Quality evaluation of brine shrimp *Artemia* cysts produced in Asian salt ponds

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Abstract

Artemia cysts produced in inoculated salt ponds in the Philippines, Thailand and India, were analyzed in comparison with the parental strains used for the inoculations. Cyst hatching efficiency, hatching rate and color changed significantly after inoculation, whereas the cyst's diameters, nauplius survival and nauplius growth remained fairly constant. The nutritional value of the *Artemia* nauplii was determined in a standard culture test with *Mysidopsis bahia* juveniles as test animals. Production results were compared with the fatty acid profiles of the *Artemia nauplii*. Low levels of the essential fatty acid 20:5ω3 in one of the produced cyst batches were probably caused by inadequate food conditions in the pond and resulted in poorer growth of the *Mysidopsis* juveniles. Based on the results of this study a distinction is made between essential and non-essential strain selection-criteria for *Artemia* inoculations and transplantations.

Introduction

Brine shrimp *Artemia* inoculations, i.e. man-managed introductions of *Artemia* in seasonal salt ponds (Persoone & Sorgeloos, 1980) appear to have large potential in the tropical belt in view of the ever increasing demands for live food in local aquaculture hatcheries and nurseries (Sorgeloos, in press). Some countries such as Thailand are very successful in applying this technique and produce *Artemia* cysts in areas which previously completely depended on high-priced imports (Tunsutapanich, 1980; Sahavchasin, 1981). Other countries in SE-Asia and Central America (e.g. Vos & de la Rosa, 1980; Naegel, 1979; Royan, 1981) are still in the experimental phase, trying to adapt available technology to local conditions.

So far most efforts in *Artemia* inoculation projects have been directed towards increased production outputs through improved pond design and management. Although it is well documented that *Artemia* strains belonging to the same genotype can differ largely in many characteristics (see reviews by Sorgeloos, 1980, in press) little or no attention has been paid to the impact of environmental effects on the quality of *Artemia* cysts produced by inoculated populations.

In this study we report about differences between inoculated *Artemia* cysts and cysts harvested after a few months production in the inoculated salt ponds in the Philippines (at Barotac Nuevo in 1978 and at Jaro in 1981), Thailand (at Bangpakong in 1979) and India (at Mundra in 1979).

The following characteristics were analyzed:
- hatching rate and efficiency;
- color of the cyst's shell which correlates with radiation sensitivity and nauplius survival (Meade, 1976);
- cyst diameter which is proportional to the nauplius length (Vanhaecke & Sorgeloos, 1980a) and has its importance with regard to the ingestibility of the nauplii by early live stages of cultured species;
- survival and growth of the nauplii as important criteria in culturing;
- biochemical composition and nutritional value of freshly-hatched nauplii for cultured species.

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Table 1. Summary of abiotic and biotic conditions in inoculated ponds.

Site	Pond surface (m²)	Water depth (cm)	Water temperature (°C)	Salinity range (‰)	Food	Origin of cysts used for inoculation	Batch identification of cysts harvested from inoculated ponds
Bangpakong (Thailand)	220	20-30	25.5-37.0	100-150	Natural productivity (regular mangrove water intake)	San Francisco Bay Brand Cy batch 1728 (SFB 1728)	BP 1979
Barotac Nuevo (Philippines)	16 000	30	25.0-37.0	80-170	Natural productivity (regular mangrove water intake)	San Francisco Bay Brand Cy batch 2596 (SFB 2596)	BN 1978
Jaro (Philippines)	1 250	30-45	25.0-37.0	100-140	Inorganic fertilisation (regular brine intake)	Barotac Nuevo batch 1978 (BN 1978)	JA 1981
Mundra (India)	3 000	40	28.0-35.0	100-160	Natural productivity (regular mangrove water intake)	San Francisco Bay Brand Cy batch 2596 (SFB 2596)	MU 1979

Methods

Inoculation and cyst production

All selected ponds were situated in privately-owned saltfarms and had previously been used as evaporation ponds. Prior to inoculation with *Artemia* most of the ponds had to be deepened by heighthening the dikes as to assure water depths of 30-40 cm in which temperatures never exceed the lethal level of 37 °C.

Seawater and brine were taken in either by gravity flow or by portable pumps. *Artemia* predators such as fish and crustacean species were eliminated from the ponds by screening the intake water and/or by keeping the salinity at levels higher than or close to 100‰ throughout the production period. Sufficient food levels were assured by either regular intakes of mangrove water or by regular fertilization with inorganic compounds (mono-ammoniumphosphate and urea, each at 50 Kg/ha/14 days).

