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# Effect of temperature on survival, growth and reproductive characteristics of *Artemia* (Crustacea: Anostraca) from Vietnam and Iran

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## Abstract

Survival, growth and reproductive performances of three *Artemia franciscana* and two Iranian *Artemia* populations were assessed at two temperatures (26 and 30°C). The former, cysts from San Francisco Bay (SFB), USA were used as a reference, were cultured after 1 year (Y1) and several years in Vinh Chau salt fields (VC), Vietnam. The latter originated from Urmia Lake (URM) and from Maharloo Lake (MAH), Iran. There was significant interaction between temperature and population for survival, growth and the majority of reproductive traits. These results indicated that populations respond differently to different temperatures where the Iranian *Artemia* populations seem not well adapted to high temperatures compared to *A. franciscana*. URM is more sensitive to high temperatures than MAH. The MAH sample appears to be close to the SFB population in most characteristics. The observations at 30°C suggest that the reproductive performance of the experimental populations follows an order of VC>Y1> SFB>MAH>URM. Therefore, the VC population may be considered as the best candidate, to be selected for inoculation. Moreover, data on survival and growth of the Y1 population indicate its relatively quick adaptation to a new habitat (Vietnam).

**Key Words:** *Artemia*, temperature, survival, reproductive characteristic

## Introduction

In view of the importance of *Artemia* as part of the live food chain for the culture of fish and shellfish larvae, and taking into account the present cyst shortage on the market, the requirement for commercial exploitation and the development of new *Artemia* sources is a real necessity (Lavens and Sorgeloos 2000). This problem can be solved either by natural harvesting from new *Artemia* sites (such as France, P.R. China, Columbia, Argentina, Canada), or by another approach that has been used during the last couples of decades in many developing countries with a monsoon climate (Thailand, Philippines, Vietnam, etc). This technique implies inoculation in man-managed salt ponds with non-endemic *Artemia* nauplii, with the objective to produce cysts. However, different populations of *Artemia* have different responses to different ecological conditions (Sorgeloos, 2001).

Vietnam has developed the seasonal culture of *Artemia franciscana* originating from San Francisco Bay, USA (SFB) in Vinh Chau saltfields since 1984 for cyst production. The efforts of the Can Tho University have eventually led to the establishment of a commercial *Artemia* cyst production in coastal salt farms of the Mekong Delta since 1990s. So far these works were demonstrated to be very successful and provided considerable socio-economic benefits (Son, 2012). The SFB population was inoculated into Vinh Chau salt works, Vietnam, where water temperatures are substantially higher than in San Francisco Bay. Cloern and Dufford (2005) conducted the survey of phytoplankton community ecology in San Francisco Bay for a decade (1992 to 2001), they reported that a broad range of water temperature was observed with average values of 8.7-22.7°C where maximum water temperatures during the summer and autumn growing season seldom exceed 24°C and are usually several degrees lower. On the contrary, the maximum water temperatures in *Artemia* ponds in Vietnam are near 37°C for the largest period of the culture season, sometimes reaching up to 40°C or more, but never below 20°C (Baert *et al*., 1997; Anh *et al*., 2010). SFB *Artemia* may have a high tolerance to a wide temperature range, but possibly after a number of generations adaptive changes occur as a response to the new environment.

In order to evaluate the potential of the *Artemia* resources in Urmia Lake, Iran, the studies of Van Stappen *et al*. (2001) and Abatzopoulos *et al*. (2006) on the reproductive and lifespan characteristics of the endemic *A. urmiana* they revealed that oviparity is the dominant reproductive mode, a favourable characteristic for commercial exploitation of the population. More and more attention is paid to alternative Iranian *Artemia* resources, like Maharloo Lake (South Iran), which are candidates for exploitation, or for inoculation at other sites. It was therefore the purpose of this study to examine the effect of different temperature regimes on survival, growth and reproductive characteristics of *Artemia* from Vietnam and Iran under laboratory conditions. For the Vietnamese cyst material, the results were compared with the original SFB population, in order to assess the degree of adaptation to the Vietnamese environment.

## Material and methods

### Source of *Artemia* cysts:

The *Artemia* cyst samples used in these experiments were obtained from the cyst bank of the Laboratory of Aquaculture & *Artemia* Reference Center, Belgium. The cyst sample of the VC population was obtained from Can Tho University, Vietnam. The five *Artemia* populations studied and abbreviations used as followed:

-San Francisco Bay, California, USA: SFB (*A. franciscana*)
-First generation production in Vinh Chau saltworks, Vietnam, from SFB 1258 inoculum: Y1
-SFB inhabiting more than 10 years Vinh Chau saltworks, Vietnam: VC
-Urmia Lake, Iran: URM (*A. urmiana*)
-Maharloo Lake, Iran: MAH (parthenogenetic population)

### Preparation of Instant Ocean® culture medium:

The culture medium was prepared by dissolving Instant Ocean® (synthetic sea salt, Aquarium Systems, Inc, Mentor, Ohio, USA) salt mixture in

deionized water. After 2-3 days with strong aeration, the salt was completely dissolved and the salinity was about 150-200 ppt. The aeration was stopped for 1 day in order to allow sedimentation of particles, then the saline water was pumped and filtered through a 5 μm filter. Finally, it was diluted with deionized water to the culture salinity of 80 ppt. A refractometer (ATAGO, S-10, Japan) was used for determining the desired salinity.

### *Artemia* culture

*Cyst hatching*:

To obtain nauplii for the culture experiment, 1 gram of cysts from each sample was incubated in 800 ml Instant Ocean® solution of 34±1 ppt in cylindroconical glass cones. Water temperature was kept at 28°C during the hatching process; strong aeration was provided through an air filter aeration pipe down to the cone bottom. Hatching conditions were according to Van Stappen (1996). After 24 hours of incubation, the instar I nauplii were transferred to the culture medium.

