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Laboratory production of early hatching *Artemia* sp. cysts by selection

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ABSTRACT

The goal of this study was to test whether it is possible to produce early hatching cysts of *Artemia franciscana* from San Francisco Bay (SFB) by truncation selection. The starting material was an SFB cyst sample, harvested in nature. After selection of early hatching nauplii had taken place, these selected individuals were cultured to maturity, and the hatching rate of their offspring (F_1) was compared with that of the parental generation and with the non-selected control F_1. The possible differences in hatching rate were used to estimate heritability.

Two different selection experiments were run, accompanied with a number of additional tests studying the influence of the hatching set-up, the hatching temperature and diapause termination using hydrogen peroxide, on the hatching rate. Also the influence of different culture salinities on hatching rate and on possible success of selection was studied.

The results revealed an influence of all those abiotic factors on the hatching rate, but also a marked interference of the hatching percentage with the hatching rate. Choosing the individuals based on their own phenotypic values (hatching within a certain time span of hatching incubation) and using those to produce the next generation, revealed a positive response. The selection in different salinities showed a different response. Depending on the strength of the selection pressure, the samples were advanced in time, starting with the highest selection pressure, and ending with the control and the parental sample. In spite of the strong interference of environmental factors, our results suggest that selection of early hatching cysts is possible.

1. Introduction

Modern aquaculture is a young industry that has shown impressive growth in the last three decades. A great deal of interest has been generated in developing an artificial larval diet as an alternative to live feed, but the artificial diet is still generally accepted much less than live food (Tandler and Kolkovski, 1991).

Several criteria determine the potential quality of an *Artemia* cyst sample for aquaculture application. Hatching rate represents the time lapse needed for complete hatching from the start of the hatching incubation onwards. Often this is quantified by determining specific time intervals within this period. High hatching synchrony means a maximum number of instar I nauplii within a short period of time, which ensures that a maximum of nauplii can be harvested before they consume too much of their energy reserves. On the other side, in case of low hatching synchrony the same hatching container needs to be harvested several times to avoid a mixture of different developing stages of nauplii (e.g. instars I, II and III). If the total incubation time exceeds 24 h, it is not possible to restock in the same hatching containers for the next day. The aquaculturist would need to spend more on containers, which implies higher infrastructural costs (Van Stappen, 1996).

The purpose of the present study is to assess to what extent the hatching rate is genetically determined by testing the possibility to produce early hatching cysts by truncation selection. Reports on heritability values and heritability in general in *Artemia* are rare. Data on the heritability of some quantitative characteristics obtained by cross-breeding, like hatching percentage, diameter of cysts, temperature resistance of nauplii and growth rate, show that there exists not only genetic control (Tackaert et al., 1987) but also a very large influence by harvesting, processing and storage conditions (Vanhaecke and Sorgeloos, 1982). Bi-directional selection for naupliar length in *Artemia franciscana* shows heritability values of moderate magnitude (Shirdhankar and Thomas, 2003), while the results from the "Vietnam Experiment" show that changes in thermal stability and heat resistance may occur extremely rapidly using genetic selection (Clegg et al., 2000; Clegg and Trotman, 2002).

To assess the heritability of the hatching rate, we chose individuals hatched within a certain time span of hatching incubation out of a parental stock population and used those to produce the next generation in the laboratory. This selected offspring group was compared with the parental generation in terms of hatching rate, and with the non-selected control, and heritability was estimated. Two different selection experiments were run accompanied with a number of additional tests to study the influence of various experimental conditions directly inherent to our

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experimental set-up on the hatching rate: the hatching set-up (type of hatching recipient), hatching temperature, hatching salinity, and treatment with hydrogen peroxide, used to break diapause in the lab-produced cysts.

2. Materials and methods

A. franciscana from San Francisco Bay, USA, ARC No. 1673, was used in the present research as original stock cyst material.

Two different selection experiments were conducted. The first used 75 ppt artificial seawater (Instant Ocean®, Aquarium Systems, France) as culture medium, while the second used three culture salinities: 40, 75 and 100 ppt. All other culture conditions were identical for all experiments: temperature 28 °C, light intensity 2000 lx 24 h per day and adequate aeration. Throughout the culture period, the animals were fed *ad libitum* everyday with *Tetraselmis suecica*.

