but this substrate did not appear to be very good for their mass culture in a laboratory. Residual foods, moulted carapaces and other small particles which settled on the sand were so small that they could not be removed completely. The particles decomposed and the water deteriorated in quality. Net-cage rearing did not have these problems. Running water was used and the cages were cleaned once a day.

It was easy to count the post-larvae. The number of glaucothöe reared in one cage was 1 000–50 000 at first, but the mortality increased suddenly at this stage and the final number of glaucothöe at the time of development into the first stage of the young crab was 210–500 in most cases. One reason for this increased mortality was cannibalism. Glaucothöe sometimes swam, but usually they remained on the bottom or held on to the net. Polypropylene films or Saran nets were used to increase their clinging areas. However, many glaucothöe gathered under these films or nets where they held on to each other and attacked each other. This method was not very useful, therefore, in protecting the glaucothöe from cannibalism. Shortage of suitable foods appeared to be another reason for increased mortality. Many kinds of foods were tried out during the glaucothöe stage. The foods provided for the fourth zoeal stage seemed to be the best of those tried, but they were still far from perfect. This stage of the king crab is very different morphologically, ecologically and physiologically from the zoeal stage, and increase in mortality may have been dependent on many factors still unknown at this time. Mass culture methods for the glaucothöe stage have not yet been studied.

The glaucothöe moult and develop into the first stage of the young crab. The young crabs were cultured up to their fifth stage using the same method as for the glaucothöe stage. From their sixth stage on (now with a carapace length of about 3 mm), they were kept in a rearing chamber ($1{\cdot}8 \times 0{\cdot}9 \times 0{\cdot}7$ m) with a sand bottom. The food used during this stage was similar to that given during the glaucothöe stage but was of larger size.

The water temperature in the rearing chamber was held under 10°C in 1971. From 1973, this stage was reared without control of the water temperature. The highest temperature was about 20°C, but the rate of survival was no different from that in 1971 (see Table I).

TABLE I
SURVIVAL RATE OF THE KING CRAB FROM FIRST ZOEAL TO ADULT STAGES IN EXPERIMENTS CONDUCTED 1970–74

Year	*Number of first-stage zoeae*	*Survival rate* (%)					
		Z_2	Z_3	Z_4	G	A_1	A_2
1970	20 000	[a]	[a]	0·4	0·2	0	0
1971	270 000	92	92	90·0	5·7	2·2	1·0
1972	20 000	[a]	92	85·0	0·4	0	0
1973	178 000	[a]	[a]	85·0	34·0	1·1	0·45
1974	1 800 000	[a]	92	90·0	90·0	0·5	0·1

[a] Not counted

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The Brine Shrimp, *Artemia salina*: A Bottleneck in Mariculture?

P. Sorgeloos

Abstract

For many years the freshly hatched nauplii of the brine shrimp (*Artemia salina*) have been the most common live food used in mariculture of finfish and crustacea. The cysts from which *A. salina* nauplii hatch are harvested from unmanaged wild populations by primitive techniques and at only a few places in the world. As a result, good quality cysts are available only in limited quantities. With expansion of mariculture, demand for these cysts is exceeding the supply, which may lead to a serious bottle-neck in many aquaculture developments.

A review of the studies on brine shrimp shows that the present critical situation can only be alleviated by revising the commercial exploitation of the cysts, as well as the practical use made of the latter in mariculture farms. In this respect it is suggested that:

(*i*) The quality of commercially available cysts can be improved and their quantity increased by the application of new harvesting techniques.

(*ii*) As salt lakes with *Artemia* are found all over the world (the collection at the State University of Ghent totals more than 60 different strains), comparative studies on the ecological characteristics and the nutritional value of different geographical races will lead to the selection of those that are best suited for aquacultural purposes.

(*iii*) Hatching of the larvae, followed by their separation from the hatching debris can be optimized by the application of new and standardized techniques. This can lead to increased survival of larvae by at least 50% and a reduction of 90% in the equipment and labour involved.

(*iv*) Biochemical analyses to determine nutrient value of the larvae indicate that freshly hatched *Artemia* nauplii should be offered to the predator as soon as possible after hatching.

