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Research

WHAT FACTORS INFLUENCE BODY SIZE VARIATIONS AND EGG PRODUCTION OF COPEPODS AND *ARTEMIA SALINA*IN THE SALTERN OF SFAX?

CHIRAZ LADHAR[1-3]*, FRANÇOISE DENIS[2-3], HABIB AYADI[1]

[1]Université de Sfax, Faculté des Sciences de Sfax, Département des Sciences de la Vie. Unité de recherche UR 11 ES 72/ Biodiversité et Écosystème Aquatiques, Route soukra Km 3,5, B.P. 1171, CP 3000 Sfax, Tunisia

[2] UMRBOREA « Biologie des Organismes et Écosystèmes Aquatiques », Muséum National d'Histoire Naturelle (MNHN), CP 26, 43, rue Cuvier, 75231 Paris Cedex 05, France

[3]Université du Maine, EA2160 Mer, Molécules, Santé, équipe biologie moléculaire et génétique évolutive, UFR Sciences et Techniques, Avenue Olivier Messiaen, 72085 Le Mans cedex 9, France

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CORRESPONDENCE AUTHOR: Dr. Chiraz Ladhar

E-mail: ladhar.chiraz@yahoo.fr

ABSTRACT:

The effect of salinity, temperature, nitrate, phosphate, and chlorophyll *a* concentration were evaluated on the body length and egg production of copepods (*Oithona nana*, *Oithona similis*, *Paracartia grani* and *Mesochralilljeborgi*) and *Artemia salina*. Sampling was conducted in four ponds of increasing salinity in solar saltern of Sfax (Tunisia) between June 2010 and May 2011. The results of the present study showed that, among the parameters considered, salinity was the major factor influencing the body length (R^2 = 0.8007) and egg production (R^2 = 0.8077) of studied species. Body size decreases as salinity increases. The results suggest that biometric and egg production of zooplankton seemed to be related to the salinity, to the nitrate concentration and chiefly to alimentation.

KEY WORDS: Artemia salina, body length, copepods, egg production, salinity, Sfax solar saltern

INTRODUCTION

Copepods (*Oithona nana*, *Oithona similis*, *Paracartia grani* and *Mesochralilljeborgi*) and *Artemia salina* are the most common metazoans in the hyper saline environment (Alajmi and Zeng 2014; Ladhar et al. 2014a). These two groups are a key component of the zooplankton communities in salty natural ecosystems such as lagoons or solar saltern-artificial ecosystems. In fact, copepods (*O. nana*, *O. similis*, *P.a grani* and *M.lilljeborgi*) and *A.salina* play an important role in aquatic ecosystem as a direct link of matter and energy transfer between primary producers and higher trophic level consumers (Wu et al. 2010; Temporoni et al. 2014). Equally important, Ladhar et al. (2014a) states that these species have a significant role in the cycle of nutrients and are considered, according to Conceição et al. (2010), as ideal live prey for various commercially exploited planktivorous fish species. *Artemiasalina*are actively implicated in the functioning of the saltern (Bruce andImberger 2009).

Salt production depends on physical process of evaporation (Vieira and Bio 2011) on the one hand and on these brine organisms and biological processes on the other hand (Davis and Giordano 1996; Davis 2000).

Despite increased research in recent years aimed to study zooplankton, particularly *O. nana*, *O. similis*, *P. grani*,*M.lilljeborgi*and *A. salina*, few of them tried to gauge the influence of physico-chemical and biological parameters on the body size and egg production of these taxa. The assessment of the influence of these parameters is essential to improve our understanding of the main factors affecting the growth and the development of copepods (*O. nana*, *O. similis*, *P.a grani* and *M.lilljeborgi*) and *A. salina*. As a consequence, the influence of factors on size and egg production of copepods and *Artemia salina* needs to be studied. According to Hirche et al. (1997), the measurement of egg production and body length of copepods has become a widely used tool in copepod ecology.

