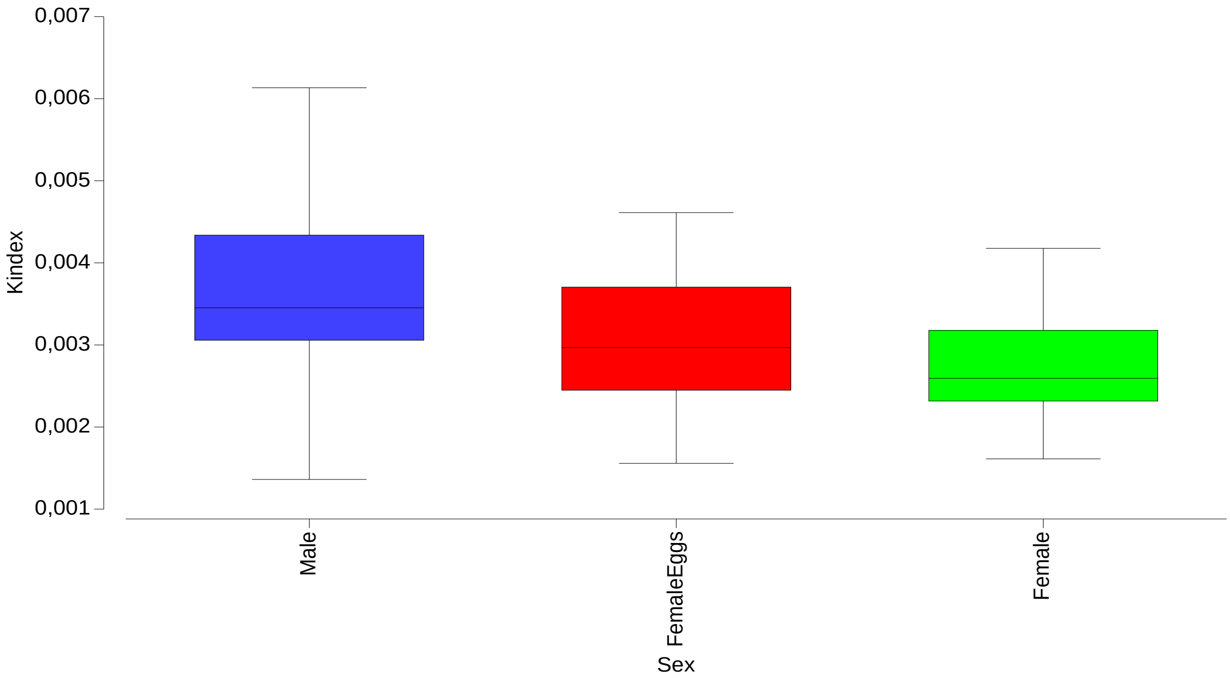

Figure 18: Condition factor for the male, female and ovigerous females (K index) during the sampling period.

When we compare, the K index values between males and females, males showed higher values than females, suggesting a better condition than all others.

4.6 Hureau Index (Fullness index)

The Hureau Index was calculated for the males, females and ovigerous females for each month. A PERMANOVA test was performed for the factors Sex, Month, and Position of Chela, and the covariate cephalothorax length (Table 3).

Table 3. PERMANOVA table of results. CL = cephalothorax length, Mo = months, Se = demography categories (female, male and ovigerous females), Ma = Position of the main chela (right or left).

Source	df	SS	MS	Pseudo-F	P(MC)
CL	1	0,059312	0,059312	0,12416	0,7279
Se	2	13,572	6,7858	14,205	0,0001
Mo	10	38,364	3,8364	8,0309	0,0001
Ma	1	0,44976	0,44976	0,94151	0,3366
Res	180	85,987	0,4777		
Total	235	160,27			

