Aquaculture, 30 (1983) 43—52
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

INTERNATIONAL STUDY ON *ARTEMIA*[1] XIX. HATCHING DATA FOR TEN COMMERCIAL SOURCES OF BRINE SHRIMP CYSTS AND RE-EVALUATION OF THE "HATCHING EFFICIENCY" CONCEPT[2]

PAUL VANHAECKE and PATRICK SORGELOOS[3]

Artemia *Reference Center, State University of Ghent, J. Plateaustraat 22, B-9000 Ghent (Belgium)*

(Accepted 4 March 1982)

ABSTRACT

Vanhaecke, P. and Sorgeloos, P., 1983. International study on *Artemia.* XIX. Hatching data for ten commercial sources of brine shrimp cysts and re-evaluation of the "hatching efficiency" concept. *Aquaculture*, 30: 43—52.

A comparative hatching study has been carried out with Reference *Artemia* Cysts as well as cysts from Argentina, Australia, Brazil, Canada, France, Italy, the People's Republic of China, the Philippines and the U.S.A. Hatching rate, percentage and efficiency vary considerably from one cyst source to another. These hatching criteria are, however, not strain-specific since significant variation is found among cyst batches from the same geographical origin.

The limitations of the "hatching efficiency" concept are discussed and a new criterion "hatching output", i.e. the biomass of nauplii expressed in mg dry weight produced per gram cyst product, is proposed for evaluation of the hatching quality of *Artemia* cyst brands.

The hatching quality of Reference Cysts and cysts from Canada and Argentina can be significantly improved by incubation of the cysts at low salinity.

For almost all commercial sources the use of decapsulated cysts results in a significant increase of the hatching output.

INTRODUCTION

At present *Artemia* cysts are commercially available from various production sources in America, Asia, Australia and Europe (Sorgeloos, 1980).

From the comparative studies of Smith et al. (1978) and Sorgeloos et al. (1978) on the hatching quality of four, and in the latter study, five commercial

[1] International Interdisciplinary Study on *Artemia* strains coordinated by the *Artemia* Reference Center, State University of Ghent, Belgium.
[2] Paper presented at the World Mariculture Society — Technical Sessions, Seattle (WA, U.S.A.), 8—10 March 1981.
[3] Research Associate at the National Fund for Scientific Research (Belgium).

cyst sources, it is obvious that there is great variation from one commercial brand to another and even among batches within the same brand. The present paper reports on the hatching quality of all commercial cyst products presently on the market.

In view of recent findings with regard to strain differences in size and weight of nauplii (Vanhaecke and Sorgeloos, 1980) the concept "hatching efficiency" i.e. the number of nauplii produced per gram of cyst product (Sorgeloos et al., 1978) can be misleading when evaluating the hatching quality of a given brand of cysts. We therefore suggest a new criterion, "hatching output", that takes into account the total naupliar biomass produced per gram of cyst product.

Considering the importance of cyst economics, this paper also reports on the effect of cyst incubation at low salinity and the use of decapsulated cysts on hatching outputs of the commercial cyst sources.

MATERIAL AND METHODS

The geographical origin of the cyst brands studied, as well as their commercial origin and batch number or year of harvest are listed in Table I. Cysts

TABLE I

List of *Artemia* sources studied

Abbreviation used	Geographical origin	Commercial origin	Batch no. or year of harvest
SFB	San Francisco Bay California—USA	San Francisco Bay Brand Cy	batch 288—2596
SPB	San Pablo Bay California—USA	San Francisco Bay Brand Cy	batch 1628
BRAZIL	Macau Brazil	Companhia Industrial do Rio Grande do Norte	batch 871172
PHIL	Barotac Nuevo Panay-Philippines	Ceramar Agro-Marine Industries	harvest 1978
GSL	Great Salt Lake Utah—USA	Sander's Brine Shrimp Cy	harvest 1977
AUSTR	Shark Bay Australia	World Ocean, Pty	batch 114
CAN	Chaplin Lake Canada	Jungle Laboratories	harvest 1978
ARG	Buenos Aires Argentina	Aquarium Products	harvest 1977
FRANCE	Lavalduc France	Compagnie des Salins du Midi et des Salines de l'Est	harvest 1979
CHINA	Tientsin P.R. China	China National Foodstuffs Export Corporation	harvest 1978
ITALY	Margherita di Savoia Italy	—	harvest 1977
R.A.C.	Reference *Artemia* Cysts	—	

from the Italian source were kindly provided by Dr. P. Trotta. Details on Reference *Artemia* Cysts can be found in Sorgeloos (1981).