Laboratory investigations have shown that temperature and food availability are the principal factors controlling the dynamic, growth and reproduction of copepod population (Kobari et al. 2010). In fact, Kobari and Ikeda (2001a, b) suggest that body size variations result from a combined effect of these two factors. It was shown that temperature is the more important environmental factor which effects variations in body length and egg production. In fact, low temperature produces larger body size in copepods (Kobari et al. 2003). The effect of salinity on the body length and egg production of *O. nana*, *O. similis*, *P.a grani*, *M. lilljeborgi*and *A. salina,* however, is not thoroughly investigated. In the study-relevant literature, egg production rate and body size were thus determined by temperature and food quantity and quality (Diel and Tande 1992).Water temperature is important factor affecting the egg production rate and size range of *Acartia lilljeborgi* (Koichi2001). Similarly, Boyer et al. (2013) report that seasonal temperature changes effect egg production of the Calanoida copepod *Paracartia grani*.

In this study, we tried address this gap in literature by examining the variations in body size and egg production of *O. nana*, *O. similis*, *P.a grani*, *M.lilljeborgi*and *A.salina* in relation to salinity, temperature, nitrate, phosphate and chlorophyll *a* in the saltern of Sfax. To our best knowledge, this is the first study to assess the relative importance of these variables as potential determinants of body size and egg production of these taxa.

MATERIALS AND METHODS

STUDY SITE AND SAMPLING

The saltern of Sfax, located in the central eastern coastline of Tunisia (34°39'N, 10°42'E), is an artificial hydro-system with a surface area of 1700 ha. This saltern is composed of shallow interconnected ponds. Four sampling ponds A5, A16, C41 and M2 were selected with an increasing salinity 42 psu, 61 psu, 96 psu and 193 psu, respectively (Figure 1).

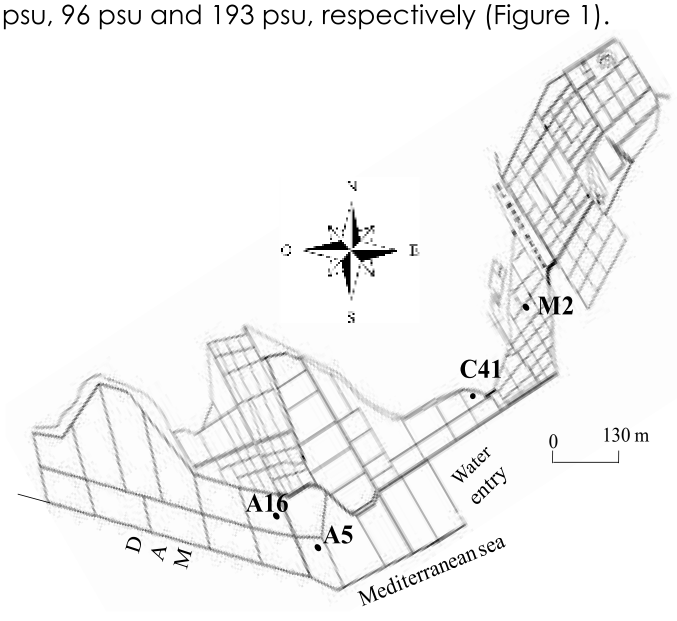

Figure 1. Study area and sampling ponds

PHYSICAL, CHEMICAL AND BIOLOGICAL PARAMETERS

Salinity and temperature were measured using refractometer and a mercury glass thermometer graduated in 0.1°C, respectively.

Water samples for nitrate (NO_3^-) and phosphate (PO_4^{3-}) measurements were filtered and immediately frozen upon collection in the dark (-20°C). These nutrients were analyzed with a Bran and Luebbeautoanalyzer type 3.

The water was filtered using a Whatman GF/C membrane (0.45 μm) and the concentration of chlorophyll a was measured using the acetone method and calculated using the equation proposed by Scor-Unesco (1966).

