

Short Research Note

## Ecological and biological notes on the brine shrimp *Artemia* (Crustacea: Branchiopoda: Anostraca) from Carmen Island, Baja California Sur, México

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Received 29 August 2005; accepted 4 September 2005

**Key words:** *Artemia*, ecology, morphometry, Carmen Island, Mexico

### Abstract

This study was carried out with a natural population of the brine shrimp *Artemia* from Bahía Salinas, in Carmen Island, Baja California Sur, México, with the purpose to determine different ecological and biological characteristics of this crustacean. The field work was performed in two periods in 1999: March to June and August to September. In both periods, the variation of temperature, pH, salinity and depth of 13 saline ponds was monitored. Brine shrimp were present in ponds with salinity between 115 and 195 g l<sup>-1</sup>. Minimum and maximum values of water temperature were 15 and 35 °C. The pH ranged from 7 to 8. The average diameter of hydrated cysts and size of newly hatched nauplii was 201.3 µm (±12.8) and 473.5 µm (±73.9), respectively. The morphometric analysis of adults showed significant differences between sexes. The population density fluctuated from 6 to 55 specimens per liter. The monthly male:female ratio was dominated by the females. Females displayed different offspring output, with ovoviviparity being dominant over oviparity. The individual fecundity oscillated between 10 and 87 cysts. The effect of water temperature and salinity on biological parameters of the adults is discussed.

### Introduction

In the New World, the genus *Artemia* is represented by the zygogenetic species, *A. persimilis* Piccinelli & Prosdocimi, 1968 (Argentina) and the *A. franciscana* Kellog, 1906 superspecies (Americas, Caribbean and Pacific Islands) (Browne & Bowen, 1991). In México, the genus *Artemia* is known to occur in at least 29 inland and coastal waters from 11 states (Van Stappen, 1996; Maeda-Martínez et al., 2002). However, according to the available literature on crossbreeding experiments, genetic data, and man-made introductions, only six Mexican populations can be determined as *Artemia franciscana* (Maeda-Martínez et al., 2002).

Field studies of the Mexican *Artemia* populations are very scarce (e.g. Castro, 1989; Del Castillo-Arias & Farfan, 1997). With respect to *Artemia* from Carmen Island, Baja California Sur, only studies on biometry of cysts and nauplii exist (Castro et al., 1987). Considering the lack of ecological and biological information relative to this brine shrimp strain, a field study was performed. The main objectives were (1) to study the physical and chemical characteristics of the habitat, such as depth, temperature, salinity, and pH, (2) to carry out a morphometric analysis on the diameter of cysts, size of instar-I nauplii, and standard morphological features of adults, and (3) to determine adult density, sexual ratio, fecundity and type of offspring output.

## Material and methods

### *Description of the study area*

The study was conducted in two periods of 1999, March to June and August to September; in Carmen Island, Baja California Sur, México (26° 03' N, 111° 08' W). This island is located 15 km off the coasts of Loreto, Baja California Sur (Fig. 1). The *Artemia* population is found mostly in the Northeast of the island, in the locality of Bahía Salinas. This zone covers an area of 7.90 km<sup>2</sup>, and is characterized by several saline ponds varying in morphometry. The sediment of the ponds is constituted mainly by slime; this condition favored the filtration of salt water from underground wells to the ponds. The weather is considered very dry or desertic and the precipitation in the island is very scarce along the year, with annual mean values of 5.0–10.2 mm (Castro et al., 1987). During the

months of observation, only once precipitation (28 mm) occurred, in September.

### *Physical and chemical data*

Thirteen saline ponds were selected to study the daily variation in temperature, salinity and pH *in situ*. These variables were monitored at 15 cm of depth in each pond using a digital thermometer (YSI 55), a refractometer (ATAGO) (salinity), and Macherey Nagel pH titer bands. All data were taken in the morning between 07:00 and 10:00 h. The mean depth of each pond was obtained measuring at least three points with a metric rule. The number of samples taken in each pond is indicated in Table 1.

