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LIVE ANIMAL FOOD FOR LARVAL REARING IN AQUACULTURE : THE BRINE SHRIMP ARTEMIA[1]

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INTRODUCTION

Intensive hatchery production of most juvenile fishes and crustaceans is still handicaped by the essential requirement for live foods (Jones and Houde, 1981). In the future this problem will most probably get solved through the use of suitable inert larval feeds (Meyers, 1979). In the meantime, however, hatchery activities, both at experimental and industrial level, still have to rely on live foods ; i.e. several species of algae and yeasts (De Pauw and Pruder, 1981) ; the rotifer Brachionus ; the cladocerans Moina, Daphnia and Diaphanosoma ; the copepods Eurytemora, Tigriopus and Tisbe (Nellen, 1981) and last but least the anostracan brine shrimp Artemia.

Brine shrimp nauplii are indeed most widely used, both in terms of cultured species (e.g. crab, shrimp, prawn, sole, sturgeon, sea bass, sea bream, etc... Kinne, 1977) and in terms of quantities : i.e. at present more than 350 metric tons live weight annualy.
For the predator larvae that can handle this 0.5 mm prey, Artemia constitutes the most practical food (Sorgeloos, 1980a). It might not be the best among live foods (copepods appear to be a better diet for marine fish larvae, Watanabe, 1979), Artemia has the unique property that it can be produced from commercially available inert powder, namely its dry cysts (Sorgeloos, 1980b). This means that, except for a 24 h hatching incubation of the cysts, one is entirely independent of live stock culture maintenance and its inherent production problems which constitute the big draw-backs with all other live foods.

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Problem number one with *Artemia* is thus availability and price of the cysts. Until about 5 years ago commercial cyst supplies were from 2 natural sources in the United States and Canada. The increasing demands for cysts by aquarium hobbyists and aquaculture hatcheries soon exceeded by far the commercial provisions of approximately 30 to 50 metric tons per year. As a consequence cyst prices skyrocketed and the quality of the product delivered became less and less reliable.
We argued at the 1976 FAO Technical Conference on Aquaculture in Kyoto (Japan) that the *Artemia* problem of that day was artificial and could be solved (Sorgeloos, 1979) ; *i.e.* more *Artemia* biotopes could be considered for exploitation, either natural sources or environments where *Artemia* can be artificially introduced ; better techniques could be adopted for cysts processing and hatching ; one could also save on the quantities of cysts needed by feeding the predator, at least from a certain larval stage onwards, with cultured *Artemia*.

It took a while before significant changes were noticed. However an *Artemia* inquiry held in preparation of this review among more than 70 aquaculture hatcheries that use *Artemia*, clearly reveals that 5 years after Kyoto the *Artemia* situation greatly has improved. The main reason for this has been the steadily growing aquaculture research interest in *Artemia*. Nationally and/or internationally financed projects are now in progress in Belgium, Brasil, Cuba, Ecuador, India, Indonesia, Mexico, New Zealand, the Philippines, Thailand, the UK, the US and for sure more countries that I am not aware off. At the 1979 first international symposium on the brine shrimp more than 40 papers were presented in relation to the use of *Artemia* in aquaculture (Persoone *et al.*, 1980). The purpose of this review is to present a brief overview of the various achievements and prospects with regard to the use of *Artemia* in aquaculture.

THE PRODUCTION OF *ARTEMIA* CYSTS

In comparison with 1976 there is now not only a larger choice of commercial cyst-products, provision is also more reliable and hatching qualities have generally improved. Refering only to those exploitations that produce more than one metric ton of cysts per year there are now 2 sources in the United States and one each in Argentina, Australia, Brasil, Canada, Colombia and the People's Republic of China (Table I).

Cyst consumption by aquaculture hatcheries is estimated at about 60 metric tons in 1981 (Department of Fisheries Thailand and UNDP/FAO Programme for the Expansion of Freshwater Prawn Farming in Thailand, 1980 and own data).
Although many people complain not to know where to buy cysts, we may say that right now there is no more shortage of cysts on the market. However, this will probably not last for long. The industrial expansion in commercial farming of *Macrobrachium* and several *Penaeus* species will provoke a very significant increase in *Artemia* cyst demands (Sandifer, 1981). According to data gathered with the *Artemia* inquiry, less than 10 major corporations will need more than 50 metric tons of cysts for their hatcheries in the next 3 to 5 years. This means that a new period of cyst shortage only can be avoided by much faster developments in *Artemia* cysts provisions than those achieved during recent years. We believe that this

is possible, the more that since Kyoto 1976 several theoretical assumptions with regard to Artemia matters have been demonstrated in practice.