Artemia cysts from the strain used for the inoculation were hatched out under laboratory conditions in natural seawater. Freshly-hatched nauplii were transferred to the culture ponds at densities ranging from 10 to 40 nauplii/l pond water. In less than 2 weeks, the inoculated brine shrimp larvae had grown to adult size and started to produce cysts (oviparous reproduction) and nauplii (ovoviviparous reproduction).

In a first phase the ovoviviparous reproduction mode was favored by maintaining the salinity levels close to 100‰; as a consequence, the continuous recruitment assured a fast increase of the population. After about 2-3 months the salinity levels were allowed to increase by evaporation and the overall mode of reproduction switched to oviparity.

Floating cysts aggregated in dense patches in the corners of the ponds from where they were regularly harvested with scoop nets. The cysts were then processed following the recommendations by Sorgeloos *et al.* (1978) and Sorgeloos (1978) to give a clean and dry product which was eventually stored under vacuum until further analysis. Production yields, extrapolated from individual ponds, ranged from 10 to 60 kg dry cysts/ha/5 month period.

More details on the production conditions at the various inoculation sites can be found in Table 1 and in the papers of De los Santos *et al.* (1980), Royan (1981), Tunsutapanit (1979) and Vos (1981).

Cyst hatching efficiency and hatching rate

The hatching efficiency was determined at 25 °C, under continuous illumination of 1 000 lux, and in 35‰ seawater according to Sorgeloos *et al.* (1978), and was expressed as the number of nauplii hatched after 48h/g dry cysts. The hatching rate was determined under the same conditions according to the

procedure mentioned by Vanhaecke & Sorgeloos (1982) and expressed as hours of incubation needed to reach 0, 50, and 90% (T_0, T_{50}, T_{90}) of the maximal hatching value.

Cyst diameter

The diameter of fully hydrated cysts was determined with Coulter Counter equipment according to the technique described by Vanhaecke *et al.* (1980).

Nauplius survival and growth

Survival and growth of the nauplii hatched from the different cyst batches were determined in the standard culture test of Vanhaecke & Sorgeloos (1980b) using San Francisco Bay batch n° 288 2596 *Artemia* as reference material. After 7 days culturing on a *Dunaliella* diet, the percent survival and the average length of the larvae were determined. The growth of the nauplii was expressed as a percentage of the growth recorded for the reference strain.

Nutritional evaluation

The nutritional evaluation studies were limited to a fatty acid analysis of the nauplii and to a culture test with the marine mysid *Mysidopsis bahia*. For the fatty acid analysis instar I nauplii were homogenized in a Ultraturax homogenizer and extracted three times with a methanol: chloroform: water (2: 1: 0.8) mixture (Bligh & Dyer, 1959). The remaining solids were extracted with acetone according to the procedure of Schauer *et al.* (1980). Saponification and methylation were carried out as described by Schauer & Simpson (1978). The separation of the fatty acid methyl esters (FAME) was performed by gas chromatography (Carlo Erba FTV 2300) on a glass column (1.8 m × 2 mm I.D.) packed with 10% Altech CS-8 on chromosorb W-AW 100-120. The gaschromatographic conditions were: 200 °C isothermal; carrier gas N_2 40 ml/min; F.I.D.-detection. The identification and quantification were done with a calibrated method on a Hewlett packard 3390 A plotter integrator. The results are presented as area-percent compositions.

The standard culture test with *Mysidopsis bahia* larvae will be described in detail by Léger & Sorgeloos (in prep.) Newborn mysid juveniles are transferred into 1000 ml glass bowls and fed daily with freshly-hatched nauplii. The survival is registered daily and after 12 days culturing, the growth and reproductive characteristics of the pre-adult mysids are analyzed. All the data were statistically analyzed in a one way analysis of variance. The Duncan's Multiple Range Test was used to determine significant differences among means. Prior to analysis, the survival data were normalized through an arcsin $\sqrt{\%}$ transformation (Snedecor & Cochran, 1967). Inoculations originating from SFB 2596 and SFB 1728 were treated separately.

Table 2. Characteristics of cysts and nauplii from parental cysts and from cysts harvested from inoculated populations (legend to abbreviations in Table 1).

	SFB 1728	BP 1979	SFB 2596	BN 1978	JA 1981	MU 1979
Hatching efficiency (nauplii/g)	100 800	304 000	267 200	214 000	339 200	236 800
Hatching rate characteristics[a]						
T_0	24.5	14.5	15.0	14.7	14.4	14.6
T_{50}	32.8	18.8	17.6	18.8	17.6	16.3
T_{90}	39.2	25.6	20.5	22.0	22.0	18.9
Color of cysts	pale	dark	pale	dark	dark	pale
diameter of cysts (μm)	225.8	232.2	224.7	228.0	225.2	222.1
standard deviation	17.3	11.8	12.4	13.0	11.7	10.8
Nauplius survival at day 7 (%)	94	96	94	86	..[c]	88
Nauplius growth at day 7[b]	100	106	100	97	..[c]	105

[a] Values refer to time-lapses (in hours) from incubation until appearance of the first nauplii (T_0) or the moment by which 50% (T_{50}) and 90% (T_{90}) of the hatching efficiency has been reached.
[b] Expressed as % recorded for *Artemia* reference strain, San Francisco Bay Brand Cy batch 288-2596.
[c] No data available.