Unless other specifications are given, hatching tests were carried out in funnel-shaped glass tubes or plastic bags in natural seawater (35 ppt) at a temperature of 25 ± 0.5°C and an illumination of 1000 lux. Cysts were kept in suspension by gentle air-bubbling at the bottom of the hatching container.

Hatching efficiency, hatching percentage and hatching rate were analyzed following procedures described in Sorgeloos et al. (1978), Bruggeman et al. (1980) and Vanhaecke and Sorgeloos (1982) respectively.

Dry weight and energy content analyses were always performed on a homogenous population of instar I nauplii following the techniques of Vanhaecke and Sorgeloos (1980) and Maciolek (1962) respectively.

The 5 ppt hatching medium, made up of natural seawater and tapwater, was buffered with 0.5 M Na_2CO_3 at a rate of 1 ml per liter.

Cyst decapsulation was carried out according to the procedures of Bruggeman et al. (1979, 1980).

COMPARATIVE ANALYSIS OF THE HATCHING QUALITY OF *ARTEMIA* CYSTS FROM DIFFERENT SOURCES

Our results show that there is considerable variation in hatching percentage, rate and efficiency among cysts from different commercial sources (Fig. 1).

For evaluation of cyst economics, the hatching efficiency concept is certainly the most practical; for example, for the production of 1 million nauplii only 3.3 g cyst product of the specific Macau batch is needed, whereas for the Great Salt Lake and the Chaplin Lake batches 9.4 g and 15.2 g of product have to be incubated respectively.

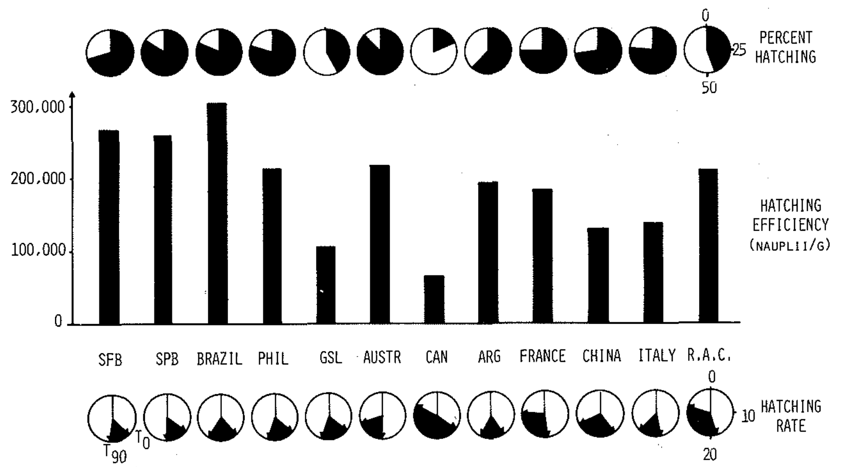

Fig. 1. Hatching characteristics of *Artemia* cysts of different geographical origins (legend to abbreviations in Table I; T_0 and T_{90} values refer to the time-lapses in hours from incubation of the cysts until appearance of the first nauplii, or the moment by which 90% of the hatching efficiency has been reached).

As already shown by Sorgeloos et al. (1978) the hatching percentage criterion is not of practical interest since it does not take into account cyst impurities and can thus be misleading; e.g. for similar hatching percentages 40% more nauplii are produced with Lavalduc cysts than with the product from Tientsin.