*Culture conditions*:

All tests were carried out at a salinity of 80 ppt. The experimental water temperatures were 26 and 30±1°C. The water temperature was regulated by a thermostat, submerged in the water bath. This experiment was performed at reduced light intensity (1 TL-lamp at a distance of about 3 meters), except during feeding and observation.

After hatching, 200 freshly hatched nauplii were carefully transferred into cylindro-conical glass tubes with 400 ml Instant Ocean® solution of 80 ppt, covered with petridishes perforated to minimize evaporation, and provided with gentle point aeration from the bottom. The initial animal density was one animal per 2 ml while from day 8 and from day 11 onwards it was reduced to one animal per 3 and 4 ml, respectively.

From day 12 onwards, sexual differentiation, with "riding couples" and gravid females, was observed in the stock cultures. These couples were separated from the stock cultures and each couple was kept in a plastic Falcon tube containing 50 ml medium of 80 ppt salinity, kept in a water bath at 26 or 30±1°C in order to observe the reproductive characteristics.

*Feeding*:

The animals were fed on a mixed diet of the alga *Dunaliella tertiolecta* (25%) and of the yeast-based formulated feed (75%) LANZY PZ from INVE Aquaculture SA, Belgium. Feeding was done once a day based on the standard feeding schedule presented in Table 1 (Coutteau *et al*., 1992).

A concentrated stock suspension of 18 x $10^6$ cells/ml was harvested from the culture and stored at 4-6°C in the fridge. Lanzy at 54 x $10^6$ cells/ml was prepared by mixing 3.9 g of the commercial product with 600 ml Instant Ocean® solution (80 ppt) and was also stored at 4-6°C. These suspensions could be used for 3-5 days. Each couple was fed daily 200 μl of a stock suspension of *D. tertiolecta* (density of 36 x$10^6$ cells/ml) and the same volume from Lanzy (density of 108 x $10^6$ cells/ml).

**Tab. 1: Feeding schedule for 200 animals (stock suspensions with concentrations of 18 x $10^6$ cells/ml *Dunaliella tertiolecta* and 54 x $10^6$ cells/ml Lanzy PZ).**

| Day of culture | *D. tertiolecta* (ml/day) | Lanzy PZ (ml/day) |
|---|---|---|
| 1 | 0.42 | 0.42 |
| 2, 3, 4 | 0.83 | 0.83 |
| 5, 6 | 1.25 | 1.25 |
| 7 | 1.65 | 1.65 |
| 8 | 2.12 | 2.12 |
| 9 | 3.40 | 3.40 |
| 10, 11 | 4.00 | 4.00 |
| 12, 13 | 5.00 | 5.00 |
| 14, 15 | 6.00 | 6.00 |
| 16, 17 | 7.00 | 7.00 |
| 18, 19 | 8.50 | 8.50 |
| 20 and onwards | 10.0 | 10.0 |

*Water exchange*:

The culture medium was renewed for 100% on day 8 and day 11, and every three to four days thereafter, until the animals reached the adult stage and started to reproduce.

For the couple cultures, water renewal was performed every one or two days.

## Experimental design

### Stock culture to sexual maturity:

The experiment was designed to determine the survival and growth rate of five experimental populations (SFB, Y1, VC, URM and MAH) on day 8 and day 11 at temperatures of 26 and 30°C. Each treatment had four replicates. After 11 days, the 4 replicates of each treatment were pooled into a single 2-liter plastic cone. This culture was maintained until sexual maturing was reached and individual couples could be isolated.

### Culture of individual couples for monitoring the reproductive characteristics:

Each experimental population had 50 replicates. A female dying within 1-3 days after incubation was replaced by another female. Beyond this period, the replicate was cancelled. A male dying was replaced by another male (Amat *et al*., 2007).

The reproductive characteristics were recorded during one month; however, the culture of a particular population was terminated if its female mortality had reached more than 50%.

### Data collection

*Determination of survival and growth rate:*

The number of animals counted at day 8 and day 11 was compared to the initial number and calculated as percentage survival.

To determine the growth rate, ten animals were taken randomly from each treatment and were fixated by adding a few drops of lugol. Body length of animals was measured from the tip of the head till the end of the telson by means of a dissection microscope, equipped with a camera lucida. The data were analyzed with a digitizer (KD 4300, Graphtec Corp., Japan).

*Determination of reproductive characteristics:*

Offspring production was checked daily, and offspring was harvested with a 50μm filter and counted under a binocular. The following reproductive characteristics were observed or calculated:

-Total pre-reproductive period (days): female was scored from hatching until first spawning (only reproducing females were taken into account for calculation).

-Total reproductive period (days): female was scored from first spawning until death or end of the experiment; *i.e*. the experiment was terminated before the post-reproductive period was attained for most replicates.

-Brood interval (days)

-Total broods per female

-Oviparous broods per female

-Ovoviviparous broods per female

-Total offspring per female

-Offspring per female per day (from the first spawning until death or the end of study)

-Percent offspring encysted

### Statistical analysis

The data of survival rate and percentage offspring encysted were normalized through an arcsin transformation before statistical treatment. A two-factor ANOVA test (STATISTICA, version 6.0) was used to detect significant interactions between population and temperature. For all treatments, results were analyzed statistically with one-way ANOVA analysis of variance to find the overall effect of the treatment. Tukey's HSD test was used to identify significant differences between the experimental sample means at a significance level of $p<0.05$. As no reproductive data were available for the culture of the URM population at 30°C, zero values were included here to complete the data set for statistical analysis. Unequal numbers of replicates were only used for the parameter "pre-reproductive period", as only reproducing females were taken into account here.

## Result

### Effect of temperature on survival and growth rate:

*Survival*

Two-factor ANOVA analysis of the survival data showed that there was highly significant interaction between population and temperature, both after 8 and 11 days of culture ($p=0.0004$ and $p=0.0001$), respectively. The survival at a given temperature is thus dependent on the population.

The survival of the experimental populations

after 8 and 11 days of culture at 26°C and 30°C is presented in Table 2. Statistical analysis showed that at 26°C there was no significant difference in survival after both culture periods, although URM had a lower survival (79.8 and 73.9%, respectively) compared to the other populations.

