

Species representation and gender proportion from mixed *Artemia franciscana* and *A. parthenogenetica* (Anostraca) commercial cysts hatched over a wide range of temperatures

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Species representation and gender proportion from mixed *Artemia franciscana* and *A. parthenogenetica* commercial cysts from the Great Salt Lake were obtained experimentally under 31 incubation temperatures from 10°C to 40°C. From 10°C to 32°C, the species representation was skewed to about 65% parthenogenetic females. From 33°C to 38°C, the zygogenetic species dominated 80–100%. Parthenogenetic cysts did not hatch at 37°C, whereas few zygogenetic cysts hatched up to 38°C. The proportion of zygogenetic males remained below 20% until the hatching temperature reached 32°C, and from 33°C to 38°C increased to about 50%. Two 'pure' zygogenetic strains, *A. franciscana* from a San Francisco Bay commercial lot and an *Artemia* sp. from a natural population in Oaxaca, México, were also studied for comparison. A significantly lower proportion of males (or a significantly higher proportion of females) can preliminarily detect the presence of parthenogenetic individuals in a sample incubated below 33°C.

Keywords: Commercial *Artemia* cysts, cyst hatching, parthenogenesis, sex ratio, zygogenesis.

Introduction

BRINE shrimp reproduction can be sexual, where an obligate mating has to occur between a female and a male to form a zygote after fertilization and approximately 50% females and 50% males compose the progeny. In the literature on brine shrimp this kind of reproduction is often named bisexual or zygogenetic. Additionally, brine shrimp reproduction can be asexual through parthenogenesis, where males are not required, females compose the progeny, and sometimes a male is found¹.

In the Americas, only zygogenetic species and populations only, whereas in the rest of the world parthenogenetic and zygogenetic species and populations coexist². Brine shrimp cysts are harvested from lakes and salt marshes. In America, the Great Salt Lake (GSL), Utah and San Francisco Bay (SFB) are the most important producers of brine shrimp cysts, which are packed in cans and bags for commercial distribution. These cysts should only contain the zygogenetic species *Artemia franciscana* Kellogg, 1906. However, in a commercial lot from the GSL, a mixture of zygogenetic and parthenogenetic cysts was identified³.

The brine shrimp *Artemia* is one of the most studied animals on earth and mixed species in a commercial lot could represent a possible mismatch of species in specific biological research, when it is assumed that only *A. franciscana* cysts are present. Brine shrimp commercial cysts are also used for educational purposes because they are an excellent biological material to study basic genetics, physiology, reproduction and crustacean morphology. For many years, brine shrimp cysts have been commercialized for hatching, and also for having them as pets and as live food for aquatic species. Therefore, one has to be aware of the possibility of having a sample with mixed brine shrimp species.

In this study, we present experimental data on species representation and gender proportion from mixed *A. franciscana* and *A. parthenogenetica* Bowen and Sterling, 1978 commercial cysts hatched over a wide temperature range.

Methods

Brine shrimp cyst source

We used one can of the GSL commercial cysts containing both the zygogenetic and the parthenogenetic species and

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one can of the SFB commercial cysts containing only *A. franciscana*. Our group has previously analysed the two sources of cysts genetically, reproductively and morphologically³. In addition, in this work we used *Artemia* sp. cysts from La Colorada, Oaxaca, México⁴.

Cyst incubation

Cysts were hatched inside a transparent 60 ml polystyrene flask with a 100 µm mesh at both the ends. The hatching containers were inside 600 ml glass flasks with aerated sea water (38 SPU). These flasks were placed in thermal baths (Cole Palmer 1266-02, Forma Scientific 2067, and Lauda E-100) with a temperature precision of $\pm 0.2^\circ\text{C}$. Two 40 W fluorescent lamps above the incubation unit provided constant light to the cysts.

Hatching temperature

Cyst hatching of all brine shrimp sources was studied at 31 different temperatures, from 10°C to 40°C . Cyst groups (0.03 g) were incubated using four replicates at each temperature. Depending on the temperature, incubation time did not last longer than 10 days below 25°C , 24 h at medium temperature ($26\text{--}33^\circ\text{C}$), and not longer than 72 h at high temperatures ($34\text{--}40^\circ\text{C}$). To avoid further development or death of nauplii, the collection of individuals incubated above 33°C was made every 4–6 h.

Species representation and gender proportion

Once nauplii hatched from each of the four incubation replicates at a given temperature, 100 individuals were randomly collected and placed in a 4 l plastic container with aerated sea water at 38 SPU and with the container in a water bath at $27 \pm 0.5^\circ\text{C}$. Nauplii grew for 10–12 days with feeding twice a day using a mixture of *Chaetoceros* sp. and *Isochrysis* sp. microalgae, which usually gave survivals above 90% and was enough to reach adulthood. The mean and standard deviation of species representation and gender proportion at each temperature were obtained. Identification of zygogenetic and parthenogenetic individual adult brine shrimp was done using body morphology³, which served to determine the species representation. Briefly, parthenogenetic females had longer antennules (first antennae), abdomen, and cercopod than bisexual females; parthenogenetic males had a semirectangular distal joint of claspers (second antennae), whereas bisexual males had claspers with a typical triangular distal joint. Phenotypic sexual dimorphism of adult individuals served to obtain the gender proportion at each temperature; males were identified with the observation of the large second antennae (claspers) and penes, and females by the presence of the brood pouch.