First of all the exploitation of natural resources. Although over 250 findspots of Artemia have been reported in scientific literature (Persoone and Sorgeloos, 1980), only less than 20 are tapped for aquaculture purposes. Most of the others have not been seriously surveyed for potential exploitation. The potential should however not be overestimated ; indeed many of these sources will be either too remote, too small or unproductive to justify commercial exploitation ; on top of that many other business factores such as property rights, export regulations and currency problems might further discourage cysts dealers to invest in such explorations.

Transplantation of brine shrimp in a suitable environment, f.ex. a solar salt work, into which Artemia has not been dispersed yet can lead to the development of a very successful Artemia business. Indeed the unique culturing properties of brine shrimp assure that in an operational salt work, only minimal investments are needed to develop an Artemia by-product operation. In Brasil for example, the more than 40 metric tons of Macau cysts that have been marketed since early 1978 all originated from a 250 gram cyst introduction in 1977 (Persoone and Sorgeloos, 1980 ; Van Tilburg, pers. comm.).

Although the basic biological principles of an Artemia transplantation are clear now, problems with regard to reduced cyst production (i.e. dominant ovoviviparous reproduction mode) in transplanted Artemia in Australia (Geddes, 1981) and Brasil (Insulata and Lai, pers. comm.) remain unsolved. Better scientific information should be generated with regard to the selection of appropriate strains of Artemia for transplantation in specific areas.

Temporal salt works that are found in monsoon-climates also can be valorized for an integrated Artemia-production.
The technical feasibility of this type of man-managed Artemia-production during the dry season has been proven first in the Philippines in 1978 (De los Santos et al., 1980 ; Primavera et al., 1980) and later in India (Royan, 1981), Thailand (Vos and Tansutapanit, 1979 ; Tunsutapanick, 1980), Costa Rica (Naegel, 1980 and 1981) and Indonesia (Djajadiredja, pers.comm.).

Commercial feasibility of such Artemia inoculation projects is however much less obvious than with the earlier cited Artemia transplantations. Since waterdepths have to be increased to assure acceptable water-temperatures for the brine shrimp (Vos and de la Rosa, 1980), extra investments are needed for pond modifications which on the other hand will reduce the salt returns per unit of surface area. In a country like Thailand, where solar salt making is a marginal venture and Artemia cysts are in high demand for the expanding Macrobrachium hatcheries, integrated salt cum Artemia production was very well received and apparently is a profitable industry (Department of Fisheries Thailand and UNDP/FAO Programme for the Expansion of Freshwater Prawn in Thailand, 1980).
In other countries like the Philippines salt making is very profitable (Vos, 1980). Local salt bed operators only will go into integrated Artemia production when the returns with brine shrimp can be maximized, which means through intensified production and harvest of cysts + biomass from fertilized ponds (see further on the potential valorisation of adult biomass in fish and crustacean hatcheries and nurseries).

It should be clear thus that one cannot generalize on both technical and economical feasibility of Artemia transplantation and inoculation projects. Site selection work is of primary importance. The feasibility of new projects should also be verified at pilot scale prior to take off on large budgets for fabulous production forecasts. In this regard the Christmas Islands Artemia project (Helfrich, 1973; Environmental Consultants, 1979) will make history as production site of golden Artemia cysts. Brine shrimp production on this remote island in the Pacific is not justified (Sorgeloos, 1980c) ; i.e. salinity levels in the so-called "hypersaline" lagoons are too low for Artemia, cheap nutrients are not available and transportation costs can never be justified.

We are convinced however that there exist many other sites especially on continents like Africa and South America that have unique potential for large scale Artemia production.
It has recently been found that several 100,000 ha of alluvial land areas in Peru might be suited for Artemia pond development. Vast amounts of salt water, infiltrated from irrigation fields are just at 30 to 50 cm depth. Chicken manure, known to be a good fertilizer for Artemia ponds (Vos and de la Rosa, 1980) is available by metric tons from nearby industrial farms. However, since one will have to start from scratch, it is clear that the development of these and similar Artemia projects will take a long time.

