Biometry of the red swamp crayfish *Procambarus clarkii* (Girard, 1852) in Ramsar sites in Morocco between 2016 and 2018

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Abstract

A population of red swamp crayfish *Procambarus clarkii*, (Girard, 1852) was studied in four wetland classified Ramsar sites in Morocco which are Merja Fouwarat, Merja Zerga, Rmel plateau and Lower Loukous. The aim of this study was to collect the first information on the size and weight of *Procambarus clarkii* for the reasons of its invasion success in the wetlands Ramsar site in Morocco. Also, comparing the size and weight of the four crayfish populations can provide valuable information on the evolution of this species in the study area, and on the degree of adaptation and survival of this population in Morocco. A total of 10,007 specimens were collected from January 2016 to December 2018, and analysed for sex, weight and total length. The study was carried out monthly and for three periods per day in order to evaluate the breeding period and identify the peak movement period of this species. The maximum weight was noted at the low Loukous wetland in 2016 which is 18 g; in the same area the maximum size has been determined which is 17.5 cm. The Merja Fouwarat and Lower Loukous wetland share the same minimum size, which is 6 cm. The minimum weight in our sample (6.3 g) was found in the Lower Loukous Wetland in 2017. The results of this study showed also that these four wetlands show a favorable habitat for the development of the red crayfish population, and the differences between the total length (TL), weight (W) and sex are statistically significant.

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Introduction

Procambarus clarkii (Girard, 1852) is an invasive freshwater species with a high ecological plasticity, which allows it to colonise different types of environments,[1] and consequently an extraordinary production.[2] This species is considered to be the most invasive of all crayfish species,[3] and its spread outside its natural habitat is an ecological mistake.[4]

Red crayfish, originating from the southern United States and northern Mexico,[5] has been introduced worldwide [6] due to the growth of international trade, which has making it a prime object of professional fishing.[7,8] The red swamp crayfish (*Procambarus clarkii*) has recently been introduced in Morocco, including the Merja Fouwarat wetland, the Merja Zerga wetland, the Rmel plateau, the Lower Loukous wetland and wells that are located in the northwestern part of the country.[9]

Comparing the size and weight of the four crayfish populations can provide valuable information on the evolution of this species in the study area for several reasons: i) adaptation to the environment: the size and weight of crayfish can be indicators of their adaptation to the specific environmental conditions of each population. For example, larger crayfish may be better adapted to habitats with more resources, while smaller crayfish could thrive in more restricted environments;[10] ii) competition and predation: size can influence crayfish's ability to defend itself against predators or to compete for resources with native species. By comparing sizes, it is possible to understand how competition between individuals or species can affect survival and reproduction, which is essential for evolution;[11] iii) reproduction: size and weight can also play a role in reproduc-

tive success. For example, larger females may produce more eggs, which could influence population dynamics. Thus, the study of these characteristics can give a better understanding of the reproductive strategies of the species; [12] iv) genetics and diversity: differences in size and weight between populations can also reflect genetic variations. By analysing these traits, we can obtain clues about genetic diversity and evolutionary mechanisms, such as natural selection;[13,14] v) response to environmental changes: variation of size and weight over time and between the study areas gives deep information about how crayfish react to environmental changes, such as pollution or habitat modifications, which is crucial for understanding their evolution.[15]

In short, although size and weight are simple measures, they can reveal complex information about the adaptation, survival and evolution of crayfish in their environment. Thus, the main objective of this study is to ascertain whether the differences in size and weight between the sexes might influence the invasiveness of the red swamp crayfish. One hypothesis is that males and females may possess divergent reproductive strategies or ecological requirements that influence their success in a novel environment. Furthermore, it is important to explore these aspects in future research, as it could enrich our understanding of the invasion dynamics and ecological impacts of this species.

Materials and Methods

Study area

The Kingdom of Morocco, situated at the extreme northwestern tip of Africa, is bordered by the Mediterranean Sea to the north, the Atlantic Ocean to the west and the Sahara to the south and east.[16] The study was carried out in Morocco at the level of four wetlands classified as Ramsar sites (Wetlands of international importance for their conservation).