(*v*) High density culturing techniques for *Artemia* larvae have been developed that are fully automatic and economically feasible. By using air-water lift operated raceways, densities of 3 000 shrimps per litre can be cultured from the first nauplius to the adult stage within 2 weeks on a diet of commercially available dried algae.

(*vi*) The use of juvenile brine shrimp larvae of appropriate sizes instead of freshly hatched nauplii offers several nutritional and economical advantages in terms of utilization by fish and crustacean predators.

La crevette de marais salant, *Artemia salina*: un problème pour la mariculture

Résumé

Depuis de nombreuses années, on a utilisé le plus souvent, en mariculture pour fournir une nourriture vivante aux poissons et aux crustacés, des nauplii nouvellement éclos de la crevette de marais salant (*Artemia salina*). Les œufs qui sont utilisés pour l'élevage de l'*A. salina* sont récoltés au moyen de techniques rudimentaires dans des populations à l'état sauvage qui ne se trouvent que dans quelques rares endroits du monde. On ne trouve donc d'œufs de bonne qualité qu'en quantités limitées. Etant donné l'extension de la mariculture, la demande d'*Artemia salina* est supérieure aux possibilités d'approvisionnement ce qui risque de faire sérieusement obstacle à son développement.

Lorsque l'on reprend les travaux théoriques et pratiques qui ont été

consacrés à la crevette de marais salant on constate que la situation difficile où l'on se trouve à l'heure actuelle ne pourrait être modifiée que par un changement dans l'exploitation commerciale des œufs ainsi que dans l'utilisation que l'on en fait en mariculture. On propose donc à cet égard les mesures suivantes:

(*i*) La qualité des œufs disponibles dans le commerce pourrait être améliorée et leur quantité accrue par l'application de nouvelles techniques de récolte.

(*ii*) Etant donné que l'on trouve dans le monde entier des lacs salés peuplés d'*Artemia* (les collections de l'Université de l'Etat de Gand en réunissent plus de 60 différentes souches), des études comparatives sur les caractéristiques écologiques et la valeur nutritionnelle des races originaires de différentes régions permettraient de choisir celles qui apparaîtraient les plus propres aux besoins de l'aquaculture.

(*iii*) En utilisant de nouvelles techniques normalisées il serait possible d'améliorer l'éclosion des larves et leur séparation des débris d'éclosion. Ce qui permettrait d'augmenter au moins de 50% la quantité de larves survivantes et de réduire de 90% le matériel et la main-d'œuvre nécessaires.

(*iv*) Les analyses biochimiques effectuées en vue de déterminer la valeur nutritive des larves montrent que les nauplii d'*Artemia* nouvellement éclos doivent être fournis aux prédateurs aussitôt que possible après leur éclosion.

(*v*) Des techniques totalement automatisées, et satisfaisantes du point de vue économique, ont été mises au point pour la culture à haute densité des larves d'*Artemia*. En utilisant des raceways actionnés à l'eau et à l'air, on peut élever jusqu'à 3 000 crevettes par litre, depuis le stade du nauplius jusqu'à l'âge adulte, en les soumettant pendant deux semaines à un régime à base d'algues séchées qui sont en vente dans le commerce.

(*vi*) Il est à bien des égards avantageux des points de vue nutritionnel et économique d'utiliser pour nourrir des poissons et des crustacés prédateurs de jeunes larves de crevettes de marais salants, parvenues à une taille suffisante, plutôt que des nauplii nouvellement éclos.

Problemas que plantea para la maricultura la limitada disponibilidad de camarón de agua salobre, *Artemia salina*

Extracto

Durante muchos años, los nauplii recién eclosionados del camarón de agua salobre (*Artemia salina*) han sido el alimento vivo más comúnmente utilizado en el cultivo de peces y crustáceos marinos. Los quistes de los que salen los nauplii de *A. salina* proceden de poblaciones silvestres, y se recogen, con técnicas primitivas, sólo en pocos lugares del mundo. Debido a ello, las disponibilidades de quistes de buena calidad son limitadas. Al extenderse la maricultura, la demanda empieza a ser superior a la oferta, lo que podría causar un grave inconveniente para muchas actividades acuícolas.