The selection in experiment 1 was done by hatching the stock cyst material in 1 L cones (see further), and isolating the nauplii hatching within the first 12 h of incubation. This fraction represented about 10% of the total number of hatchable cysts and was further cultured to maturity, after which the F_1 cysts were collected. The experiment was continued until the F_2 generation, but this time the hatching (and selection) was performed in Petri dishes, in view of the smaller number of cysts available. Nauplii of the F_1 generation, hatching within the first 20 h were isolated, representing 17% of the population.

In experiment 2 selection was done in Petri dishes by isolating nauplii hatching within the first 20 h (corresponding with 12 and 14% of the population in the set-up with hatching salinity 40 and 75 ppt, respectively). The third salinity, 100 ppt, was used to determine the influence of abiotic factors on hatching, but the number of cysts produced was insufficient to allow selection.

A number of additional tests were run to study the impact of environmental conditions on the hatching rate and hatching percentage. Therefore, the ARC 1673 sample was hatched under the same controlled conditions in different set-ups (cones and Petri dishes) and at three different temperatures (22, 26 and 28 ± 0.5 °C) for each set-up. Similarly, cysts were subjected to a treatment with 6, 12 and 24 ppm of hydrogen peroxide, again in both set-ups (cones and Petri dishes). The effect of all these treatments on the hatching rate was evaluated.

As described above, hatching was done either in 1 L cones or in 20 cm wide Petri dishes. Cones were set set-up in triplicate. In each cone 1.6 g of cysts was incubated. They were provided with air-lines aerating from the bottom. The hatching rate was determined by counting the number of nauplii and the number of umbrellae every hour, with the first observation approximately 11 h after the start of the hatching incubation, and the last one around 42 h, depending on the sample. The counting was done by taking six 0.25 ml subsamples from each of the three cones.

When Petri dishes were used, the available cysts were distributed equally over 3 replicate dishes (without determining the exact weight). The Petri dishes were filled with strongly aerated Instant Ocean® seawater, and no additional aeration was supplied during hatching incubation itself. For determination of the hatching rate, the same procedure as for the cones was applied, but since the number of cysts used for incubation in Petri dishes was smaller, at each observation all nauplii hatched so far were counted and removed.

The unhatched cysts, in both cones and Petri dishes, were decapsulated (to distinguish them from empty cyst shells) using a few drops of NaOH and approximately 1 ml of NaOCl, and the number of embryos was counted. The hatching percentage (H%) at any time of observation was thus calculated as follows

$$H\% = (\text{No. of nauplii}/\text{No. of nauplii} + \text{No. of umbrellae} + \text{No. of embryos})*100. \quad (1)$$

The results of all tests were compared statistically by the Fit Nonlinear Model Using Generalized Least Squares at probability of 0.05. All tests were performed using the computer program S-Plus 6.1. The hatching rate curve for one sample was explained by formula:

$$y = 100/1 + e^{-Z(t-Q)} \quad (2)$$

in which

t	time
Z	slope
Q	the onset of the hatching

and that model was expended to second formula to compare slope and onset of hatching between two curves:

$$y = 100/1 + e^{-(Z_1+Z_2)(t-Q_1-Q_2)} \quad (3)$$

in which

t	time
Z_1	slope of the first curve
Z_2	slope of the second curve
Q_1	the onset of the hatching for the first curve
Q_2	the onset of the hatching for the second curve.

All analyses were done comparing curves two by two. Unknown variables Z_1 and Z_2, and Q_1 and Q_2 were calculated using Generalized Least Squares.

The hatching synchrony (T_s) was calculated as follows (Van Stappen, 1996):

$$T_s = T_{90} - T_{10} \quad (4)$$

in which

T_{90}	incubation time till appearance of 90% of total hatchable nauplii
T_{10}	incubation time till appearance of 10% of total hatchable nauplii.

Realized heritability was estimated using the following formula (Falconer, 1981):

$$H^2 = \mu' - \mu/\mu_s - \mu \quad (5)$$

in which

μ	duration of hatching incubation, needed for parental generation to reach a certain hatching percentage (in our calculations 25, 50 or 75%)
μ'	duration of hatching incubation, needed for the selected F_1 generation to reach a certain hatching percentage (25, 50 or 75%)
μ_s	duration of hatching incubation of selected parents (in our calculations 12 or 14% of the population depending on the test).