As suggested earlier by Smith et al. (1978), hatching rates can vary widely. The present data reveal that most commercial strains start to hatch after 15 to 20 h incubation. However, the time lapse until 90% hatching efficiency may vary from 20 to over 30 hours. This can place a serious burden on the practical use of the slow hatching cyst brands, especially with regard to the timing of incubation periods and the harvesting of only instar I nauplii that contain the highest energy content (Benijts et al., 1976).

The hatching results reported in Fig. 1 may not be extrapolated for all batches from these cyst sources since significant variation can sometimes be noted (see Fig. 2). Another striking example can be given for Great Salt Lake cysts:

harvest 1977 (unspecified batch) : 106,000 nauplii per gram
harvest 1979 (batch 185) : 192,000 nauplii per gram

Both criteria, hatching rate and hatching efficiency are indeed sensitive to varying conditions prior to and at cyst harvesting and/or cyst processing and storage (Sorgeloos et al., 1976; Vanhaecke and Sorgeloos, 1982). The data reported in Fig. 1 are thus not strain- or brand-specific but should merely be seen as an illustration of, and a warning for, potential variation in hatching quality parameters among different cyst products.

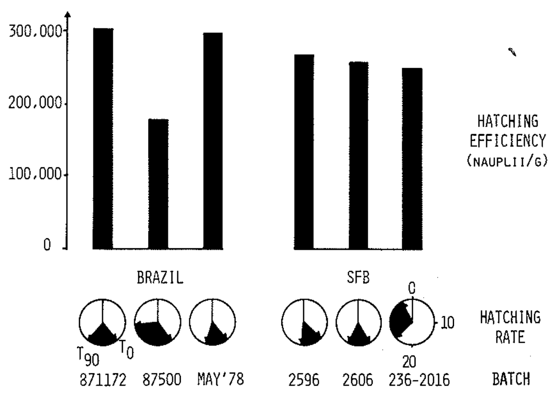

Fig. 2. Hatching rate and efficiency for 3 different batches of *Artemia* cysts from Macau (Brazil) and San Francisco Bay (SFB) (legend to abbreviations as in Fig. 1).

THE NEW CONCEPT OF "HATCHING OUTPUT"

Recent studies on the biometrical characteristics of different *Artemia* strains have revealed very substantial differences in the size of cysts and nauplii (Vanhaecke and Sorgeloos, 1980). It appears from Table II that naupliar dry weights vary from a minimum in San Francisco Bay *Artemia* (1.63 μg) up to more than double this figure in Italian brine shrimp (3.33 μg). Naupliar energy contents, on the other hand, show only minor differences, overall variation for the 12 cyst sources studied being less than 10%.

TABLE II

Individual dry weight and energy content of *Artemia* nauplii hatched in standard conditions (35 ppt, 25°C) from different *Artemia* cyst sources

Source of cysts	Individual dry weight (μg)	s[1]	Energy content (10^3 J/g nauplii)	s[1]
San Francisco Bay	1.63	0.11	22.48	0.15
San Pablo Bay	1.92	0.08	22.33	0.34
Macau	1.74	0.08	22.52	0.51
Barotac Nuevo	1.68	0.03	22.74	0.44
Great Salt Lake	2.42	0.11	22.35	0.29
Shark Bay	2.47	0.13	23.33	0.09
Chaplin Lake	2.04	0.18	21.94	0.41
Buenos Aires	1.72	0.07	22.02	0.29
Lavalduc	3.08	0.12	21.76	0.16
Tientsin	3.09	0.11	22.05	0.20
Margherita di Savoia	3.33	0.18	21.76	0.52
Reference *Artemia* Cysts	1.78	0.05	22.62	0.37

[1] Standard deviation

In view of these differences in naupliar dry weight, it is now clear that the hatching efficiency criterion underestimates the food quantity per unit of cyst weight that is provided by a cyst source with large nauplii. We therefore suggest the new concept of "hatching output", i.e. the amount of naupliar biomass, expressed in milligram dry weight, that in standard conditions (35 ppt, 25°C) can be hatched from 1 gram of cyst product. Since naupliar dry weights are strain-specific (Vanhaecke and Sorgeloos, 1980), the hatching output of a specific cyst batch can easily be computed from the hatching efficiency figure and the naupliar dry weight for the corresponding cyst source (see Table II); i.e. hatching output (in mg/g) = hatching efficiency × individual dry weight of nauplius.

