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International Study on *Artemia**. XIV. Growth and Survival of *Artemia* Larvae of Different Geographical Origin in a Standard Culture Test

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ABSTRACT: For characterization of strains of the brine shrimp *Artemia* of different geographical origin, a standard culture test has been developed in order to compare statistically growth and survival of larvae of different strains. 25 geographical strains have been studied so far – including, for 3 strains, analyses of cysts harvested at different times. Important differences in rates of growth and survival were observed between strains but not among batches of the same strain. Best performances were noted for strains from Bahia Salinas (Puerto Rico), Buenos Aires (Argentina), Chaplin Lake (Canada), Great Salt Lake (Utah, USA), Galera Zamba and Manaure (Colombia).

INTRODUCTION

The use of *Artemia**** as live food for larval fishes and crustaceans has, thus far, been mostly restricted to nauplii (for details consult 'Marine Ecology', Volume III: Kinne, 1977). Although it was demonstrated that, as predators grow, better survival and growth are obtained with brine shrimp larvae (Walne, 1967; Sick and Beaty, 1974; Purdom and Preston, 1977), nauplii are still preferred because of the simplicity of hatching procedures, as opposed to more labour, equipment and food costs required for raising larvae (Brouillet, 1977 in Girin, 1979). However, due to recent developments in *Artemia* cultivation on cheap diets (Sorgeloos et al., 1980; Dobbeleir et al., 1980) in easy-to-operate raceway systems (Bossuyt and Sorgeloos, 1980), the use of *Artemia* larvae and preadults in aquaculture hatcheries is gaining interest (Sorgeloos, 1980), not only because of the improved output of fish and crustacean hatcheries, but also because of substantial savings in the quantity of *Artemia* cysts needed for hatchery operations (Fujimura, 1978).

Within the framework of the 'International Study on *Artemia*', whose goal is to improve the use of brine shrimp in aquaculture through characterization and selection studies of various geographical strains of *Artemia* (Sorgeloos, 1980), a comparative study of growth rates and conversion efficiencies is presently in progress. Although there is evidence that growth rates can vary significantly from one strain of brine shrimp to another (Gilchrist, 1960; Sorgeloos, 1975; Tobias et al., 1980) more information is needed, preferably obtained under standardized experimental conditions.

This paper reports on the rates of growth and survival of 25 different *Artemia* strains (3 of which were analyzed after differential harvesting in a standard culture test.

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*** The binomen *Artemia salina* L. is taxonomically no longer valid (see reviews in Bowen and Sterling, 1978; Bowen et al., 1980). In view of the important genetical differences between parthenogenetical strains of brine shrimp, (Abreu-Grobois and Beardmore, 1980) species definition in the genus *Artemia* has become unclear. One of the main conclusions of the 'International Symposium on the Brine Shrimp, *Artemia salina* L.' (Corpus Christi, Texas-USA, Aug. 20–23, 1979) was that, unless the exact sibling species can be identified (20 bisexual strains have been classified so far into 5 sibling species by Bowen et al., 1978) and until speciation in brine shrimp is better understood, only the genus designation *Artemia* should be used (Persoone et al., 1980)

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MATERIALS AND METHODS

For each *Artemia* strain approximately 100 mg of cysts were incubated under optimum hatching conditions (Sorgeloos, 1980). The culture experiments were carried out in test tubes filled with 25 ml of 0.2-μm filtered natural seawater and kept in darkness at 25 °C ± 0.5 C°. Each tube was inoculated with 10 instar-I nauplii.

The *Artemia* larvae were fed once daily with a defined number of algal cells *(Dunaliella viridis)*: Day 1, 12×10^5 cells; Days 2, 3 and 4, 24×10^5 cells; Days 5 and 6, 36×10^5 cells; Day 7, 48×10^5 cells. This optimal feeding schedule has been determined by Vanhaecke and Cooreman (1979) in preliminary culturing tests with nauplii hatched from San Francisco Bay (California-USA) cysts (San Fancisco Bay Brand Company, Batch 288–2596).

The algae were cultured according to De Pauw et al. (1978), harvested in their exponential growth phase by centrifugation, then resuspended in filtered seawater and diluted to a final concentration of 12×10^6 cells ml^{-1}. The suspension was then stored in darkness at 4 °C during the whole experimental period. Algal suspension was distributed with an automatic micropipet (100, 200, 300 or 400 μl $tube^{-1}$ d^{-1}) according to feeding schedule. After each feeding the test tubes were shaken to assure good food distribution.

The number of surviving brine shrimp were counted on Day 8, fixed with lugol's solution (100 μl per tube). These were then transferred to microscopic slides where their length (from the anterior tip of the head along the intestine to the base of the furca) was determined by camera-obscura drawing at a magnification of 50x.

The minimum number of replicate test tubes necessary for statistical analysis for each geographical strain was determined according to Cohran and Cox (in Sokal and Rohlf, 1969) using data from preliminary experiments (Vanhaecke and Cooreman, 1979). Four replicate tubes assured 95 % probability for detecting a 15 % difference from the mean at the 0.05 level. Since higher mortalities can be expected with some experimental strains (the San Francisco Bay strain gives an average of over 90 % survival) 5 replicate test tubes per strain were used. Test tubes with a survival below 70 % were not taken into consideration for growth evaluation.

The culture tests are designed to enable statistical interpretation by means of a one way analysis of variance, Model I (Sokal and Rohlf, 1969). The survival data are normalized through an arc sin $\sqrt{\text{percent}}$ transformation. The standard culture test was repeated for the strains from Shark Bay, Macau (harvested in March 1978), and from Adelaide and Great Salt Lake (harvested in 1977).

