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Reproductive performance of three *Artemia franciscana* Kellogg (Crustacea, Anostraca) populations in north-eastern Brazil pond culture conditions

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Artemia franciscana Kellogg (Crustacea, Anostraca) is found on a year-round and permanent basis in the State of Rio Grande do Norte (RN) in north-eastern Brazil as a result of inoculations made in Macau saltworks in 1977 with cysts from San Francisco Bay (California, USA). Inoculation of *Artemia* in the saltworks of RN was initially followed by high cyst yields. However, recent data indicate that these feral brine shrimp populations reproduce predominantly ovoviviparously. In the present study, the reproductive performance of three populations from Macau (5° 06′ S; 36° 38′ W), Areia Branca (4° 57′ S; 37° 08′ W), and Grossos (4° 58′ S; 37° 09′ W) in RN was examined under similar pond culture conditions. A higher incidence of ovoviviparous than oviparous females for all experimental populations was found throughout ten culture cycles carried out in ponds of 0.72 ha. Furthermore, no significant variability in reproductive mode (ovoviviparity *versus* oviparity) or in fecundity (brood size) was observed ($p > 0.05$). These results presumably reflect the homogeneity of local populations and are consistent with the suspected decrease of genotypic diversity of feral *Artemia franciscana* in the saltworks of RN in north-eastern Brazil.

Key words: *Artemia franciscana*, inoculation, reproductive output, saltworks, Brazil.

INTRODUCTION

Natural and cultured populations of the brine shrimp *Artemia* (Crustacea, Anostraca) can produce either cysts (dormant embryos) or nauplii (free-swimming larvae) and combine both types of reproductive mode (oviparity and ovoviviparity) with ratios varying widely among them (Lenz & Browne, 1991). Although it is difficult to relate environmental, physiological, or genetic factors to the onset of a specific reproductive mode in *Artemia* (Wear *et al*., 1986; Abatzopoulos *et al*., 1993; Mura, 1995; Baxevanis & Abatzopoulos, 2004; Baxevanis *et al*., 2004), dependence on oviparity (cyst production) is commonly seen in populations experiencing a seasonal cycle of either temperature or salinity, or living in ephemeral habitats (Browne & Bowen, 1991; Lenz & Browne, 1991; Gajardo *et al*., 2002). Ovoviviparity (nauplii production), on the other hand, is associated with stable environmental conditions (Wear *et al*., 1986; Lenz, 1984, 1987; Lenz & Dana, 1987; Wear & Haslett, 1987; Dana *et al*., 1990, 1995; Mura, 1995; Van Stappen *et al*., 2001; Wurtsbaugh & Gliwicz, 2001; Torrentera & Dodson, 2004). For both modes of reproduction, however, factors such as brood size, hypoxia, photoperiod, availability/type of food, and maternal heterozygosity can be also of importance (Browne & Bowen, 1991; Lenz & Browne, 1991; Gajardo *et al*., 2002 and references therein).

Among the six bisexual species within the anostracan genus *Artemia*, two exist in the New World: *Artemia persimilis* Piccinelli & Prosdocimi and *Artemia franciscana* Kellogg. *Artemia persimilis* is restricted to Argentina and some localities in Chile (Cohen *et al*., 1999; Amat *et al*., 2004; Gajardo *et al*., 2004), whereas the bisexual *A. franciscana* super-

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species is endemic to the Americas and the Caribbean, with various populations established in South American countries, either by deliberate inoculation or natural dispersal (Van Stappen, 2002).

In north-eastern Brazil, *A. franciscana* is found on a year-round and permanent basis in the State of Rio Grande do Norte (RN) as a result of inoculations made in Macau saltworks in 1977 with cysts from San Francisco Bay (California, USA) (Persoone & Sorgeloos, 1980). The inoculation of *Artemia* in the saltworks of RN was followed by high cyst yields in the beginning (Camara & Rocha, 1987). However, recent data indicate that these feral brine shrimp populations reproduce mainly ovoviviparously (Camara, 2001).

In this paper, the reproductive performance of three *A. franciscana* populations of different origin in north-eastern Brazil was examined under similar pond culture conditions.