The sequence in hatching quality has changed when referring to the concept of hatching output instead of hatching efficiency (Fig. 3). Considering the criterion hatching efficiency, the best result was obtained with the Macau batch; however, in terms of biomass produced, Macau ranks only third (529.0 mg) after Lavalduc (561.8 g) and Shark Bay (537.5 g) since both strains produce a bigger nauplius (3.08 μg respectively 2.47 μg) than Macau *Artemia* (1.74 μg). It should be emphasized however, that the hatching output data reported are not brand-specific, e.g. the 1977 harvest of Great Salt Lake provides 256.5 mg naupliar biomass whereas for the 1979 harvest (batch no.

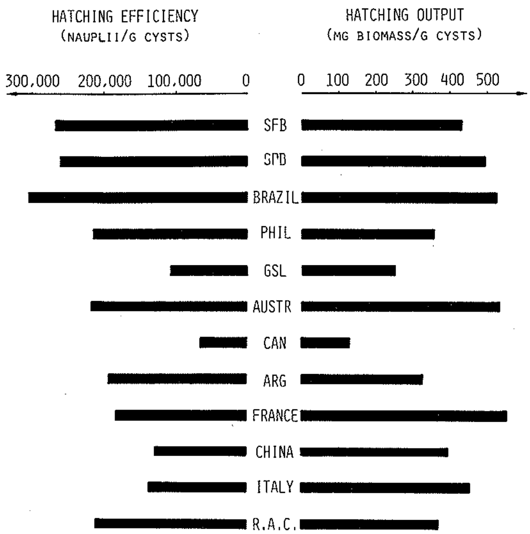

Fig. 3. Hatching efficiency and hatching output of different *Artemia* cysts sources incubated in standard conditions (legend to abbreviations in Table I).

185) of the same strain one gram of cysts yields 467.0 mg of naupliar biomass. These differences are due to the previously mentioned variability in hatchability among cyst batches.

As long as nauplius size does not interfere with the ingestion mechanism of the predator, one might expect that the use of larger nauplii with higher individual dry weight and energy content will be beneficial. The predator will indeed spend less energy in taking up a smaller number of larger nauplii to fulfill its food requirements. In this regard the hatching output is a more realistic criterion than the hatching efficiency in evaluating the quantity of food that can be produced per weight unit of cyst product.

A high hatching output does not necessarily assure success in using a specific cyst product as larval food source. Indeed, recent findings by the International Study on *Artemia* have revealed that aside from the hatching quality, other criteria should be taken into account in evaluating the food value of a given *Artemia* cyst brand, e.g. the size of the freshly hatched nauplii as a reference for potential uptake by the predator (Beck et al., 1980; Vanhaecke and Sorgeloos, 1980), the biological effectiveness of the nauplii for a given predator (see review in Beck and Bengtson, 1980) as a function of the naupliar fatty acid pattern (Schauer et al., 1980) or the level of contamination of the nauplii with pesticides and heavy metals (Olney et al., 1980).

INCREASED HATCHING OUTPUT BY INCUBATING CYSTS AT LOW SALINITY OR BY USING DECAPSULATED CYSTS

Regarding *Artemia* from Chaplin Lake, Reference strain and Buenos Aires, hatchabilities increase significantly as a result of the lowering of the salinity of the hatching medium (see Fig. 4). Significantly higher dry weight values were obtained for the nauplii from Chaplin Lake, Buenos Aires, Shark Bay, the Philippines and San Pablo Bay.