3. Results

3.1. Influence of abiotic hatching incubation factors on hatching rate and hatching percentage

Variability among replicates in these and the following tests generally ranged between 2 and 5% (CV 0.02–0.05), and is thus not shown on the plotted curves for reasons of visual clarity.

The set-up of hatching (1-L cones *versus* Petri dishes) had an influence on the hatching rate, but not on the hatching percentage: statistical comparison between cones and Petri dishes showed that the use of Petri dishes resulted in a significantly ($P<0.05$) delayed hatching rate (Fig. 1a).

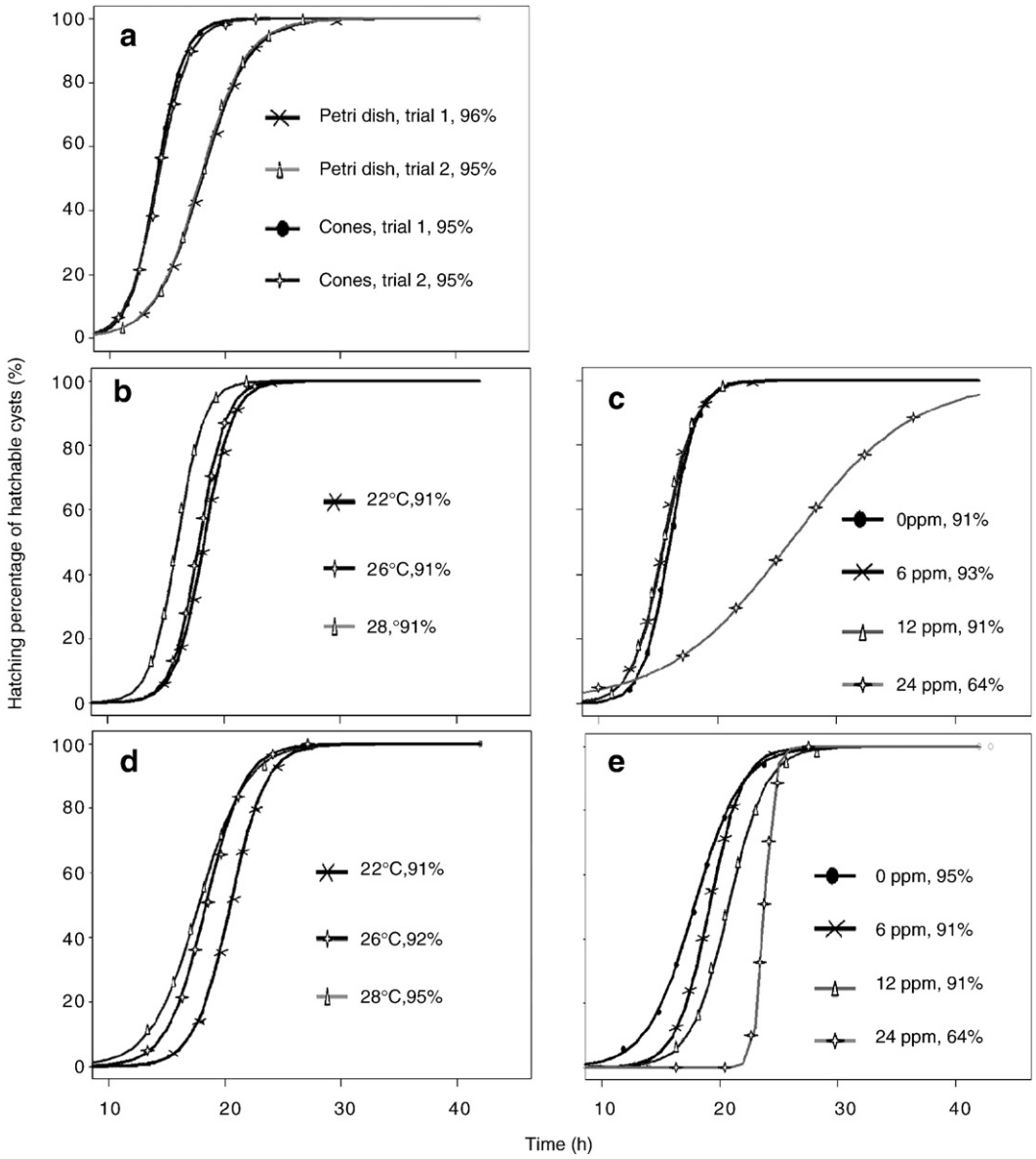

Fig. 1. Influence of abiotic factors on sample ARC No. 1673: a) different hatching set-ups (cones and Petri dishes), b) in cones at different temperatures (22, 26 and 28 °C), c) in cones with different hydrogen peroxide concentrations (6, 12 and 24 ppm), d) in Petri dishes at different temperatures(22, 26 and 28 °C), and e) in Petri dishes with different hydrogen peroxide concentrations (6, 12 and 24 ppm).

