

## Influence of Some Physicochemical Parameters on *Artemia* Biomass and Cyst Production in Some Thalassohaline Aquatic Environments in the Colombian Caribbean

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**Abstract.**—From July 1998 to June 2000 four thalassohaline aquatic environments along the Colombian Caribbean coast (Manaure, Galerazamba, Salina Cero, and Tayrona) were surveyed monthly to determine the influence of salinity, percent O<sub>2</sub> saturation, pH, temperature, and nutrients (NO<sub>2</sub><sup>-</sup>, NO<sub>3</sub><sup>-</sup> and PO<sub>4</sub><sup>-3</sup>) on *Artemia* (Crustacean, Anostracan) biomass production and cyst production potential. The effects of the regularly measured physicochemical parameters on biomass and cyst production potential were analyzed using univariate analysis of variance (SPSS V10.0). The influence of physicochemical parameters on biomass production was not significant ( $P > 0.05$ ). In contrast, there was a significant interaction ( $P < 0.05$ ) of salinity, percent O<sub>2</sub> saturation, and nitrate (used as a proxy for chlorophyll *a*) on cyst production potential. In addition, for all four locations nitrate levels were directly proportional to salinity. This might be explained by the fact that in saltworks numerous organisms are trapped and slowly die as salinity increases progressively in the evaporating basins; thus, organic matter accumulates and decomposes. Consequently, the concentration of the nitrogenous compounds, first nitrite and later nitrate, increases through time as salinity increases. Moreover, decreasing nitrate levels seem to increase cyst production potential; thus supporting the notion that when insufficient food is available cyst production increases.

Increasing sampling efforts in Latin America for potential *Artemia* (Crustacean, Anostracan) sites during the mid 1990s have resulted in numerous reports of *Artemia* populations and the characterization of

*Artemia* habitats (Camargo et al. 2000; Camargo 2002; Camargo et al. 2002a, 2003), particularly with respect to remote thalassohaline aquatic environments.

Lenz (1987) observed that zooplankton population dynamics are influenced by abiotic factors (salinity, temperature, and nutrients concentration) and by biological interactions (predation, competition, and grazers). Further, Por (1980) and Lenz (1987) showed that biological interactions are limited in hypersaline communities where species diversity is low, and abiotic parameters, particularly those that regulate seasonal characteristics, are dominant. Lenz (1987) identified two critical factors that determine the population dynamics of *Artemia* and they are: 1) habitat conditions allowing the survival of the animals throughout the year; and 2) predictability of the seasonality of the environment.

Bisexual and parthenogenetic *Artemia* species are characterized by an extensive genetic variation resulting in difference in their life history traits (Lenz and Browne 1991). Under laboratory conditions, longevity, fecundity, and reproductive period vary one order of magnitude among *Artemia* species and populations (Browne 1980,

1988; Lenz 1984, 1987; Dana and Lenz 1986; Browne and Hoopes 1990). Browne et al. (2002) measured reproductive and life span traits for two obligate parthenogenetic types and three sexual (two *A. franciscana* and one *A. sinica*) brine shrimp populations and determined that, for all traits studied, the environmental component was greater than the genetic component measured. However, every trait has a genetic component that can potentially be acted upon by selection. The average genetic component of variation for the 10 traits was 23.44%, ranging from 5.26% for number of cysts to 44.87% for number of nauplii.

Ecological and physiological differences have been previously reported among several populations of *A. franciscana* (Sorgeloos et al. 1975; Abreu-Grobois and Beardmore 1982; Bowen et al. 1985). These ecological and physiological differences have important impacts on *Artemia* populations by influencing reproductive strategies through biomass and cyst production.

