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Impact of Pollution on the Biometry of the Harpacticoid Copepod *Euterpina acutifrons* from the Oum Er Rbia Estuary (Atlantic Coast, Morocco)

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ABSTRACT

This study evaluated the effects of the proximity of a domestic discharge on the biometric characteristics of the harpacticoid copepod *Euterpina acutifrons* in the Oum Er Rbia estuary (Atlantic coast, Morocco). Using comparative sampling between two locations - one near the discharge, potentially facing high pollution levels, and a less affected one - we measured six biometric traits of adult males and females under a light microscope. The Mann-Whitney test was utilized to analyze biometric variations. The results showed that at the discharge location (station S1), female total lengths ranged from 531.60 to 675.16µm (average 585.87± 54.70), and male lengths ranged from 451.20 to 578.94µm (average 502.45± 52.64). In contrast, at station S2, further from the discharge, female lengths varied from 553.46 to 634.46µm (average 596.78± 29.79), and male lengths varied from 472.97 to 641.54µm (average 578.83± 71.57). These measurements indicated a significant reduction in all mean biometric values, except head width, in males close to the discharge. No significant variations were found in females. A stronger positive correlation between biometric parameters was observed in males than in females. These findings are crucial for estuary conservation and management, highlighting the importance of monitoring pollution's effects on planktonic populations.

INTRODUCTION

Estuaries are ecosystems that occupy an intermediate situation between continental and marine waters. They are areas of a complex mixture of substances of various origins: (1) autochthonous, produced by internal biological activities of the environment, or (2) allochthonous, such as soil leaching and substances of anthropogenic origin **(Williams, 1981)**.

The Oum Er Rbia estuary is one of the main Moroccan estuaries suffering from the degradation of the quality of its waters under the effect of domestic effluents, leaching, and runoff from agricultural land **(ABHOER, 2020)**. This degradation can have an influence on the living beings of the environment such as the copepods, which represent a

very important part of the plankton and constitute an essential link of the aquatic food chains **(Trégouboff & Rose, 1957; Mollo & Noury, 2013)**. The copepods are Entomostracan crustaceans of small size (not exceeding a few millimeters), with sexual reproduction and which can live in marine, brackish, and freshwaters **(Rose, 1933; Trégouboff & Rose, 1957; Durand & Lévêque, 1980)**.

Euterpina acutifrons (Dana, 1848) is a free benthic harpacticoid copepod with pronounced sexual dimorphism **(Rose, 1933; Durand & Lévêque, 1980)** and with a very wide distribution in the seas and oceans (such as the North Sea, Atlantic, English Channel, Mediterranean, Adriatic, Red Sea, Pacific, and the Indian Ocean) **(Rose, 1933; Razouls *et al.*, 2005)**.

Studies on *E. acutifrons* are numerous and diverse, covering various aspects of its biology and ecology, including sexual dimorphism in males **(HAQ, 1965; Stancyk & Moreira, 1988)**, resistance to temperature and salinity variations **(Tundisi & Tundisi, 1968; Moreira *et al.*, 1982)**, respiration rates **(Coull & Vernberg, 1970; Moreira & Yamashita, 1973; Vernberg & Moreira, 1974)**, growth and development rates **(Nassogne, 1970; Zurlini *et al.*, 1978; Sciandra, 1986; Carlotti & Sciandra, 1989)**, fecundity **(Nassogne, 1970; Zurlini *et al.*, 1978; Guisande *et al.*, 1996; Hopcroft & Roff, 1996)**, seasonal fluctuations in abundance **(Viñas & Gaudy, 1996; Ara, 2001; Nandy & Mandal, 2020)**, feeding rates **(Sautour & Castel, 1998; Jasmine *et al.*, 2016; Tarangkoon *et al.*, 2017)**, and population structure **(Da Costa *et al.*, 2011; Pinheiro *et al.*, 2013; Nandy & Mandal, 2020; Guerreiro *et al.*, 2021)**. However, few studies have been devoted to the biometry of the species **(Rose, 1933; Viñas & Gaudy, 1996; Ara, 2001; Conway *et al.*, 2003)**.