Merja Zerga is a large coastal lagoon located in northwest Morocco (34°51'N 006°16'W), it has an area of 7,300 ha, and was classified as a Ramsar site since 1980.[17]

The Rmel Plateau is also located in north-west of Morocco (35°02'N 006°14'W), near the town of Larache city. It is a set of three freshwater coastal lakes, sandy beach and adjacent coastline, inter-dune marshes and irrigated areas. Its area is 1,300 ha and this site was classified as a Ramsar site in 2005.[18]

The Lower Loukous Complex is located in the north of Morocco, 35°07'N 006°00'W; it is a unique wetland complex on the Atlantic coast of Morocco, comprising estuarine waters, shallow marine waters, salt steppes, freshwater marshes and floodplains, in addition to rice paddies in drained areas and a number of abandoned salt works on an area of 3,600 ha. It was classified as a Ramsar site in 2005.[19]

Merja Fouwarat, located at 34°14'N 06°31'W, is a shallow swamp that is believed to be a remnant of the vast wetland complex that once covered the Gharb Plain in northwest Morocco. The site covers an area of 502 hectares and was recently designated a Ramsar site in 2018.[20,21]

Sampling

In choosing the sampling stations, we drew on our experience in the field, particularly in the context of this study. We know that crayfish prefer to avoid areas where the water is very deep and that they do not like hard, rigid ground. This is why our selection of random stations was based on several criteria: the abundant presence of nutrients, a soil suitable for crayfish mobility, and a water depth favorable to the life of this species.

In addition, we felt that it was sufficient to choose two 50 m² sampling stations per site, given the relatively small surface area of these Ramsar sites and the anthropogenic impact to which these areas are subject. We therefore opted for pristine stations, far removed from this anthropogenic pressure, in order to best represent the crayfish's natural habitat. This approach allowed us to obtain more reliable and relevant data for our study.

The sampling was conducted in each wetland over an area of 100 m^2 for each wetland with an altitudinal gradient of 50-100 m, using a Catch Per Unit Effort (CPUE) of 60 min[22] and a 3 mm mesh net. A total of 10,007 individuals of *Procambarus clarkii* were caught at 4 stations, with two samples taken at each station covering 50 m^2 each. In total, 100 m^2 were sampled at each station. The population of *Procambarus clarkii* was captured on a monthly basis, from January 2016 to December 2018, for three periods during the day at 7 a.m., 2 p.m. and 7 p.m. to gather data on their activity patterns.

Caught crayfish were preserved in a 5% Formalin solution in the laboratory. We identified the sex, measured the Total Length (TL) (from the tip of the rostrum to the rear edge of the telson), and evaluated the body Weight (W) of each specimen. We used a digital caliper with an accuracy of 1 mm to measure TL, and an electronic balance (5161-LED, Rabat, Morocco) with an accuracy of 0.1 g to measure body weight.

The males were identified by the presence of hooks,[5] and mature females were distinguished by the presence of dark yellow, orange, brown ovaries. Body color is also a distinguishing factor between adults and juveniles.[23,24]

Statistical analysis

Descriptive quantitative analysis was used to examine the laboratory data. Mean values (M) and Standard Deviation (SD) were calculated, and the differences between the morphometric variables were tested using the ANOVA test.

The null hypothesis was rejected at a minimum level of significance of $P<0.05$. The statistical analyses were conducted using R software.[25]

A multivariate test of differences between groups was performed using Discriminant Function Analysis (DFA). In our study, DFA was used to test the differences between colonized habitats by *Procambarus clarkii* in four sites, based on the mean values of the quantitative variables.

Results

Between January 2016 and December 2018, a total of 10,007 individuals of *Procambarus clarkii* were collected from four Ramsar wetland sites: Merja Fouwarat, the Lower Loukous Complex, Merja Zerga, and Rmel Plateau.

Upon examining the total number of crayfish caught annually in these sites, a decrease in crayfish population in 2017 and 2018 when compared to the number caught in 2016 in the Merja Zerga (Figure 1) and Rmel Plateau wetlands was observed (Figure 2). As evidenced in Table 1, the documented sex ratio indicates a decline in the number of males and females from 2016 to 2018.

In Merja Fouwarat, the crayfish population decreased in 2017 compared to 2016 and remained constant in 2018 (Figure 3). In the Lower Loukous area the crayfish population slightly increased since 2016 (Figure 4).