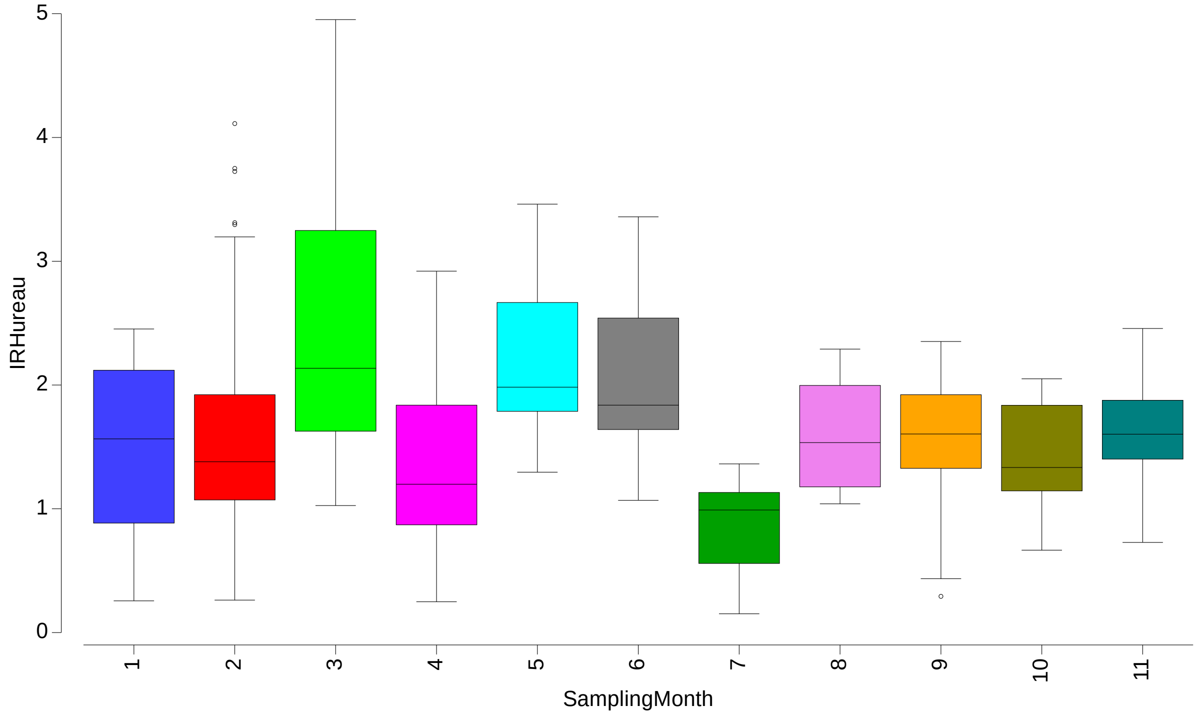

Figure 19: Fullness index variation during sampling period for A. qatari

Among these factors, Sex and Month showed significant differences. Overall, the fullness index (FI) of the stomach showed the highest values in autumn (Figure 19) and, among the different demographic categories, the highest values occurred in females (Figure 20).