LENGTH AND EGG PRODUCTION MEASUREMENTS OF ZOOPLANKTON

Specimens of copepods (*Oithona nana*, *Oithona similis*, *Paracartia grani* and *Mesochralilljeborgi*) and *Artemia salina* were collected every two month between June 2010 and May 2011. Samples were taken with a80 μm mesh screen, fixed and preserved immediately after collection with 4% formaldehyde. To identify the zooplankton groups, various keys were used: Rose 1933; Bradford-Grieve 1994 and Boxshall and Halsey 2004.

Copepods (*O. nana*, *O. similis*, *P.a grani* and *M.lilljeborgi*) and *A. salina* were measured with an ocular micrometer (100 subdivisions) at a magnification of 10×. Species used in the study were selected because of their abundance and their constant presence in each pond (Table1).

Table 1. Distribution of copepods and *Artemiasalina* samples

Species	Sampling ponds
Oithona similis	A5 and A16
Oithona nana	A5 and A16
Paracartia grani	A5 and A16
Mesochralilljeborji	C41
*Bryocamptus*sp.	C41
Artemia salina	M2

Length of *A.salina* was measured as the distance from the head to the base of the caudal furca. Total body length, between the top of the cephalosome and the end of the furca of all copepod specimens (*O. nana*, *O. similis*, *P.a grani* and *M.lilljeborgi*) was measured. Only adult developmental stage of *Artemia salina* and copepods was treated. Thirty individuals were sorted according to each sex and species. Eggs of *O. nana*, *O. similis* and *M. lilljeborgi* were counted for each individual.

STATISTICAL ANALYSIS

Mean and standard deviation (SD) were reported when appropriate. The relations between the variables analyzed (length, egg production measurements of zooplankton and physico-chemical parameters) were statistically tested with a normalized principal component analysis (PCA) (Chessel et al. 1992). Calculations and statistical analyses were performed using XL stat software. Correlation coefficients were calculated using the regression program Origin 6 and Tcwin.

RESULTS

PHYSICO-CHEMICAL AND BIOLOGICAL FACTORS

Variations in temperature, salinity, nitrate (NO_3^-), phosphate (PO_4^{3-}) and chlorophyll*a* concentration were shown in Figure 2.

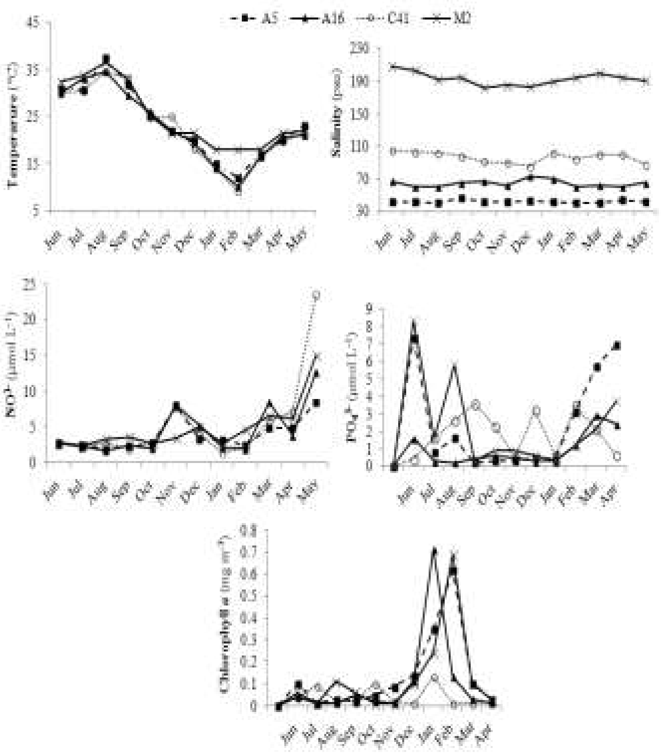

Figure 2. Spatiotemporal variations of salinity, temperature, phosphate, nitrate and chlorophyll *a* in saltern of Sfax