The sex ratio of *Procambarus clarki* catches varies over time

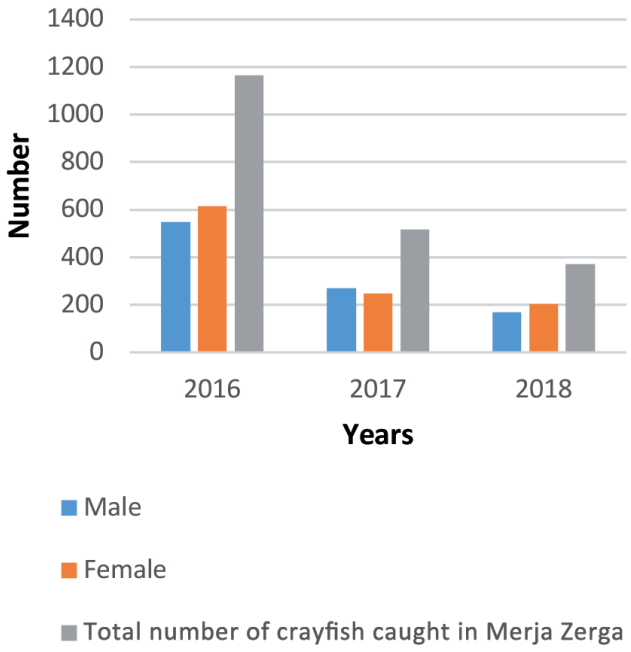

Figure 1. Total number of *Procambarus clarkii* captured (male and female) in 2016, 2017 and 2018, Merja Zerga, Morocco.

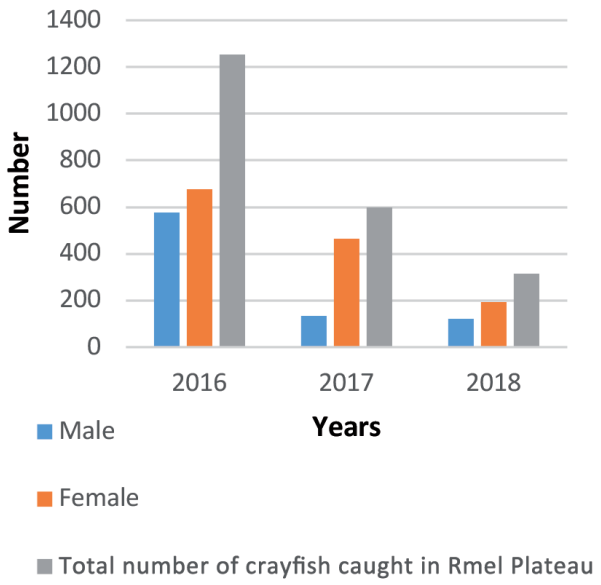

Figure 2. Total number of *Procambarus clarkii* captured (male and female) in 2016, 2017 and 2018, Rmel Plateau, Morocco.

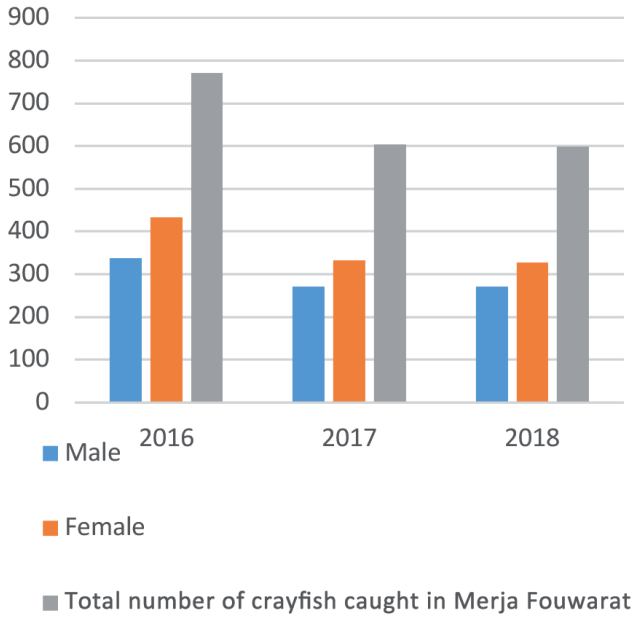

Figure 3. Total number of *Procambarus clarkii* captured (male and female) in 2016, 2017 and 2018, Merja Fouwarat, Morocco.