Table 3. Results of *Mysidopsis* feeding-test with *Artemia* nauplii from different cyst sources (legend to abbreviations in Table 1).

Mysis results	Artemia source: SFB 2596	MU 1979	BN 1978	JA 1981	SFB 1728	BP 1979
Survival (%)	93.3[a]	86.7[a]	94.0[a]	75.0[b*]	81.3	90.0
s	5.2	13.8	5.5	10.6	11.0	8.9
Dry weight (μg)	318.1[a]	371.9[a]	385.8[a]	264.0[b**]	306.6	311.9
s	44.1	47.7	48.2	31.8	22.4	37.7
Length (μm)	4467[b*]	4479[a]	4369[b*]	3192[c**]	4748	4758
s	185	181	170	193	166	145
Reproductive data (%)						
♂i/♂♂	4.6	0	0	0	0	0
♀i/♀♀	50.6	13.3	8.0	48.2	48.8	30.3
♀°/♀♀	49.4	73.5	76.0	51.9	51.2	51.5
♀m/♀♀	0	13.3	16.0	0	0	18.2

Legend to symbols
[a,b,c] Means with a different superscript (a,b,c) are significantly different at the *α:0.05 or **α:0.01 level.

♂i: immature male
♀i: immature female
♂♂: total number of males
♀♀: total number of females
♀°: female with eggs in ovaria
♀m: female with eggs in marsupium

Results

The characteristics of cysts and nauplii from the various cyst products are summarized in Table 2. Apparently the hatching efficiencies, hatching rates and cyst colors were altered after inoculation. On the other hand the cyst's diameter, nauplius survival and nauplius growth showed only minor changes as a result of the new environmental conditions. The most pronounced differences were noted for the hatching quality criteria. The results showed that upon inoculation not only poorer (e.g. Barotac Nuevo) but also better qualities (e.g. Bangpakong & Jaro) may be harvested. The cyst's color changed from pale to dark in some inoculated products (Bangpakong & Barotac Nuevo) but remained dark in the Jaro and pale in the Mundra-inoculations.

Production performances of the mysid larvae fed with the various *Artemia* products are summarized in Table 3. High survival rates were observed for all the nauplii products. After 12 days of culturing, all mysids reached sexual differentiation. The survival, growth and reproductive characteristics obtained with a diet of *Artemia* from Mundra, Barotac Nuevo & Bangpakong were at least as good as the data recorded for mysids fed with parental *Artemia* material (SFB 2596 and SFB 1728 respectively). Significant differences however in survival and growth were noticed between test-animals fed Jaro and those fed the parental *Artemia* from Barotac Nuevo.

From Table 4 it is clear that the fatty acid profile varied considerably among *Artemia* nauplii from the different sources. In view of the importance of the highly unsaturated fatty acids for marine animals (Schauer *et al.*, 1980; Watanabe *et al.*, 1980; Léger *et al.*, in prep.) the deficiency for the essential fatty acid (E.F.A.) 20:5ω3 in *Artemia* nauplii from Jaro is obvious.

Discussion

It is very likely that the variations observed in the quality of cyst hatching between the parental cysts and those harvested from the inoculated populations are due to differences in cyst harvesting-, processing- and storage techniques.

From the studies of Sorgeloos *et al.* (1976, 1978) and Vanhaecke & Sorgeloos (1982, 1983) it appears indeed that essential variables in this respect are the regularity of harvesting; the percentage composition of the product in full cysts, empty cysts and debris; the water content of dry cysts; the method used for drying and storage, etc. Up to now, no decisive evidence of eventual effects of the envir-

Table 4. Procentual composition of fatty acid methyl esters (F.A.M.E.) of *Artemia* nauplii from the different cyst sources.