Hatching at different temperatures resulted in different hatching rates for the three treatments (Fig. 1b and d): at 28 °C cysts started to hatch first, than at 26 °C and finally at 22 °C. The same pattern of hatching was found in both set-ups (cones and Petri dishes). Comparing the results of Fig. 1b and d shows that there was an overall delay in hatching rate when using Petri dishes (in agreement with the results of Fig. 1a).

As for the treatment with hydrogen peroxide, different concentrations of this chemical in the cones showed significantly different ($P<0.05$) results in hatching rates for all samples except between 6 and 12 ppm (Fig. 1c). The hatching started first in 6 and 12 ppm, followed by the untreated control, and finally by 24 ppm. In Petri dishes (Fig. 1e), the sequence of onset of hatching was: control, 6, 12 and 24 ppm. The highest concentration, 24 ppm, showed a big delay and lowest synchrony in both set-ups. The hatching percentage was more or less constant (91–95%) in all treatments, except for 24 ppm, which showed only 64% hatching in both set-ups as well. Again, an overall delay in hatching rate occurred when Petri dishes were used.

3.2. Selection experiment 1

The results of the selection experiment 1 revealed that neither control nor selected group started or finished hatching before the parental group. The parental group was the fastest, followed by the selected F_2, the selected F_1, and the controls. All groups were significantly different except F_2 and F_1 controls. Variability among the replicates decreased from 30 to 2% (CV 0.3–0.02) as hatching progressed. The hatching percentages of the different groups, however, varied considerably (Fig. 2).

3.3. Selection experiment 2

The observations from the selection experiment 1 made clear that the parental generation showed a faster hatching rate than any of its descendants, selected or not. As nothing was known about the production conditions of this parental sample (produced and harvested

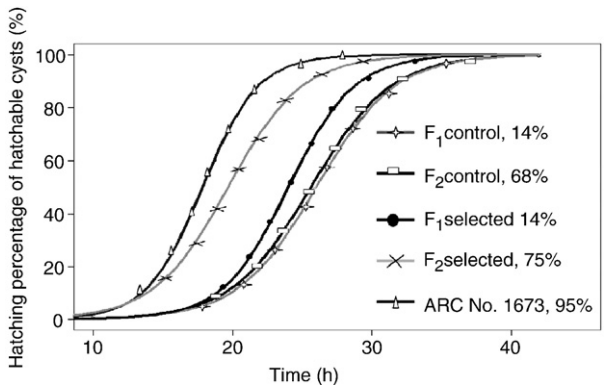

Fig. 2. Comparison of hatching rates in selection experiment 1: F_1 and F_2 generations.

in nature), the parental generation of experiment 2 was produced under controlled laboratory conditions at two different salinities, i.e. 40 and 75 ppt. The results of the selection at 40 ppt indicated that the selected group was the fastest. After the selected group followed the parental and control F_1 groups (Fig. 3a). The pattern of hatching was significantly different ($P<0.05$) between all groups, and so was the hatching synchrony: 10, 13 and 14 h for the control F_1, the parental generation and the selected group, respectively.

As for selection at 75 ppt, the results were more pronounced than at 40 ppt. The selected group was the fastest followed by the parental and control F_1. The selected and the parental group showed the same hatching synchrony, which was 5 h shorter than control F_1 (Fig. 3b). The values of the hatching synchrony were 14, 14 and 9 h for the selected group, the parental generation and the control F_1, respectively. Variability among the replicates was similar as in selection experiment 1.