The salinity ranged between 40 psu in the first studied pond (A5, August 2010) and 208 psu in the last studied pond (M2, June 2010). Water temperature differed slightly from pond to pond with seasonal variations similar in all ponds. The lowest value of about 9°C was recorded in January 2011 (C41), whereas the highest value was 37.5°C (A5, August 2010). Nitrate concentration varied between1.3 µmol l^{-1} (C41, January 2011) and 14.9 µmol l^{-1}(M2, May 2011). Phosphate values varied from 0.2 µmol l^{-1} (A5, September 2010 and A16, August 2011) to 8.3 µmol l^{-1} (January, M2).Chlorophyll *a* concentration ranged from 0.01 mg m^{-3} (in all studied ponds) to 0.71 mg m^{-3} (January, A16).

VARIATIONS IN BODY LENGTH AND EGG PRODUCTION OF ZOOPLANKTON

Temporal variations in the body length of males and females of copepod species (*Oithona nana*, *Oithona similis*, *Paracartia grani*, *Mesochralilljeborgi*)and *Artemia salina*were illustrated in Figure 3. Thirty males and females per species were used in the data analysis.

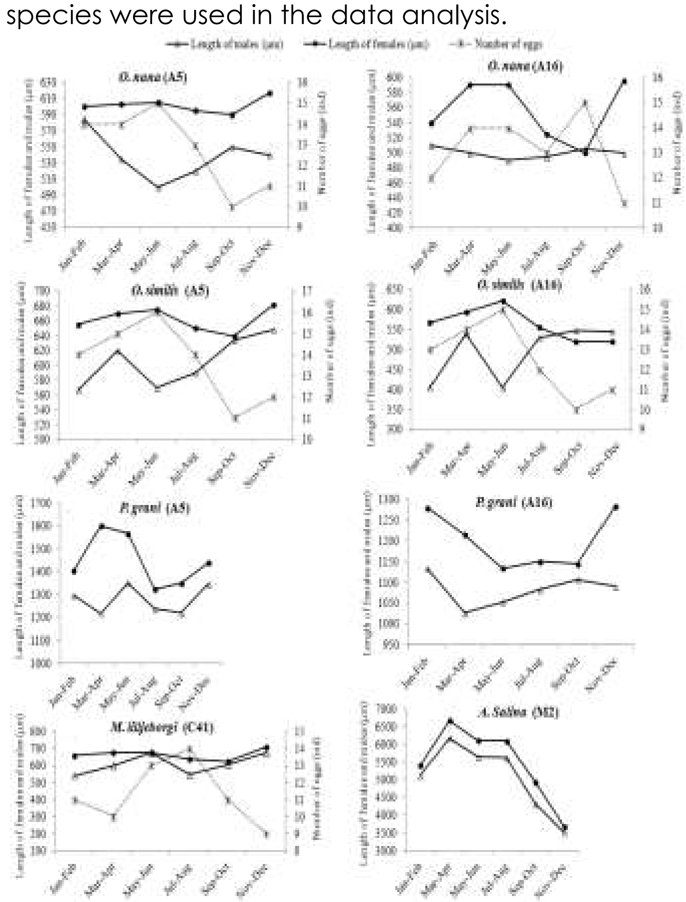

Figure 3. Variations in egg production and length of females and males of copepods (*O. nana*, *O. similis*, *P.a grani* and *M.lilljeborgi*) and *A.salina*

The highest figures in length of *Artemia salina*, both males and females, were recorded in March-April (6181 µm and 6669 µm, respectively), and the lowest figures were recorded in November-December (3510 µm and 3666 µm, respectively). Mean lengths of females of *O. nana* (A5), (A16) and *O. similis* (A5) were the greatest in November-

December (617 µm, 595 µm and 681 µm, respectively).
Maximum egg production was observed in *O. similis* (A5) in summer (May-June, 16 eggs) and minimum egg production was seen in *M. lilljeborgi* (C41) in winter (November-December, 9 eggs).