Much quicker developments can be expected from the integration of Artemia production in operational solar salt works. Salt farmers have indeed realized that their vast areas of evaporation ponds can be valorized for more than just salt production. For a long time these chemists were afraid that,"...Artemia might eat the salt", better evidence is now available that the presence of Artemia in salinas assures not only more salt production, but even a better quality salt (Davis, 1980; Jones et al., 1981; Sorgeloos, 1979). Knowing that in the tropical-subtropical belt several 100,000 ha of solar salt works are in operation, these areas might be very well suited for vertically integrated aquaculture projects.

THE PRACTICAL USE OF ARTEMIA CYSTS IN AQUACULTURE HATCHERIES

The hatching quality of commercial cyst products has greatly improved since Kyoto'76 (Smith et al., 1978; Sorgeloos et al., 1978; Vanhaecke and Sorgeloos, 1981a). Cyst processors have indeed adopted improved techniques for harvesting and cleaning of their cysts from sand, cracked shells and other debris.

Hatching techniques have also been improved (Sorgeloos, 1980a) as a confirmed by about 80% of the aquaculture hatcheries that were recently questionned with regard to Artemia uses. Better standardisation, illumination of the cysts, change of equipment, incubation at lower salinity and decapsulation of the cysts appear to be the most important innovations.

In addition, better criteria have been developed for evaluating the hatching quality. One now considers hatching rate and synchrony, hatching efficiency and hatching output. Incubated at 25°C in 30 ppt seawater, the first nauplii should appear after 15 to 20 hours cyst incubation and the last nauplii should have hatched out within a time-laps of less than 10 hours (Vanhaecke and Sorgeloos, 1981a). The hatching efficiency criterium (Sorgeloos et al., 1978) is widely used (Dye, 1980; Prescot, 1980). However, since it has been shown that the nauplii from different geographical sources greatly differ in size and weight (Vanhaecke and Sorgeloos, 1980) it became obvious that the hatching efficiency criterium underestimates the quality of a batch of large cysts. This lead to the introduction of the concept hatching output (Vanhaecke and Sorgeloos, 1981a), which precises the total weight of nauplii that can be produced from 1 gram cysts.

As appears from an at random testing of commercial cyst batches (Table II) there is still much room for improvement. Most cyst-customers that completed the Artemia questionnaire considered that an improved service by commercial cyst-dealers is a high priority need for future Artemia-developments. Cyst distributors could certainly avoid a lot of frustrations and arguing by a more explicit reference to the guaranteed hatching quality of their cyst products and to its keeping qualities.

The use of Artemia cysts in aquaculure hatcheries can further be improved by application of the cyst decapsulation technique, i.e. during a short exposure to a hypochlorite solution, the hard cyst shell is dissolved without affecting the viability of the embryo (Sorgeloos et al., 1979; Tunsutapanich, 1979; Bruggeman et al., 1980). This technique not only eliminates all problems with regard to the separation of the freshly hatched nauplii from the empty cyst shells, it has many other advantages not the least the fact that for many fish and crustacean larvae decapsulated cysts appear to be as good a food source as the freshly hatched nauplii (Bruggeman et al., 1980; Mock et al., 1980; Royan, 1980a). The handicap however is that decapsulated cysts do not stay in the water column but sink.
The main rationale for the present using of decapsulated cysts in aquaculture hatcheries is the increased hatching output (Vanhaecke and Sorgeloos, 1981a). The Artemia inquiry furthermore revealed that the present technique for cyst decapsulation is still too complex. In this regard we are wondering if commercial availibility of decapsulated cysts might not be a better solution for this technical problem at the hatchery level.