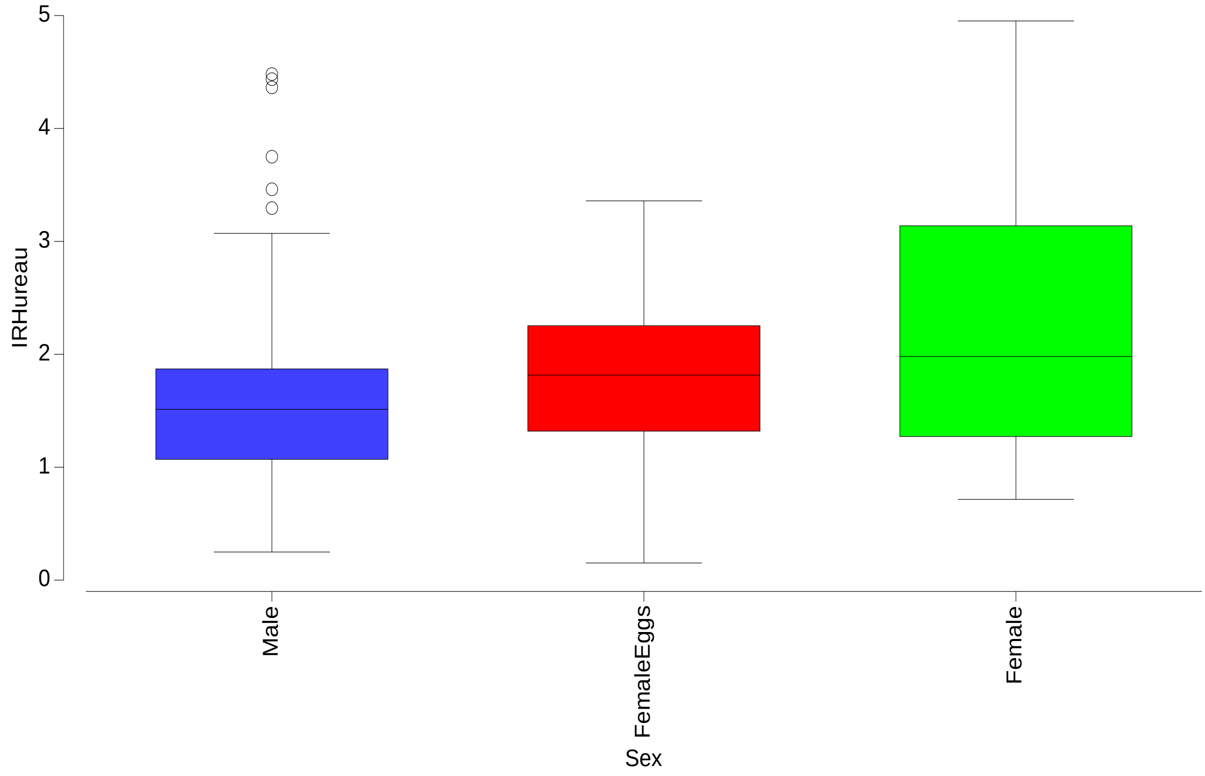

Figure 20: Fullness index for the male, female and ovigerous females during the sampling period for all organisms

5 Discussion

The family Alpheidae has one of the highest diversity of species among marine invertebrates, several species complexes and a large diversity of species niche-restricted to specific substrates, zones, depths and marine provinces (Giraldes et al, 2021). Those authors identified *A. qatari* as a new species in the southwestern province of the Persian Gulf. This species was also recorded in the Bay of Cadiz (SW Spain) in 2015 (González-Ortegón, personal communication). Both regions (Persian Gulf and Bay of Cadiz) are shallow productive zones and are characterized by the presence of halophyte plants adapted to variations in the water salinity (Martínez.,*submitted*). However, the environmental conditions are very different in both regions, suggesting that *A. qatari* can adapt to a wide range of environments. Martínez (*submitted*) analyzed time series of meteorological (air temperature, rainfall, solar radiation, wind, and atmospheric pressure) and oceanographic (sea surface temperature, salinity, and total suspended matter) variables between 1993 and 2020 (Table 4). While both regions present a marked seasonal variability, the Persian Gulf is considerably warmer, with an average maximum temperature of 36.19ºC and minimum of 15.60ºC, while the maximum (minimum) average temperature in the Bay of Cadiz is 25.90ºC (11.63ºC). The high temperatures and extremely low precipitation in the Persian Gulf are also reflected in the higher sea surface temperature and salinity mean values in comparison with the Gulf of Cadiz. In both regions, the air and sea surface temperatures present a positive trend, indicating that they have increased between 1993 and 2020. The temperature increase in the Bay of Cadiz supports the idea that this species, native of a warm ecosystem, will be able to establish in the Bay of Cadiz.

Table 4. Range and trend of several oceanographic and meteorological variables for the period 1993 - 2020 at the Persian Gulf and the Bay of Cadiz. Green (red) numbers indicate a positive (negative) trend. (Table adapted from Martínez., *submitted*).

