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International Study on *Artemia** XXIV. Cold Storage of Live *Artemia* Nauplii from Various Geographical Sources: Potentials and Limits in Aquaculture

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ABSTRACT

Freshly-hatched Artemia *nauplii from various geographical sources survived storage in a refrigerator (2–4°C) at densities of 2000 per ml and above. Except for* Artemia *from Chaplin Lake and Buenos Aires, naupliar viability was very high even after 48 h storage, and did not decrease significantly after a 24 h post-storage transfer to 25°C. Neither the naupliar dry weight nor biochemical composition changed significantly during refrigeration for most strains tested. Comparative culture-tests with stored and freshly-hatched nauplii as food for juvenile marine mysids* Mysidopsis bahia *M. and larval carp* Cyprinus carpio *L. revealed similar production performances.*

INTRODUCTION

Artemia is a practical and suitable larva food for both marine and freshwater crustaceans and fishes (Kinne, 1977). Brine shrimp are commonly used as freshly-hatched nauplii, because older unfed nauplii lose their nutritional value (Morris, 1956; Maddox and Manzi, 1976; Watanabe

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et al., 1978; Dye, 1980). It is not clear if this is due to biochemical changes (Benijts *et al.*, 1976), critical size increase (Hentschel, 1968; Smith, 1976) or increased swimming activity (Miller *et al.*, 1979). Although it is obvious that freshly-hatched nauplii are a preferred food, little attention has been paid to the instar-stage at which *Artemia* larvae are offered to the preying larvae. The optimal use of *Artemia* nauplii as a source of live food implies the daily incubation of cysts and harvesting of nauplii. Methods of optimizing *Artemia* use and reducing cysts-hatching operations by storing freshly-hatched nauplii at low temperature was therefore studied.

MATERIALS AND METHODS

The *Artemia* strains used in the experiments are listed in Table 1. Cysts were incubated under optimal hatching conditions (Sorgeloos,

TABLE 1

Percent Survival of *Artemia* Nauplii from Different Geographical Sources Stored at 2–4°C for 24 h and 48 h at Densities of 2 000 or 8 000 per ml

Source of Artemia *cysts*	*24 h* 2–4°C	*48 h* 2–4°C
Macau (Brazil) batch no. 871172	96·3	96·1
Macau (Brazil) batch no. 971051	94·0	91·4
Macau (Brazil) batch no. 971051 – 8 000 nauplii/ml	92·6	91·8
San Francisco Bay (San Francisco, USA) batch no. 2596	93·0	86·7*
Shark Bay (Australia)	94·5	93·9
San Pablo Bay (San Francisco, USA) batch no. 1628	99·1	99·1
Tientsin (People's Republic of China)	100	97·0
Reference *Artemia* cysts (Sorgeloos, 1981)	94·0	93·0
Great Salt Lake (Utah, USA)	95	95·1
Chaplin Lake (Canada)	12·4**	7·1**
Buenos Aires (Argentina)	71·6**	73·7**
Lavalduc (France)	97·8	95·6

*Significantly different at the level 0·05.

**Significantly different at the level 0·01.

1980), and a homogenous instar-I population was harvested after different cyst incubation periods (Vanhaecke and Sorgeloos, 1982*a*). The nauplii were separated from the hatching debris in a cylindrical separator box (Persoone and Sorgeloos, 1972), rinsed with chilled seawater (2–4°C), concentrated at densities of 2000 or 8000 nauplii per ml seawater, and transferred to cylindro-conical vessels (150 ml content). These were placed in a (thermostatic) refrigerator (2–4°C) and constantly aerated. The percentage survival of nauplii was calculated by subsampling from a uniform suspension with an automatic micropipette after 24 h and 48 h. Extra subsamples of a few strains were removed and held for 24 h at 25°C in petri dishes after which time survival of the nauplii was determined. The instar-stages were determined according to Hentschel (1968).