A comparison between all strains tested was possible by using the San Francisco Bay strain, Batch 288–2596 as inner standard; the growth for each strain (up to 5 strains were tested per experiment) was expressed as percentage of the growth recorded for the reference strain.

RESULTS

For the reference strain, average larval length after 7 days is 3.16 ± 0.17 μm; the mean percent survival was 94.2 ± 2.8 %. Growth and survival data for the 25 geographical strains tested are summarized in Table 1.

Significant differences in growth rates occur between the geographical strains studied. Compared to the reference strain, brine shrimp form Larnaca Salt Lake, Santa Pola, Salin du Giraud and an unknown locality in China grow significantly slower. In contrast, higher growth rates prevail for *Artemia* from Adelaide, Manaure, Bahia Salinas, Great Salt Lake, Buenos Aires, Galera Zamba and Chaplin Lake. For 14 other strains no significant differences from the reference strain could be observed. The variations between different harvests from the same strain and between the 4 repeated experimental runs are small and not significant. Furthermore, the differences in growth rates between the San Francisco Bay strain and the strains originating from either the San Francisco Bay transplantations in Macau and Barotac Nuevo (Sorgeloos et al., 1979) or the P_1 generation of San Francisco Bay cysts produced under laboratory conditions (Versichele and Sorgeloos, 1980) are statistically not significant.

With regard to percent survival, mortality rates significantly higher than in the reference strain prevail for *Artemia* from Bonaire, Salin du Giraud, Great Salt Lake 1966, Larnaca Salt Lake, Lavalduc, Buenos Aires, San Felix and Santa Pola. Variations between different harvests are small with the exception of the Great Salt Lake samples 1966 and 1977. However, during the last decade this biotope has been subjected to considerable ecological changes (Stephens and Gillespie, 1972).

DISCUSSION

The standard culturing test, outlined above and tested on 25 strains, appears to be applicable for comparing *Artemia* strains on the basis of larval growth and mortality. Since it has been reported earlier that the optimal temperature-salinity combination can vary from one *Artemia* strain to another (Sorgeloos et al., 1976) it is possible that some differences in growth and

Table 1. *Artemia*. Percent growth and survival of different geographical strains of brine shrimp under standard culture conditions

Geographical strain	Survival at Day 7 (%)		Growth expressed as % recorded for *Artemia* reference strain, San Francisco Bay, Batch 288–2596		
Larnaca Salt Lake (Cyprus)	70*		88		*
Santa Pola (Spain)	76*		88		
Salin du Giraud (France)	66*		90		
China (unknown locality)	84		91		
Shark Bay (Australia)	90	84[a]	95	96[a]	
San Francisco Bay (California/USA; cysts produced in the laboratory)	92		96		
San Francisco Bay (California/USA; Batch No 236–2016)	98		96		
Macau (Brazil; harvested in March 1978)	84	96[a]	96	98[a]	
Barotac Nuevo (Philippines)	86		97		
Tientsin (China)	96		98		
San Francisco Bay (California/USA; Batch No 933–235)	94		99		
Aigues Mortes (France)	90		99		
Izmir (Turkey)	96		101		
Margherita di Savoia (Italy)	96		102		
San Felix (Spain)	74*		102		
Macau (Brazil; harvested in May 1978)	94		103		
Bonaire (Netherlands Antilles)	66*		104		
San Pablo Bay (California/USA; Batch No 1628)	90		105		
Port Araya (Venezuela)	90		107		
Eilat (Israel)	92*		107		
Lavalduc (France)	70*		109		
Adelaide (Australia)	88	88[a]	113*	113[a]	
Manaure (Colombia)	90		115		
Bahia Salinas (Puerto Rico)	88		122		
Great Salt Lake (Utah/USA; harvested in 1977)	94	94[a]	119	125[a]	
Buenos Aires (Argentina)	72*		126		**
Galera Zamba (Colombia)	98		126		
Great Salt Lake (Utah/USA; harvested in 1966)	66*		127		
Chaplin Lake (Canada)	88		130		

[a] result of replicate test in time
* significantly different from the reference strain at the 0.05 level
** significantly different from the reference strain at the 0.01 level

survival between the strains studied have been masked by our present culture technology (25 °C; natural seawater of ca 35 ppt). In any case, the order of growth performance reported by Sorgeloos (1975) for three strains (San Francisco Bay, Great Salt Lake and China) is identical to the sequence obtained here for the same strains.

In this study, all *Artemia* strains that grew significantly faster than the reference strain were bisexual. However, Gilchrist (1960) reported that a parthenogentic strain from La Palme (France) grew faster than a bisexual *Artemia* from San Diego (California, USA). It appears from our results that this observation cannot be ascribed to a difference in growth rate related to the mode of reproduction. Our study provides further evidence that growth rates of *Artemia* larvae are strain-dependent. There are indications that this phenomenon might be genetically controlled: the strains produced from San Francisco Bay inoculations in Brazil and the Philippines, or from a San Francisco Bay population maintained under laboratory conditions exhibited growth performances similar to the parental strains.

While brine shrimp strains between which the genetic distance is known to be large (e. g. Buenos Aires and Chaplin Lake; Abreu-Grobois and Beardmore, 1980) can have similar growth rates, different rates may be observed in strains of the same sibling species, e. g. from Chaplin Lake and San Francisco Bay, which are both *Artemia franciscana* (Bowen et al., 1978). It is furthermore interesting to note that no correlation exists between growth rates and biometrical characteristics of nauplii from corresponding strains (Vanhaecke and Sorgeloos, 1980).

With regard to the practical use of *Artemia* in aquaculture, the results reported here provide a guideline for the selection of specific geographical strains. While data on growth rates are useful, addi-

tional criteria should be taken into consideration. Accordingly, our comparative analyses have now been extended to include biomass production, food conversion efficiency and temperature-salinity preferences.

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