In saltworks, *Artemia* occur abundantly in the evaporation ponds at salinity levels between 80 to 220 g/L (depending on the strain and/or species), and in rare occasions have been found at salinities up to 340 g/L (Post and Youssef 1977). At these salinity levels high ionic concentrations are a limiting factor due to the energy requirements for osmoregulation. *Artemia* populations are favored by the absence of predators and food competitors and develop successfully in these extreme biotopes often at high densities. Further the dominance of these hypersaline habitats is shared with archaeobacterial halophiles (Grant and Ross 1986) and halotolerant microalgae as the concentration of salts approaches saturation. Further, a relatively small number of these halobacterias and microalgae species might serve as a food source to this very efficient, non-selective filter feeder (Sorgeloos 1980).

Some *Artemia* strains are predominantly oviparous at high salinities (i.e., Lake Urmia, Lake Grassmere, some strains from Spain, and Manaure strains—Berthélémy-

Okazaki 1986; Camargo et al. 2000), while other strains primarily opt for the ovoviviparous mode of reproduction at high salinities (i.e., Great Salt Lake, Salina Cero strains—Amat 1982; Camargo et al. 2000). Depending on the strain and the hydrobiological conditions of the pond (i.e., high water retention, high evaporation, and primary productivity), cyst production is induced by changes in brood number, salinity, photoperiod, temperature, hypoxia, and iron rich foods (Dutrieu 1960; Baker 1966; Bowen et al. 1969; Sorgeloos et al. 1975; Provasoli and Pintner 1980; Amat 1982; Berthélémy-Okazaki 1986). Cyst production can be produced seasonally or annually.

Studies *in vitro* revealed that optimum temperature and salinity varies between *Artemia* populations (Vanhaecke et al. 1984; Wear et al. 1986; Browne et al. 1988; Camargo, unpublished data). *A. franciscana* populations have a high reproductive capacity up to senescence (Browne et al. 1984). Browne et al. (1988) further revealed that *A. franciscana* is the most plastic (flexible) *Artemia* species with respect to temperature followed by the parthenogenetic type.

In nature, *Artemia* is found in neutral to alkaline waters. According to Vos (1979), nauplii growth decreases and the overall appearance of adults deteriorates with pH values below 7.0, and they concluded that the optimum pH for *Artemia* growth ranges from 8.0 to 8.5. Additionally, Sato (1967) determined that cyst hatching efficiency was greatly compromised at pHs below 8.0.

The aim of this research was to identify physicochemical variables that influence monthly *Artemia* biomass production *in vivo* and monthly cyst production potential of some wild Colombian populations (Manaure, Galerazamba, Tayrona, and Salina Cero) for potential use in aquaculture.

### Study Areas

**Manaure.** Located west of the town of Manaure (11°46'32"N 72°29'27"W), in the

center of La Guajira department, near the city of Riohacha. This saltwork is a thalassohaline shallow aquatic ecosystem that extends over 4,000 ha. This zone was originally a natural lagoon, still surrounded in some small areas by mangroves.

**Galerazamba.** Located approximately 20 km to the north of the city of Cartagena (10°47'38"N 75°14'48"W). This small thalassohaline saltwork has five ponds (three to obtain brine and two crystallizers) with a total extension of 220 ha. This saltwork was built in a natural saline lagoon, surrounded by some mangroves.

**Tayrona or Chengue salt pond.** This small natural thalassohaline saltwork of approximately 2.5 ha is situated (11°19'03"N, 74°08'13"W) approximately in the middle of the Tayrona National Natural Park. The salt pond is flooded, during most of the year, by the often prolonged winter and serves as a saltwork during summer.

**Salina Cero or Cienaga Prieto.** This small thalassohaline lagoon of only 18 ha is located at only 3 km of Galerazamba (10°46'29"N, 75°15'55"W). For several decades, salt has been manually extracted occasionally once or twice per year.

### Materials and Methods

Twenty *Artemia* samples were taken randomly in a given pond (Camargo et al. 2000; Camargo 2002; Camargo et al. 2002b) at each site (Manaure, Galerazamba, Tayrona, and Salina Cero) every month with a 14-L acrylic box (Haslett and Wear 1985). The samples were collected in both shallow and deeper water sectors. Samples were preserved in a formalin-alcohol solution (4% borax buffered), and maintained in a cooler until they arrived at the laboratory where they were counted and classified using a stereoscope to determine population distribution (females, males, juveniles, and nauplius).