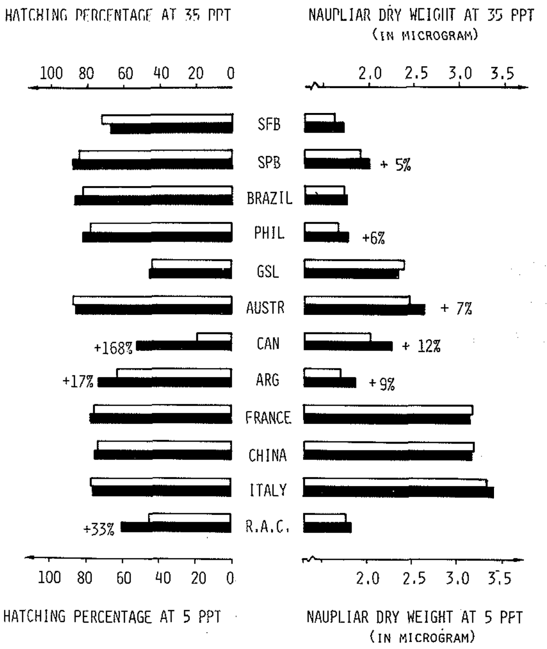

Fig. 4. Effect of incubation at low salinity on hatching percentage and individual nauplius weight for *Artemia* cysts of different geographical origin (legend to abbreviations in Table I).

As shown in Fig. 5, the use of decapsulated instead of untreated cysts results in a significant increase in hatchability for almost all *Artemia* sources studied. Naupliar dry weights however are significantly higher for only a few strains, i.e. Buenos Aires, Margherita di Savoia and Barotac Nuevo.

From these data one might expect an even more beneficial effect on hatchabilities and naupliar dry weights by incubating the decapsulated cysts at low salinity. However, with the exception of the Chaplin Lake cysts (231% increase in hatching percentage), no further increase in hatchability was recorded. On the contrary, for most strains hatching percentages dropped considerably as a result of a precocious breaking of the E-1 embryos.

The data provided in Table III for five selected cyst sources reveal that neither the use of decapsulated cysts nor the incubation at 5 ppt have a significant effect on the naupliar energy content.

From Table IV it is clear that for all strains studied the use of decapsulated cysts or the use of a low salinity medium assures increased hatching outputs. This means that opportunities are provided for some important cyst savings in the aquaculture hatcheries depending on the cyst source used.

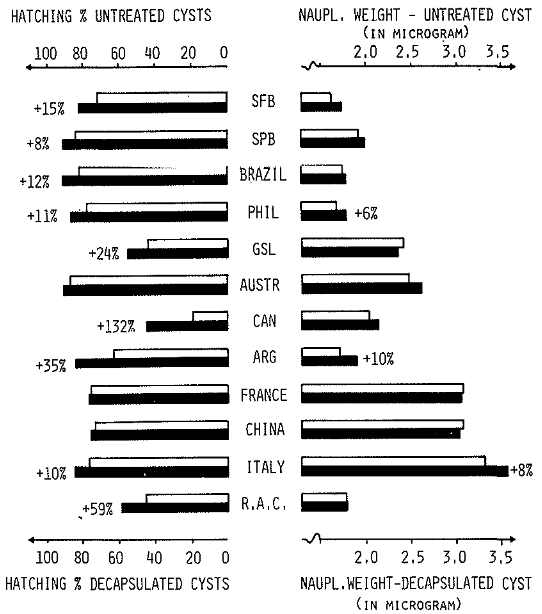

Fig. 5. Effect of cyst decapsulation on hatching percentage and individual nauplius weight for *Artemia* cysts of different geographical origin when incubated at 35 ppt salinity (legend to abbreviations in Table I).