**Tab. 2: Survival (%) after 8 and 11 days of culture at 26°C and 30°C (a: effect of population ; b: effect of temperature)**

**a)**

| | Population | | | | |
|---|---|---|---|---|---|
| | **VC** | **SFB** | **Y1** | **URM** | **MAH** |
| ***26°C*** | | | | | |
| Survival day 8 | 89.1±12.9[a] | 92.4±5.3[a] | 91.6±5.8[a] | 79.8±14.3[a] | 92.1±2.9[a] |
| Survival day11 | 88.4±13.2[a] | 83.4±4.8[a] | 88.6±6.3[a] | 73.9±12.0[a] | 90.8±4.8[a] |
| ***30°C*** | | | | | |
| Survival day 8 | 97.0±1.1[a] | 91.1±0.9[a] | 92.8±5.8[a] | 52.9±5.0[b] | 68.3±8.0[c] |
| Survival day11 | 96.3±1.7[a] | 75.3±9.3[b] | 92.0±5.4[a] | 31.3±8.6[c] | 67.1±7.6[b] |

Values on the same row sharing different superscript are significantly different ($p<0.05$).

**b)**

| | Survival at day 8 | | Survival at day 11 | |
|---|---|---|---|---|
| **Population** | **26°C** | **30°C** | **26°C** | **30°C** |
| VC | 89.1[a] | 97.0[a] | 88.4[a] | 96.3[a] |
| SFB | 92.4[a] | 91.1[a] | 83.4[a] | 75.3[a] |
| Y1 | 91.6[a] | 92.8[a] | 88.6[a] | 92.0[a] |
| URM | 79.8[a] | 52.9[b] | 73.9[a] | 31.3[b] |
| MAH | 92.1[a] | 68.3[b] | 90.8[a] | 67.1[b] |

Values on the same column sharing different superscript are significantly different ($p<0.05$).

The results for survival at day 8 at 30°C showed that the survival of VC, SFB and Y1 were similar while significant differences ($p<0.01$) in survival existed between the *A. franciscana* populations (VC, SFB and Y1) on one hand (survival >90%) and the Iranian populations (URM and MAH) on the other. Furthermore, URM had a significantly lower survival as compared to MAH ($p<0.05$) at day 8. However, the survival at day 11 showed a significantly lower value for SFB than for VC and Y1 ($p<0.01$). A sharp decline in survival (31.3%) was obtained for URM at 30°C. According to the analysis of the temperature effect, (Table 2b) the *A. franciscana* populations (VC, SFB and Y1) do not show any significant effect. In contrast, the Iranian populations (URM and MAH) show a significantly lower survival at 30°C, compared to 26°C.

*Growth*

A two-factor ANOVA analysis of the growth data on day 8 and day 11 revealed a significant interaction between population and temperature ($p=0.0011$ and $p=4.4\times10^{-7}$). Table 3a shows the growth after 8 and 11 days of culture at 26°C and 30°C of the different test populations. The URM population was significantly longer than all other populations at day 11, both at 26°C (8.6 mm) and 30°C (7.7 mm). The long abdomen of this population was obvious also by visual observation.

At 26°C, significant differences among the *A. franciscana* samples only occurred at day 11 (SFB being smaller than both VC and Y1). Length at 30°C showed a similar pattern, with VC being significantly longer than both SFB and Y1. For nearly all samples, growth at 26°C was better than or equal to growth at 30°C; only the VC *Artemia* grew into bigger animals, when cultured at 30°C, compared to 26°C (Table 3b).

**Tab. 3: Growth (mm) after 8 and 11 days of culture at 26°C and 30°C. (a: effect of population; b: effect of temperature)**

**a)**

| | Population | | | | |
|---|---|---|---|---|---|
| | **VC** | **SFB** | **Y1** | **URM** | **MAH** |
| ***26°C*** | | | | | |
| Length day 8 | $4.3\pm0.2^{abc}$ | $3.8\pm0.^{2ab}$ | $4.5\pm 0.3^{bc}$ | $6.5\pm0.3^{d}$ | $6.2\pm0.2^{d}$ |
| Length day 11 | $6.1\pm0.1^{a}$ | $4.9\pm0.2^{b}$ | $6.2\pm0.2^{a}$ | $8.6\pm0.5^{c}$ | $6.8\pm0.3^{d}$ |
| ***30°C*** | | | | | |
| Length day 8 | $4.6\pm0.1^{cde}$ | $3.5\pm 0.2^{bc}$ | $4.1\pm0.5^{abc}$ | $6.3\pm0.5^{d}$ | $5.0\pm0.3^{de}$ |
| Length day 11 | $6.5\pm0.1^{a}$ | $4.9\pm0.1^{b}$ | $5.8\pm0.2^{c}$ | $7.7\pm0.2^{d}$ | $5.6\pm0.2^{c}$ |

Values on the same row sharing different superscript are significantly different ($p<0.05$).

**b)**

| | Length at day 8 | | Length at day 11 | |
|---|---|---|---|---|
| **Population** | **26°C** | **30°C** | **26°C** | **30°C** |
| VC | $4.3^{a}$ | $4.6^{b}$ | $6.1^{a}$ | $6.5^{b}$ |
| SFB | $3.8^{a}$ | $3.5^{a}$ | $4.9^{a}$ | $4.9^{a}$ |
| Y1 | $4.5^{a}$ | $4.1^{a}$ | $6.2^{a}$ | $5.8^{b}$ |
| URM | $6.5^{a}$ | $6.3^{a}$ | $8.6^{a}$ | $7.7^{b}$ |
| MAH | $6.2^{a}$ | $5.0^{b}$ | $6.8^{a}$ | $5.6^{b}$ |

Values on the same column sharing different superscript are significantly different ($p<0.05$).

## Effect of temperature on reproductive characteristics

The statistical data of the reproductive characteristics are summarized in Table 4. In the URM population, all mature males and females died after a few hours, when exposed to 30°C. In the statistical analysis, the corresponding reproductive characteristics were represented by zero values. The mean values for the different reproductive and lifespan characteristics are grouped in Table 4.