Samples containing a known number of adults (males and females) were strained and dried in an oven at 80 C for 24 h (Camargo et al. 2000; Camargo 2002). Individ-

ual average weight from each of the four locations was calculated according to Camargo et al. (2000) by dividing the sample dry weight by the number of adults in each sample. Finally, to calculate total dry biomass (production), the individual dry weight was multiplied by the number of adults counted at each of 20 stations in the 14-L box and finally multiplied by the total respective pond volume. Our assumption for the mean weight is that all individuals from each population were the same weight (Camargo et al. 2000; Camargo 2002).

Additionally, the following physicochemical parameters were determined, at each station and location, simultaneously with the *Artemia* samples collection: salinity (temperature compensated refractometer); percent O<sub>2</sub> saturation and temperature (Oxymeter WTW® 330 calibrated prior to use, WTW Measurement Systems, Inc., Ft. Myers, Florida, USA); pH (pHmeter WTW® 330 calibrated prior to use, WTW Measurement Systems, Inc., Ft. Myers, Florida, USA); nitrates, nitrites, and phosphates (colorimetric method within an hour of water sample collection using Hatch® DREL 2010 spectrophotometer, Hach Company, Loveland, Colorado, USA); and chlorophyll *a* (we use the seston method, preserving samples in a cooler while arriving to the laboratory; samples were then filtered using Whatman® GF/C filter papers and acetone for destruction and chlorophyll *a* extraction and finally read using a Hatch® DREL 2010 spectrophotometer, Hach Company, Loveland, Colorado, USA). We used an indirect method to measure primary productivity, by correlating a limiting nutrient (nitrate) to primary productivity. Further, the N salt form (nitrate) was used rather than nitrites or phosphates, since nitrites as well as phosphates showed very low values, sometimes below detection levels (Fig. 1). Besides, no trend was established statistically between nitrite or phosphate and cyst and biomass production (Figs. 2a, 2b, 2i, 2j).

Reproductive experiments were conduct-

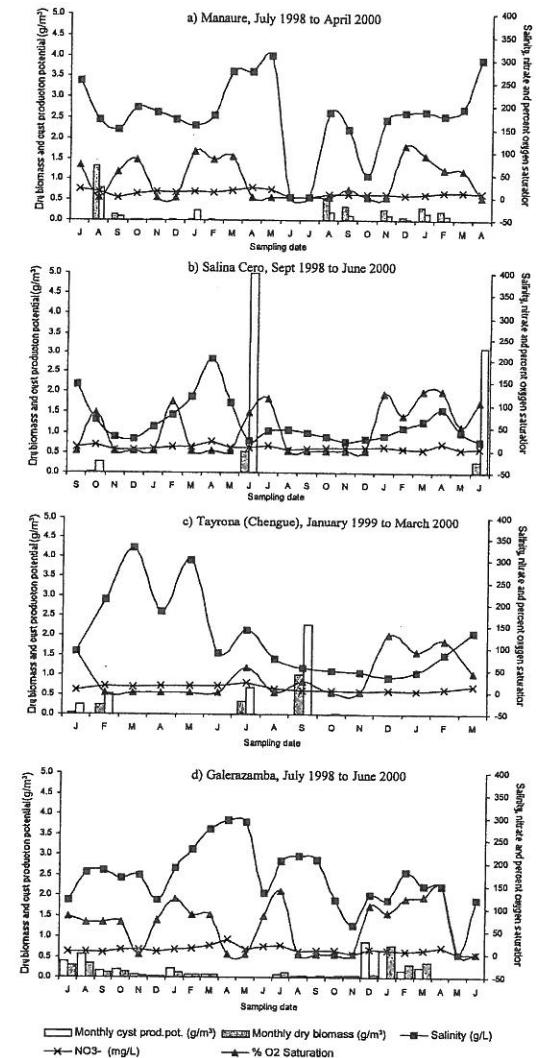


FIGURE 1. Interaction of significant physicochemical parameters over monthly *Artemia* biomass production and cyst production potential at the four locations.