	Range		Trend (Unit/decade)	
	Persian Gulf	Bay of Cadiz	Persian Gulf	Bay of Cadiz
Sea surface temperature (ºC)	[17.89, 34.65]	[14.57, 24.22]	+0.26	+0.23
Sea surface salinity (psu)	[38.22, 40.12]	[36.25, 36.76]	-0,0027	+0,0019
Total suspended matter (g/m^3)	[2.4, 6.34]	[1.1, 13.09]	-0.12	+0.65
Air temperature at 2m (ºC)	[15.60, 36.19]	[11.63, 25.90]	+0.44	+0.21
Total precipitation (m)	[0, 0.003]	[~0, 0.013]	-3.00×10^{-5}	-1.40×10^{-4}
Solar radiation (J/m^2) x10^7	[1.16, 2.80]	[0.71, 2.98]	-8.97×10^{-4}	$+4.51 \times 10^{-3}$

Atmospheric pressure at sea level (mbar)	[995.60, 1021.80]	[1009.50, 1027.80]	-0.04	+0.06
Zonal wind component at 10m (m/s)	[-1.77, 3.02]	[-4.88, 5.66]	-0.01	+0.01
Meridional wind component at 10m (m/s)	[-4.45, 0.06]	[-2.93, 2.75]	+0.09	-0.05

The population traits of *A. qatari* in the San Pedro River are similar to other *Alpheus* populations. According to the results obtained, the sex ratio of the *Alpheus qatari* is not significantly different from the 1:1 ratio characteristic of other *Alpheus* species. This condition was in fact observed during the sampling and has also been verified in populations of *A. schmitti* (Chace, 1972; Werding, 1990), *A. angulatus* (Mathews, 2002) and *A. armillatus* (Mossolin et al, 2006). This has been attributed to the formation of male and female pairs that commonly occur in alpheid species (Mossolin, et al, 2006). The nearly 1:1 sex ratio and the overall size class distributions indicate that pairs are formed by individuals of approximately similar size with females slightly larger than males. Interestingly, ovigerous females were recorded during the entire sampling period. Continuous reproduction and multiple recruitments per single year were also verified in *A. normanni* in the seagrass meadows of Puerto Rico (Bauer, 1989). This could explain that this population is expanding in the Bay of Cadiz. Moreover, the continuous food availability would allow the San Pedro River population to increase its size. In addition, males are significantly larger than females, in agreement with other studies (Mossolin et al, 2006). Subsequently, females benefit from large males because they can better defend the burrow against predators than smaller males, resulting in the formation of stable mating pairs during more than one reproductive cycle (Azofeifa-Solano, et al, 2020). In addition, males benefit from mating with large females, which produce more offspring than smaller females (Anger & Moreira, 1998; Wehrtmann & Lardies, 1999).

In this study, most of the individuals collected were adults and only few of them had a small size, similar to other populations (Moraes et al, 2020). According to Costa-Zouza et al (2018), adults and juveniles are available during the whole annual period with a dominance of adults in most months. Thus, it could be assumed that adults are reproducing in another ground and migrate to the sampling area to grow.

On the other hand, cohorts were identified in the seasons where a high secondary production occurs in temperate waters (summer and spring), similar to other study where two possible cohorts for the *A. armillatus* in April-May and July-August were identified (Mossolin et al 2006).

The significant correlation between the total weight and total length was found to be a power function in both sexes of *A. qatari* in the San Pedro River with a high correlation between both covariates. For instance, the values obtained from the length/weight relationship (LWR) of some shrimp species in the Iko River estuary showed that there was a high and significant correlation between both covariates (Udoinyang, et al, 2016). Generally, sexual dimorphism related to size is observed in the shrimp species, where female individuals are mainly bigger and heavier than male individuals, and is probably related to the reproductive process and considered an adaptation that increases the energy investment during reproduction (Hartnoll, 1982). Although, in this species males are bigger than females, the difference in the slopes between regression lines for males and females represents that male and female *A. qatari* have different growth patterns, and it can indicate a clear sexual difference in the growth type in this species. Different energy investment in growth between males and females of this species A.qatari

The most conspicuous feature of the *Alpheus* species is the snapping claw or chela (Schmitz and Herberholz (1998)). Male organisms have a larger snapping claw than females. This large chela produces a unique sound and is used for capturing and handling preys, as well as for agonistic interactions and digging. Our results revealed that *A. qatari* displayed a sexual dimorphism of the snapping claw with males having larger claws than females and showing a steeper allometric claw-body relation than females.