*Female pre-reproductive period:*

Two-factor ANOVA test for the prereproductive period reveals that there was no significant interaction between temperature and population ($p=0.2374$).

At 26°C, the prereproductive period of VC, Y1 and MAH populations was similar, ranging between 17.9 and 18.6 days. SFB showed a significantly higher intermediate value (20.1 days), while URM had the highest value (27.1 days). At 30°C, all populations showed the same trend as at 26°C. No significant differences were found between the values at both temperatures (Table 4b), except for VC, which had a significantly shorter period at 30°C (17.7 days) as compared to 26°C (18.6 days).

*Female reproductive period:*

A two-factor ANOVA ($p=1.6\times10^{-23}$) indicated highly significant interaction between population and temperature for this parameter.

In general, *A. franciscana* samples had a much longer reproductive period than the Iranian populations at both temperatures ($p<0.01$). In particular, URM showed the shortest period (1.4 days) with highly significant difference ($p<0.01$) as compared to the other samples. MAH had a significantly longer reproductive period at 26°C (8.5 days), compared to 30°C (3.3 days). Within the *A. franciscana* samples, significant differences were not found at 26°C, although the Y1 population showed a slightly higher value (13.5 days) than VC (12.7 days) and SFB (10.7 days). In contrast, at 30°C the value for VC was significantly higher, as compared to Y1

and SFB ($p<0.01$) with the order of VC>Y1>SFB. Comparison of the effect of temperature within VC, SFB and Y1, at 30°C VC had a significantly longer reproductive period than at 26°C. For SFB, the opposite effect was found while there was no effect for Y1.

*Total broods per female:*

There was a highly significant interaction between population and temperature (two-factor ANOVA, $p=1.8x10^{-26}$).

The number of broods per female showed the same trend as the reproductive period: the Iranian populations had lower numbers of brood (in the range 0.2-2.8) compared to the *A. franciscana* samples (range 3.1-7.3) at both 26°C and 30°C ($p<0.01$). At 26°C, the values for VC and Y1 were similar, and SFB had a significantly lower value than VC and Y1. On the contrary, at 30°C these values showed a highly significant difference ($p<0.01$), with the same rank of the reproductive period VC>Y1>SFB. VC had the highest number of broods at 30°C while Y1 and SFB had similar numbers as compared to 26°C. MAH showed a significantly lower value at 30°C than at 26°C. In general, populations with a longer reproductive period had a higher number of broods.

*Oviparous broods per female:*

Two-factor ANOVA detected an interactive effect ($p= 0.0220$) for oviparous broods. The numbers of oviparous brood per female was overall low in all cases (highest value: 1.3) especially at 30°C (all values <1.0); URM reproduced exclusively ovoviviparously at 26°C. No significant differences were found between the other populations.

*Ovoviviparous broods per female:*

Two-factor ANOVA showed a highly significant interaction between population and temperature ($p= 8.3x10^{-19}$) for this parameter. The VC and Y1 populations had the same number (3.5) of ovoviviparous brood at 26°C; SFB and MAH were similar and significantly lower than VC and Y1 at 26°C. The lowest numbers were found at both temperatures for the Iranian populations, especially URM. At 30°C, the *A. franciscana* samples showed values in the following order: VC>Y1>SFB. At 26°C, a lower number of ovoviviparous broods was found than at 30°C for all populations except MAH. Particularly, VC had a significantly higher number (6.6) at 30°C ($p<0.01$).

*Total offspring per female:*

A highly significant interactive effect (two-factor ANOVA, $p=1.9x10^{-18}$) was observed, and may reflect the reproductive performance of these populations following an order of VC>Y1>SFB>MAH>URM at both 26°C and 30°C.

The *A. franciscana* samples had a significantly higher total offspring per female than the Iranian populations at both temperatures. At 26°C the value for SFB was significantly lower than for VC and Y1; at 30°C these three populations had significantly different values, with VC> Y1>SFB. URM showed an extremely low value (11.6 at 26°C), while MAH showed intermediate values (at both temperatures) between URM and the *A. franciscana* samples. At 30°C, MAH, SFB and Y1 had a lower number of offspring than at 26°C, while VC had a significantly higher value ($p<0.01$).

*Offspring per female per day:*

By two-factor ANOVA analysis for this characteristic, no significant interaction between population and temperature was found ($p= 0.1063$).

At 26°C, all populations showed similar values ($\pm$40), except for URM with a significantly lower value (9.9). At 30°C, MAH had a significantly higher number (49.3) than all other populations (30-39). Again, values for the *A. franciscana* populations were similar, both at 26°C and 30°C. Although for the *A. franciscana* populations the values at 30°C were lower than at 26°C, and the opposite for MAH, differences between values at both temperatures were not significant for each single population.

*Percent offspring encysted:*

There was a significant interaction between population and temperature (two-factor ANOVA, $p=0.0022$). At 26°C, no clear differences were found between the respective populations (highest value

**Table 4 Reproductive and lifespan characteristics for experimental *Artemia* populations at 26°C and 30°C. (a: effect of population; b: effect of temperature)**