This study aimed to collect quantitative data on the biometry of *E. acutifrons* in the Oum Er Rbia estuary and to examine the impact of proximity or distance from a potential source of pollution on the biometry of this species. In addition, it was organized to explore the interaction of the environmental variables with these biometric parameters.

MATERIALS AND METHODS

1. Description of the study area

The Oum Er Rbia estuary (33°17'N and 8°20'W) is located on the Moroccan Atlantic coast about 16km northeast of the city of El Jadida. On its left bank is the city of Azemmour. Sampling was conducted in summer 2018 at two stations in the estuary (Fig. 1):

- Discharge station (S1): located about 1.1km downstream from the first bridge, 150m upstream from the main domestic discharge of Azemmour City, and about 2.5km upstream from the river mouth with a depth of 2.30m at high tide.

- Comparison station (S2): located about 900m from S1, 2km downstream of the first bridge, 750m downstream of the discharge, and 1.6km upstream of the river mouth with a depth of 6m at high tide.

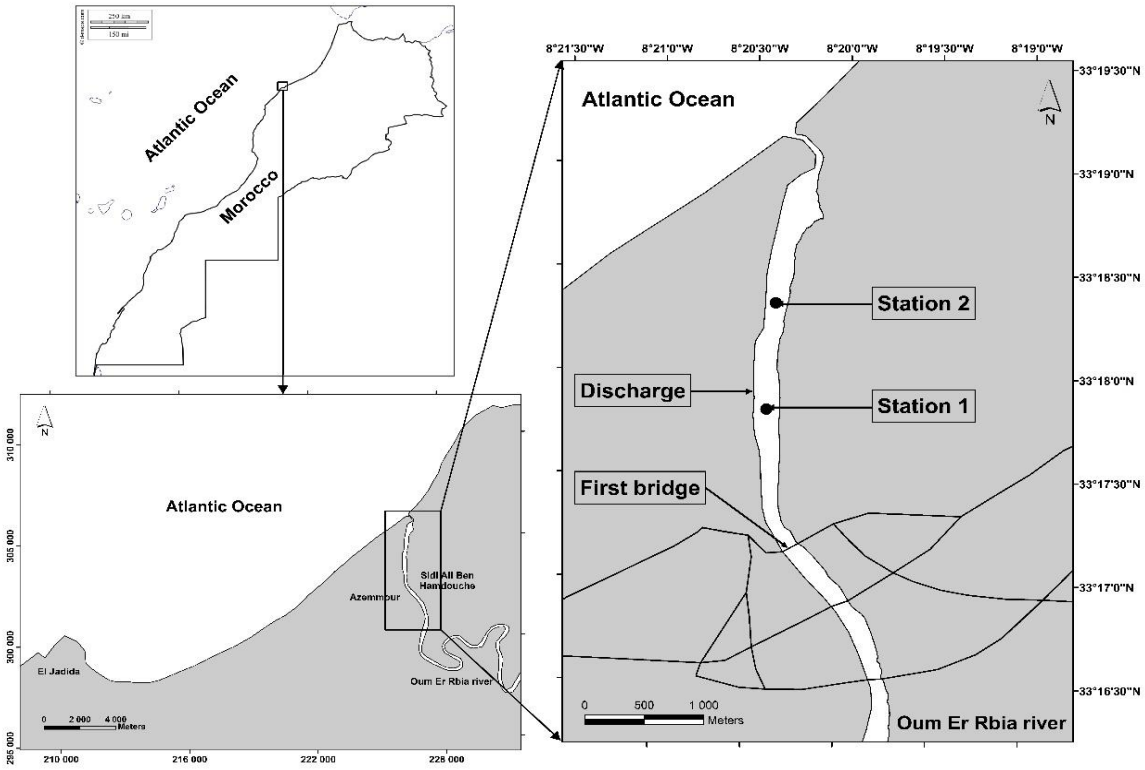

Fig. 1. Location of sampling stations in the Oum Er Rbia estuary

2. Sampling and sample processing

The samples have been carried out with a Juday-type plankton net, made of 80µm mesh blutter nylon. The captured plankton was immediately fixed and preserved in 5% formulated seawater.