Examinando los estudios fundamentales y aplicados que se han hecho sobre el camarón de agua salobre, es evidente que la actual situación crítica sólo puede aliviarse revisando el sistema de explotación comercial de los quistes y su empleo práctico en maricultura. A este propósito, se sugiere que:

(*i*) Podría aumentarse la calidad y cantidad de quistes disponibles comercialmente aplicando nuevas técnicas de recolección.

(*ii*) Dado que se encuentran en todo el mundo lagos de agua salada con *Artemia* (la colección de la Universidad estatal de Gante comprende más de 60 variedades diferentes), podrían hacerse estudios comparativos de las características ecológicas y el valor nutricional de las diversas razas geográficas para seleccionar las más adecuadas para la acuicultura.

(*iii*) La salida de las larvas del quiste y su separación de los restos de éste, puede perfeccionarse aplicando técnicas nuevas y uniformes. Podría obtenerse así un aumento de la supervivencia de las larvas de al menos el 50%, y una reducción del 90% del equipo y la mano de obra necesarios.

(*iv*) Los análisis bioquímicos hechos para determinar el valor de las larvas desde el punto de vista nutricional indican que los nauplii de *Artemia* deben ponerse a disposición de los depredadores lo antes posible después de eclosionados.

(*v*) Se han preparado técnicas de cultivo de larvas de *Artemia* en grandes densidades, que son plenamente automáticas y económicamente viables. En canales con un sistema de elevación de agua y aireación pueden criarse en dos semanas hasta 3 000 camarones por l, desde la primera fase de nauplius hasta la fase adulta, con una dieta de algas secas que pueden adquirirse comercialmente.

(*vi*) El empleo de juveniles de camarón de tamaño adecuado en vez de nauplii recién eclosionados ofrece varias ventajas nutricionales y económicas por lo que se refiere a su utilización por los peces y crustáceos depredadores.

1 Introduction

Seale (1933) and Rollefsen (1939) described the high value of nauplii of *Artemia salina* as food for fish fry and thus effectively changed the direction of controlled indoor fish culture. Indeed, it was soon found that the cysts of the brine shrimp, which accumulate along the shores of salt lakes, are a practical food source. When kept vacuum-dry, the cysts remain viable for years; they can easily be exported all over the world and, on immersion in sea water, produce nauplii within approximately 24 hours.

During the last decade, the research effort as well as the practical implementation of commercial fish and crustacean farming have increased considerably. As a consequence, the demand for brine shrimp cysts, which are harvested for commercial purposes from only a few salt lakes in North America, has increased markedly and now exceeds the supply. Not only is the price for cysts rising continuously, but the acute dependence of mariculture farms on provision of good quality *Artemia* cysts places a considerable burden on further expansion of maricultural activities.

In view of this situation, a thorough analysis of the *Artemia* problem should be given high priority in order to prevent shortage of brine shrimp cysts becoming a bottleneck in mariculture development.

2 Commercial exploitation of brine shrimp cysts

As controlled production of brine shrimp for the harvesting of cysts has not been accomplished on a commercial basis, the only source of *Artemia* eggs is still from cyst-deposition by unmanaged wild populations that are subject to major environmental perturbations. In fact, production of brine shrimp has been primarily a by-product of salt-production plants. Water management for salt production is often unfavourable for the development of *Artemia* and the high production of brine shrimp and their eggs occurs unpredictably when the environmental conditions are accidentally suited for these halophilic organisms.

The techniques for harvesting the cysts are quite primitive: driven by wind and currents, the floating cysts accumulate along the shores of salt lakes, where they are ladled out into bags. The time lapse, however, between washing ashore till harvesting is not constant and is often spread over several weeks. At each rainfall during this period, cysts can undergo a hydration and dehydration cycle, which results in a temporary activation of cyst metabolism. Fundamental studies related to this subject have shown that the energy content of cysts which have been subjected to repeated hydration-dehydration cycles, decreases and that the size of the nauplii hatching from these cysts is reduced significantly (Sorgeloos, 1975). In the extreme case of multiple exposure to hydration-dehydration cycles, the energy content of the cysts drops below a critical level and subsequent hatching becomes impossible.