**a)**

| | 26°C | | | | | 30°C | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Characteristics** | **VC** | **SFB** | **Y1** | **URM** | **MAH** | **VC** | **SFB** | **Y1** | **URM** | **MAH** |
| Pre-reproductive period* (d) | 18.6$^{a}$ (1.9) | 20.1$^{b}$ (2.5) | 18.6$^{a}$ (1.9) | 27.1$^{c}$ (2.4) | 17.9$^{a}$ (2.3) | 17.7$^{a}$ (2.1) | 20.5$^{b}$ (3.5) | 18.8$^{a}$ (2.2) | 0.0$^{c}$ | 18.1$^{a}$ (2.1) |
| Reproductive period (d) | 12.7$^{ab}$ (3.9) | 10.7$^{abc}$ (4.9) | 13.5$^{ab}$ (4.5) | 1.4$^{d}$ (1.3) | 8.5$^{bc}$ (7.2) | 20.6$^{a}$ (4.7) | 8.2$^{b}$ (4.0) | 13.4$^{c}$ (3.3) | 0.0$^{d}$ | 3.3$^{e}$ (2.9) |
| Total broods per female | 4.3$^{a}$ (1.4) | 3.4$^{b}$ (1.4) | 4.6$^{a}$ (1.4) | 0.2$^{c}$ (0.5) | 2.8$^{b}$ (1.8) | 7.3$^{a}$ (1.7) | 3.1$^{b}$ (1.3) | 4.8$^{c}$ (1.2) | 0.0$^{d}$ | 1.7$^{e}$ (1.0) |
| Oviparous broods per female | 0.8$^{a}$ (1.3) | 1.3$^{a}$ (1.5) | 1.1$^{a}$ (1.2) | 0.0$^{b}$ (0.0) | 1.1$^{a}$ (1.2) | 0.7$^{ab}$ (1.3) | 0.4$^{abc}$ (0.9) | 0.6$^{ab}$ (1.1) | 0.0$^{bc}$ | 0.7$^{ab}$ (0.8) |
| Ovoviviparous broods per female | 3.5$^{a}$ (1.7) | 2.1$^{b}$ (1.8) | 3.5$^{a}$ (1.8) | 0.2$^{c}$ (0.5) | 1.7$^{b}$ (1.5) | 6.6$^{a}$ (1.9) | 2.7$^{b}$ (1.5) | 4.2$^{c}$ (1.6) | 0.0$^{d}$ | 1.0$^{e}$ (1.0) |
| Total offspring per female | 557.5$^{a}$ (220.4) | 388.3$^{b}$ (211.0) | 551.8$^{a}$ (244.9) | 11.6$^{c}$ (27.8) | 258.6$^{d}$ (203.5) | 787.4$^{a}$ (184.5) | 194.8$^{b}$ (113.1) | 495.1$^{c}$ (149.1) | 0.0$^{d}$ | 126.0$^{e}$ (82.1) |
| Offspring per female per day | 43.4$^{a}$ (16.1) | 38.2$^{a}$ (20.6) | 40.1$^{a}$ (12.1) | 9.9$^{b}$ (24.0) | 44.7$^{a}$ (35.5) | 39.0$^{a}$ (6.8) | 30.0$^{a}$ (23.7) | 38.1$^{a}$ (11.1) | 0.0$^{b}$ | 49.3$^{c}$ (29.4) |
| Percent offspring encysted | 21.1$^{ac}$ (31.5) | 41.4$^{bc}$ (41.8) | 27.1$^{abc}$ (30.5) | 0.0$^{d}$ | 38.8$^{bc}$ (37.9) | 11.8$^{a}$ (18.1) | 14.7$^{a}$ (31.1) | 15.8$^{a}$ (26.5) | 0.0$^{a}$ | 42.8$^{b}$ (45.2) |
| Brood interval (d) | 2.5$^{a}$ (1.0) | 3.1$^{a}$ (2.2) | 2.5$^{a}$ (0.7) | - | 3.1$^{a}$ (1.4) | 2.2$^{a}$ (0.5) | 2.5$^{a}$ (1.3) | 2.5$^{a}$ (1.3) | - | 2.1$^{a}$ (1.8) |

(*) Pre-reproductive period was only calculated for those females producing offspring. The numbers within parenthesis indicate standard deviation.
For each temperature, means with different superscripts within the same row are significantly different (P< 0.05).

**b)**

| Population | Pre-reproductive period (day) | | Reproductive period (day) | | Total broods per female | | Oviparous broods per female | | Ovoviviparous broods per female | | Total offspring per female | | Offspring per female per day | | %Offspring encysted | | Brood interval (day) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **26°C** | **30°C** | **26°C** | **30°C** | **26°C** | **30°C** | **26°C** | **30°C** | **26°C** | **30°C** | **26°C** | **30°C** | **26°C** | **30°C** | **26°C** | **30°C** | **26°C** | **30°C** |
| **VC** | 18.6$^{a}$ | 17.7$^{b}$ | 12.7$^{a}$ | 20.6$^{b}$ | 4.3$^{a}$ | 7.3$^{b}$ | 0.8$^{a}$ | 0.7$^{a}$ | 3.5$^{a}$ | 6.6$^{b}$ | 558$^{a}$ | 787 | 43.4$^{a}$ | 39.0$^{a}$ | 21.1$^{a}$ | 11.8$^{b}$ | 2.5$^{a}$ | 2.2$^{a}$ |
| **SFB** | 20.1$^{a}$ | 20.5$^{a}$ | 10.7$^{a}$ | 8.2$^{b}$ | 3.4$^{a}$ | 3.1$^{a}$ | 1.3$^{a}$ | 0.4$^{b}$ | 2.1$^{a}$ | 2.7$^{a}$ | 388$^{a}$ | 195$^{b}$ | 38.2$^{a}$ | 30.0$^{a}$ | 41.4$^{a}$ | 14.7$^{b}$ | 3.1$^{a}$ | 2.5$^{a}$ |
| **Y1** | 18.6$^{a}$ | 18.8$^{a}$ | 13.5$^{a}$ | 13.4$^{a}$ | 4.6$^{a}$ | 4.8$^{a}$ | 1.1$^{a}$ | 0.6$^{b}$ | 3.5$^{a}$ | 4.2$^{b}$ | 552$^{a}$ | 495$^{a}$ | 40.1$^{a}$ | 38.1$^{a}$ | 27.1$^{a}$ | 15.8$^{a}$ | 2.5$^{a}$ | 2.5$^{a}$ |
| **URM** | 27.1 | - | 1.4 | - | 0.2 | - | 0.0 | - | 0.2 | - | 11.6 | - | 9.9 | - | 0.0 | - | - | - |
| **MAH** | 17.9$^{a}$ | 18.1$^{a}$ | 8.5$^{a}$ | 3.3$^{b}$ | 2.8$^{a}$ | 1.7$^{b}$ | 1.1$^{a}$ | 0.7$^{a}$ | 1.7$^{a}$ | 1.0$^{b}$ | 259$^{a}$ | 126$^{b}$ | 44.7$^{a}$ | 49.3$^{a}$ | 38.8$^{a}$ | 42.8$^{a}$ | 3.1$^{a}$ | 2.1$^{b}$ |