In parallel to planktonic fishing, physicochemical parameters were measured *in situ*: temperature, salinity, conductivity, and pH of the water using a WTW 340i multi-parameter analyzer. Other parameters were measured in the laboratory,viz. dissolved oxygen (Winkler method) and chlorophyll-*a* **(Aminot & Chaussepied, 1983)**.

3. Data acquisition and analysis

The sagittal plane lengths of the body parts (cephalosome, pedigerous somites, urosome, caudal rami, and total length) as well as the cephalosome width in the dorsal view of 40 adult specimens of *Euterpina acutifrons* (20 males and 20 females distributed over the two stations) were measured under a Motic BA210 microscope at x40

magnification. The microscope was equipped with a Moticam 3: 3.0 MP camera connected to a computer running Microsoft Windows 10 Professional. The Motic Image Plus 2.0 ML software was used to acquire the images in jpeg (jpg) format and to take the biometric measurements.

Graphical representations, statistical tests, and correlations between the measurements of the body parts of *E. acutifrons* for the two stations were carried out by the packages ggplot2 **(Wickham, 2016)**, prettyR **(Lemon & Grosjean, 2019)**, corrplot **(Wei & Simko, 2021)**, ggpubr **(Kassambara, 2023)**, and the freeware R **(R Core Team, 2023)**.

RESULTS

1. Physicochemical parameters

The different values of the physicochemical parameters measured *in situ* and in the laboratory are reported in Table (1).

Table 1. Physicochemical parameters of the two study stations

Measured parameter	S1	S2
Water temperature (ºC)	25.8	23.0
Salinity (‰)	34.5	35.1
Conductivity ($mS.cm^{-1}$)	52.3	53.2
pH	7.36	7.62
Dissolved oxygen ($mg.l^{-1}$)	3.59	7.53
Chlorophyll-*a* ($mg.m^{-3}$)	0.54	0.27

At S1, water temperature is higher (25.8 ºC ºC versus 23.0 ºC ºC at S2), which may influence oxygen solubility. Salinity is slightly higher at S2 (35.1‰ versus 34.5‰ at S1), and conductivity follows this trend, suggesting a higher ion concentration at S2. The pH at S2 is slightly more alkaline (7.62 compared to 7.36 at S1). Dissolved oxygen is clearly more abundant at S2 ($7.53mg.l^{-1}$ compared with $3.59mg.l^{-1}$ at S1), indicating a better water quality or less oxygen-consuming biological activity. Finally, the chlorophyll-*a* concentration was lower at S2 ($0.27mg.m^{-3}$ compared with $0.54mg.m^{-3}$ at S1), potentially reflecting a reduced primary productivity.

2. Biometry of *Euterpina acutifrons*

The distributions of the different measured parameters of males and females in the two sampling stations are shown in Fig. (2).

Females of station S1 have a cephalosome length ranging from 203.29 to 295.02µm (246.78± 39.54); a pedigerous somites length ranging from 184.38 to 204.72µm (194.46± 6.16); a urosome length ranging from 126.71 to 176.35µm (144.63± 18.48); caudal rami length ranging from 20.08 to 23.97µm (22.12± 1.36); a total length ranging from 531.60

to 675.16µm (585.87±54.70), and a cephalosome width ranging from 138.97 to 189.10µm (163.04± 16. 38). Whereas at station S2, female's cephalosome length ranged from 250.90 to 279.19µm (265.93± 11.14); pedigerous somites length varied from 154.22 to 215.80µm (185.90±2 2.00); urosome length ranged from 137. 94 to 151.67µm (144.95± 4.82); caudal rami length ranged from 21.32 to 25.70µm (23.45± 1.45); a total length ranged from 553.46 to 634.46µm (596.78± 29.79), and cephalosome width varied from 154.40 to 185.58µm (165.13± 10.90).