MATERIALS AND METHODS

The experimental data were obtained during ten culture cycles carried out from July 2001 to November 2002 in an experimental *Artemia* farm owned by the Brazilian Shrimp Farmers Association (ABCC) in north-eastern Brazil. The farm is located in the municipality of Grossos (4° 58′ S; 37° 09′ W) in the State of Rio Grande do Norte (RN) (Fig. 1). Although primarily designed to test the feasibility of cyst and biomass production in a multi-cycle culture system in north-eastern Brazil (Camara *et al*., 2004), the ABCC farm provided a unique opportunity for the concurrent study of reproductive performance of *A. franciscana* in a sort of 'natural laboratory'. The farm consists of a fertilization/evaporation area of 1.44 ha (two ponds of 0.72 ha), a production area of 2.16 ha (three production ponds of 0.72 ha), a pumping station, inflow and outflow channels, and a small laboratory.

The populations of *A. franciscana*, duration of culture cycles in the experimental ponds, and corresponding abbreviations used in this study, are indicated in Table 1. The experimental populations were hatched out from the following cyst sources in RN: a cyst batch harvested in September 2000 from saltworks located in the municipality of Macau (5° 06′ S; 36° 38′ W), a cyst batch harvested in June 2001 from the saltworks of Areia Branca (4° 57′ S; 37° 08′ W), and a cyst batch harvested in September 2001 from saltworks in Grossos (4° 58′ S; 37° 09′ W). The location of saltworks used as cyst sources in this study is depicted in Fig. 1. The cysts were incubated for 24 hours following standard protocols (Lavens & Sorgeloos, 1996), and the resulting brine shrimp nauplii were stocked in the ponds at a density of 20 nauplii/l. Precautions taken to avoid cross-contamination of experimental populations included meticulous cleaning of hatching apparatus, pond bottom drying between culture trials, and independent culture cycles for each one of the *A. franciscana* populations.

The environmental conditions of the experimental ponds were monitored daily. The following schedule for recording abiotic parameters was used: dis-

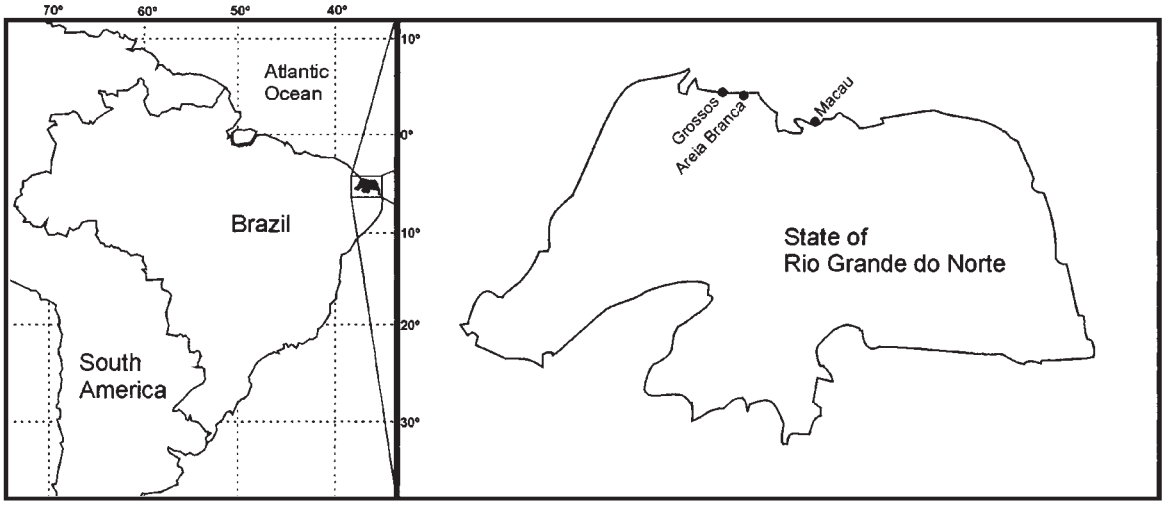

FIG. 1. Location of saltworks in the State of Rio Grande do Norte (RN), north-eastern Brazil, used as cyst sources in this study (Macau, Areia Branca, and Grossos).

solved oxygen and temperature at 05:00 and 16:00, salinity at 16:00, and transparency at 12:00. Dissolved oxygen (mg L^{-1}) and temperature (°C) were measured with a field probe (YSI model F-1055; Yellow Springs Instrument Company, Yellow Springs, Ohio, USA), and salinity (ppt) with a hand refractometer (Atago model S-28; Atago Company Ltd., Tokyo, Japan). Transparency levels (cm) were measured with a Secchi disc.