TABLE III

Effect of incubation at low salinity or cyst decapsulation on naupliar energy content for *Artemia* cysts of different geographical origin

Source of cysts	Naupliar energy content (10^3 J/g nauplii) (in parentheses: % difference from control)		
	Untreated cysts at 35 ppt (control)	Untreated cysts at 5 ppt	Decapsulated cysts at 35 ppt
San Pablo Bay	22.33	22.28 (−0.2)	22.78 (+2.0)
Macau	22.52	22.53 (0.0)	22.44 (−0.4)
Chaplin Lake	21.94	21.50 (−2.0)	21.84 (−0.5)
Buenos Aires	22.02	22.08 (+0.3)	21.94 (−0.4)
Margherita di Savoia	21.76	22.06 (+1.4)	21.74 (−0.1)

In order to verify the effects of improved hatching quality at low salinity or with decapsulated cysts, additional hatching tests were performed with a few more batches from 3 cyst sources. Although the trends remain strain-specific, some variation was noted in quantitative effects, e.g. no significant increases for any of the two effects with another batch of Tientsin cysts; no improved hatching at low salinity with two Great Salt Lake batches but extra yields of 54.6 mg and 44.8 mg respectively on using decapsulated cysts from

TABLE IV

Data on the hatching output of different *Artemia* cyst sources in standard conditions, incubated at low salinity or upon decapsulation

Source of cysts	Naupliar biomass (in mg) produced from 1 g untreated cysts (in parenthesis: % difference from control)		
	Incubated at 35 ppt (control)	Incubated at 5 ppt	Decapsulated and incubated at 35 ppt
San Francisco Bay	435.5	440.2 (+1.1)	534.6 (+22.8)
San Pablo Bay	497.7	544.1 (+9.3)	555.6 (+11.6)
Macau	529.0	563.7 (+6.6)	603.8 (+14.1)
Barotac Nuevo	359.5	400.9 (+11.5)	426.3 (+18.6)
Great Salt Lake	256.5	257.0 (+0.2)	311.1 (+21.3)
Shark Bay	537.5	563.3 (+4.8)	590.0 (+9.8)
Chaplin Lake	133.8	400.4 (+199.3)	326.1 (+143.7)
Buenos Aires	333.0	424.2 (+27.4)	494.9 (+48.6)
Lavalduc	561.8	566.6 (+0.8)	570.3 (+1.5)
Tientsin	400.5	406.0 (+1.4)	409.8 (+2.3)
Margherita di Savoia	458.2	463.0 (+1.0)	544.1 (+18.7)
Reference *Artemia* Cysts	375.6	515.7 (+37.3)	485.1 (+29.2)

this source; 354% and 243% increases of the hatching output of another batch of Chaplin Lake cysts incubated at low salinity, respectively upon decapsulation.

ACKNOWLEDGEMENTS

This study has been supported by the Belgian National Science Foundation (N.F.W.O.) through Grant F.K.F.O. – 2.0010.78.

REFERENCES

Beck, A.D. and Bengtson, D.A., 1981. International study on *Artemia*. XXII. Nutrition in aquatic toxicology: diet quality of geographical strains of the brine shrimp *Artemia* spp. In: J.G. Pearson, R. Forster and W.E. Bishop (Editors), Aquatic Toxicology and Hazard Evaluation: Fifth Conference. Am. Soc. Testing & Materials, STP 766, Philadelphia, PA, pp. 139–147.

Beck, A.D., Bengtson, D.A. and Howell, W.H., 1980. International study on *Artemia*. V. Nutritional value of five geographical strains of *Artemia*: effects on survival and growth of larval Atlantic silverside, *Menidia menidia*. In: G. Persoone, P. Sorgeloos, O. Roels and E. Jaspers (Editors), The Brine Shrimp *Artemia*. Vol. 3. Ecology, Culturing, Use in Aquaculture. Universa Press, Wetteren, Belgium, pp. 249–259.

Benijts, F., Vanvoorden, E. and Sorgeloos P., 1976. Changes in the biochemical composition of the early larval stages of the brine shrimp, *Artemia salina* L. In: G. Persoone and E. Jaspers (Editors), Proc. 10th European Symposium on Marine Biology. Vol. 1. Universa Press, Wetteren, Belgium, pp. 1–9.