Both males and females of *O. nana*, *O. similis* and *P. grani* sampled from A16 (61 psu) were smaller than those sampled from A5 (42 psu) (Table 2).

Table 2. Mean length and mean egg number ± SD of copepod species and *Artemia salina*

Species	Ponds	Sex	Number of individuals used in data analysis	Mean length (µm)	Mean egg number (individual)
A. salina	M2	Male	30	5071 ± 989	
		Female	30	5481 ± 1077	
M. lilljeborgi	C41	Male	30	607 ± 34	
		Female	30	662 ± 16	11 ± 2
O. similis	A5	M Male	30	605 ± 34	
		Female	30	662 ± 16	14 ± 2
	A16	Male	30	495 ± 70	
		Female	30	563 ± 40	12 ± 2
O. nana	A5	Male	30	583 ± 29	
		Female	30	602 ± 9	13 ± 2
	A16	Male	30	500 ± 7	
		Female	30	557 ± 40	13 ± 1
P. grani	A5	Male	30	1277 ± 62	
		Female	30	1447 ± 113	
	A16	Male	30	1082 ± 38	
		Female	30	1201 ± 68	

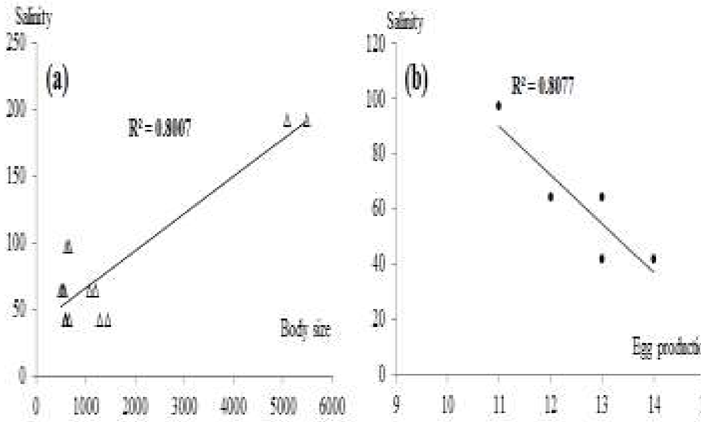

Figure 4. Correlation between salinity and body length (a) and between salinity and egg production (b) Length of *O. nana*, *O. similis* and *P. grani*, males and females, in studied ponds, were negatively correlated with salinity (r = -0.808, r = -0.807, p < 0.05, for *P. grani* and *O. nana* male, respectively). However, salinity was, positively correlated with body length of *A. salina* males and females (r = 0.941, p< 0.05). Nitrate was positively correlated with body length of males and females of *M. lilljeborgi* and with number of egg production of the same species (r = 0.785, p< 0.05). However, thisnutrient (NO_3^-) was negatively correlated with the length and egg production of other copepods (r = -0.906, r = -0.914, r = -0.911, p< 0.05, for *O. nana* male, *O. similis* males and *P. grani* females, respectively).

Incopepod taxa, *P. grani*female (A5) showed the highest length (1447 ± 113 µm). The maximum and minimum of mean egg production was observed in *O. similis* (A5, 14 ± 2 eggs) and *M. lilljeborgi* (C41, 11 ± 2 eggs) (Table 2).A significant functional relationship was found between salinity and length of males and females of *O. nana*, *O. similis*, *P.a grani*, *M.lilljeborgi*and *A. salina* (R^2 = 0.8007) and between salinity and egg production for female of copepods (R^2= 0.8077) (Figure 4).

Chlorophyll *a* was negatively correlated with body length of males and females of *M. lilljeborgi* and with the number of produced eggs of this species (r = -0.937, p< 0.05).