The mode of reproduction (ovoviviparity *versus* oviparity) and average brood size (fecundity) of oviparous and ovoviviparous females were used to assess the reproductive performance of the experimental *A. franciscana* populations. *Artemia* sampling was initially carried out fifteen days after stocking (day 15), and thereafter at weekly intervals. Samples were collected with a scoop net (size of 30 cm × 30 cm; 500 mm mesh size) dragged from the surface to the bottom of the water column in the vicinity of the sluice pond gate. Collected animals were placed in glass vials, anaesthetized with chloroform-saturated water, and then fixed in 5% formalin. In the laboratory, subsamples from each glass vial were placed under a stereo microscope (Olympus model SZ3060; Olympus Optical Company Ltd., Tokyo, Japan) and adult females separated according to their reproductive status in ovigerous (full ovisac/oviducts) or non-ovigerous (empty ovisac/oviducts) females. The first 100 ovigerous females were dissected and accounted as oviparous (females bearing encysted embryos) and ovoviviparous (females bearing fully developed embryos at pre-larval stage), and the number of cysts and embryos was determined. The individual dissection of ovigerous females confirmed their reproductive mode; the number of cysts or embryos provided data on the fecundity of oviparous and ovoviviparous females, respectively.

Reproductive differences among *A. franciscana* populations from Macau, Areia Branca, and Grossos were analyzed by a standard single factor ANOVA for unequal sample sizes (Sokal & Rohlf, 1981).

RESULTS

In the present study, culture cycles lasted from 28 (ABR3) to 63 (GRO3) days (Table 1). Throughout ten culture cycles, average values for dissolved oxygen ranged from 4.2 (GRO4) to 9.7 mg L^{-1} (ABR2), temperature from 26.2 (MAC1) to 28.4°C (MAC3), salinity from 81 (GRO1) to 97 ppt (MAC1), and transparency from 42 (GRO3) to 85 cm (ABR2) (Table 2). Range values for pond water temperature and dissolved oxygen followed diurnal fluctuations: minimum water temperature (21.1°C at MAC1) and oxygen (1.2 mg L^{-1} at GRO4) were recorded during early morning hours (05:00) and maximum values (32.3°C and 15.6 mg L^{-1} at MAC1) at mid-afternoon (16:00). Minimum and maximum values for salinity and transparency, on the other hand, were recorded in the beginning (77 ppt at ABR2; 22 cm at ABR2 and GRO3) and towards the end of each culture cycle (119 ppt at GRO3; 87 cm at ABR2), respectively.

The experimental brine shrimp populations of Macau (MAC), Areia Branca (ABR), and Grossos (GRO) did not display any significant variability ($p > 0.05$) in their reproductive mode (ovoviviparity *versus* oviparity) or in fecundity (brood size) under similar pond culture conditions (Table 3). These *A.*

TABLE 1. Populations of *Artemia franciscana*, duration of culture cycles in experimental ponds, and corresponding abbreviations used in this study

Population	Pond	Duration (days)	Abbreviation
Macau	1	39	MAC1
	2	31	MAC2
	3	32	MAC3
Areia Branca	1	30	ABR1
	2	43	ABR2
	3	28	ABR3
Grossos	1	42	GRO1
	2	42	GRO2
	3	63	GRO3
	1	36	GRO4

TABLE 2. Average and range values for dissolved oxygen, water temperature, salinity, and transparency recorded throughout ten culture cycles in the experimental *Artemia* farm (range values are given in parentheses). For identification of cycles and populations see Table 1