In view of the observed differences in hatching quality from one cyst source to another, it is logic to suspect differences in nutritional value of the nauplii for specific cultured species. A detailed characterization study of Artemia strains was initiated at an interdisciplinary level in 1978 (Sorgeloos et al. 1979) and is actually in progress with 5 laboratories from the U.S., the U.K., Spain and Belgium. The more than 15 papers that have been published so far all reveal very significant differences for most characteristics studied. Of interest for this review here are the feeding tests with 3 crustacean and 4 fish species as well as the detailed biochemical analyses that were performed with 10 commercial strains :

- naupliar size variation (Vanhaecke and Sorgeloos, 1980) appears to be the first criterion that at least with some predator species determines the ingestibility of specific Artemia products (Beck et al., 1980 ; Beck and Bengtson, 1981) ;
- San Pablo Bay Artemia is very low in the essential fatty acid 20:5ω3 (Schauer et al., 1980; Léger et al., 1981) and provokes high larval mortalities in all marine fishes and crustaceans tested so far (Beck et al., 1980; Johns et al., 1980 and 1981b; Klein-MacPhee et al., 1980;

Léger et al., 1981; see also Goy and Costlow, 1980). A similar correlation between low levels of 20:5ω3 in different Artemia strains and poor performances with cultured marine fish was reported by Fujita et al., (1980). Although San Pablo Bay Artemia was also found to be contaminated with chlorinated hydrocarbons (Olney et al., 1980), it is an acceptable diet for freshwater fishes (Usher and Bengtson, 1981; Vanhaecke and Sorgeloos, 1981b) ;

- High mortalities are noticed with a diet of Artemia from Great Salt Lake origin for shrimp (review in Sorgeloos, 1980a), crabs (Sorgeloos, 1980a, Johns et al., 1980 and 1981a) and flatfishes (Sorgeloos, 1980a and Klein-MacPhee et al., 1980 ; but mysid (Johns et al., 1981b), carp (Vanhaecke and Sorgeloos, 1981b) and atlantic silverside (Beck et al., 1980) do very well on the same Artemia. The exceptional report of successful culturing of crabs with Great Salt Lake Artemia (Goy and Costlow, 1980) has recently been clarified : i.e. the batch of cysts received by Goy and Costlow had been harvested from a distinct source in the Great Salt Lake (Sanders, per. comm.) and was significantly lower in copper content as compared to the levels found in commercial batches (Blust, 1981), which so far always have been harvested at the same spot (Sanders, pers. comm.).
- except with Chaplin Lake Canadian Artemia which give intermediate results, the other Artemia strains tested (Lavalduc-France, Tientsin-PR China, Macau-Brazil, San Francisco Bay-USA, Margherita di Savoia-Italy and Shark Bay-Australia) assure acceptable survival and growth for the marine and freshwater test species (Beck and Bengtson, 1981 ; Klein-MacPhee et al., 1981 ; Léger and Sorgeloos, 1981; Seidel et al., 1981 ; Vanhaecke and Sorgeloos, 1981b)

Since over 2 years now these differences in nutritional value of Artemia from various geographical sources have been the subject of several publications and were repeatedly discussed at international meeting. It nonetheless appears from the Artemia inquiry that many of the participants are unaware of these results. It is high time that hatchery people realize that there is Artemia and Artemia ; that aside from hatching quality one should also consider nutritional quality ; in other words that one should select the most appropriate Artemia sources for the specific predator one is working with. We know of at least 6 major hatcheries in the UK, the US and the Philippines were post factum mass mortalities of marine fishes and crabs could be related to the source of the Artemia used ! As long as specific literature data are lacking on the nutritional value of different Artemia strains for commercially important fish and crustacean species, one should be very careful in deciding about an Artemia product to be used, i.e. a comparative bioassay should be run with an intercalibration product such as Reference Artemia Cysts (Sorgeloos, 1981).

THE USE OF ONGROWN ARTEMIA AS FOOD SOURCE IN AQUACULTURE

It is known from scientific literature that in fact (pre)-adult Artemia have a higher nutritive value than freshly hatched nauplii (Sorgeloos, 1980a) ; i.e. adults contain 60 % protein, are rich in all essential amino acids, high in poly-unsaturated fatty acids and have an ash content of 10 %. However aside from a few experimental tests (see also Palmegiano and Trotta, 1981) feeding with adult Artemia has never been verified for large scale application. This might soon change as a result of following recent findings and developments :

- a fish farmer in the Philippines has demonstrated that his milkfish production per surface area and per year can be significantly increased as a result of supplementary feeding of the Chanos fry during their 3 week stay in the nursery ponds with adult Artemia; survival at the end of the nursery stage has not only increased, the milkfish has also grown faster and are thus more resistant which assures better results during consequent ongrowth (De los Santos, pers. comm.);
- a commercial shrimp farm in Brasil reports significant improvement in their Penaeus hatchery-nursery output since extra-feeding with adult Artemia has been applied in routine fashion (Guimaraes, pers. comm.).