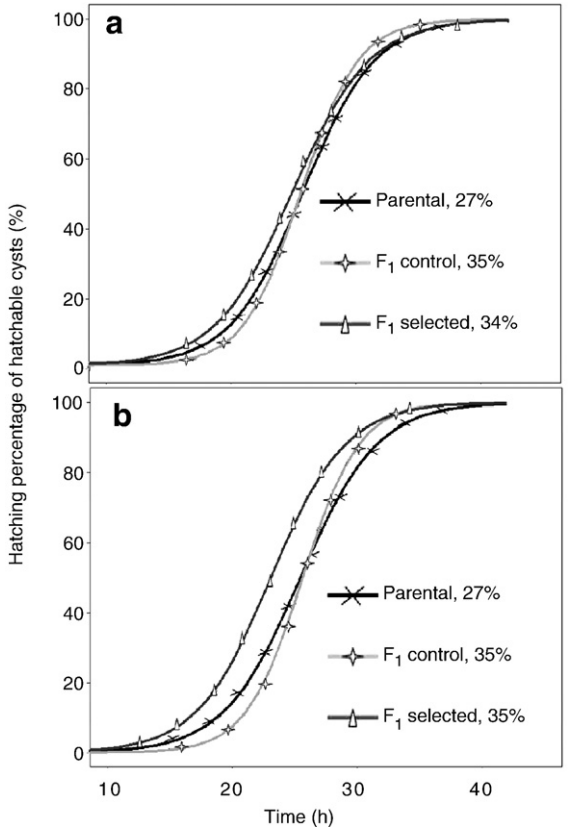

Fig. 3. Comparison of hatching rates in selection experiment 2: a) parental and F_1 generations produced in 40 ppt, and b) parental and F_1 generations produced in 75 ppt.

Table 1
Hatching percentage of samples stored at 6 °C

Sample	Storage time at 6 °C (months)	Hatching percentage (%)
Selection experiment 1		
Control, F_1, one hydration–dehydration cycle	6	90
Control, F_1	1	14
Control, F_1	3	64
Selected, F_1	1	14
Selected, F_1	3	36
Control, F_2	1	68
Selected, F_2	1	75
Selection experiment 2		
Parental, 40 ppt	1	27
Parental, 75 ppt	1	28
Parental, 100 ppt	1	12
Control, F_1, 40 ppt	1	35
Selected, F_1, 40 ppt	1	34
Control, F_1, 75 ppt	1	35
Selected, F_1, 75 ppt	1	35

3.4. Diapause deactivation

Diapause deactivation in our experiments was done by storage of the lab lab-produced cysts in brine at 6 °C. One month of storage in these conditions resulted in only partial diapause breaking: for all samples the hatching percentage did not exceed 35%, except for the samples of F_2 from experiment 1 (68–75%) (Table 1). As the storage period was extended, an increased hatching percentage was observed.

The selected group and control of experiment 1 were tested twice, revealing different hatching results. At first they showed slower hatching rate as well as lower hatching percentage (14%). After two more months of storage the hatching started earlier and the hatching percentage increased to 36% in the case of the selected group and to 64% in the control (Fig. 4a).

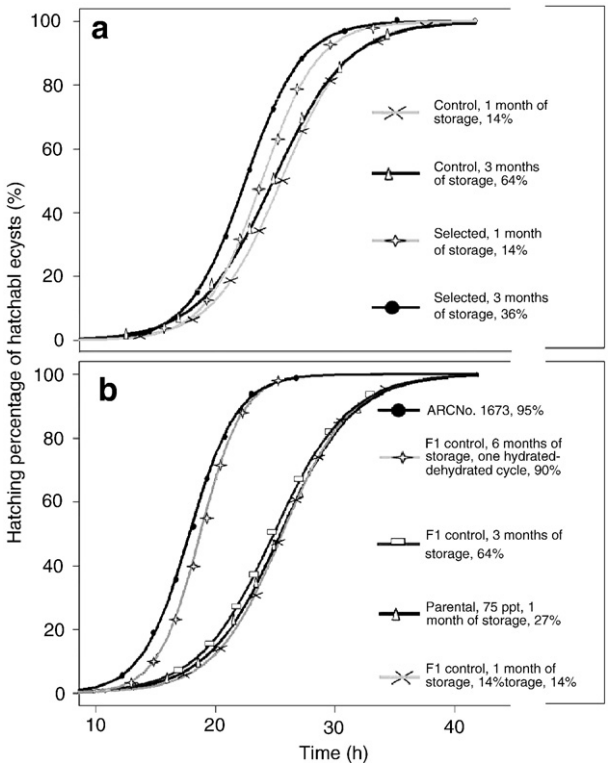

Fig. 4. Comparison of hatching rates and hatching percentages: a) samples of experiment 1 after one and after three months of storage, and b) controls from selection experiment 1, original stock cyst material (ARC No. 1673) and parental generation of selection experiment 2 in Petri dishes.

