Effects of Cultivation Techniques on the Characteristics of Cysts from a Salina in Kenya

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Cysts produced after inoculation with San Francisco Bay (SFB) strain Artemia were harvested and analysed at *Artemia* Reference Centre. Results obtained after nutritional analysis indicate a potential source of high quality cysts. Cyst hatching percentage, hatching rate and hatching efficiency was 61%, 24h and 173,400n/g respectively. Hatching percentage (H%) varied slightly with variation in salinity and peroxide treatment. Highly unsaturated fatty acids (HUFA) content was representative of high quality cysts. This was also supported by the good growth performance in test with the marine Mysid, *M. bahia* M.

Les kystes produits d'une culture inoculée des souches d'Artemia de San Francisco Bay (SFB) ont été récoltés et analysés au Centre de Référence d'Artemia. Les résultats obtenus après une analyse nutritionnelle font preuve d'une source potentielle des kystes de haute qualité. Le pourcentage, le taux et la capacité d'éclosion des kystes ont été respectivement de 61%, 24h et 173.400n/g. Le pourcentage de H s'est légèrement modifié en variant la salinité et le traitement péroxyde. La teneur en acides gras hautement non-saturés (AGHN) a permis d'obtenir des kystes de haute qualité. Ce résultat a été également confirmé par la performance de croissance bonne enregistrée à l'observation réalisée sur le Mysid marin, *M. bahia* M.

Introduction

The brine shrimp *Artemia* has been the most widely accepted live-feed in aquaculture for the culture of larval stages of marine fish and crustaceans. Due to its wide range of biotopes, *Artemia* has been introduced in such countries like Brazil, Thailand, Phillippines and Kenya with great success. However, this has also led to an influx of different *Artemia* strains in the international market, some of which are inferior in quality (Vanhaecke and Sorgeloos, 1983; Léger *et al.*, 1986). This has necessitated the creation of a body (International Study on *Artemia*) to evaluate *Artemia* cysts for the purpose of standardization.

Artemia from San Francisco Bay (SFB) was introduced in Kenya in 1985 (Rasowo and Radull, 1986). Since then there has been a continuous population in the salinas after the initial inoculation. As observed by Léger *et al.* (1986) it is necessary to evaluate nutritional value of *Artemia* as a food to the predator. Knowledge of hatching quality parameters of the cysts such as hatching effeciency (HE), hatching percentage (H%), and hatching rate (HR) are also vital to the aquaculturists. This is especially so for large scale farmers, for ease of operation and the determination of the cost effectiveness of the cysts. In view of the importance of this exercise, cysts were tested for fatty acid composition and hatching quality at the Artemia Reference Centre (ARC) State University of Ghent, Belgium.

This paper reports the findings of the analysis and makes a comparison with findings from quality tests on other cyst strains in order to establish the suitability of cysts from inoculation in Kenya for aquaculture.

Materials and Methods

Cysts for the analysis were collected at the salinas of Fundisha saltworks. No modification was done on the ponds since Artemia introduction and there was no fertilization of the ponds. Processing of the cysts was done following the techniques described by Sorgeloos (1978; 1979). The dry product was stored in sealed synthetic bags. No hydrogen or vacuum treatment for preservation was used. Analysis for cyst hatching efficiency, hatching rate and hatching percentage was done two months post-harvest. Cyst diameter and chorion thickness were measured and nutritional analysis carried out.

Cyst hatching efficiency, hatching rate and hatching percentage

These parameters were determined under standardized conditions of 1,000 lux in 35% artificial sea-water for 48h according to the methods of Sorgeloos (1978). Hatching efficiency was expressed as the number of nauplii hatched per gram dry cysts, while hatching rate as hours of incubation needed to

reach 0%, 50% and 90% (T0, T50 and T90) of total hatching efficiency. For H%, 100 full cysts were incubated and the total hatch expressed as a percentage. Experimentss were also performed to determine the effects of peroxide and salinity variation on the hatching percentage of the cysts. Peroxide was used in concentrations of 0.05% and 0.5% while salinities were 10% and 35% artificial seawater.

Cysts diameter and chorion thickness

Cysts were re-cleaned using bi-floatation method and hydrated for 2h at 25°C; a light intensity of 1,000 lux and in 10% artificial sea-water. Lugol's solution (1%) was added to prevent metabolic activity.

Some of the hydrated cysts were decapsulated following the method of Bruggeman *et al.* (1980). Both decapsulated and non-decapsulated cysts were also incubated in 1% Lugol's solution in the dark for 24h before measurement. An optical microscope was used to measure the diameters after calibration with Reference Artemia Cysts batch (SFB 188-2590).