Dry-weight analyses were performed by the procedure of Vanhaecke and Sorgeloos (1980), and determinations of total lipid were carried out according to the method of Schauer and Simpson (1978). Separation of fatty acid methyl esters was performed by gas chromatography on a glass column (1·80 m × 2 mm I.D.) packed with 10% Altech CS-8 on Chromosorb W-AW 100–120. Gas chromatographic conditions were: 200°C isothermal; carrier gas N_2 at 40 ml/min; F.I.D.-detection. Identification and quantification was performed using a calibrated method on a HP 3390A plotter–integrator. Nutritional evaluation tests were carried out according to the standard culture test procedure with *Mysidopsis bahia* M. (Léger and Sorgeloos, 1982*a*) and the culture test with *Cyprinus carpio* L. (Vanhaecke and Sorgeloos, 1982*b*).

Except for the fatty acid analysis, all data were treated statistically in a one way analysis of variance. Duncan's Multiple Range Test was used to determine significant differences among means. Before analysis, survival data were normalized through an $\arcsin\sqrt{\%}$ transformation (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

For most *Artemia* strains tested, storage of nauplii in densities up to 8000 per ml at 2–4°C did not significantly ($P > 0{\cdot}05$) affect their survival (Table 1). The high mortality rates found for Chaplin Lake and Buenos Aires *Artemia* confirm earlier findings that nauplii from the

same strains do not survive well at 20°C and 30°C; this appears to be correlated with lower caloric contents of the nauplii of these two strains (Vanhaecke *et al.*, 1982).

After 48 h storage, the nauplii were still at the instar I stage. Upon transfer to 25°C their metabolism was enhanced and molting into instar II and III stages occurred, apparently without any significant ($P > 0{\cdot}05$) effect on their survival (Table 2).

After 24 h of storage at 2–4°C, the naupliar dry-weight losses were insignificant ($P > 0{\cdot}05$). After 48 h storage, the differences were still very small as compared with the dry weight losses after 24 h at 25°C (Table 3).

As compared to a 26% decrease in total lipid content when kept at 25°C for 24 h (Benijts *et al.*, 1976), *Artemia* nauplii apparently did not metabolize their lipid reserves when stored at 2–4°C, and the fatty acid patterns were not significantly changed during cool storage (Table 4).

The survival, growth and reproductive development of mysid juveniles were at least as good when fed nauplii stored for 24–48 h as when fed freshly-hatched *Artemia* nauplii (Table 5).

TABLE 2
Percent Survival of *Artemia* Nauplii from Different Geographical Sources Stored at 25°C for 24 h Post-storage

Source of Artemia	*Storage conditions*			
	24 h, 2–4°C	*24 h, 2–4°C + 24 h, 25°C*	*48 h, 2–4°C*	*48 h, 2–4°C + 24 h, 25°C*
Macau, no. 971051	94·0	93·4	91·4	89·5
Macau, no. 971051 (8 000 nauplii per ml)	92·6	94·5	91·8	88·6
Reference *Artemia* cysts	90·6	89·2	88·4	87·8
Shark Bay	94·5	94·8	93·9	94·2
San Pablo Bay, no. 1628	99·1	97·0	99·1	100
Tientsin	100	95·8	97	94·1

TABLE 3
Individual Dry Weights (μg) of *Artemia* Nauplii from Different Geographical Sources Stored Under Various Conditions

Source of Artemia	*Freshly-hatched nauplii*	*Stored nauplii*		
		24 h, 2–4°C	*48 h, 2–4°C*	*24 h, 25°C*[a]
Great Salt Lake	2·42	2·36 (–2·5%)	2·22* (–8·0%)	1·59 (–34·3%)
San Pablo Bay, no. 1628	1·92	1·87 (–2·6%)	1·75* (–8·0%)	1·36 (–29·2%)
Tientsin	3·09	3·02 (–2·3%)	2·85* (–7·8%)	2·37 (–23·3%)

*Significantly different at the 0·05 level.
[a] From Vanhaecke *et al.* (1982).