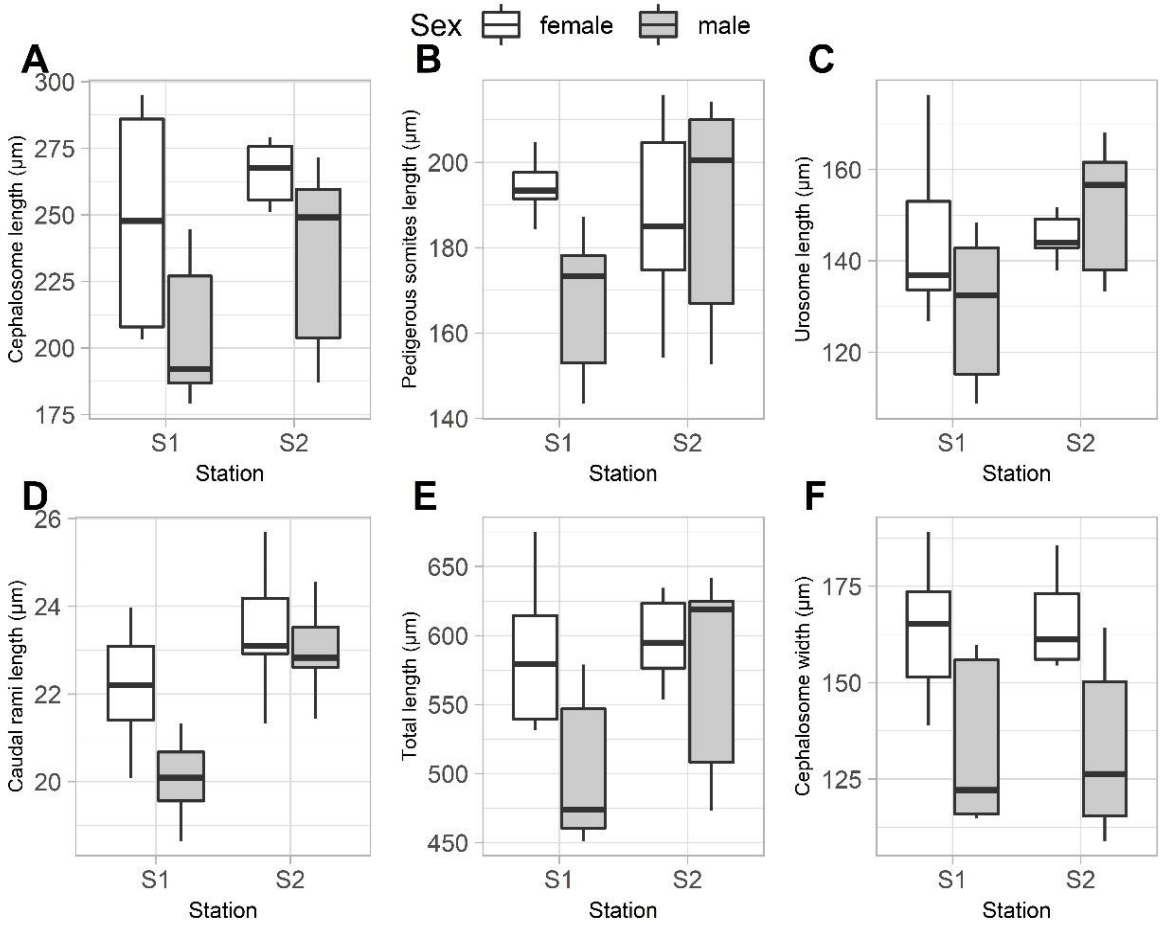

Fig. 2. Distributions of the different biometric parameters for males and females in the two stations (A = Cephalosome length, B = Pedigerous somites length, C = Urosome length, D = Caudal rami length, E = Total body length, F = Cephalosome width)