Cycle	Dissolved oxygen (mg L^{-1})	Water Temperature (°C)	Salinity (ppt)	Transparency (cm)
MAC1	7.7 (2.2 – 15.6)	26.2 (21.1 – 32.3)	97 (84 – 112)	50 (30 – 63)
MAC2	6.4 (3.2 – 10.3)	26.5 (22.5 – 31.9)	94 (90 – 114)	59 (40 – 68)
MAC3	4.4 (2.0 – 11.4)	28.4 (21.5 – 32.3)	88 (83 – 118)	40 (24 – 74)
ABR1	5.3 (3.1 – 8.0)	27.1 (22.8 – 30.8)	82 (80 – 110)	71 (30 – 82)
ABR2	9.7 (2.8 – 13.7)	27.4 (22.8 – 29.6)	85 (77 – 102)	85 (22 – 87)
ABR3	5.5 (2.1 – 9.8)	28.3 (22.7 – 30.2)	87 (80 – 104)	53 (27 – 69)
GRO1	4.8 (1.8 – 9.7)	26.4 (21.2 – 32.0)	81 (83 – 101)	45 (28 – 73)
GRO2	4.7 (1.9 – 11.9)	26.9 (21.7 – 31.9)	96 (83 – 115)	43 (27 – 74)
GRO3	5.1 (1.7 – 12.3)	27.2 (21.3 – 31.4)	95 (83 – 119)	42 (22 – 70)
GRO4	4.2 (1.2 – 13.1)	26.8 (22.1 – 31.5)	87 (83 – 111)	49 (26 – 79)

TABLE 3. Reproductive performance of *Artemia franciscana* females sampled throughout ten culture cycles in the experimental farm. Mode of reproduction is expressed in mean percentage values of oviparous (% Ovip.) and ovoviviparous (% Ovov.) females. Fecundity is expressed in mean ± standard deviation (SD) number of cysts and nauplii for each corresponding set of oviparous and ovoviviparous females (range values are given in parentheses). For identification of cycles and populations see Table 1

Cycle	Mode		Fecundity	
	% Ovip.	% Ovov.	Cysts ± SD	Nauplii ± SD
MAC1	31.30	68.70	33.5 ± 21.4 (11 – 76)	37.2 ± 19.6 (73 – 115)
MAC2	37.42	62.58	29.7 ± 19.6 (19 – 110)	26.9 ± 13.5 (17 – 81)
MAC3	31.68	68.32	33.1 ± 13.6 (22 – 191)	39.2 ± 17.3 (16 – 224)
ABR1	33.08	66.92	24.4 ± 14.7 (12 – 183)	29.4 ± 19.7 (11 – 110)
ABR2	34.32	65.68	36.9 ± 20.0 (21 – 76)	29.5 ± 13.2 (18 – 82)
ABR3	33.94	66.06	33.4 ± 12.0 (14 – 158)	30.0 ± 12.1 (11 – 150)
GRO1	34.74	65.26	38.2 ± 19.6 (21 – 198)	41.7 ± 15.8 (23 – 141)
GRO2	29.83	70.17	37.2 ± 7.1 (12 – 101)	25.8 ± 8.1 (10 – 132)
GRO3	33.24	66.76	34.6 ± 7.1 (17 – 59)	39.7 ± 7.8 (18 – 71)
GRO4	33.97	66.03	28.9 ± 17.2 (20 – 128)	34.7 ± 13.3 (17 – 217)

franciscana populations were predominantly cyst-producing throughout all culture cycles in the experimental farm. For Macau, the percentage of the population producing nauplii varied from 62.58 (MAC2) to 68.70% (MAC1). Nauplii-producing females ranged from 65.68 (ABR2) to 66.92% (ABR1) for Areia Branca, and from 65.26 (GRO1) to 70.17% (GRO2) for Grossos. Regarding fecundity, the average brood size for oviparous females ranged from 29.7 (MAC2) to 33.5 (MAC1) cysts for Macau, from 24.4 (ABR1) to 36.9 (ABR2) cysts for Areia Branca, and from 28.9 (GRO4) to 38.2 (GRO1) cysts for Grossos. For ovoviviparous reproduction, the average number of offspring per female varied from 26.9 (MAC2) to 39.2 (MAC3) nauplii for Macau, from 29.4 (ABR1) to 30.0 (ABR3) nauplii for Areia Branca, and from 25.8 (GRO2) to 41.7 (GRO1) nauplii for Grossos.