### *Artemia sampling and processing*

Samples of cysts were collected periodically from the surface of all ponds, and preserved in saturated

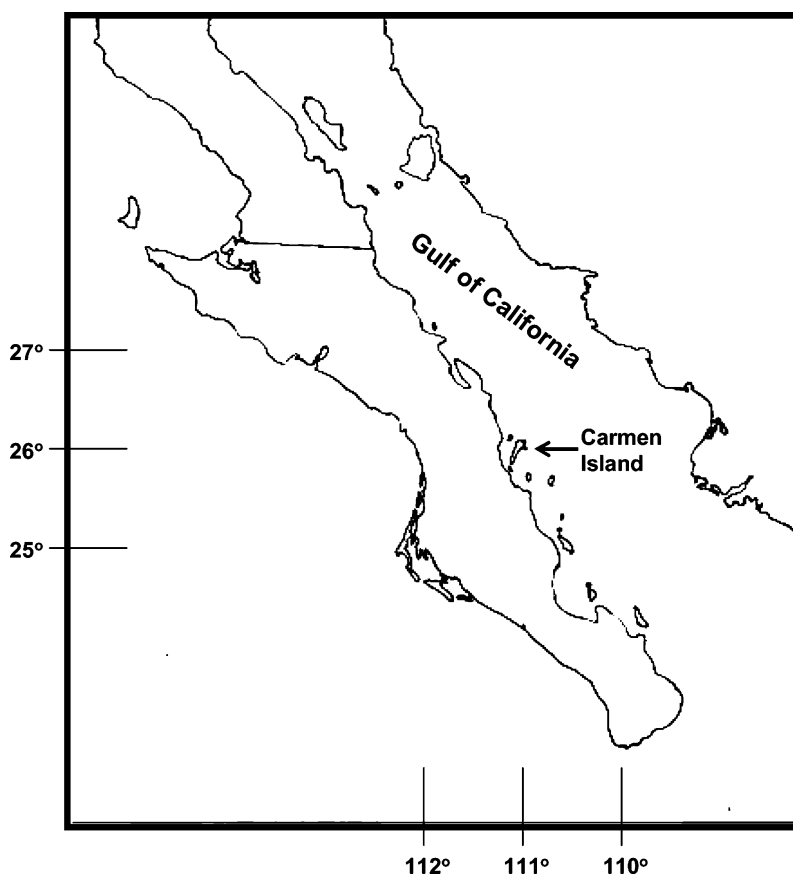


Figure 1. Localization of Carmen Island in the Gulf of California, México.

Table 1. Mean values of depth (cm), temperature (°C) and salinity (g l<sup>-1</sup>) of saline ponds from Carmen Island, Baja California Sur

Pond	Period 1 (n = 69) (March–June)			Period 1 (n = 39) (August–September)		
	Depth	Temperature	Salinity	Depth	Temperature	Salinity
1 <sup>a</sup>	25.0 ± 2.5	21.6 ± 3.0	116.1 ± 13.6	DP	DP	DP
2 <sup>a</sup>	80.0 ± 7.0	23.5 ± 2.8	115.9 ± 15.4	80.0 ± 6.8	30.3 ± 1.0	104.7 ± 5.5
3 <sup>b</sup>	130.0 ± 16.0	23.5 ± 3.5	99.4 ± 13.4	125.0 ± 29.7	30.8 ± 1.3	98.3 ± 9.7
4 <sup>b</sup>	110.0 ± 10.5	22.9 ± 3.2	106.3 ± 10.9	100.0 ± 10.7	30.8 ± 1.4	106.6 ± 3.1
5	25.0 ± 8.8	23.9 ± 2.6	126.3 ± 9.6	DP	DP	DP
6 <sup>b</sup>	90.0 ± 15.2	22.3 ± 2.8	143.8 ± 12.7	90.0 ± 9.6	30.0 ± 1.2	135.2 ± 4.3
7 <sup>b</sup>	70.0 ± 5.5	22.9 ± 3.1	112.6 ± 12.1	70.0 ± 3.7	30.3 ± 1.3	113.9 ± 11.8
8	50.0 ± 3.5	21.1 ± 2.5	81.1 ± 2.7	50.0 ± 4.0	29.8 ± 0.9	84.3 ± 3.7
9 <sup>b</sup>	65.0 ± 11.5	24.3 ± 2.8	122.5 ± 10.6	65.0 ± 13.9	30.7 ± 1.3	108.3 ± 7.4
10	50.0 ± 4.0	21.8 ± 2.3	79.0 ± 4.2	50.0 ± 5.2	29.8 ± 1.0	85.3 ± 3.2
11	50.0 ± 17.5	21.0 ± 2.7	91.7 ± 5.3	45.0 ± 20.1	28.6 ± 0.9	79.3 ± 2.8
12	115.0 ± 5.0	26.4 ± 3.2	147.6 ± 25.5	115.0 ± 79.4	31.9 ± 1.6	140.4 ± 15.0
13	60.0 ± 22.5	23.7 ± 2.5	130.6 ± 11.4	DP	DP	DP