ed in the laboratory according to Vanhaecke et al. (1984) under controlled conditions (salinity, temperature, DO, pH, illumination, and supplying artificial food *ad libitum*) at two salinities (80 and 120 g/L) in

order to determine the mean cyst production potential for each site (Newmark 1988; Duran and Garcia 2001). The density of females in the pond was determined by multiplying the number of females counted in

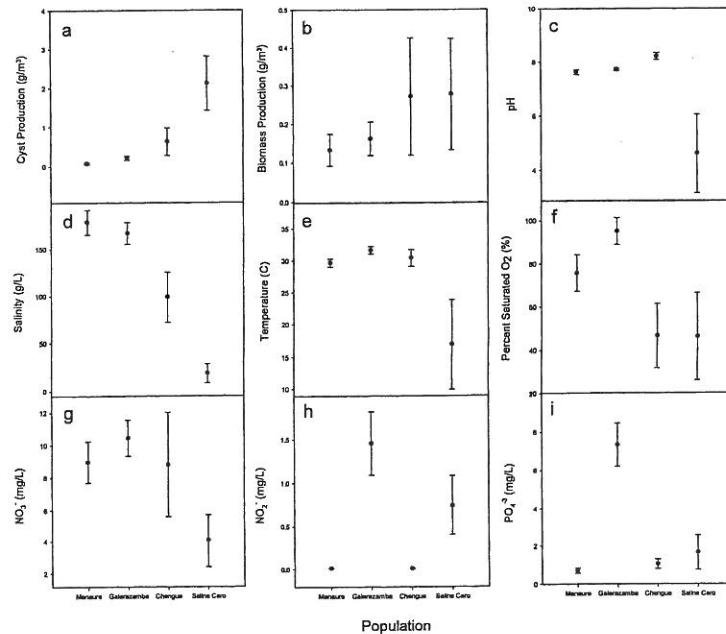


FIGURE 2. Mean values with standard errors for a) *Artemia* cyst production potential, b) *Artemia* biomass production (DW), c) pH, d) salinity, e) temperature, f) percent  $O_2$  saturation, g) nitrate, h) nitrite, and i) phosphates. (Chengue: Tayrona NNP).

the 20 samples by the pond volume at each site (Camargo et al. 2000; Camargo 2002). Pond volume was estimated from pond mean depth and surface area. Total cyst production potential was calculated at each respective site by multiplying the mean number of cysts per female (previously obtained

*in vitro*) of a given population by the total number of females in the pond.

Finally, the effects of the measured physicochemical parameters (Table 1) on biomass production and cyst production potential were analyzed using univariate ANOVA with Tukey post-hoc tests (SPSS V10.0,

SPSS Inc., Chicago, Illinois, USA) among populations. Variables (salinity, percent  $O_2$  saturation, nitrate, and temperature) selected for the analyses were based on previously observed relationships (Camargo et al. 2000) between physicochemical parameters and dependent variables. Water temperature was excluded from the final analysis because its correlation with cyst and biomass production was insignificant. In order to apply the univariate statistical analysis to the field data, we had to use a scoring method for physicochemical parameters ranges, rather than reporting partial missing data or deleting rows of incomplete data, because of the periodically partial or total (some or no water in the pond) crystallization of some locations and its negative effect on the analysis. Dependent variables (biomass and cyst production) can have values equal to zero, contrasting with independent variables (i.e., pH, temperature, salinity, and percent  $O_2$  saturation), which could not be measured upon crystallization of the pond, thus presenting a blank. The values for each variable were scored consistently from 1 to 3, as follows: each physicochemical parameter analyzed was divided into three data groups (containing 0 to the maximum value recorded in the field), where crystallization of the basin (0 or no data) was represented by rank No. 1 and the subsequent measured values within the two ranges were set to 2 and 3, respectively. The scoring was done, rather than using the original values, in order to determine possible relationships. The alpha ( $\alpha$ ) was set at 0.05 for all analyses.