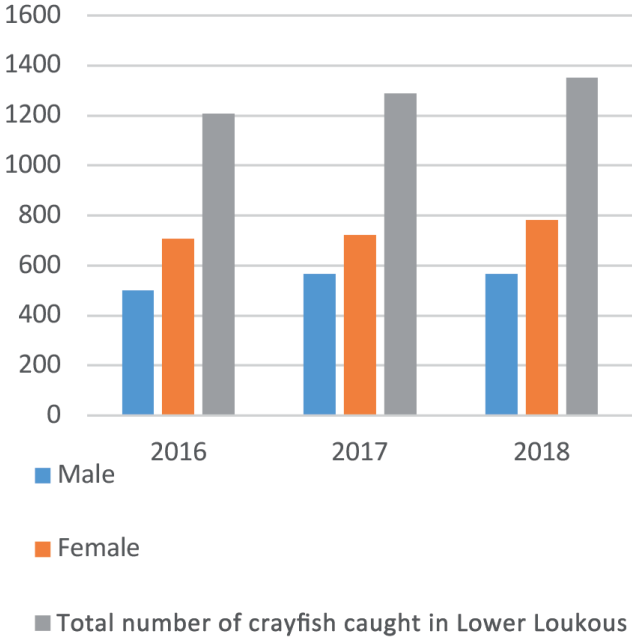

Figure 4. Total number of *Procambarus clarkii* captured (male and female) in 2016, 2017 and 2018, Lower Loukous, Morocco.

Table 1. Total number of males and females caught in Ramsar sites, Morocco.

	Male	Female	Sex ratio
2016	1964	2432	0.8
2017	1244	1763	0.7
2018	1127	1507	0.74

(Table 1). Over the entire period 2016 to 2018 the sex ratio slightly favored females, with 57% females and 43% males. The sex ratio for the entire population over the three year period was 0.76.

The monthly catches over three years at the four Ramsar sites indicate a variation in abundance. Crayfish are generally more abundant in spring and summer, but less abundant in winter and autumn in Merja Fouwarat (Figure 5a), Lower Loukous (Figure 5b), and Rmel Plateau (Figure 5c).