Complaints of mariculture operators on the significant differences in hatchability from one batch of cysts to another can be at least partly explained by the repeated activation-inactivation of metabolism during the period the cysts are on shore prior to their harvest. Cyst quality could be optimized and kept constant by either more frequent harvesting or by preventing the cysts from accumulating on the shore. In the latter case, although immersed, the cysts will remain in the dormant stage and will only hatch when the salinity drops below 70 ppt.

Despite the fact that salt lakes with natural populations of brine shrimp are found throughout the world, they are commercially exploited for *Artemia* cysts in only a few places, mainly in North America, *ie*, Great Salt Lake, Utah, USA; salt pans in the San Francisco Bay area, California, USA; and salt lakes in Saskatchewan, Canada.

In the State University of Ghent laboratory, a collection of over 60 different strains from all continents has been made. A comparative study of these strains has revealed differences in important characteristics, such as the hatching rate, size of nauplii, viability, optimal temperature, and salinity ranges for larval growth and survival, *etc.* (Sorgeloos *et al.* 1975).

Further studies on the determination of the ecological characteristics as well as the nutritional value of these strains should lead to the selection of those best suited for aquacultural purposes. This can lead to transplantation experiments with inoculation of particular strains into biotopes where, for example, the autochthonous *Artemia* races have poor characteristics with respect to their use in mariculture.

The application of both the selection studies and better harvesting techniques can alleviate the present critical demand for *Artemia* cysts. Not only could the yearly yield of commercial exploitation in North America be greatly increased but the harvesting could also be extended to many other sites on other continents.

3 Optimization of the use of brine shrimp cysts

Despite the present limited supply of *Artemia* cysts, their use is far from optimal. In most cases, survival from hatching is poor. One factor which contributes to this poor survival is inefficient separation of nauplii from the hatching debris. Feeding of nauplii to predators is not always at the appropriate larval stage, and efficient techniques for high-density culturing of *Artemia* larvae are not available.

It is considered that important benefits can be obtained by the application of several recent findings and new techniques.

3.1 *Hatching cysts*

Various hatching devices are commonly used: *eg*, rectangular boxes; water baths, and plastic vats with flat or funnel-shaped bottoms. It originally appeared from studies on *Artemia* that the cysts can be hatched out within a wide range of temperature, salinity and oxygen concentration (Sorgeloos and Persoone, 1975). Therefore, it was considered that the hatching of cysts at high densities did not require any standardization or special control. However, from the scarce data available it now appears that the hatching efficiency obtained with the various devices in common use is low and does not exceed an output of 50% from cyst to larvae in most cases. The principal rules which guarantee maximum hatching efficiency have now been summarized by Sorgeloos (1975) and Sorgeloos and Persoone (1975) as follows:

(*i*) Hatching rate is dependent on temperature and salinity, and hydrated cysts must be kept under strictly controlled conditions in order to obtain a maximum number of nauplii within a well defined immersion period.

(*ii*) Cysts must be kept in suspension in oxygen-saturated sea water. Cysts accumulating on the bottom of hatching apparati will often be exposed to anaerobic conditions and do not hatch under these conditions.

(*iii*) Hydrated cysts must be illuminated for specific time periods after immersion in order to trigger cyst metabolic activity. The critical triggering period differs from one strain to another (*eg*, 10 minutes at 2 000 lux for cysts from Great Salt Lake and only 5 minutes for cysts from San Francisco Bay).

(*iv*) Maximum cyst densities for optimal hatching efficiency are approximately 12–15 g/l. At higher cyst densities, the vigorous aeration needed to keep the water saturated with oxygen affects the larvae mechanically and leads to mortality.

The following standard procedure is proposed for hatching cysts. Densities of about 10 g cysts/l are hatched out in natural sea water in transparent cylindrical tubes with a funnel-shaped bottom. Continuous aeration from the bottom of the cylinder keeps all the cysts in suspension and a few drops of a non-toxic antifoam additive should be used. The cylinder is illuminated by a fluorescent light tube for 1 hour after immersion of the cysts and water temperature is kept constant at 28°C. This technique has been applied for many years in the laboratory giving hatching percentages of 70% with Great Salt Lake cysts, and as high as 90% with San Francisco Bay cysts, which means a harvest of approximately $2{\cdot}5 \times 10^6$ nauplii/l of water.