Of course the basic question in this regard is where to obtain cheep Artemia.
As is done at the brasilian farm, live Artemia can be collected from an operational solar salt work where it is mostly available on a year-round basis and by the metric tons (Sorgeloos, 1979). If not available from a nearby salt work cheap Artemia can be produced in either extensive or intensive culture systems.
As is applied by the Philippino fish farmer extensive Artemia culturing can be done in small ponds adjacent to the nursery ponds. Lime and crude salt are added to the seawater to create an acceptable medium for monoculturing of brine shrimp. Phytoplankton development is enhanced by weekly additions of chicken manure and commercial inorganic fertilizer (see also Dwivedi et al., 1980; Royan, 1980b; Tunsutapanich, 1980; Vos and de la Rosa, 1980).
In tropical climates like in the Philippines, production yields of more than 10 g brine shrimp per square meter and per day can easily be maintained. This means that, in the Philippines for example, large scale practice of this type of extensive Artemia production could lead to a very substantial increase in milkfish productions. Such Artemia could also be used as a suitable food for other cultured species, even for freshwater organisms like Macrobrachium. Upon transfer in freshwater adult Artemia stay alive for several more hours, enough time thus to be captured and eaten by the predator.

Extensive Artemia productions, however, are limited to warm climated and dry seasons. In this regard intensive culturing of brine shrimp in controlled conditions is much more versatile in applicability.
Since Kyoto 1976 techniques for intensive Artemia culturing have grown from lab scale testing into pilot scale and, in some situations already into industrial application.

The major breakthrough in intensive culturing of brine shrimp has been the finding that cheap agricultural byproducts (Dobbeleir et al., 1980; James et al., 1981; Sorgeloos et al., 1981) such as e.g. ricebran and whey-powder, can be successfully used as food source. Ingestibility of the food is a critical factor ; therefore particle sizes mostly have to be reduced below the 50 mikron limit (Dobbeleir et al., 1980).

The most widely applicable culture techniques is the batch culturing from nauplius to adult stage without any water renewal in air-water-lift operated raceways (Bossuyt and Sorgeloos, 1980) :

- air lifts assure optimal aeration and circulation without harming the animals;
- faecal pellets are selectively settled out in a plate separator which is

connected to the raceway;

- food distribution is semi-automatic, the only adjustment needed being the frequency and rate of distribution as a function of the turbidity changes within the culture tank.

Present production figures that are obtained in routine operations amount to 5 kg brine shrimp per cubic meter after 2 weeks culturing on ricebran at 25°C (Bossuyt and Sorgeloos, 1981). The pilot plant of the Artemia Reference Center at the Belgian coast, which consist of 4 raceways of $5m^3$ each, can be operated by 1 technician only and can produce an average of 50 kg pre-adult Artemia per week. Variations on this culture technique in batch conditions have been reported by James (1980a and b) and Rallo et al. (1981)

More intensive Artemia culturing can be achieved with flowthrough culturing. The basic requirement of course is the availability of large volumes of warm seawater or brine effluents ; e.g. thermal effluents from desalination plants.
An interchangeable and self-cleaning screen system that retains the animals in the culture tank but allows drainage of water and faecal pellets is the key item that makes high density frow through culturing of brine shrimp technically feasible (Tobias et al., 1979). This culture system is very useful for converting algal biomass into animal protein in artificial upwelling projects (Roels et al., 1979) or, more realistically, in tertiairy treatment systems (Milligan et al., 1980).
Using the effluent of a geothermal well, it has recently been demonstrated that with inert diets such as e.g. grinded ricebran, brine shrimp can be cultured in densities up to more than 10,000 animals per liter (Brisset et al., 1981). Production figures are indeed substantially higher than with raceways operated under batch conditions. Both with algae as with ricebran one can easily produce more than 20 kg Artemia in a $1m^3$ tank over a culturing period of 2 weeks only (Sharfstein et al., 1979 respectively Brisset et al., 1981).