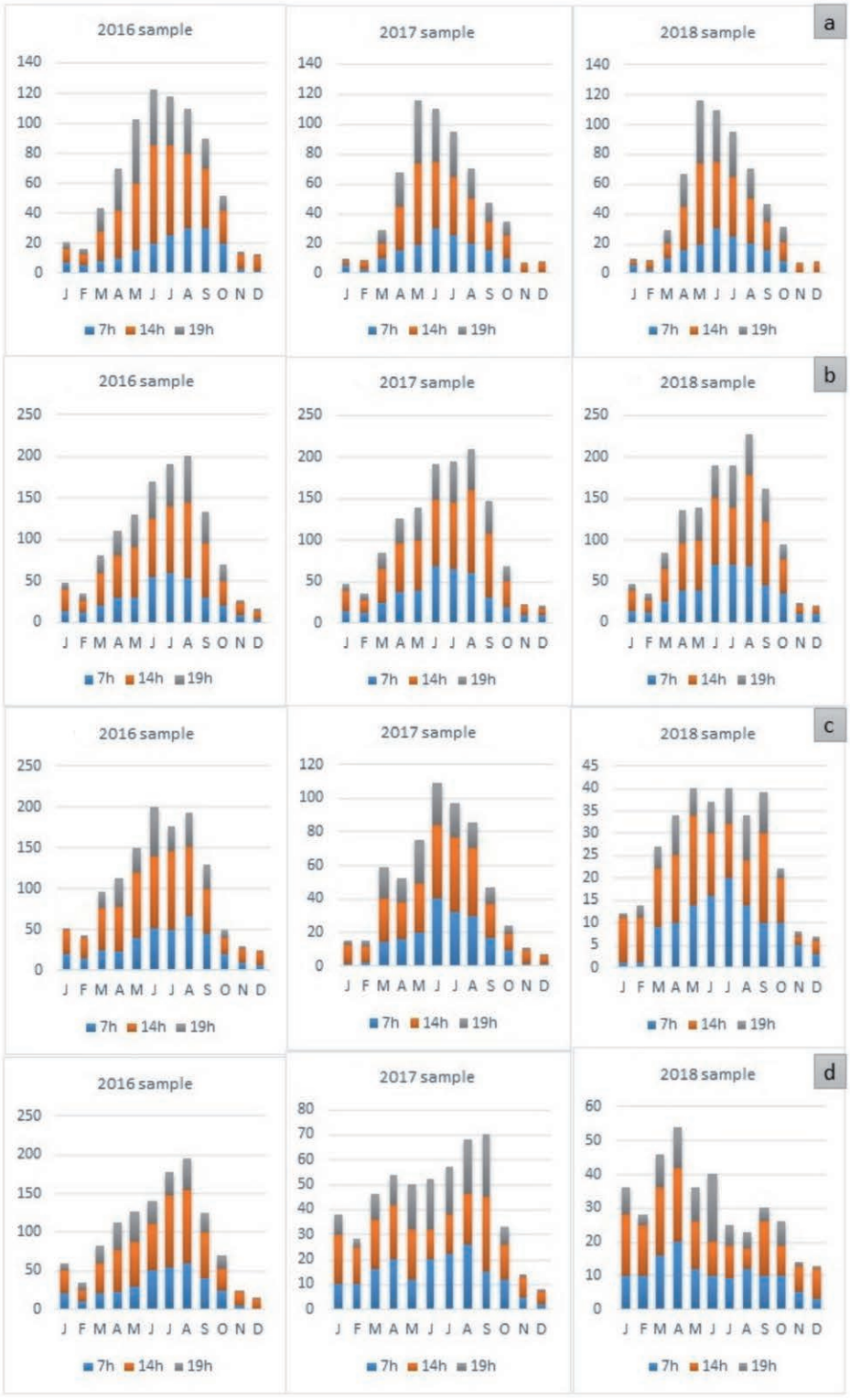

Figure 5. Number of *Procambarus clarkii* caught per month and per hour of capture, a) Merja Fouwarat, b) Lower Loukous, c) Rmel Plateau, and d) Merja Zerga 2016, 2017 and 2018, Morocco.

Crayfish were found to be abundant in all seasons at the Merja Zerga wetland during 2017 and 2018, with the exception November and December (Figure 5d).

The results of the hourly catch indicate that the number of crayfish during the 60-minute period starting at 14:00 is higher compared to the catches time at 7:00 and 19:00 (Table 2).

Based on these results, it appears that crayfish are more active in the water during the 14-hour period. Thus, this increased activity may be due to various factors such as the water temperature obeying optimal at that time, crayfish searching for prey, or a decrease in the fishing activity.at that time. It is important to note that these are objective observations and not subjective evaluations.

The average length (in cm) and average weight (in g) at the four Ramsar sites were measured over a period of three years (Table 3).

The population dynamics of crayfish caught in the four Ramsar sites indicate a pre-breeding stage, with a higher number of large animals (average body length of 10.47 cm and average individual weight of 11.35 g) than small animals.

The results of the descriptive statistics of two morphometric variables measured for the entire sample are summarized in Table 4.

The smallest specimen found in the net was 6 cm total length (male) and was present in the Merja Fouwarat wetland in 2017 as well as in the Lower Loukous in 2016. The longest specimen was a female with 17.5 cm in total length, and was found in the Lower Loukous in 2016. The maximum weight recorded was 18 g (female) in the Lower Loukous in 2016, while the minimum weight of our sample was 6.3 g (male) found in the same area in 2017.

The ANOVA test finding show statistically significant differences ($p < 0.001$) in total length (TL) and weight (W) between sexes at the four Ramsar sites (Table 5).

The analysis of variance shows a significant effect for the three variables investigated, namely station, year and gender. However,

Table 2. Statistical description of the entire sample in Merja Fouwarat, Merja Zerga, Lower Loukous, and Rmel Plateau sites, 2016, 2017 and 2018, Morocco.