## Results

### Population Site Characteristics

At Manaure the overall distribution of females (F), males (M), juveniles (J) and nauplius (N) was 22:16:28:35 (F:M:J:N), with a female to male (F:M) ratio of 1:0.84. Biomass production in two evaporation areas of this very large saltwork occurred for 21 mo (at two different time intervals) out of

the 24 surveyed (Fig. 1a), presenting the lowest mean biomass production ( $0.15 \text{ g/m}^3 \text{ DW}$ ) of all four sites (Fig. 2b). The highest monthly biomass value of  $1.33 \text{ g/m}^3 \text{ DW}$  was recorded in August 1998, when the salinity was  $170 \text{ g/L}$ , percent  $O_2$  saturation ranged from 56 to 99%, and  $NO_3^-$  was  $11.75 \text{ mg/L}$  (Table 1).

At Salina Cero the proportion of adults (males and females) to the juveniles and nauplii was lower than that of the four sites. From September 1998 to June 2000, mean population composition was 11: 8:26:55 (F:M:J:N), and the F:M ratio was 1:0.84. Twenty-two inspections were conducted, collecting *Artemia* biomass in only three of them, although this site presented the highest mean biomass production ( $0.28 \text{ g/m}^3 \text{ DW}$ ) of all sites surveyed (Fig. 2b). Salina Cero had the lowest monthly biomass production (Fig. 1b), but reproduction experiments indicated that Salina Cero presented the highest mean cyst production of all four sites. In June 1999, monthly biomass production reached its maximum value with  $0.53 \text{ g/m}^3 \text{ DW}$ , salinity was  $22 \text{ g/L}$ , percent  $O_2$  saturation of 84%, and  $NO_3^-$  was  $4.85 \text{ mg/L}$  (Table 1).

At Tayrona the mean population distribution from November 1998 to June 2000 presented a high composition of adults (males and females) compared to that of juveniles and nauplius being 36:46:10:8 (F:M:J:N), but with a low F:M ratio of 0.88: 1, contrary to the other three populations. Eighteen sampling campaigns were conducted, collecting *Artemia* biomass in only six occasions, and very rarely cysts. This site presented the second highest mean biomass production ( $0.27 \text{ g/m}^3 \text{ DW}$ ) of all sites surveyed (Fig. 2b). Production reached its maximum value (Fig. 1c) in September 1999 with a monthly biomass of  $0.99 \text{ g/m}^3 \text{ DW}$ , salinity was  $55 \text{ g/L}$ , very low percent  $O_2$  saturation of 23.3% and  $NO_3^-$  was  $3.7 \text{ mg/L}$  (Table 1).

At Galerazamba the population distribution, between July 1998 and April 2000, was 20:16:26:38 (F:M:J:N) and F:M ratio

TABLE 1. Results for the field determination of biotic and abiotic parameters of four Colombian *Artemia* populations. All values are ranges for sampling period (July 1998–June 2000) except precipitation (maximum).

Parameters	Manaure	Galerazamba	Tayrona	Salina Cero
Salinity (g/L)	148–275	65–295	34–330	19–204
pH	7.6–7.9	7.2–8.1	7.9–8.8	6.7–8.6
Percent $O_2$ sat.	56–99	70–150	23–131	53–131
Temp. (C)	24.9–31.3	26.6–35.5	23.4–33.8	27.5–35.1
$NO_2^-$ (mg/L)	0.005–0.025	0.120–0.005	0.002–0.018	0.003–0.115
$NO_3^-$ (mg/L)	0.29–20.45	1.4–33.7	2.15–22.1	0.4–18.75
$PO_4^{3-}$ (mg/L)	0.05–1.27	0.33–1.98	0.32–2.83	0.21–5.05
Precipitation (mm)	79.6	326.7	288.2	326.7
Chl. <i>a</i> (mg/m <sup>3</sup> )	ND	0.01–0.11	0.01–0.39	0.09–3.04