Comparison of controls from selection experiment 1, original stock cyst material (ARC No. 1673) and parental generation of selection experiment 2 revealed that a higher hatching percentage corresponded with a faster hatching rate. With one exception (the parental generation of selection experiment 2 at 75 ppt *versus* the F_1 control of selection experiment 1 after one month of storage) hatching rates of all samples were significantly different from each other (Fig. 4b).

3.5. Heritability

Since selection experiment 1 shows different hatching percentages for the different groups, and refers to offspring collected at different dates as well, it was not possible to estimate heritability values for this experiment. In experiment 2 the parental generations were produced under controlled conditions, and their diapause status was similar as in their offspring (1 month in brine at 6 °C), which enabled to estimate the heritability for T_{25}, T_{50} and T_{75}; heritability values were 0.5, 0.16 and 0.11, respectively for 40 ppt culture salinity, and 0.95, 0.45 and 0.26, respectively, for 75 ppt culture salinity.

4. Discussion

4.1. General outlines

Artemia, in all stages of the life cycle, is a suitable food for most diversified groups of aquatic animals. Especially the nauplii are a crucial live food in marine larviculture. High hatching synchrony should ensure that a maximum of nauplii can be harvested before they consume too much of their energy reserves, and that the same hatching container does not need to be harvested several times to avoid a mixture of different developing stages of nauplii (e.g. instars I, II and III). A total hatching incubation time not exceeding 24 h makes it possible that the same hatching container is stocked for the next day (Van Stappen, 1996). Although hatching characteristics, including hatching rate, differ considerably from strain to strain (Vanhaecke and Sorgeloos, 1982), also environmental factors, like temperature (Browne et al., 1984; Royan, 1975; Vanhaecke and Sorgeloos, 1989), salinity (Browne et al., 1984; Royan, 1975), oxygen levels and water chemistry (Browne et al., 1984) have been shown to be of great importance; this problem might be overcome using selective breeding techniques. The formulation of any selective breeding program calls for a proper understanding of the genetic architecture of the economically important traits of the population under study. However, there is a lack of information regarding heritability estimates in *Artemia*, except for the study by Browne et al. (1984) to separate out the genetic component of a number of traits in 12 strains of *Artemia*. Furthermore, Shirdhankar et al. (2004) studied the heritability values for growth and reproduction traits of *A. franciscana*, and estimated heritability using parent–offspring regression.

Thus, in the present study an attempt was made to produce early hatching cysts of *Artemia* sp. by truncation selection, as well as to estimate the heritability of this trait and the influence of abiotic factors.

4.2. Influence of abiotic factors during hatching on hatching rate and hatching percentage

Temperature (Royan, 1975; Vanhaecke and Sorgeloos, 1989) and salinity (Royan, 1975) are known to be important abiotic parameters affecting and conditioning the life processes in aquatic organisms. This includes the hatching process in *Artemia* cysts. Furthermore, commercial producers and users of *Artemia* cysts apply several techniques to terminate the diapause, e.g. treatment with hydrogen peroxide (H_2O_2) (Van Stappen, 1996).

An additional factor which might affect hatching rate was the type of hatching set-up: as sufficient amounts of sample 1673 were available, the standard hatching set-up in cones could be used for this material (Van Stappen, 1996). The small quantities of cysts, however, produced in the laboratory cultures forced us to apply an alternative set-up. For these samples, hatching was performed in 20 cm-diameter Petri dishes. Comparison of the hatching rate of sample 1673 in different set-ups demonstrated that the synchrony in the cones was higher (5 h) than in Petri dishes (9 h). In cones the hatching was advanced as compared to the Petri dishes, and this was confirmed by repeating the experiment in time (Fig. 1a).