	7H	14H	19H
Sum	2946	4582	2511
Min	1	2	1
Max	70	110	60
M±SD	20.46±17.15	31.82±25.93	17.44±15.42
Variance	294.138	672.204	237.800

Min, minimum; Max, maximum; M, mean; SD, standard deviation.

Table 3. Average size and average weight of the entire sample at the four Ramsar sites.

	Weight (g)		Length (cm)	
	Average	SD	Average	SD
Merja Fouwarat	11.81	1.55	10.79	1.60
Lower Loukous	11.24	1.77	10.5	1.77
Merja Zerga	11.68	1.42	10.66	1.45
Rmel Plateau	11.63	1.64	10.57	1.65

SD, standard deviation.

Table 4. Statistical description of the entire sample at the four Ramsar sites.

	Females				Males			
	Max	Min	Mean	SD	Max	Min	Mean	SD
TL (cm)	17.5	7	10.602	1.708	16	6	10.311	1.611
W (g)	18	7	11.638	1.683	17.3	6.3	11.392	1.606

TL, total length; W, weight: SD, standard deviation.

Table 5. Analysis of variance of length (cm) and weight (g) of *Procambarus clarkii* in the four studied sites: Merja Fouwarat, Merja Zerga, Lower Loukous, and Rmel Plateau sites in 2016, 2017 and 2018, Morocco.

Parameters	Dependent variable: weight in (g)			Dependent variable: size in (cm)		
Source of variation	DF	Mean square	F	P	Mean square	F
Sites	3	71.788	34.871	.000	131.224	65.160***
Years	2	284.924	138.402	.000	289.200	143.604***
Sexes	1	1605.493	779.866	.000	1702.997	845.637***
Sites - year	6	215.401	104.631	.000	209.500	104.029***
Sites - sexes	3	244.916	118.968	.000	249.080	123.683***
Year - sexes	2	27.460	13.339	.000	33.921	16.844***
Sites - year - sexes	6	31.771	15.433	.000	27.539	13.675***

***, Significant variation at $p<0.001$; DF, degrees of freedom; F, F value.

the gender effect (F= 845.637) is clearly more discriminating than the other two effects (station and year). The interaction station-year-sex is moderately significant (F=13.675) indicating that the three effects are independent and vary separately.

Through discriminant function analysis (DFA), we compared the morphological difference of *Procambarus clarkii* in four through its size, total length and sex over three years Ramsar sites.

Discriminant function analysis (DFA) shows that there is not a remarkable difference between the four sites according to the measured parameters (Figure 6).

Discussion

The low catch rate observed in the Merja Fouwarat compared to Merja Zerga, Rmel Plateau and Lower Loukous can be explained by four scenarios. The first scenario is that colonization by *Procambarus clarkii* is probably limited considering that this area is an urban wetland and geographically bounded by cities.[26] In the second scenario, it is possible that this habitat supports a greater number of birds predatory of this species.[27,28]

The third scenario is less likely because this species is known by its high ecological plasticity,[29,30] meaning that the characteristics of its habitat only have a moderate influence on its distribution.[31] As the fourth scenario, it is highly unlikely that the minimum water temperatures were too low to induce the activity of *Procambarus clarkii*, as this area belongs to the sub humid to temperate winter stage.[32]

The decline in the crayfish population caught in 2017 and 2018, observed in Merja Zerga and Rmel Plateau, can be attributed to the over export of this species that began in 2017 in these two areas.

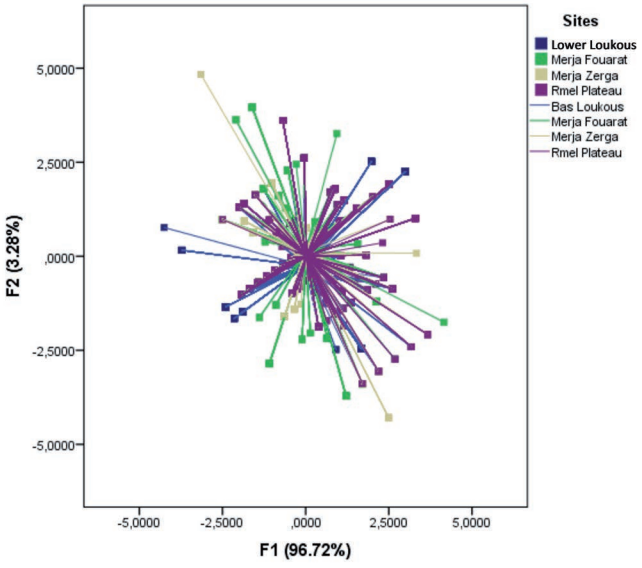

Figure 6. Discriminant analysis of the four study sites based on the means of length and weight traits of *Procambarus clarkii* in 2016, 2017 and 2018.