Fulton's condition factor is widely used in fisheries studies and this factor is calculated from the relationship between the organism's weight and its length (Nash et al, 2006). The Condition factor (CF) is useful in expressing the wellbeing of the shrimp. It differs with sex, maturity stage, and time of the year. The differences observed in the condition factor between males and females could be due to the presence of gravid females or due to higher weight of the female's gonads, which are lacking in their male counterparts. The Condition factor of the *Alpheus qatari* was calculated for each demography category and each sampling month from July 2021 to May 2022. The highest condition factor was recorded in summer, especially in August 2021.. Male chela was heavier than the female ones at a similar size. These differences could explain that they are capable to reproduce or mate several times per year and protect females in their galleries.

The fullness index value in this study also showed the fullness of gut of the organisms and, according to the results, the highest gut fullness value occurred in summer-autumn and the lowest one in winter, similar to other studies (Garrido, 2008). During spring and summer, the seasonal food availability increases and enhances productivity (primary and secondary) within the coastal area, perhaps explaining the greater feeding intensity of the *Alpheus* species. In fact, in previous studies, the feeding intensity observed was lower in winter and moderate in spring and autumn (Ouakka et al, 2017). According to Nikolsky (1963), the food supply changes during the year is closely linked to these changes to a very significant degree. The energy accumulated in summer will be spent for their reproductive activity (Ouakka et al, 2017).

6 Conclusion

New species to the San Pedro River right bank, Cadiz, Spain is belonged to the Family Alpheidae and identified as *Alpheus qatari*. Specimens collected by hand picking method, from the 275m long stretch of the river bank and carried alive to the laboratory at the Institute of Marine Science of Andalusia (CSIC) for further analysis. The sampling periods covered all four seasons: Summer (June to September), Autumn (September to December), Winter (December to March), and Spring (March to May). Totally 383 organisms were collected and, total length, carapace length, weight, chelea length and width were measured. Sex of each specimen were determined. Among the collected organisms, 188 were males, 174 were females (50 non-ovigerous and 124 ovigerous). The sea ratio between male: female is 1: 0.92. its value did not differ significantly from the expected equal proportion. Carapace length of the males and females were compared and there was significantly difference between males and females, males were slightly larger than females. But there was no significant difference between ovigerous females and non-ovigerous females.

When consider about the population structure, the percentage of females increased from July -to December in 2021. The maximum percentage of females was recorded during December 2021. With regards to males, the percentage increased from July to October and gradually decreased up to December. In the case of Females with eggs, the percentage of individuals was higher in summer and in spring. Two cohorts could be identified in males and females from July to October and, from November to May of the following year.

The relationships between weight and lengths and the correlation coefficients were analyzed for all the demographic categories of *Alpheus qatari* during the 11-month study period at the San Pedro River. The significant correlation between the total weight and cephalothorax length was found to be a power function. R^2 for the regressions of the females was slightly higher than of males and growth pattern of the organisms was negatively allometric, when the increase in length of this species was accompanied by a smaller increase in weight.

Major, well developed chela of the male is very prominent feature of this family and which produce unique snapping sound.

Relative chela length of males and females were analysis. A Kruskal-wallis test was performed to determine significant difference of the relative chela length between the three demography categories. Males showed a significantly larger chela than females. Female and ovigerous females did not show significant differences among their length.

Fulton's condition factor described condition of the organisms. The Fulton´s condition factor (K index) was calculated for the males, females and ovigerous females at each month. Among the analyzed factors, Sex, Month and cephalothorax length showed significant differences, and there was no evidence for a two and three-way significant interactions. The highest Fulton's condition factor (K index) was recorded for the summer months July and August 2021, both in males and in females. Males showed higher values than females, suggesting a better condition than all others.

The Hureau Index was calculated for the males, females and ovigerous females for each month. Among the analyzed factors, Sex and Month showed significant differences. Overall, the fullness index (FI) of the stomach showed the highest values in autumn and, among the different demographic categories, the highest values occurred in females.

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