DP = Dried Ponds. <sup>a</sup>Live adult *Artemia* present only in the period 1, <sup>b</sup>Live adult *Artemia* present in both periods.

brine ( $\geq 300$  g l<sup>-1</sup>). In the laboratory the cysts were washed with distilled water and dried in an oven at 38 °C. Finally, each sample was kept in a dark container. Six ponds were selected for the monthly population monitoring of *Artemia* adults. In each pond, the specimens were collected with a plastic container (20 l) which was introduced between 15 and 30 cm of depth, and the samples obtained were filtered in an 80  $\mu$ m mesh size zooplankton net to filter the volume of the container. This procedure was repeated three times per pond. *Artemia* adults only were counted and separated (not younger stages) by sex to determine the density and sexual ratio. All specimens were fixed in 10% formalin. Additionally, related fauna such as aquatic insects was determined taxonomically.

### Biometry

After rehydration in artificial sea water, the diameter of the cysts was measured according to Vanhaecke & Sorgeloos (1980) and Castro et al. (1997). Instar-I nauplii ( $n = 101$ ) obtained of the incubation of cysts were fixed in a 10% lugol solution and measured using an eye-piece micrometer mounted on the microscope. Adult morphometric analysis was carried out by examining 20 males and 20 females of each sampled pond. Kolmogorov–Smirnov tests ( $p < 0.05$ ) were

performed to test the normality of the data of cysts, instar-I nauplii and adults. The biometry of *Artemia* adults was performed according to the procedures described by Hontoria & Amat (1992). The biometric data analyzed were: total length (TL), abdominal length (AL), width of Ovisac (WO), length of Furca (LF), abdominal width (AW), width of head (WH), length of first antenna (LFA), diameter of eyes (DE) and distance between eyes (DBE). Mean values of each parameter were compared between sexes by means of the Independent-Samples “t” test (Zar, 1996). Calculations were performed with the statistical Package SPSS version 8 (1997). Morphometrical and physicochemical variables were correlated using the Pearson’s correlation coefficient to find the degree of association among them.

### Fecundity

Two saline ponds (numbers 2 and 6) were selected to estimate the fecundity and type of offspring output. One hundred and 150 ovigerous females were collected during the first (pond 2) and second (pond 6) period, respectively. These females were individually separated and preserved with 10% formaldehyde in vial tubes. In the laboratory the ovigerous sacs were dissected under the microscope and the number of cysts or nauplii was

counted. The relationship between TL and fecundity was analyzed by means of a potential regression equation ( $Y = aX^b$ ) using log-transformed data (Zar, 1996).

## Results

### Physical and chemical data

The mean depth of each pond showed a range between 25 and 130 cm for both sampling periods. In the second period (August to September) three ponds were found to be totally dry (Table 1). Temperatures of the ponds ranged between 15 and 35 °C, and mean values per pond were between 21.0 and 31.9 °C (Table 1). During the second period (August–September), there was a significant increase in water temperature of the ponds over 30 °C.

The minimum and maximum salinity observed in the ponds during the first period was 70 and 252 g l<sup>-1</sup>. For the same period, the mean pond salinity ranged between 79.0 and 147.6 g l<sup>-1</sup>. For the second period the mean values ranged between 79.33 and 140.4 g l<sup>-1</sup> (Table 1). Monthly pH values by pond ranged between 7 and 8.

### Biometry

The size of hydrated cysts, hatched instar I nauplii and adults was normally distributed ( $p < 0.05$ ). The mean diameter of the hydrated cysts was 201.3 ± 12.9 μm. For instar I nauplii the mean length was 473.5 ± 74.0 μm. The mean values of TL, AL, LF, AW and WH showed significant differences between both sexes ( $p < 0.05$ ) (Table 2). The correlation between the measured morphological parameters vs. temperature and salinity showed in all cases a significant positive and negative association, respectively ( $p < 0.05$ ). The highest value was observed for the relationship of TL with temperature for males ( $r = 0.548$ ).