Males in station S1 have a cephalosome length ranging from 178.97 to 244.68µm (205.82± 26.35); a pedigerous somites length ranging from 143.37 to 187.29µm (167.00± 16.10); a urosome length ranging from 108.66 to 148.47µm (129.63± 15.49); caudal rami length ranging from 18.65 to 21.32µm (20.04± 0.83); total length ranging from 451.20 to 578.94µm (502.45± 52.64), and cephalosome width ranging from 114.72 to 159.76µm (133.60± 20. 61); whereas for station S2, male's cephalosome length ranged from 187.10

to 271.60µm (235.70± 33.76); pedigerous somites length varied from 152.57 to 214.26µm (190.86± 25.49); urosome length ranged from 133. 30 to 168.13µm (152.28± 13.96); caudal rami length varied from 21.43 to 24.57µm (22.95± 0.93); total length from ranged 472.97 to 641.54µm (578.83± 71.57), and cephalosome width ranged from 108.97 to 164.33µm (131.93± 20.23).

3. Statistical analysis

The Mann-Whitney test (Wilcoxon rank sum exact test) was used to compare the biometric characteristics of the specimens and determine if they are significantly different at the two stations. The comparison was made for all specimens (including males and females) (Table 2) and for specimens of each sex (Table 3).

Table 2. Mann-Whitney test results for specimens from each station

Biometric characteristic	Wilcoxon rank sum exact test			
	Mean (µm)		W	*P*-value
	S1	S2		
Cephalosome length	226.2985	250.8120	123	**0.03752**
Pedigerous somites length	180.7305	188.3800	147	0.1572
Urosome length	137.1325	148.6135	32	0.1903
Caudal rami length	21.0785	23.2005	28	0.1051
Total length	544.1615	587.8055	105	**0.009484**
Cephalosome width	148.3185	148.5310	209	0.8201

Statistically significant differences are shown in bold (P<0.05).

Comparing the biometric characteristics for all the specimens at the two stations, it was observed that only the cephalosome length and the total length have significantly different distributions with low values at station S1 compared to the other station (Wilcoxon rank sum exact test, P< 0.05).

Table 3. Mann-Whitney test results for specimens of each sex at both stations

Biometric characteristic	Wilcoxon rank sum exact test							
	Females				Males			
	Mean (µm)		W	*P*-value	Mean (µm)		W	*P*-value
	S1	S2			S1	S2		
Cephalosome length	246.777	265.928	40	0.4813	205.820	235.696	20	**0.02323**
Pedigerous somites length	194.462	185.904	68	0.1903	166.999	190.856	20	**0.02323**
Urosome length	144.631	144.948	32	0.1903	129.634	152.279	12	**0.002879**
Caudal rami length	22.118	23.446	28	0.1051	20.039	22.955	0	**1.083e-05**
Total length	585.87	596.78	39	0.4359	502.453	578.831	13	**0.003886**
Cephalosome width	163.040	165.132	52	0.9118	133.597	131.930	57	0.6305

Statistically significant differences are shown in Bold (P<0.05).

Comparing the biometric characteristics for each sex at the two stations, it can be seen that only the males reveal significantly different distributions for all the measured characteristics, except for the cephalosome width, with lower values at station S1, while for the females, the biometric values reveal practically no significant differences between the two stations (Wilcoxon rank sum exact test, $P< 0.05$).

The correlations (Spearman's rank correlation rho) between the measured biometric characteristics as well as the *P*-value of the significance test were calculated. The correlation matrix is displayed by a correlogram in Fig. (3).