Vanhaecke and Sorgeloos (1989) studied 32 different geographical strains of brine shrimp and revealed that the hatching incubation temperature significantly affects cyst hatching percentage in all *Artemia* strains studied. They reported that the hatching percentage was always maximal in the range 25–30 °C, but no research was done on the effect of temperature on the hatching rate. Sorgeloos (1980), however, stated that temperatures below 25 °C slow down the hatching rate and therefore are not appropriate for use in aquaculture. Consequently two hatching temperatures were tested which are in the range currently used in hatcheries (26.0 and 28.0±0.5 °C), and additionally one lower temperature well below this range (22.0±0.5 °C), in order to check their effect on the hatching rate. Our results revealed that a higher temperature advances the hatching process (Fig. 1b and d). In line with the initial tests in different set-ups, there was an overall delay in hatching rate when using Petri dishes, as compared to the cones.

Incubation in a hydrogen peroxide (H_2O_2) solution has been proven to be successful in termination of diapause, but the sensitivity of the strain (or batch) to this chemical is generally difficult to predict, hence the need for preliminary tests to provide information about the optimal dose/period to be applied (Van Stappen et al., 1998). Thus the effect of this chemical on the hatching rate was tested as well. The use of 3 different concentrations of H_2O_2 (6, 12 and 24 ppm), applied in the hatching medium in both set-ups, affected the hatching rate and hatching percentage (Fig. 1c and e). Generally the onset of hatching was delayed as the H_2O_2 concentration increased. An overall delay in hatching rate occurred when Petri dishes were used. The hatching percentage was more or less constant (91–95%) in all treatments, except for 24 ppm, which showed only 64% hatching in both set-ups as well. This latter observation, and the incidence of high numbers of umbrellae in the hatching medium, not developing into the instar I stage when using 24 ppm, can be interpreted as an indication of toxicity of this concentration for the hatching *Artemia* embryo.

4.3. Selection experiment 1

Various reports on heritability estimates are available in aquatic animals such as in *Oreochromis niloticus* (Tave and Smitherman, 1980) and *Cyprinus carpio* (Brody et al., 1981). However, literature data on heritability estimates in *Artemia* are scarce.

Selection experiment 1 indicated that as the selected F_2 hatched faster than the selected F_1, both hatching faster than the control F_1 and F_2, might be the effect of additive genetic variance. In contrast, the parental generation was faster than any of its descendants, selected or not. As the production conditions of this parental sample (produced and harvested in nature) were unknown, we assumed an effect of abiotic production conditions on the hatching rate, i.e. drying, diapause termination, salinity, temperature etc.

4.4. Selection experiment 2

The aim of selection experiment 2 was thus to produce a parental generation in controlled laboratory conditions, and to use this as starting material for selection experiments. Additionally, by producing different parental groups, cultured in different salinities (40, 75 and 100 ppt), we intended to study the influence of this abiotic factor on hatching as well as on selection.

The hatching rate of these three parental groups proved very similar (results not shown), suggesting the absence of an effect of the production salinity on the hatching rate. The hatching percentage, on

the other hand, was very similar for the 40 and 75 ppt parental groups (27 and 28%, respectively), but lower for 100 ppt (12%) (Table 1).

Furthermore, the results of the selection in different salinities showed a much bigger influence of the selection at 75 ppt than at 40 ppt, and a faster hatching of the selected F_1 in both salinities than the parental and control groups. These results suggest that selection of early hatching cysts was possible when the diapause status of the parental and offspring groups was similar (in our tests after 1 month in brine at 6 °C), and when parental and F_1 generations were produced under the same controlled laboratory conditions (Fig. 3a and b).

4.5. Diapause deactivation

Evidently diapause could be broken by storage in brine, but the storage period needed to reach 90–95% hatching (ensuring that the resulting population was maximally representative) was not known. The sample with the highest hatching percentage (90%) was stored for more than six months in total; it was hydrated after a first two and a half months in order to check its level of diapause, and was then returned in brine for four more months (Table 1); this supplementary treatment may also have affected the hatching percentage, in accordance with the findings of Browne (1980), who reported that many cysts do not hatch until after the first hydration–dehydration cycle. Apart from this observation, the highest hatching was obtained after four and a half months storage (79%).

Furthermore, the hatching percentages of F_2 in experiment 1 were much higher after one month of storage (68–75%) than in F_1 (14%), possibly due to the fact that selection had also taken place for cysts quickly emerging from diapause. A similar, but lower, increase was observed in experiment 2 from the parental generation (27–28%) to F_1 (34–35%); here the selection pressure may have been lower, because the animals for the next generation were taken from the first 27% of hatchable cysts, as compared with 14% in selection experiment 1.