F.A.M.E.	SFB 2596	MU 1979	BN 1978	JA 1981	SFB 1728	BP 1979
14 : 0	1.3	0.9	1.7	1.4	1.8	0.7
14 : 1	0.9	2.4	1.4	1.5	0.9	2.6
15 : 0	0.3	0.9	1.4	1.5	1.3	
15 : 1	0.2	1.4	0.7	0.8	0.7	0.7
16 : 0	13.0	12.7	14.4	11.4	14.4	10.1
16 : 1ω7	21.9	8.9	15.9	13.7	16.3	10.3
16 : 2ω7 17 : 0		1.6	1.9	2.1	1.3	
16 : 3ω4 - 17 : 1ω8	0.8	2.3	4.0	4.7	5.3	1.3
18 : 0	3.0	2.8	3.3	3.9	3.3	2.9
18 : 1ω7 ω9	34.1	27.9	29.6	27.0	28.0	31.4
18 : 2ω6	4.7	12.0	9.1	15.0	4.5	5.5
20 : 0 [a]	7.8	14.6	4.2	12.9	9.2	23.3
18 : 3ω3 ω6 [a]						
18 : 4ω3	1.9	3.0	1.2	1.1	1.1	3.2
20 : 2ω6 ω9	0.2	0.3	0.3	0.5	0.4	0.3
20 : 3ω3 ω6	0.1	0.2	0.2		0.2	0.1
20 : 4ω3 ω6	1.9	1.5	2.1	1.3	2.5	1.7
22 : 1	0.3	0.8	0.3	0.1	0.2	0.6
20 : 5ω3	7.9	5.3	8.6	1.9	13.8	5.3
22 : 2 21 : 5	-	0.3	-	-	-	-
24 : 0	-	0.2	-	-	-	-
22 : 6	-	0.1	0.3	-	-	-

[a] More than 99% 18 : 3ω3.

onment on the cyst hatching quality is available. The results prove, however, that even an inoculation with low quality cysts can produce excellent cysts. The hatching quality of the parental material should therefore not be considered as an important strain selection-criterion for inoculation.

In the Philippine and Thai inoculations, the harvested cysts always had a dark brown color which indicates a high hematine content in the cyst's shell (Gilchrist & Green, 1960) which in turn is probably correlated with a high hemoglobin concentration in the mother-animal. The environment plays an obvious role through the Fe-content of the ingested food and low oxygen concentrations of the water. Thus the marked difference between San Francisco Bay parental cysts and Barotac Nuevo and Thai cysts on one hand, and the persistence of the dark color of Barotac Nuevo cysts through the Jaro inoculation on the other hand, could be explained by the much higher average water temperatures and consequently lower oxygen concentration in these tropical conditions as compared to the San Francisco Bay environment. The effect of the salinity concentrations can be neglected in this comparison since the San Francisco Bay cysts are produced in similar salinity ranges (Carpelan, 1957; Baker, 1966; pers. observ.). The same reasoning applies to the dark colored Macau (Brazil) cysts which are produced since 1978 from another San Francisco Bay transplantation (Sorgeloos *et al.*, 1979). It is, however, not a general rule that in hot tropical biotopes darker cysts are produced, because the Mundra cysts which were exposed to equally hot and saline conditions as the Thai and Philippine inoculations, turned out to be as pale as their parental SFB strain. It is therefore difficult to predict the cyst's color because it seems to be controlled by a whole set of environmental factors, but it certainly is not a strain-dependent characteristic.

The constancy of the cyst's diameters (overall range: 222–232 μm) and the growth of nauplius (97–106% of growth recorded for the reference strain) confirms the earlier conclusion of Vanhaecke & Sorgeloos (1980a, b) that these characteristics are strain dependent and, except for small differences, are unaltered by a new environment. These characteristics are therefore essential criteria to be considered in selecting suitable strains for inoculation and transplantation purposes.

The poorer nutritional value of nauplii from Jaro

Artemia cysts for *Mysidopsis bahia* correlates with a lower content of the E.F.A. 20:5ω3. This confirms the finding of Johns *et al.* (1981) and Léger *et al.* (in prep.) that *Artemia* nauplii with low levels of 20:5ω3 give poor production results when offered as food to *Mysidopsis* juveniles. The lower content of particular fatty acids in the nauplii is probably explained by similar profile differences of the food ingested by the cyst-producing adults (Hinchcliffe & Riley, 1972). Food management of the ponds is therefore of crucial importance. Up to now, however, no specific management guidelines exist in this respect because local conditions vary considerably. The Jaro inoculation was the only one where the fertility of the water (brine from the adjacent salt-farm) was supplemented with inorganic fertilization. This resulted in blooms of reddish-colored algae which might not have been an ideal food for *Artemia*. It therefore seems that the safest results may be obtained in those inoculations where the available food has a great composition diversity, such as in inoculations using the natural fertility of intake water (e.g. mangroves, estuaria) and/or organic fertilizers as is presently routinely done in Thailand with excellent results.

In view of the importance of sufficient levels of 20:5ω3 in *Artemia* nauplii, and considering the fact that fatty acid profiles are mainly determined by the composition of the assimilated food, one cannot assure that inoculation with good quality cysts will result in cysts of an equally high nutritional value.

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