As compared to results from literature, the proposed technique gives much better hatching efficiences with considerably higher cyst densities in inexpensive and simple hatching cylinders. This results not only in more economic use of brine shrimp cysts but also, as the hatching density can be increased by a factor of 10 compared to other techniques, in an important saving of manpower and equipment (pumps, heaters, water *etc*).

3.2 *Harvesting hatched nauplii*

As empty cyst shells and non-hatched cysts can be harmful when ingested by young fish or crustacean larvae, free-swimming *Artemia* nauplii are most often separated from the remaining debris immediately after hatching.

To date, the technique utilized in most mariculture farms to separate cysts from larvae is quite primitive: after hatching, the aeration is turned off in order to separate the sinking cyst shells from the swimming nauplii. After a certain period of time (which can be up to 75 minutes), and sometimes after concentrating the phototactic larvae around a light source, the suspension containing the swimming nauplii is drained off.

Immediately after the hatching peak, the associated bacterial flora consumes more than 50% of the oxygen in the hatching medium in a few minutes. In order to avoid oxygen depletion with subsequent mortality of the most sensitive larvae, the medium is always renewed prior to separation by filtering off the whole suspension on a plankton gauze, rinsing it a couple of times, and resuspending it in fresh sea water.

The separation efficiency (qualitative and quantitative) can be greatly increased by using a cylindrical separator box as described by Sorgeloos and Persoone (1975).

It was discovered recently that the outer layer of the cyst shell, which contains haematin, can be removed by an oxidation technique. This may eliminate the need for separation of cyst shells from larvae, as the remaining hatching debris would only consist of very thin transparent membranes which might be harmless to the predators of *Artemia* nauplii. This possible application is now being investigated.

4 Feeding brine shrimp to fish and crustacean larvae

Freshly hatched brine shrimp nauplii are presently one of the best live food sources for fish and crustacean larvae. Fundamental research on the moulting rate and the energy content of the non-fed nauplii revealed that the nauplii have to be fed to the predator as soon as possible after hatching. In fact, holding the nauplii without feeding results in a considerable decrease in their energy value; within 24 hours after hatching at 28°C, the larvae moult into the second and third larval stages which is associated with a decrease in individual weight and caloric content of 24 and 28% respectively (Remiche-Van der Wielen and Sorgeloos (in press); Benijts, Van Voorden and Sorgeloos, 1975).

In most mariculture farms, only hatched brine shrimp nauplii are fed to larvae of fish and crustaceans. However, in many cases, adult *Artemia* and older and larger larvae that have been fed on an algal diet could be a more appropriate and highly nutritive food for fish and crustacean larvae. Since it has been demonstrated by Sorgeloos (1973), Cognie (1975) and Person-le Ruyet (1976) that *Artemia* can be cultured on different types of inert foods, controlled mass culturing of their larvae is now economically feasible.

Recently an automatic culturing technique has been developed with air-lift operated raceways in which densities up to 3 000 larvae/l can be cultured up to the adult stage within two weeks (Sorgeloos, Bossuyt and Baeza-Mesa, MS). The only manual work involved is replenishment of the food container twice a week.

The use of older larvae cultured to the appropriate size as prey organisms instead of freshly-hatched nauplii has several advantages: (*i*) The quantity of cysts needed will be significantly reduced as the predator will need fewer of the larger larvae as food. (*ii*) The food quality (*eg*, protein content) of the older larvae is higher than that of the first instars. (*iii*) The predator spends much less energy in ingesting a specific biomass of older larvae or adults as compared to ingestion of an identical biomass of nauplii. (*iv*) Strains of *Artemia* whose first nauplii stages appear to be unsuitable for mariculture purposes, *eg*, because of contamination by pollutants (Great Britain, 1967; Little, 1969; Bookhout and Costlow, 1970; Wickins, 1972) or because they are too large to be swallowed by their predators (Provasoli, 1969), can become an appropriate food for large cultured species by spreading the feeding of nauplii over a number of days (Wickins, 1972). In the case of contamination of the nauplii by a pollutant, it is clear that the relative concentration of the toxicant decreases as the organism grows. Consequently, the quantity of toxicant ingested by the predators decreases if they are fed later stages of *Artemia*.

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