Carp larvae grew well when fed freshly-hatched and 24 h stored *Artemia* nauplii (Table 6). The growth rate was, however, 8% lower when fed 48 h stored nauplii; after this long storage, the nauplii had probably lost resistance to survive the extra stress created by the transfer into freshwater and died off rapidly. The survival of carp was not affected by storage conditions of the *Artemia* (Table 6).

CONCLUSIONS

Artemia nauplii can be stored for 24–48 h without losing their nutritional value for fish and crustacean larvae; densities up to 8000 nauplii per ml can be kept in moderately aerated cylindro-conical containers at a temperature of 2–4°C.

This is valid for *Artemia* from Macau (Brazil), San Francisco Bay (USA), Tientsin (People's Republic of China), Shark Bay (Australia), Lavalduc (France) and Great Salt Lake (Utah, USA), but not for Chaplin Lake (Canada) and Buenos Aires (Argentina) brine shrimp that die off rapidly during storage.

This technique of cool storage of *Artemia* nauplii offers unique advantages for application in aquaculture hatcheries, e.g.:

1. the frequency of *Artemia* cyst-hatching and nauplii-harvesting can be considerably reduced (half to quarter of present activities);

TABLE 4

Procentual Composition of Fatty Acid Methyl Esters (FAME) and Percent Total Lipid (on a Dry Weight Basis) of *Artemia* Nauplii from Reference *Artemia* Cysts Stored Under Various Conditions

FAME	*Storage conditions*				
	0 h	*24 h, 2–4°C*	*48 h, 2–4°C*	*24 h, 25°C*	*48 h, 25°C*
14:0	1·86	1·89	1·84	1·89	1·59
14:1	2·23	2·23	2·20	2·33	1·25
15:0	0·83	0·85	0·85	0·87	0·72
15:1	0·94	0·94	0·95	1·05	0·65
16:0	13·65	13·50	13·16	13·02	12·04
16:1ω7	16·39	16·18	15·85	15·89	11·88
16:2ω7–17:0	2·22	2·28	2·18	2·27	1·50
16:3ω4–17:1ω8	3·66	3·89	3·86	3·76	2·21
18:0	3·24	3·28	3·34	3·91	6·10
18:1ω7/ω9	31·19	31·45	32·32	31·80	36·86
18:2ω6	9·78	9·41	10·00	8·96	8·84
20:0 18:3ω3/ω6[a]	1·30	1·17	1·08	1·30	1·37
20:1ω7/ω9	0·94	0·91	0·79	0·90	1·11
18:4ω3	–	–	–	–	–
21:0	0·31	0·31	0·32	0·33	0·10
20:2ω6/ω9	–	–	–	–	–
20:3ω3	0·16	0·08	0·06	0·05	–
20:4ω3/ω6	4·24	4·28	4·20	4·50	5·99
22:1	0·06	trace	–	–	–
20:5ω3	7·05	7·07	7·07	7·22	8·07
% Total lipid/dry weight	20·94 ±0·82	20·47 ±1·06	20·70 ±1·15	[b]	[b]

[a] More than 99% – 18:3ω3.

[b] No data available.

TABLE 5

Results of the Mysid Culture Test with *Artemia* Nauplii (Macau no. 871051) Stored Under Various Conditions

	Storage conditions of Artemia *nauplii*		
	0 h	*24 h, 2–4°C*	*48 h, 2–4°C*
Percent survival	89·7 ± 8·9	96·5 ± 13·5	93·3 ± 13·6
Individual dry weight (μg)	254 ± 28·0[a]	352·6 ± 55·3[b]	335·8 ± 33·8[b]
Individual length (μm)	4 376 ± 233	4 591 ± 200	4 593 ± 220
Reproductive characteristics			
% Sexual differentiation	100	100	100
% ♂ᵢ/♂♂	14·6	6·7	3·7
% ♀ᵢ/♀♀	53·0	33·0	18·3
% ♀*/♀♀	43·6	53·1	78·0
% ♀‡/♀♀	3·3	13·9	3·7