The selected group and control of experiment 1 were tested twice for their status of diapause, revealing different hatching rate results. After a first one month of storage these samples showed a slower hatching rate and a lower hatching percentage (14%) as compared to two more months of storage (hatching percentage 36% in the selected group and 64% in the control, Fig. 4a). These results thus suggest that the slowest hatching cysts were released from diapause first (i.e. after the shortest storage period).

In view of the relationship between the hatching percentage and the hatching rate, as shown above, the hatching rates of the different controls with different hatching percentage are presented in Fig. 4b. The samples used in this comparison had thus undergone no treatment, except for one (experiment 1 control F_1), stored for six months, which had been hydrated and then dehydrated. This comparison confirms that a higher hatching percentage corresponds with an earlier onset of hatching. Only the two samples with the lowest hatching percentages were not significantly different. Another general observation is the considerable delay of all samples *versus* ARC No. 1673 and control F_1, stored for six months. The history of production and diapause termination of ARC No. 1673 (harvested in the field) was not known. Lavens and Sorgeloos (1987) suggested that several dehydration cycles may have a negative effect on the hatching rate when the treated cysts are stored for a long time. Cysts exposed to two dehydration–hydration cycles initially hatched faster, but after several weeks of storage needed 2 h extra incubation to reach hatching (Sorgeloos et al., 1976; Vanhaecke and Sorgeloos, 1982). However, our results revealed that the hatching rate of the control F_1, stored for six months and one hydration–dehydration cycle, was much faster than others, which lacked this extra treatment.

4.6. Heritability

The results of the selection experiments showed that selection in favour of a faster hatching rate could be obtained. However, this parameter is influenced by numerous environmental factors, and there is also interference with the status of diapause. The importance of heritability in a breeding experiment lies in its predictive role in expressing the reliability of a phenotypic value as a guide to the breeding value. So in selection experiment 2, with a similar diapause status of the parental generation and the offspring and both generations (parental and F_1) being produced under the same controlled laboratory conditions, the realized heritability H^2 was estimated for the parameters T_{25}, T_{50} and T_{75}: H^2 values were 0.50, 0.16 and 0.11, respectively for 40 ppt culture salinity, and 0.95, 0.45 and 0.26, respectively, for 75 ppt culture salinity, indicating the highest additive genetic variance for the earliest hatching phase (T_{25}). It should again be emphasized that these H^2 values correspond to a hatching percentage of 27–35%, and that the selection pressure corresponded to the isolation of the 12–14% earliest hatchers of the total hatchable cysts.

Comparison of the H^2 values for 40 and 75 ppt shows a much bigger influence of the selection on hatching rate in the latter case, in spite of the slightly lower selection pressure (12 *versus* 14% of hatchable nauplii for 40 and 75 ppt, respectively), suggesting again not only genetic variance, but also environmental variance affecting the hatching rate. To minimize the interference between hatching rate and hatching percentage (hardly any of our samples produced in the laboratory resulted in a hatching percentage in the range 90–95%), our experiments should be repeated using samples which show at least 90% of hatching, and hence calculate heritability.

5. Conclusions

Environmental factors have a major effect on the hatching rate, as shown in the comparison between different hatching set-ups (cones *versus* Petri dishes): the hatching starts earlier in cones, whereas the set-up does not influence the hatching percentage. Different hatching temperatures (22, 26 and 28 °C) have an influence too: the higher the temperature, the earlier the hatching starts in both set-ups. Furthermore a diapause termination treatment with different hydrogen peroxide concentrations (6, 12 and 24 ppm) influences hatching rate as well. Production of the cysts in different salinities (40, 75 and 100 ppt) has a limited influence on their hatching rate.

The selection of early hatching cysts is possible (existence of additive genetic variance) and the response is higher at a culture salinity of 75 ppt than at 40 ppt. The state of diapause termination, reflected in the hatching percentage, interferes with the hatching rate: cysts terminating diapause after the shortest storage period in brine at 6 °C, display a relatively delayed hatching rate. Cysts requiring a longer storage period in these conditions tend to show a relatively advanced hatching rate. The conditions of breaking diapause, like the use of hydration–dehydration cycles, have an important influence on hatching rate as well.

Further selection tests should be done with cyst batches showing maximal diapause termination. Our results also suggest that it might be possible to select cysts, which are more easily and rapidly released from diapause.

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