Nutritional evaluation

Nutritional evaluation studies were limited to fatty acid analysis of freshly hatched nauplii and culture test with a marine mysid, *Mysidopsis bahia* (Molenock, 1966). Freshly hatched nauplii were homogenized in a Ultraturax homogenizer and extracted three times with a methonol:chloroform: water (2:1:0.8) mixture following the method outlined in Vos et al. (1984). The remaining solids were extracted with acetone as per the procedure of Schauer *et al.* (1980). The qualitative fatty acid composition of the cysts was analysed by gas chromatography (Carlo Erba FTV 2300) and the identification and quantification were done with a calibrated method on a Hewlett Packard 3390A Plotter-integrator. The results were expressed as area-percent compositions.

The culture test with *Mysidopsis bahia M.* was done following the method described by Léger *et al.* (1987). A homogeneous population of 24h old mysid juveniles were cultured under standard conditions and fed on freshly hatched *Artemia* from different strains. After 12 days, results on survival, individual length, dry weight and reproductive characteristics were analysed. Though strains from different climatic zones were tested for nutritional quality for *M. bahia*, only results from tropical and subtropical strains and the parental SFB strains were considered in this paper. Data was analysed by a one-way analysis of variance. Percentage survival data were normalized through arcsine (arcsin $\sqrt{\%}$) transformation, prior to statistical analysis. The Duncan multiple range test was applied to detect significant differences among means (Sorkal and Rohlf, 1966). A two-way analysis of variance was used to detect any interactions between the salinity and peroxide treatments.

Results

Results from the cyst hatching quality experiment are shown in Tables 1 and 2. The HE was 173.400 nauplii/g(n/q) while HR was 24h and H% was 61%. While the HR ranges between 20-30h, the H% varied with different treatments for specific diapause deactivation requirements. The best hatching results were obtained in salinities of 10% artificial sea-water. Individually, both salinity and peroxide treatments had marked effects on Artemia hatching (Table 1). A two-day analysis of variance indicated no significance in interaction of the two parameters. At higher peroxide levels, there was evident decline in H%. Cyst diameter was 237±25µm while the chorion thickness was 8µm.

Table 1. Hatchability (%) of cysts under different salinity-peroxide treatments.

		Salinity (%)	
		10	35
Peroxide	0	66.0 ± 5.62	61.0 ± 4.64
	0.05	65.0 + 6.51	64.0 + 6.11 (% conc.)
	0.5	61.0 + 6.07	49.0 + 9.04

Table 2. Biometrics of cysts from Kenya and from parental[1] and other inoculated strains.

	SFB (1728)	BP (1979)	SF (2596)	BN (1978)	KE (1987)
H.R. (n/g)	100.800	304.000	267.200	214.000	173.400
H.R. (hours)					
T_0	24.5	14.5	15.5	14.7	14.0
T_{50}	32.8	18.8	17.6	18.8	17.5
T_{90}	39.2	25.6	20.5	22.0	24.0
Cyst diameter	225.8	232.7	224.7	228.0	236.7
Std. dev.	17.3	11.8	12.4	13.0	25.1

T_0 time taken from incubation to appearance of first nauplii
T_{50} time at which 50% of full cysts have hatched (i.e. 50% of H.E)
T_{90} time at which 90% of full of cysts have hatched (90% of H.E.)
SFB San Francisco Bay *Artemia*
BN Barotac Nuevo (Phillipines) *Artemia*
KE Kenya *Artemia*
(n/g) nauplii/gram
SFB (1728,2596) cyst batch number
[1]fromVos *et al.* (1984)

Fatty acid profile (Table 3) shows that cysts from the inoculation are similar in composition to other cysts produced in the Phillipines (BN) and Thailand (BP). Small differences in quality of the different fatty acids are present. The total lipid composition was 22.46% while the total HUFA was 8.7%. A preliminary analysis of cysts collected from the same

source in 1985 showed a higher percentage of HUFA (10.6%) and total lipid composition of 29.06%.

Quality evaluation performances with the mysid larvae are summarized on Table 4 and Fig. 1. Compared to GSL *Artemia* and *Artemia* from the Artemia Reference Centre (RAC) of SFB origin, mysids fed on Kenya *Artemia* supported good growth and survival. It supported the best individual dry weight. Maturation after 12 days culture was best but not significantly better than RAC for those mysids fed on the Kenyan *Artemia*.

Table 4. *Mysidopsis bahia* survival and growth for juveniles after 12 days of feeding on freshly hatched *Artemia* from different origins.