Results

The species representation from the commercial cyst lot containing mixed *A. franciscana* and *A. parthenogenetica*, hatching from 10°C to 40°C is shown in Figure 1. The proportion was skewed about 65% to parthenogenetic females at temperatures from 10°C to 32°C . When the temperature reached a threshold of 33°C , the hatching proportion of the parthenogenetic species decreased, leaving mostly the zygogenetic species (*A. franciscana* from the GSL) at temperatures between 33°C and 36°C . A few parthenogenetic cysts hatched at 36°C but did not hatch at 37°C . A few zygogenetic cysts from the GSL hatched up to 38°C . A few zygogenetic cysts of the *A. franciscana* sample from the SFB hatched up to 38°C and a few zygogenetic cysts of *Artemia* sp. from Oaxaca hatched up to 39°C .

Figure 2 shows the proportion of zygogenetic males observed at the incubation temperatures from 10°C to 40°C . In the mixed-species commercial lot, the proportion of zygogenetic males remained below 20% until the temperature reached 32°C . Then this increased to values around

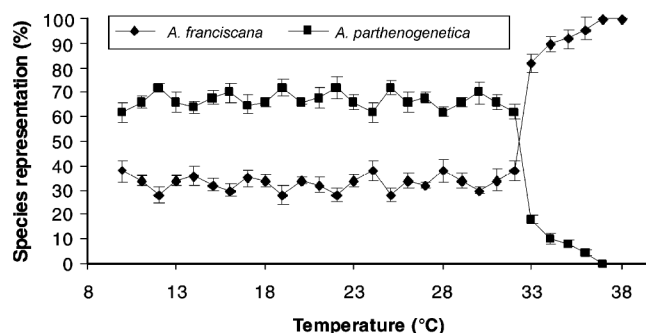


Figure 1. Species representation (mean and standard deviation for each species) from a commercial sample in the Great Salt Lake hatched from 10°C to 40°C .

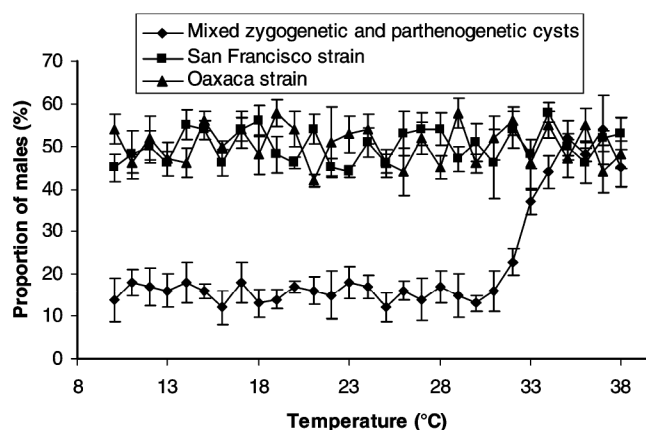


Figure 2. Mean and standard deviation of the proportion of zygogenetic males hatched from 10°C to 40°C from a commercial sample containing mixed *Artemia franciscana* and *A. parthenogenetica* cysts in the Great Salt Lake and two pure zygogenetic species, *A. franciscana* from San Francisco Bay and an *Artemia* sp. from Oaxaca.

50% at temperatures between 33°C and 38°C. The proportion of zygogenetic males from the two zygogenetic strains (SFB and Oaxaca) had values around 50% over the temperature range studied.

Discussion

Although native zygogenetic strains had similar hatching profiles, it was possible to differentiate the tropical Oaxaca strain (39°C) from the temperate GSL and SFB strains (both 38°C) by only 1°C of hatching temperature.

The species representation was slightly skewed to parthenogenetic species (65%) at temperatures from 10°C to 32°C, similar to that reported by Campos-Ramos *et al.*³, who found that this commercial lot contained approximately 60% parthenogenetic and 40% zygogenetic species at 27 ± 0.5°C. In contrast, species representation of the parthenogenetic species decreased sharply from 33°C and was absent at 37°C, which indicated a lower tolerance of hatching at higher temperatures than *A. franciscana*. Our finding also suggests that high temperature suppresses the hatchability of parthenogenetic cysts, as observed by Triantaphyllidis *et al.*⁵. The low proportion of males at temperatures up to 32°C indicated the presence of parthenogenetic females in the commercial lot. The increase in zygogenetic males hatching from 33°C again confirmed the suppression of hatching of the parthenogenetic species at high temperatures.

In our study, the low proportion of zygogenetic males below 33°C was so obvious that no statistical test was needed. However, it is expected that the species representation among the commercial lot could vary and a simple statistical test, such as a chi-square test, should be used. Zygogenetic males from the SFB and the Oaxaca strains maintained an expected gender ratio of 1 : 1 over the temperature range (Figure 2).

Skewed gender ratios are sometimes observed in natural zygogenetic *Artemia* populations. For example, Cuellar⁶ measured a skewed gender ratio to females during a specific season of the year in the GSL. Rodríguez-Almaraz *et al.*⁷ measured a monthly gender ratio with a dominance of females at Isla Carmen, México. However, these

measurements have been made under environmental variables and biological interactions in a particular natural habitat. Laboratory conditions are standardized by controlling all variables but one, such as temperature. Therefore, we conclude that it is simple and preliminarily to identify mixed parthenogenetic and zygogenetic cysts by means of a significantly lower proportion of males, or a significantly higher proportion of females, in a sample incubated below 33°C. Identification of parthenogenetic individuals should also follow morphology, reproduction and mitochondrial DNA analyses³.

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