In the Merja Zerga study of 2015, the proportion of males was significantly higher than that of females.[33] This result was similar to our study in 2017, and contrary to other areas where the number of females was higher than males. This difference may be explained by differences in the reproductive behavior of *P. clarkii.*[29]

The sex ratio of our entire sample in Morocco was lower than the ratio found in Lake Trasimeno in Italy which was 1.15:1,[34] and in the Merja Zerga area of Morocco in 2015 which was 1.29:1.[33]

The sex ratio in our case is 0.76:1 close to 1:1 which is consistent with other ratios.[11,35,36]

The present study revealed a significant geographical and seasonal variation in the sex ratio of the *Procambarus clarkii* population. These findings align with those previously documented in southern Tuscany.[37]

The abundance of crayfish in most areas decreased during autumn and winter, as explained by Bravo *et al.* and Frutiger *et al.*[38,39] This decrease is likely due to the fact that most mature females become ovigerous during this season and remain in their borrowings, suggesting that reproduction probably takes place in autumn. In other studies, females were less abundant in months when ovarian egg maturation was maximal such as October, November and January.[34,40] *Procambarus clarkii* individuals have two mating periods, one in spring and one in summer.[41]

Procambarus clarkii produces at least two generations annually in its natural habitat[40] and in parts of its invasive range such as in southern Europe.[42-44]

The number of *Procambarus clarkii* caught per season and per hour is mainly influenced by water regime and water temperature.[29]

The population of *Procambarus clarkii* consisted mainly of individuals ranging in size from 6 to 17.5 cm. This size range is larger than that recorded in north-western France, which ranged from 1 cm to 10 cm in the Brière Park,[45] and in northwest Morocco which ranged from 4.5 cm to 12.1 cm in the Merja Zerga and Canal Nador in 2015.[33] Also, it is larger than the maximum size recorded in Lake Trasimeno, Italy which was 15 cm.[34]

In the National Park of Doñana, Spain the largest crayfish caught was 10.5 cm,[38] while in Portugal São Miguel *Procambarus clarkii* did not exceed 9.8 cm as a maximum size.[41]

The smallest specimen in the sample measuring 6 cm is larger than the smallest specimen caught in Italy, which measured 4 cm.[46]

The maximum weight recorded in the four Ramsar sites in Morocco is only 18 g, which is significantly lower than the weight of a specimen collected in Lake Trasimeno, which had a weight greater than 76 g,[34] and the maximum weight noted in the Nador channel in 2015, which is 62.74 g[33].

The mean size of our sample is 10.47 cm, which is greater than that observed in the specimens of the marshes of Brière, in France (8.5 cm).[44] However, the average weight of the sample set is much lower (11.35 g) compared to the average weight of the individuals recorded in Lake Trasimeno, in Italy, which was 22.2 g.[34]

There were significant differences in the number of catches of *P. clarkii i*n the four zones. These results demonstrate that the occurrence of the species is not affected by variations in period and habitat, confirming its high adaptability to different habitats.[29,31,47]

Conclusions

The reasons for the variation of crayfish density between sites are unclear; it is possible that the habitat type plays a role in limiting or increasing density. In the relatively fresh, humid and temperate climate of northern and northwestern Morocco, the crayfish population may experience a prolonged phase of development and spread.

Further research is necessary to investigate the dynamics in Morocco.

The study found no significant difference in the relationship between male and female weight and body length across the various studied sites, indicating the strong adaptation and acclimatization of *Procambarus clarkii* in Morocco. To slow the spread of this species, it is necessary to implement stricter control strategies, increase education, and raise awareness.

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