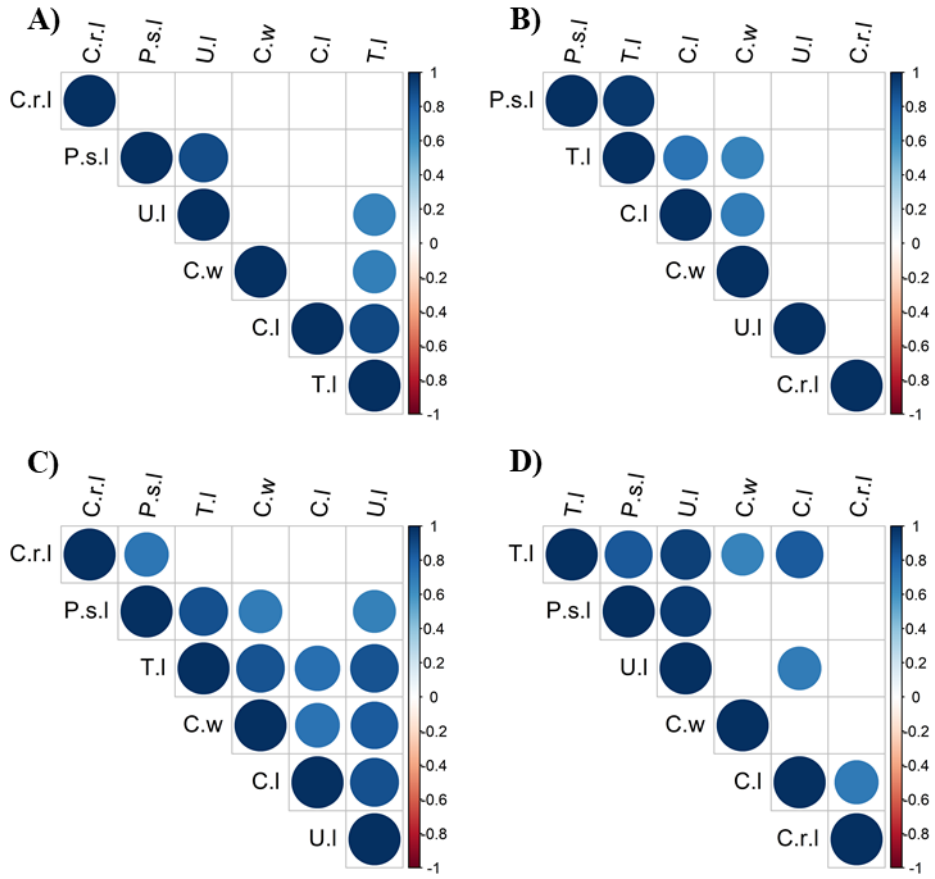

Fig. 3. Graphical representation of the correlation matrix (correlogram). **A:** Females from S1. **B:** Females of S2. **C:** Males from S1. **D:** Males of S2. (C.l = Cephalosome length, P.s.l = Pedigerous somites length, U.l = Urosome length, C.r.l = Caudal rami length, T.l = Total length, C.w = Cephalosome width). Significant correlations with a $P< 0.05$ are represented by circles of color intensity and size proportional to the correlation coefficients, while non-significant correlations with a $P> 0.05$ are replaced by white boxes

For females at station S1, the only existing correlations are those of the total length with urosome length and cephalosome length and width. Additionally, the pedigerous somites length is positively correlated with the urosome length. For station S2, the total

length is correlated with the pedigerous somites and cephalosome lengths, and cephalosome width. Cephalosome length and width are positively correlated with each other.

For males at station S1, all characteristics are positively correlated with each other except for caudal rami length, which is only correlated with pedigerous somites length. While, the cephalosome length is not correlated with the pedigerous somites length. At S2, except for caudal rami length, which is only correlated with cephalosome length, and cephalosome width which is not correlated with cephalosome, pedigerous somites, and urosome lengths, and cephalosome length which is not correlated with pedigerous somites length, all other characteristics are positively correlated with each other.

DISCUSSION

This study is a characterization of several biometric characteristics for males and females of *Euterpina acutifrons* as a function of proximity or distance from the discharge. The main results obtained show that females are not affected while males tend to decrease in length the closer they get to the discharge.