Bruggeman, E., Baeza-Mesa, M., Bossuyt, E. and Sorgeloos, P., 1979. Improvements in the decapsulation of *Artemia* cysts: In: E. Styczynska-Jurewicz, T. Backiel, E. Jaspers and G. Persoone (Editors), Cultivation of Fish Fry and its Live Food. EMS Spec. Publ. No. 4. Institute for Marine Scientific Research, Bredene, Belgium, pp. 309—315.

Bruggeman, E. Sorgeloos, P. and Vanhaecke, P., 1980. Improvements in the decapsulation technique of *Artemia* cysts. In: G. Persoone, P. Sorgeloos, O. Roels and E. Jaspers (Editors), The Brine Shrimp *Artemia*. Vol. 3. Ecology, Culturing, Use in Aquaculture. Universa Press, Wetteren, Belgium, pp. 261—269.

Maciolek, J.A., 1962. Limnological organic analyses by quantitative dichromate oxidation. Fish. Res. Rep., 60: 1—61.

Olney, C.E., Schauer, P.S., McLean, S., You Lu and Simpson, K.L., 1980. International Study on *Artemia*. VIII. Comparison of the chlorinated hydrocarbons and heavy metals in five different strains of newly-hatched *Artemia* and a laboratory-reared marine fish. In: G. Persoone, P. Sorgeloos, O. Roels and E. Jaspers (Editors), The British Shrimp *Artemia*. Vol. 3. Ecology, Culturing, Use in Aquaculture. Universa Press, Wetteren, Belgium, pp. 343—352.

Schauer, P.S., Johns, D.M., Olney, C.E. and Simpson, K.L., 1980. International Study on *Artemia*. IX. Lipid level, energy content and fatty acid composition of the cysts and newly hatched nauplii from five geographical strains of *Artemia*. In: G. Persoone, P. Sorgeloos, O. Roels and E. Jaspers (Editors), The Brine Shrimp *Artemia*. Vol. 3. Ecology, Culturing, Use in Aquaculture. Universa Press, Wetteren, Belgium, pp. 365—373.

Smith, T.I.J., Hopkins, J.S. and Sandifer, P.A., 1978. Development of a large-scale *Artemia* hatching system utilizing recirculated water. In: J.W. Avault, Jr. (Editor), Proc. 9th Annu. Meeting WMS, Louisiana State University, Baton Rouge, LA, pp. 701—714.

Sorgeloos, P., 1980. Improvements in availability and use of *Artemia* as food source for *Macrobrachium*. In: Giant Prawn 1980, Bangkok, Thailand, June 1980. IFS Provisional Report No. 9. International Foundation for Science, Stockholm, Sweden, pp. 123—132.

Sorgeloos, P., 1981. Availability of Reference *Artemia* Cysts. Aquaculture, 23: 381—382.

Sorgeloos, P., Baeza-Mesa, M., Benijts, F. and Persoone, G., 1976. Current research on the culturing of the brine shrimp *Artemia salina* L. at the State University of Ghent, Belgium. In: G. Persoone and E. Jaspers (Editors), Proc. 10th European Symposium on Marine Biology, Vol. 1. Universa Press, Wetteren, Belgium, pp. 473—495.

Sorgeloos, P., Persoone, G., Baeza-Mesa, M., Bossuyt, E. and Bruggeman, E., 1978. The use of *Artemia* cysts in aquaculture: the concept of "hatching efficiency" and description of a new method for cyst processing. In: J.W. Avault, Jr. (Editor), Proc. 9th Annu. Meeting WMS, Louisiana State University, Baton Rouge, LA, pp. 715—721.

Vanhaecke, P. and Sorgeloos, P., 1980. International Study on *Artemia*. IV. The biometrics of *Artemia* strains from different geographical origin. In: G. Persoone, P. Sorgeloos, O. Roels, and E. Jaspers (Editors), The Brine Shrimp *Artemia*. Vol. 3. Ecology, Culturing, Use in Aquaculture. Universa Press, Wetteren, Belgium, pp. 393—405.

Vanhaecke, P. and Sorgeloos, P., 1982. International study on *Artemia*. XXIII. The hatching rate of *Artemia* cysts — a comparative study. (submitted for publication).