was 1:0.88. *Artemia* biomass was collected in the same pond, in 19 out of 24 sampling campaigns realized in this saltwork; thus, achieving the most constant biomass production estimates of all sites. This site presented a low mean biomass production ( $0.16 \text{ g/m}^3 \text{ DW}$ ) of all sites sampled (Fig. 2b). This saltwork showed an increase in biomass and salt production in the second semester of the second sampling year. In January 2000, monthly biomass production reached its maximum value (Fig. 1d) with  $0.79 \text{ g/m}^3 \text{ (DW)}$ , salinity was  $120 \text{ g/L}$ , percent  $\text{O}_2$  saturation of 91%, and  $\text{NO}_3^-$  was  $9.8 \text{ mg/L}$  (Table 1).

The results of the in vitro experiment for the number of *Artemia* cysts produced per female at two salinities (80 and  $120 \text{ g/L}$ ) showed that Galerazamba, Tayrona, and Salina Cero females produced more cysts (14.9, 57.8, and 127.6 cyst/female, respectively) at the higher salinity ( $120 \text{ g/L}$ ) than Manaure (34.0 cyst/female at  $80 \text{ g/L}$ ).

The influence of physicochemical parameters (Fig. 2) on mean biomass production was not significant ( $P > 0.05$ ). Conversely, the interaction of salinity, percent  $\text{O}_2$  saturation, and nitrate on cyst production was significant ( $P < 0.05$ ). Salina Cero had the highest mean cyst production potential at the lowest salinity, percent  $\text{O}_2$  saturation, and nitrate concentration (Fig. 2) and Tayrona was characterized by higher salinity and nitrate concentration but low percent  $\text{O}_2$  saturation. In addition, Manaure and Galerazamba had a relatively low cyst production potential at higher salinity, percent  $\text{O}_2$  saturation, and nitrate concentrations.

### Discussion

The results of this study demonstrated that variation in *Artemia* biomass among different thalassohaline aquatic environments in the Colombian Caribbean were less pronounced than estimates of variations in cyst production potential derived from controlled laboratory measurements of reproduction. Variation in physicochemical conditions between the sites examined did

not appear to influence biomass production. However, the results of this study were consistent with previous observations that *Artemia* populations respond to food shortages, associated with changes in physicochemical conditions, by increasing cyst production (Ballardin and Metalli 1963; D'Agostino and Provasoli 1968; Amat 1985; Román and Rodríguez 1986).

The relatively low proportion of adults presented at Salina Cero (19%) may have been due to the relatively low salinity (Table 1) presented by this site compared to Manaure (38%), Galerazamba (36%) and Tayrona (82%). Further, the high proportions of adults at Tayrona may have been due to poor recruitment (J:N) resulting from a possible environmental impact at this site.

Relatively stable physicochemical conditions at Manaure may have suppressed cyst production compared to the other three sites (Galerazamba, Salina Cero, and Tayrona) which presented less stable conditions. According to the criteria of Wear et al. (1986), Lake Grassmere is indicative of a relative environmental stability which results in a low cyst production and a high ovoviviparity level, which might be selectively advantageous for *Artemia* to be able to maximize the success of intraspecific competence. Moreover, the encysting mechanism might cause a delay in population growth that could be disadvantageous under relatively stable conditions (Browne 1980). Further, in the Manaure saltwork, Colombia (Camargo et al. 2000) an abundant cyst production was reported for some years (Rozo and Pinzón 1983; Bengtson and Simpson 1989) before Hurricane Joan in 1988. The floods caused by the hurricane damaged the levees considerably; therefore, joining several evaporation ponds in the salt production circuit and causing salinity and temperature to fluctuate very little for several years. Consequently, *Artemia* primarily exhibited the ovoviviparous mode of reproduction for several years. Only until recently, salinity has increased in the same evaporation ponds, above the tolerance lim-

it for *Artemia*, thus producing appreciable amounts of cysts. These types of relatively stable environments can result in a low but permanent reproduction capacity and a multiplicity of asynchronous generations each year (Lenz 1987).