Measurements show that, near the discharge (station S1) the total length of females ranges from 531.60 to 675.16µm (585.87± 54.70), and the length of males varies from 451.20 to 578.94µm (502.45±5 2.64). Whereas moving away from the discharge, the (station S2) female length ranged from 553.46 to 634.46µm (596.78±29.79) and male length varied from 472.97 to 641.54µm (578.83± 71.57). These values are comparable with the biometric measurements conducted on *E. acutifrons* showing that female length ranges from 0.50 to 0.75mm, while male length fluctuates from 0.50 to 0.56mm **(Rose, 1933; Conway *et al.*, 2003)**. In the Gulf of San Matias, the length of females is between 0.42 and 0.54mm and that of males is between 0.30 and 0.38mm, while in the Gulf of Marseille, the length of females varies from 0.38 to 0.5 mm and that of males ranges from 0.265 to 0.325mm **(Viñas & Gaudy, 1996)**. **Razouls *et al.* (2005)**, in reviewing several works related to the biometry of this species, proposed a wide spectrum of body lengths that can range from 0.38 to 0.86mm for females and from 0.36 to 0.76mm for males.

These size differences in the two stations can be explained by the differences in environmental conditions, especially temperature, salinity, and chlorophyll-*a* concentration (25.8ºC, 34.5‰ of salinity, and 0.54mg.m^{-3} of chlorophyll-*a*) at S1 and (23.0 ºC, 35.1‰ of salinity, and 0.27mg.m^{-3} of chlorophyll-*a*) S2.

Our results are consistent with the observations and conclusions of several authors. In the North Sea, the size of the planktonic copepod *Temora longicornis* (Müller) is closely related to the water temperature and food supply **(Evans, 1981)**. In the laboratory, **Breteler and Gonzalez (1988)** showed that the prosome length of the copepods *Temora longicornis* (Müller) and *Pseudocalanus elongatus* (Boeck) was positively correlated with food concentration and negatively correlated with the temperature. On the other

hand, **Viñas and Gaudy (1996)** reported that the final size of the adults of *E. acutifrons* decreased with increasing the temperature. Hence, the copepods from San Matias were significantly larger than those from Marseilles owing to the minimum temperatures in San Matias, recording values lower than those of Marseilles. **Ara (2001)** has found that the body length of the juvenile stage of *E. acutifrons* correlated negatively with the temperature and chlorophyll-*a* concentration and positively with salinity.

Variations in salinity and temperature can directly influence the metabolic and physiological processes of these organisms, potentially limiting their growth. In addition, the increase in chlorophyll-*a* suggests an increased eutrophication of the water, often associated with an excessive supply of nutrients. This can lead to algal blooms such as those reported by **Bengriche *et al.* (2023)** which, although initially providing an abundant food source, can subsequently reduce the water quality and oxygen availability, and thus adversely affecting the planktonic fauna. In addition, further research is needed to explore in more detail the specific mechanisms by which variations in temperature, salinity and chlorophyll-*a* affect these copepod populations in order to develop more targeted and effective mitigation strategies.

Since *E. acutifrons* is known to be a prey for some economically important fish ***(*Kraul *et al.*, 1993; Viñas & Ramírez, 1996)**, the decrease in its size can have an impact on planktivorous animals.

The comparison of the biometric characteristics for both sexes at the two stations revealed that males close to the discharge were significantly smaller with respect to five characteristics (lengths of cephalosome, pedigerous somites, urosome, caudal rami, and total length) compared to males found more distant from the discharge (Wilcoxon rank sum exact test, $P< 0.05$). For females, for all measured biometric characteristics and males for the cephalosome width, no significant effect has been recorded for these characteristics as a result of proximity or distance from the discharge (Wilcoxon rank sum exact test, $P> 0.05$). The correlation between the biometric characteristics under study showed that males exhibited a more marked positive correlation for most characteristics compared to females, especially in the proximity of the discharge.

The significance of this study is that it is not limited to the total length of the specimens but considers five additional biometric characteristics, considering the measurements of environmental parameters and setting a comparison between both sexes. However, there are potential limitations to consider.

CONCLUSION

In conclusion, our results suggest that the size of the copepod *Euterpina acutifrons*, particularly in males, decreases significantly in proximity to domestic discharge. This observation could be attributed to several environmental factors linked to the anthropogenic activity, in particular the increase in temperature and chlorophyll-*a* concentration, as well as the decrease in salinity. The presence of these environmental

changes in the discharge zones highlights the importance of monitoring and managing the ecological impacts of domestic discharges on the aquatic ecosystems.

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