(Reproductive period: 1 month). For each temperature, means with different superscripts within the same column are significantly different (P< 0.05).

for SFB: 41.4%; lowest value for VC: 21.1%); in particular, URM reproduced exclusively ovoviviparously and thus had 0% encystment. At 30°C, the results for the *A. franciscana* samples were similar (range 11.8-15.8%); these values were significantly lower than the MAH value (42.8%) at this temperature. Comparison between the two temperatures for each single *A. franciscana* population shows a decline at 30°C. However, for Y1 this decrease is not significant. Conversely, MAH had a slightly higher value at 30°C than at 26°C, although there was no significant difference between both values.

*Brood interval:*

The interaction between population and temperature was not significantly different (two-factor ANOVA, p=0.0869) for the brood interval. All populations showed similar values for this parameter, both at 26°C and at 30°C. Though at 30°C the brood interval was slightly shorter for each population than at 26°C, a significant difference was only found for the MAH population.

## Discussion

The results from this study indicated that temperature had an important effect on the survival, growth and reproductive characteristics of the experimental *Artemia* samples that are three crucial parameters in the selection of populations for inoculation in a new habitat. Furthermore, the effect of temperature on the survival, growth and reproductive performance varied as a function of the geographical origin of the *Artemia* populations. This result is in agreement with the results of Vanhaecke and Sorgeloos (1989) who studied the effect of temperature on cyst hatching, larval survival and biomass production for different geographical populations of *Artemia* spp. and the results of Browne *et al*. (1988) where they examined the effect of temperature on reproductive performance of different geographic origin.

### Survival:

The results showed that the *Artemia franciscana* populations (SFB, Y1 and VC) had a significantly higher survival than the Iranian populations (URM and MAH) at high temperature (30°C). Also, Vanhaecke and Sorgeloos (1989) observed that *A. franciscana* populations are most resistant whereas *A. salina* and *A. persimilis* populations are very sensitive to high temperatures. At 26°C, the survival for both the *Artemia franciscana* and the Iranian samples were not significantly different (ANOVA, p>0.05). This temperature tolerance range may refer to the natural environment, although according to Browne and Wanigasekera (2000) for all *Artemia* populations the common temperature optimum was 25°C.

The survival of the *Artemia franciscana* populations (SFB, Y1 and VC) was more than 90% after 8 days of culture at 26°C and 30°C (Table 2a). After 11 days, the survival at 26°C was similar to the values of Vanhaecke *et al*. (1984), and at 30°C even higher (75.3-96.3%), as these authors found that the SFB population had more than 90% survival at a range of 18 to 25°C and 50% mortality occurred after a period of nine days at 29-30°C.

Comparing the survival for SFB, Y1 and VC populations revealed the different responses to both temperatures studied. The SFB population, originating from San Francisco Bay (USA), appears to be less tolerant to high temperature (30°C) than Y1 and VC. This difference is probably related to the temperature of the natural habitat; *i.e*. the mean annual temperature of San Francisco Bay is about 25°C with extremes between 8°C in January and 33°C in August (Cloern, and Dufford, 2005). The water temperatures in *Artemia* ponds in Vinh Chau (Vietnam) on the contrary, fluctuate from 22°C up to >40°C, depending on the water depth and salinity (Anh *et al.*, 2010). The Y1 population, a SFB population inhabiting one year Vinh Chau saltfields, showed a survival intermediate to SFB and VC at 30°C. This response could be related to a heat shock protein, present in the population; in this case the concentration of this protein might increase after a number of generations in the new habitat with higher average ambient temperature. Crack *et al*. (2002) illustrated that heat shock proteins may be involved in the production of thermotolerance and possibly other forms of stress resistance, including

dehydration stress in *Artemia* cysts and larvae.

In addition, Clegg *et al*. (2000) compared thermal resistance, developmental rate and heat shock proteins (hsp) in brine shrimp, *A. franciscana*, from San Francisco Bay and southern Vietnam. They found that adults produced in the laboratory from SFB cysts were much less resistant to high temperatures than adults from VC cysts; *e.g*. at a lethal temperature the time required to kill 50% of the animals was a little over 60 minutes for VC adults, and about 35 minutes for SFB animals. This indicated that the VC cysts retained the developmental potential to produce heat-resistant adults, even though cultured under the same conditions as SFB animals, and at considerably lowers temperatures than they experienced in the ponds in Vietnam. However, this difference was not related to the levels of the hsp70 family, which were similar in both groups of animals. These authors also suggested that the difference in thermal tolerance between SFB and VC populations were maintained in the second generation, the natural selection process in the Vietnamese ponds has created adults with higher thermal resistance.

Other study from Clegg *et al*. (2001) who conducted in the laboratory for resistance to high temperature and relative contents of three stress proteins (Hsp-70, artemin and p26) between VC and SFB cysts. They found that thermal adaptation took place rapidly, during the first growing season. The increase in thermal tolerance was reflected in an overall increase in stress protein content, compared to SFB cysts used for the initial inoculation. Our results are thus in agreement with the results of Clegg *et al*. (2000; 2001).

The Iranian populations (URM and MAH) also showed a different performance at different temperatures (Table 2a and 2b). At 30°C, the survival of URM was significantly lower than of MAH ($p<0.01$). This result is comparable to the results of Triantaphyllidis *et al*. (1994), who illustrated that *A. urmiana* is less tolerant to high temperatures than *A. franciscana*; *e.g*. at 35°C the average hatching percentage of *A. franciscana* was 77.9% while *A. urmiana* was 3.6%. The difference in temperature tolerance between the URM and MAH samples may be related to their seasonal changes in temperature. For example, the water temperature in Urmia Lake ranges from -1°C to 26°C while Maharloo Lake fluctuates in the range 12-32.5°C (Abatzopoulos *et al*., 2006).