The results of the reproductive experiment (mean cyst production per female) do not entirely agree with the estimated cyst production potential at each site and may be due to a combination of certain parameters (i.e., salinity, percent  $\text{O}_2$  saturation, low nitrate, and/or starvation of the adult *Artemia* population after reaching a high density, particularly of the female portion) on cyst production, particularly in the case of Salina Cero. An additional explanation to these contradictory results may be due to the controlled conditions where *Artemia* was fed ad libitum (constant food availability). In contrast, these conditions do not occur naturally in the field. The latter may be supported, in part, by the observation of low nitrate levels during the peak cyst production potential during this study. Further, nitrate levels and cyst production show a negative trend and do not add to cyst production, thus providing evidence that food limitation might contribute to cyst production. According to Amat (1985) at low salinities and percent  $\text{O}_2$  saturation, the factor influencing *Artemia* oviparity was invariably hypoalimentation, particularly prolonged starvation periods (Ballardin and Metalli 1963). Román and Rodríguez (1986) affirm that cyst production in Cadiz saltworks (Spain) is given when the chlorophyll *a* and *Artemia* density ratio decreased. Similarly, according to Newman (personal communication, 2001) food quantity (density) might be the determining factor for *Artemia* to select an oviparous mode of reproduction rather than food quality. There are times wherein a particularly poor algae strain dominates and *Artemia* cannot break down the cell wall and at that point they will most likely switch reproductive modes, prior to their demise. However, D'Agostino and Provasoli (1968) also rec-

ognized that food quality and quantity could induce oviparity. Moreover, according to Hernandonena (1974), with increasing salinity, *Artemia* energy requirements decrease and protein intake for albumin increases.

In addition, for all four locations nitrate levels were directly proportional to salinity (Fig. 2d, 2g), this might be explained by the fact that in saltworks numerous organisms are trapped and slowly die as salinity increases progressively in the evaporating basins; thus, organic matter accumulates and decomposes, both of animal (i.e., crustaceans, fish, insects) and plant origin (i.e., leaves, phytoplankton). Consequently, the concentration of the nitrogenous compounds, first nitrite and later nitrate (through the process of nitrification), increases through time as salinity increases. Similarly, Nyongje et al. (1995) showed that in the saltworks at Gongoni (Kenya) chlorophyll *a* increased with increasing salinity and related this to elevated levels of nitrates in the evaporation ponds. The same phenomenon which occurs in saltworks (chlorophyll *a* being dependent on nitrate and salinity) also is present in the sea as a cycle (summer vs. winter). According to Slinn (1974) in the North-West Irish Sea, nitrate disappeared during the summer and thus would be the limiting nutrient. Even more, the lack of nitrate limits photosynthetic activity in the surface ocean layers (Knapp-USGS in Conomos and Gross 1968).

To summarize we were able to show a relationship between three physicochemical parameters (nitrate, percent  $\text{O}_2$  saturation, and salinity) and *Artemia* cyst production potential for the four locations surveyed. In contrast, no significant relationship could be established for any physicochemical variable and *Artemia* biomass production. Further, nitrate (chlorophyll *a*) and salinity were shown to be negatively correlated to cyst production potential. The previous observation may be supported by indications that insufficient food plays an important role on cyst production (food shortage hy-

pothesis) as shown by Ballard and Metalli (1963), D'Agostino and Provasoli (1968), Amat (1985), and Román and Rodríguez (1986).

Finally, the implications of these results might be valuable to balance the conservation and exploitation of natural populations of *Artemia* to supply the demand of the local and global aquaculture industry.

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