♂ᵢ, Immature male; ♀ᵢ, immature female; ♂♂, total number of males; ♀♀, total number of females; ♀*, female with eggs in ovaria; ♀‡, female with eggs in marsupium.
[a,b] Means with a different superscript (*a*,*b*) are significantly different ($P \leq 0{\cdot}05$).

TABLE 6

Results of the Carp Culture Test with *Artemia* Nauplii (Macau no. 871051) Stored Under Various Conditions

Carp results	*Storage conditions of* Artemia *nauplii*		
	0 h	*24 h, 2–4°C*	*48 h, 2–4°C*
Percentage survival	95·7 ± 1·4	95·7 ± 1·4	93·3 ± 0·8
Individual wet weight (mg)	173 ± 2·9[a]	169·5 ± 6·4[a]	159·3 ± 3·2[b]

[a,b] Means with different superscript (*a*,*b*) are significantly different ($P \leq 0{\cdot}05$).

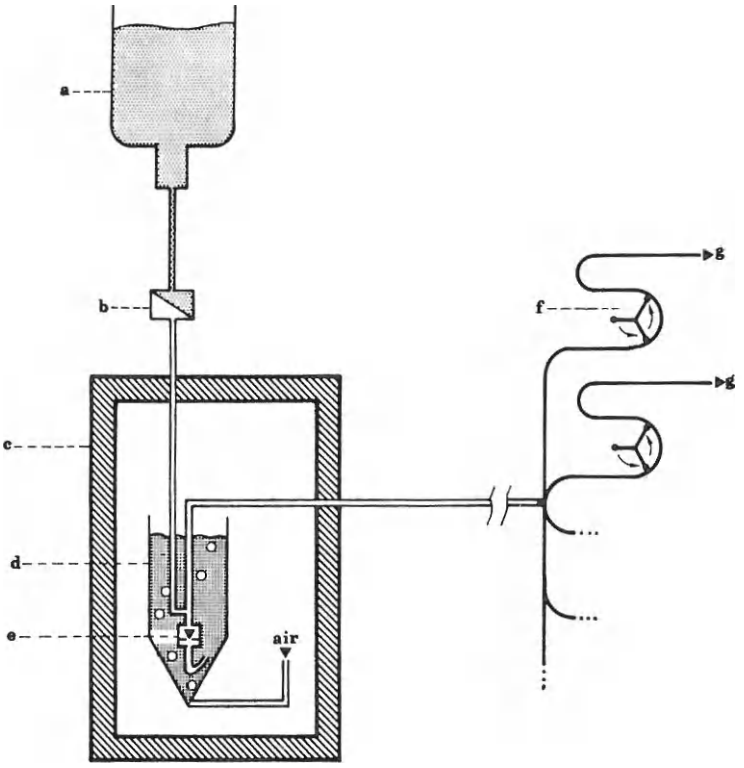

Fig. 1. Schematic diagram of automatic distribution system for cooled *Artemia* nauplii (modified from Léger and Sorgeloos, 1982*b*). (a) Tank filled with rinsing water; (b) electromagnetic valve; (c) refrigerator; (d) stock-cylinder with *Artemia* nauplii; (e) one-way valve; (f) peristaltic pump; (g) to culture tank.

2. nauplii distribution from a cooled stock to the preying larvae can be automated, e.g. with the system outlined in Fig. 1. The frequency of live-food distribution can be increased at will, assuring shorter retention times of the *Artemia* nauplii in the culture tanks thus providing a higher quality food for the preying larvae;
3. *Artemia* leftovers are not wasted but can be stored for later use.

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