**Growth rate:**

Within physiological temperature range, the growth rate of organisms increase with temperature and growth performance was affected by temperature (Vanhaecke and Sorgeloos, 1989). The Iranian *Artemia* individuals were significantly longer than *A. francicana* populations at 26°C, whereas growth rate in SFB, URM and MAH was retarded at 30°C. The URM population was significantly longer than all other populations at day 11, both at 26°C and 30°C. This could be attributed to morphological characteristics; *i.e*. *A. urmiana* has a body with very long abdomen (Van Stappen, 1997). Our results seem to confirm the findings of Vanhaecke and Sorgeloos (1989) and Barata *et al*. (1996) that growth is a population specific characteristic.

Difference in growth rate between *A. franciscana* populations at 26°C and 30°C may be due to genetic adaptation to the habitat temperature. The growth response of these populations to temperatures is similar to the observations made for survival. For all populations, only the VC population had a better growth at 30°C than at 26°C (the other populations showed a similar or slower growth) illustrating the adaptation of the VC population to the high temperature environment.

**Reproductive characteristics:**

Variation in temperature can have major effects on such life-history traits as the maturation period, length of the reproductive period, total life span, brood size, brood numbers, brood interval and total offspring production (Browne and Wanigasekera 2000). The reproductive output varies greatly among populations (Gajardo *et al*., 2001; Amat *et al*., 2007). According to Lenz (1987), females with a short reproductive period will tend to concentrate their reproductive efforts in a small number of large and closely spaced broods. Longer lived ones will distribute their reproductive effort.

Under laboratory conditions, the reproductive characteristics varied between *A. franciscana* and the Iranian populations at 26°C and 30°C. The ANOVA analyses showed that most reproductive characteristics did not show significant differences at 26°C while highly significant differences were mainly found at 30°C.

The length of the pre-reproductive period may be considered as a key factor in determining population growth rate. Browne and Wanigasekera (2000) compared the lifespan components and reproductive values for four species of *Artemia* (*Artemia franciscana*, *A. salina*, *A. sinica*, *A. persimilis*) and parthenogenetic population cultured at different temperatures (15, 24 and 30°C) and salinities, author found that all *Artemia* populations matured more rapidly at 30°C than 15 and 24°C. This is comparable with the VC population, having a shorter maturation time at 30°C than at 26°C. However, values for the other populations were not significantly different at both temperatures. URM had the longest development time to produce offspring (27.1 days) at 26°C. Our laboratory data for the *A. franciscana* samples for this parameter are much longer (18-20 days), compared to the observations of *Artemia* cultured in Vinh Chau saltfields, ranging from 10 to 15 days (Baert *et al*., 1997; Anh *et al*., 2010).

The reproductive period is an important factor that also reflects the animals' tolerance to high temperature. At a given temperature, the *Artemia* adults can survive and reproduce offspring, but the level of these performances may be much less than the optimal temperature range, as is illustrated by the Iranian populations, as compared to the *A. franciscana* populations (Table 4) at the two temperature regimes studied. For all populations except VC, the reproductive period was significantly shorter at 30°C than at 26°C. URM had the shortest period at 26°C. Browne *et al*. (1988) found that the length of the reproductive period for six populations studied (including SFB) reached its peak at 24°C, significantly decreased with increasing temperature (30°C), and was positively correlated to reproductive output. Similar finding was reported by Browne and Wanigasekera (2000), five experimental species had the longest reproductive period at 24°C.

In the present study, at 30°C, the value for the Y1 population (13.4 days) was in-between VC (20.6 days) and SFB populations (8.2 days). This means that Y1 population showed a better response to high temperature than SFB, but was still inferior to the VC population.

The values for total broods per female were well related with the reproductive period (Table 4a). Populations with a longer reproductive period had a higher number of broods. In this study, the numbers of broods for the *A. franciscana* samples were significantly higher (3.1-7.3) than for the Iranian *Artemia* samples (0.0-2.8) at both temperatures. The VC population produced the highest number of broods (7.3) at 30°C whereas URM had the lowest number (0.2) at 26°C and did not reproduce due to early mortality at 30°C. Our results indicated that the effect of temperature on this trait seems to be similar to the reproductive period.

Ovoviviparous broods were dominant in all experimental populations at both temperatures. However, the opposite pattern existed between VC and MAH: for VC the number of ovoviviparous broods at 30°C was more than two times the value at 26°C, while the value for MAH at 30°C was slightly lower than at 26°C. Since laboratory environmental conditions remained constant throughout the culture period, our results seem to be in good agreement with Wear *et al*. (1986), who reported that there is relative environmental stability in lake Grassmere, which results in low cyst production and high levels of ovoviviparity. This selective advantage allows *Artemia* to maximize its success in intraspecific competition. In addition, similar results were observed in Laysan Lagoon, Hawaii, USA (Lenz and Dana, 1987). On the contrary, dominance in oviparity in optimal culture conditions has been observed in Vinh Chau salt fields (Baert *et al*., 1997; Anh *et al*., 2010). Furthermore, Lenz (1987); Berthélémy-Okazaki and Hedgecock (1987) found that the major factors controlling the mode of reproduction in *Artemia* from San Francisco Bay were maternal age, photoperiod, temperature and salinity, and no single factor controls the switch from ovoviviparity to oviparity. However, Kappas *et al*. (2004) found that

switching of reproduction mode does not depend exclusively on environmental cues but is also under genetic control. Moreover, Browne (1983) demonstrated that the incidence of ovoviviparity decreased in a laboratory *A. franciscana* culture which was kept in a hydration: dehydration cycle of 4:8 months for 25 years. They concluded that this decrease resulted from selection against ovoviviparity. Therefore, genetic variation in reproduction mode among *Artemia* populations may be the result of natural selection.