### Adult density and sex ratio

In the saline ponds monitored the density of *Artemia* adults ranged between 6 and 55 specimens per liter. In each pond, *Artemia* adults showed a patchy distribution. The number of

adults monthly collected was highest in March and the lowest value was observed in September. Females predominated throughout the observation period with a maximal value (1:1.86 males/females) in August (Table 3).

### Type of reproduction and fecundity

In the first sampling period (dry season) oviparity (78%) dominated over ovoviviparity (22%). In the second period, females switched to ovoviviparity (87.4%). The fecundity was low as maximum values observed were only 87 and 68 for the first and second sampling periods, respectively. The females with broods ranged in size from 5.4 to 12.0 mm. The relationships between fecundity (F) and size of females (TL) were  $\ln F = \ln 0.119 + 2.731 \ln TL$  and  $\ln F = \ln 1.818 + 1.417 \ln TL$ , for the first and second period, respectively. The

Table 2. Mean values of morphometric characters analyzed for *Artemia* adults from Carmen Island, Baja California Sur (in mm)

Parameter	Females	Males
TL	7.88 ± 1.35	6.27 ± 0.94
AL	3.73 ± 0.85	2.79 ± 0.55
WO	1.39 ± 0.33	–
LF	0.27 ± 0.05	0.23 ± 0.04
AW	0.40 ± 0.07	0.35 ± 0.07
WH	0.66 ± 0.13	0.56 ± 0.12
LFA	0.78 ± 0.21	0.79 ± 0.16
DE	0.26 ± 0.06	0.25 ± 0.04
DBE	1.34 ± 0.21	1.34 ± 0.21

For each variable  $n = 290$ .

Table 3. Number of adults monthly collected and male/female ratio of *Artemia* from Carmen Island, Baja California Sur, México

Month	Total by month	Sex ratio (male/female)
March	2927	1:1.38
April	1904	1:1.18
May	17,702	1:1.23
June	1541	1:1.66
August	2227	1:1.86
September	828	1:1.64
Total	11,129	1:1.45

slopes of both equations were significant (P1,  $F=525.93$ ;  $p<0.005$ ; P2,  $F=37.69$ ;  $p<0.005$ ). However, the fecundity showed the highest correlation with TL ( $r^2=0.782$ ) in the first period.

#### *Aquatic insects present in the saline ponds*

Eleven ponds of the 13 saline ponds monitored were inhabited by the insects *Ephydra* sp. (dipteran), *Graptocorixa californica* (hemipteran), and *Acilius semisulcatus* (coleopteran). *Ephydra* sp. was most common, it was found in 10 ponds where the salinity ranged between 70 and 180 g l<sup>-1</sup>. The coxixid *G. californica* was recorded in six ponds with salinities that fluctuated between 70 and 110 g l<sup>-1</sup>. *A. semisulcatus* was only found in two ponds with salinities between 70 and 110 g l<sup>-1</sup>.

## Discussion

#### *Physical and chemical data*

The dryness of some ponds in the 2nd observation period was the result of high air temperatures (43 °C). The scarce rain observed during only three days in September did not contribute significantly to the water volume registered in the ponds. The main supply of water was the infiltration of seawater caused by the tides and winds.

Variations in water temperature (15–35 °C) were commonly observed among the ponds for the same sampling date. These values were taken during the morning and probably the water temperatures could have been higher in the afternoon. Previous studies showed that the brine shrimp inhabits water bodies with temperature fluctuations between 6 and 35 °C (Van Stappen, 1996). The rise of temperature, mainly during the second period provoked a reduction in the water volume, dryness of some ponds and the reduction or absence of *Artemia*. With this respect, De Donato & Graziani (1993) mentioned that variations of water temperature in natural saline ponds can affect the presence of *Artemia*.