In the present study, no relationship was found between temperature, egg production and body length (R^2 = 0.06). However, a significant relationship (R^2= 0.8077) was detected between salinity and egg production in female of copepods. There is an inverse relationship between salinity and egg production.

DISCUSSION

Seasonal variations in body size and fecundity varied among ponds, species and sex in saltern of Sfax. In our study, the greatest lengths of copepod species were observed in low salinity (A5, 42 psu) during colder months, and the lowest lengths were observed in high salinity (A16, 61 psu and C41, 96 psu).According to Ara (2002), copepod body lengths are generally larger in winter and smaller in summer. The maximum egg production of copepods species was observed in summer in A5 (*O. similis*) and the minimum was found in winter in C41 (*M. lilljeborgi*). Our results suggest that zooplankton body length is inversely related to salinity. In fact, copepod body lengths are generally larger in low salinity and smaller in high salinity. Our data support an inverse interaction between body length and salinity as seen with temperature in the study of Bozhurt and Can (2014).Some scientists suggest that temperature is the primary determinant of body size at maturity and that it is a key abiotic factor regulating the growth and reproductive potential of copepods in marine and freshwater systems (Bozhurt and Can 2014). The complicated spatial and temporal variations in ecological environmental factors such as temperature, salinity, food quantity and quality may result in the wide range of copepods egg production rate (Diekmann et al. 2012; Aguilera et al. 2013), but the major influence of temperature and food availability has been pointed out (Huntley and Lopez1992; Kleppel et al. 1996 a, b; Mauchline 1998).

Some studies showed that female size affects egg production by influencing clutch size (Runge and Plourde 1996), whereas temperature affects egg production by influencing the frequency of spawning (Hirche 1990; Hirche et al. 1997; Bozhurt and Can 2014).

Among factors affecting copepod body size and fecundity, temperature and food concentration were found to be highly important (Viitasalo et al. 1995; Kobari et al. 2010).

A negative correlation was found between chlorophyll *a* concentration and body length in *M. lilljeborgi*. A significant correlation between chlorophyll *a* concentration and egg production was detected in this species. While some authors have reported a negative relationship (Moraitou-Apostolopoulou et al. 1986) between chlorophyll *a* concentration and copepod body length, others have found a positive relationship (Klein Bretelerand Gonzales 1982; Sander and Moore 1983; Ban 1994). This shows that, despite earlier works done, the effect of chlorophyll *a* concentration on copepod body length still remains confuse and controversial.

The concentration of NO_3^- seems to be among the factors controlling growth and egg production rates of copepods. The analysis highlights that nitrate was positively correlated with body size and egg production rate of the Harpacticoida *M. lilljeborgi* (C41). It appears that *M. lilljeborgi*is known to feed a variety of food sources including green algae (Ladhar et al. 2014b).The concentration of algal cells of >5 µm seems to be one of the main factors controlling egg production rates (Bozhurt and Can 2014).NO_3^- was negatively correlated with body length and egg production level of *O. nana*, *O. similis* and *P. grani* in ponds A5 and A16, and this can be due to a carnivore-based diet such as dinoflagellates (Ladhar et al. 2014b). So, there is a close relationship between size, egg production rates and feeding activity (Hirche et al. 1997).However, as discussed by Halsband and Hirche (2001), it may be difficult to discern the effects of these factors independently in the field, where the effects of one factor may be overridden by others. Thus, interactions between food, nutrients, salinity, temperature, and body size on the one hand and egg production rates on the other hand may prove to be complex.

CONCLUSION

Results suggest that salinity is a more important environmental factor than nitrate and chlorophyll *a* concentration in its effect on body length and egg production of copepods (*Oithona nana*, *Oithona similis*, *Paracartia grani*, *Mesochralilljeborgi*) and *Artemia salina* in Sfax solar saltern. At low rate, nitrate can influence body size and egg production, and this is directly related to the diet of studied species.

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