Browne *et al*. (1984) found that the total reproductive output reflects a combination of brood size, brood interval, and length of reproductive period. Our results indicated that total offspring per female was well in line with the reproductive period and that this parameter is the most important one that may reflect the different tolerance of experimental *Artemia* populations under the temperatures studied. The VC population appeared to be better adapted to high temperature, producing more than twice as many offspring as SFB and Y1, and much more than URM and MAH at 30°C. At higher temperature more energy may be used for thermoregulation and less energy is saved for reproduction, which reduces reproductive output. Our results matched those of Browne *et al*. (1988), who reported that SFB had maximal offspring production at 24°C and a decrease at 30°C. The differential response of VC, Y1 and SFB is possibly due to local adaptation. This result is in agreement with Vos *et al*. (1984), who found that temperature resistance in *Artemia* populations, originating from the same parental material (San Francisco Bay) but produced in biotopes (tropics) with higher average water temperatures, appears to be significantly higher as compared to the parental material.

The Iranian populations also showed important differences in temperature response. MAH produced at least half the amount of offspring at 30°C, compared to 26°C, while URM's production was zero due to early mortality after maturation.

For VC and MAH, the number of offspring produced per female per day (reproductive period) appeared to be not positively related to the total offspring production per female, and did not follow the same tendency as the reproductive period and the total broods produced. The VC sample, which had a longer reproductive period and higher total number of broods, produced fewer offspring per day, compared to MAH at 30°C. All populations except URM produced a similar number of offspring per day at 26°C (Table 4a). Our results were different from the results of Browne *et al*. (1988), who obtained higher values with females having higher total offspring production in most populations.

The effect of temperature on percent offspring encysted was similar to the effect on the number of oviparous broods per female. For the *A. franciscana* populations a significantly lower percent encystment was found at 30°C than at 26°C, whereas the value for the MAH population (42.8%) was significantly higher than for the *A. franciscana* samples (11.8-15.8%) at 30°C ($p<0.01$). In particular, URM had no production of cysts in this study. This is not comparable with the available literature, where a high percent of encystment was found in the URM population (Browne *et al*., 1984, Van Stappen, 1997). Possibly both experimental temperatures may be out of the optimal temperature range for URM, which might be the reason for a poor reproductive performance at 26°C (only 9 of 50 females produced one brood of offspring while at 30°C all gravid females died). A certain environmental variation is known to affect encystment rate (Browne *et al*. 1984; Lenz, 1987, Anh *et al*., 2010). On the other hand, Browne (1980) stated that large differences in the percent encystment were found between different populations cultured under the same experimental conditions, and cyst production is more costly than ovoviviparous reproduction.

The values of brood interval were similar (2.2-3.1 days) for all populations, and showed little variation between both temperatures; only MAH showed a significantly shorter brood interval at 30°C. Perhaps the range of temperature tested was not extreme enough to produce different results. Browne and Wanigasekera (2000) found that *A. franciscana* showed a shorter time inbetween broods and lower number of offspring per brood as the temperature is increasing (from 15°C to 30°C). This is probably related to the metabolic rate of the animal as the

metabolic rate depends on the environment or culture temperature. Therefore, an increase of ambient temperature also increases the metabolic rate. As a consequence, an enhanced reproduction frequency was observed (*i.e*. a shortened brood interval).

A two-factor ANOVA test found that the reproductive period, total broods per female, oviparous broods, ovoviviparous broods, total offspring per female and percent encystment had significant interaction between population and temperature illustrating that populations respond differently to different temperatures. The population-temperature interactions found for some of the life-history traits also indicated that there is a genetic basis in the response to temperatures between populations (Browne *et al*., 1988).

Comparing the performance of the Iranian populations with *A. franciscana* in this study, which can be stated that URM had the longest pre-reproductive period, the shortest reproductive period, the smallest number of broods per female, and the lowest number of total offspring. However, for MAH the performance of reproductive characteristics seems to be close to the SFB population, although the *A. franciscana* samples had much higher values at 30$^{o}$C.

Data for the *A. franciscana* populations clearly demonstrated difference in tolerances within the same sibling species. This is not unlikely since a marked degree of genetic differentiation may occur among populations belonging to the same sibling species (Abreu-Grobois and Beardmore, 1982; Clegg *et al*., 2001). On the other hand, the animals may have genetically adapted to their specific niche. Since the VC and Y1 *Artemia* populations in fact are of San Francisco Bay origin, but inhabit saltworks with higher water temperatures (Brands *et al*., 1995; and Anh, *et al*., 2010), the increased temperature resistance of Y1 and VC *Artemia* could be considered as a heritable adaptation to high temperature. The performance of *A. franciscana* populations followed the order: VC>Y1>SFB, which is in agreement with the findings of Clegg *et al*. (2000 and 2001).

Furthermore, it was found that most suitable sites for *Artemia* inoculation are located in the tropical and sub-tropical belt, where water temperatures of 30$^{o}$C or more are very common (Van Stappen, 2005). Therefore, the success of inoculation trials is greatly dependent on the selection of *Artemia* populations with high temperature resistance and good production performance at high temperatures. In this regard, the VC population should be selected. On the basis of their temperature resistance the Y1, SFB and MAH may also taken into consideration.

## Conclusions

-In terms of survival and growth, the experimental populations respond differently to different temperatures (26$^{o}$C and 30$^{o}$C).

-The Iranian populations seem not well adapted to high temperatures, compared to *A. franciscana*. URM is more sensitive to high temperatures than MAH. The MAH sample appears to be close to the SFB population in most characteristics.

-For the *A. franciscana* samples, the rank for the response of survival and growth to high temperature was VC>Y1>SFB. It could be extrapolated that only after a few generations they may be completely adapted to high temperatures.

The data obtained are too limited to make a predictable equation about how many generations of SFB population, subjected to Vietnamese water temperature conditions, are needed to reach the tolerance of the VC sample. The reproductive period was observed for only one month; post-reproductive period and total lifespan were not determined. Further research is needed to clarify these aspects.

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