DISCUSSION

Average values recorded for water temperature, salinity, and dissolved oxygen in the present study were all within the range considered appropriate for culturing *Artemia* (Tackaert & Sorgeloos, 1991). Salinity and transparency increased towards the end

of each culture cycle. The gradual increase in salinity reflected the management adopted in the pilot farm as well as the high (> 6 mm day^{-1}) evaporation rates typically found in Grossos (RN), north-eastern Brazil (Camara, 2001). The grazing pressure of the fast growing *Artemia* populations prevented blooming concentrations of microalgae and accounted for the decreasing of transparency levels observed towards the end of culture cycles. Indeed, these high transparency measurements denoted restricted food availability conditions and indicated that culture cycles should be terminated.

Artemia franciscana populations from Macau (MAC), Areia Branca (ABR), and Grossos (GRO) reproduced predominantly by ovoviviparity in the present study. Interestingly, populations from MAC, ABR, and GRO exhibited a rather similar pattern of ovoviviparous reproduction. The higher incidence of ovoviviparous to oviparous females for all experimental *A. franciscana* populations is probably related to the relatively stable pond rearing conditions: a combination of adequate levels of temperature, dissolved oxygen, salinity and food. Therefore, the predominance of ovoviviparity would allow the rapid colonization of culture ponds through the production of a large number of nauplii within the shortest possible period. In line with the results on reproductive mode, fecundity was relatively homogeneous within and among MAC, ABR, and GRO populations. The brood sizes observed in the present study match well to the scarce data previously reported for feral *A. franciscana* populations under pond culture conditions. After pond culturing of *A. franciscana* from Macau (Brazil) and Great Salt Lake (USA) in Vietnam for 30 days, Quynh & Lam (1987) found average values of offspring/brood/female of 43.3 ± 31.2 and 51.6 ± 21.4, respectively. More recently, Baert *et al*. (1997) reported average brood sizes of oviparous *A. franciscana* females ranging from 50 to 75 cysts in multi-cycle culture ponds in Vinh Chau (Vietnam).

The absence of significant differences in reproductive mode and fecundity among the experimental brine shrimp populations probably reflects the similarity of pond rearing conditions. However, an alternative explanation for the predominance of ovoviviparous reproduction might be found in the origin of the cysts used in the experiment. Although it is well-accepted that a number of environmental factors, including dissolved oxygen, salinity, temperature, light and iron levels, affect encystment rates in *Artemia* (Browne & Bowen, 1991; Lenz & Browne, 1991; Gajardo *et al*., 2002 and references therein), the influence of a genetic component, which can potentially be acted upon by selection, has been also pointed out in numerous studies (Amat, 1983; Browne, 1982, 1983; Browne *et al*., 1984; Browne & Hoopes, 1990; Abatzopoulos *et al*., 1993; Gajardo *et al*., 2001; Baxevanis & Abatzopoulos, 2004; Baxevanis *et al*., 2004; Kappas *et al*., 2004). Indeed, Gajardo & Beardmore (1989), documented that encystment is under genetic control and is associated, at least in part, with the level of heterozygosity (determined electrophoretically) in the females. Populations of *A. franciscana* in the areas of Macau, Grossos, and Areia Branca reproduce predominantly by ovoviviparity (Camara, 2001). This reproductive pattern might have resulted from the removal of heterozygous genotypes predisposed towards oviparity caused by over-harvesting of cysts (for use in local aquaculture) in these biotopes (Gajardo *et al*., 2002). In this regard, the low percentage of oviparous females observed in the present study might be interpreted as a sort of insurance retained by these *A. franciscana* populations against unstable or stressful conditions. Altogether, these results presumably reflect the homogeneity of MAC, ABR, and GRO populations and are consistent with the suspected decrease of genotypic diversity (heterozygosity) of feral *A. franciscana* in the local saltworks. In perspective, molecular investigations are strongly recommended to shed light on microevolutionary changes that are likely to have occurred since the inoculation of *A. franciscana* in north-eastern Brazil.

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