It is to be expected that in the near future, the use of adult Artemia in aquaculture hatcheries and nurseries will contribute to improved production results. There is urgent need however for further research on the nutritional value of different products of adult brine shrimp ; in this regard it is highly likely that for intensive culturing on inert feeds one might have to consider diet formulations for the Artemia as to assure an optimal nutritional value for specific predators (Dobbeleir et al., 1980; Metailler et al., 1981; Sorgeloos et al., 1981).

As production techniques both at the extensive and the intensive level further will develop it will become more and more realistic to consider mass scale production and use of Artemia meal as a protein-ingredient for artificial feed formulations (Anonymous, 1978 ; Sorgeloos, 1980a; Webber and Sorgeloos, 1980).

CONCLUSION

5 years after the FAO Technical Conference on Aquaculture in Kyoto it can be said that the overall Artemia situation has improved (Pillay, 1981) and that there is enough evidence now for an optimistic outlook into the future. This does not mean however that no action has to take place, i.e.:

- extra cyst provisions have to be found in the near future ;

- better cooperations between cyst dealers and customers should be finalized;
- nutritional differences between Artemia sources should be taken into account;
- nursery feeding with adult Artemia should be better explored.

The potential with Artemia has by far not been realized yet. It takes time however, not the least to learn the a,b,c, of its culturing biology. The availability of inert cysts is one thing; one should not give up there, Artemia has many other unique characteristics that should be exploited for the benefit of aquaculture production.

Table I. Updated list of major Artemia cyst dealers

- AQUAFAUNA BIO-MARINE INC.
  P.O. Box 5, Hawthorne, CA 90250, USA
  (distributor of Great Salt Lake, UTAH-USA

- AQUARIUM PRODUCTS
  180L Penrod Court, Glen Burnie; MD 21061, USA
  (harvestor-distributor of sources from Argentina and Colombia)

- ARTEMIA INC.
  P.O. Box 2891, Castro Valley, CA 94546, USA
  (harvestor-distributor of Shark Bay-Australia ; distributor of Macau-Brazil)

- CHINA NATIONAL CEREALS, OILS & FOODSTUFFS IMPORT & EXPORT CORP. - TIENTSIN BRANCH
  No. 134 Chih Feng Road, Tientsin, PR China
  (harvestor-distributor of Tientsin-PR China)

- HL MARINOCULTURA LTDA.
  Caixa Postal 25, Macau, RN 59500 Brasil
  (harvestor-distributor of Macau-Brasil)

- JUNGLE LABORATORIES CORPORATION
  P.O. Box 66, Comfort, TX 78013, USA
  (harvestor-distributor of Chaplin Lake-Canada)

- SANDERS BRINE SHRIMP CO.
  1255 West 4600 South, Ogden, UT 84403, USA
  (harvestor-distributor of Great Salt Lake, UT-USA)

- SAN FRANCISCO BAY BRAND INC.
  8239 Enterprise Drive, Newark, CA 94560, USA
  (harvestor-distributor of San Francisco Bay, CA-USA)

Table II. Hatching characteristics of different commercial batches of *Artemia* cysts (from Vanhaecke and Sorgeloos, 1981a)

source of cysts	hatching efficiency[1] (nauplii/g)	nauplius dry weight (in µg)	hatching output (mg/g cysts)
San Francisco Bay (USA)	267,200	1.63	435.5
San Pablo Bay (USA)	259,200	1.92	497.7
Macau (Brasil)	304,000	1.74	529.0
Barotac Nuevo (Philippines)	214,000	1.68	359.5
Great Salt Lake (USA)	106,000	2.42	256.5
Shark Bay (Australia)	217,600	2.47	537.5
Chaplin Lake (Canada)	65,600	2.04	133.8
Buenos Aires (Argentina)	193,600	1.72	333.0
Lavalduc (France)	182,400	3.08	561.8
Tientsin (PR China)	129,600	3.09	400.5
Margherita di Savoia (Italy)	137,600	3.33	458.2

[1] Hatching efficiency and hatching output data are not source but batch specific!

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