Parameter	RAC	GSL *Artemia*	Kenyan *Artemia*
% Survival (±Std. dev.)	57.6 ± 11.9	47.3 ± 13.4*	70.6 ± 12.3
Indl. dry wt.	426.8 ± 24.5	404.7 ± 60.3	404.4 ± 45.0
Indl. length (um ±)	5250.6 + 413.3	5213.2 + 133.7	5693.8 ± 131.1

*Sign at 0.05 level.
RAC - Reference *Artemia* Cysts
GSL - Great Salt Lake *Artemia*

Discussion

The results obtained in experiments with cysts from the inoculation in Kenya indicated a potential source of good cyst material. The relatively poor hatchability of these cysts as compared to other commercial cysts may be attributed to differences in harvesting, processing and storage techniques. Food availability to the parents appear to be the factor of primary importance in determining the hatching quality of the cysts produced (Lavens *et al.*, 1986). The studies of Sorgeloos (1978); Vanhaecke and Sorgeloos (1983) and Lavens and Sorgeloos (1985) also list the percentage composition of full cysts, the water content of the cysts, regularity of collection, storage and specific diapause deactivation requirements as other essential variables. The collection of the cysts used in this experiment was not done on a regular basis thus exposing some of them to cyclic hydration and dehydration processes in the production ponds before they could be harvested. This may have led to untimely diapause deactivation and commencement of metabolic activity. Another factor to note is that the cysts were stored in sealed polythene bags but with no vacuum or nitrogen, leading to a possible metabolic activity within and ultimate degradation of the cysts. The inconsistency of cyst diameter (237µm±25µm) indicates the possibility of contamination of the parental SFB strain with other yet unidentified strain. On the other hand it may also be due to errors accruing from the use of the optical microscope as the method for the determination of cyst diameter. According to Vanhaecke and Sorgeloos (1983) Vos *et al.* (1984), cyst hatching characteristics are strain dependent and as such there should be no great variability of inoculated cysts from the parental strain. The chorion thickness was not at variance with that of the parental strain.

Table 3. Procentual composition of fatty acid methyl series esters (FAME) from different strains and total HUFA composition (%).

FAME	SFB* 2596	BN* 1978	SFB* 1728	BP* 1979	KE 1985	KE 1987
14:0	1.3	1.7	1.8	0.7	2.3	1.1
14:1:14:2	0.9	1.4	0.9	2.6	1.0	2.0
15:0	0.3	1.4	1.3	-	1.9	1.0
15:1	0.2	0.7	0.7	0.7	0.4	0.9
16:0	13.0	14.4	14.4	10.1	14.8	14.8
16:2ω7:0	-	1.9	1.3	-	2.5	1.6
16:3ω7:1µ8	0.8	4.0	5.3	1.3	7.2	2.8
18:0	3.0	3.3	3.3	2.9	3.0	4.3
18:1ω7,ω9	34.1	28.6	28.0	31.4	29.8	29.4
18:2ω6	4.7	9.1	4.5	5.5	8.1	13.1
20:0	7.8	4.2	9.2	23.3	2.3	7.2
18:4ω3	1.9	1.2	1.1	3	0.3	0.4
20:2ω6,ω9	0.2	0.3	0.4	0.3	-	-
20:3ω3,ω6	0.1	0.2	0.2	0.1	0.1	0.2
20:4ω3,6	1.9	2.1	2.5	1.7	3.7	1.6
22:1	0.3	0.3	0.2	0.6	0.1	-
20:5ω3	7.9	8.9	13.8	5.3	6.2	6.3
22:2:21:5	-	-	-	-	0.1	-
22:6ω3	-	0.3	-	-	-	0.3
HUFA>20:3µ3	10.4	11.5	18.0	7.7	10.3	8.4

*SFB, BN, BP, KE - see table 2

The similarity in general fatty acid profile between the Kenyan cysts and those produced in other countries as outlined in Table 3 is quite evident. However it is also evident that there are differences in quality among the different cysts. These differences in the fatty acids of *Artemia* may be attributed to the different locations where there are seasonal variations in food for *Artemia*. The difference in the content of the fatty acid methyl esters between the cyst batches collected in the Kenyan salinas in 1985 and 1987 is also evident. The total HUFA content was higher in the 1985 batch than the latter while the latter had the 22:6 ω3 HUFA which was not detected in the former.