The salinity range observed (70 and 252 g l<sup>-1</sup>) is similar to those values reported by Bowen et al. (1988), who mentioned that North American *Artemia* habitats range in salinities from 30 to 300 g l<sup>-1</sup>. However, specimens of *Artemia* were

only observed in ponds with salinities between 115 and 195 g l<sup>-1</sup>. With this regard, Tackaert & Sorgeloos (1991) mentioned that the development of the brine shrimp is optimal when salinity fluctuates between 100 and 180 g l<sup>-1</sup>, as a way to avoid the presence of predators, such as aquatic insects. The values of pH observed in the saline ponds were in the tolerance range for *Artemia* populations (6.5–8) reported by other authors as Dhont & Lavens (1996).

#### *Biometry*

The cyst size previously recorded for this strain by Castro et al. (1987), was 179.6 μm. Higher values of hydrated cysts were found in this study (201.3 ± 12.9 μm). However, both records indicate that the cyst size observed corresponds to the smallest values for *Artemia* cysts from North America, including Mexico. Van Stappen (1996) mentioned that biometrical parameters, in particular cyst diameter, are good tools to characterize *Artemia* strains. The mean length of nauplii (473.5 μm) from Carmen Island was within the range for nauplii from Mexican coastal waters, described in literature (379.7–758.9 μm) (Torrentera & Dodson, 1995; Castro et al., 2000).

Previous studies of *Artemia* strains from Baja California Peninsula showed a substantial difference in size between sexes (e.g. Del Castillo-Arias & Farfan, 1997; Naegel & Rodríguez, 2002). The same pattern was also observed with *Artemia* adults from Carmen Island, where females were larger than males.

The salinity is the most important factor affecting the biometric characteristics of *Artemia* (Amat, 1982). With this respect, Amat et al. (1991) and Naegel & Rodríguez (2002) found that in higher salinity *Artemia* individuals show lower size values. This pattern was confirmed in our study. Naegel & Rodríguez (2002) mentioned that the main reason for the decrease in size of adults is because, at high salinity levels (200–250 g l<sup>-1</sup>) the food becomes a limiting factor, and *Artemia* needs more energy for osmoregulation.

#### *Adult density and sex ratio*

There is a paucity of data on biomass density of *Artemia* adults in Mexican habitats (e.g. Del

Castillo-Arias & Farfan, 1997; Naegel & Rodríguez, 2002). Adult densities reached values up to 56 individuals/l in March. Lenz & Browne (1991) mentioned that in hypersaline lakes and ponds the highest density of *Artemia* adults from North America occurred in the summer with values up to 10 ind./l. The monthly monitoring of adults revealed that the females were more abundant than the males.

#### *Type of reproduction and fecundity*

The differences in the reproduction type for both periods could be associated with the changes of temperature and salinity. This could be explained, because, the lowest values of salinity during the second period coincide with the ovoviviparity. To this respect, Lenz & Browne (1991) and Van Stappen (1996) mentioned that the salinity is considered as one of the main ecological factors affecting the mode of reproduction. The maximum fecundity recorded for American strains of *Artemia* ranged between 100 (Lenz & Browne, 1991) and 300 nauplii or cysts (Van Stappen, 1996). The low fecundity observed for the *Artemia* strain from Carmen Island could be attributed to environmental factors as salinity, low productivity in the ponds, as stated by the transparency of the water column and accidental loss of eggs during the collection of the females.

#### *Aquatic fauna*

As in many *Artemia* habitats in the world, the dipteran *Ephydra* sp. was the most common insect in the saline ponds of Carmen Island. *Ephydra* larvae and *Artemia* were found at the same time in most of the ponds. In saline biotopes from Baja California Peninsula, the family Corixidae was found at salinities of 141 g l<sup>-1</sup> (Del Castillo-Arias & Farfan, 1997). In this study, the corixid *G. californica* was observed in lower salinities (70 and 110 g l<sup>-1</sup>). The coleopteran *A. semisulcatus* was found in similar conditions of salinity as other coleopterans from Baja California (Del Castillo-Arias & Farfan, 1997). The occurrence of *G. californica* and *A. semisulcatus* in some ponds could affect the presence of *Artemia*, because both species are considered as predators (Merritt & Cummins, 1984).

#### **Acknowledgements**

This study was supported in part by Organización Vida Silvestre, A.C. (OVIS) and the project CN276-99 from the Programa de Apoyo a la Investigación Científica y Tecnológica (PAICYT) from the Universidad Autónoma de Nuevo León (UANL), México.

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