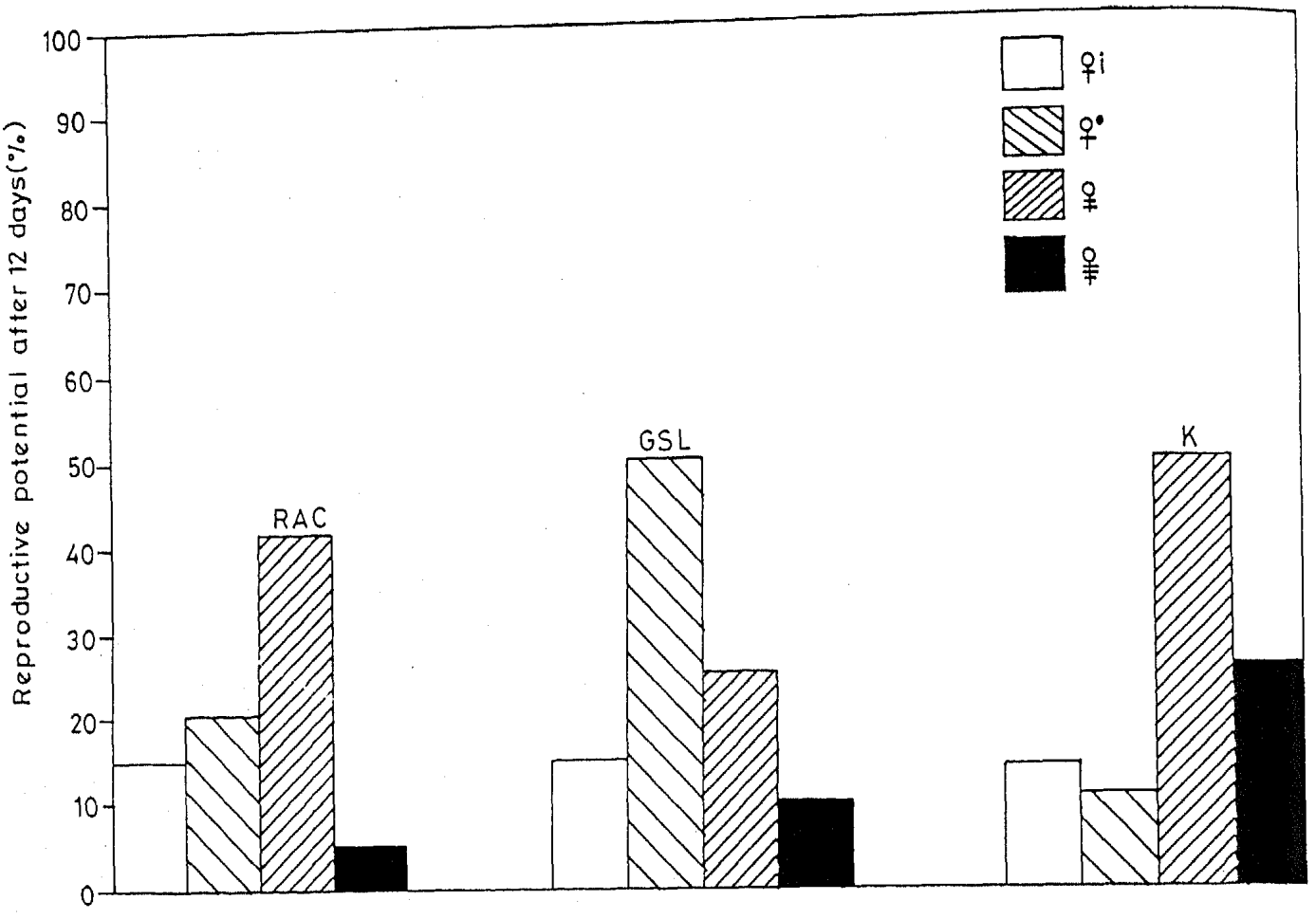

Fig.1. Mysidopsis bahia reproductive potential after 12 days of cultivation using Artemia from different origins as food source. ♀i imature females, ♀• eggs in ovaries, ♀ eggs in broodpouch, ♀ǂ non eyed juveniles in broodpouch.

Cysts from Kenya have a good combination of 18:3 ω3 EFA for fresh water fishes and the HUFA 20:5 ω3 and 22:6 ω3 essential for marine fish and crustaceans (Watanabe, 1980). This essentially means that the Kenya cysts are suitable for both the marine and freshwater fish and Crustaceans. The essential fatty acid content of the Kenya cysts compares well with cysts from other sources e.g., SFB, Phillipines and Vietnam as indicated in Table 3. Growth test with the marine mysid *M. bahia* confirms the already established good quality of the cysts from the inoculation in Kenya. It suffices to say that lower body weight gain of mysids fed on nauplii from Kenyan cysts may be attributed to factors other than the quality of the *Artemia*. In a previous study, Vos *et al.* (1984) reported that poor nutritional value of cysts for *M. bahia* correlates with a lower content of the HUFA 20:5 ω 3, which is not the case in Kenyan cysts.

Conclusions

The hatchability of cysts from the inoculation in Kenya is good as evidenced by the good hatching rate, hatching efficiency and hatching percentage despite lack of standard collecting, and storage facilities and pond management. There is an urgent need to examine the reasons for the varying cyst diameter in the cysts from Kenya to establish whether any contamination could have occurred. As shown by the results from the nutritional quality analysis and mysid growth, test cysts from Kenya are as good as the parental strain. And finally, it surfices to say that with proper pond management and more regularized cyst collection and fertilization, Kenyan cysts have the potential of being one of the best quality cysts in the international cyst market.

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