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PRODUCTION, UTILIZATION AND MANIPULATION OF *Artemia* AS FOOD SOURCE FOR SHRIMP AND FISH LARVAE

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Summary

Larval culture of fish and shrimp can be seriously improved thanks to the results of recent research and development in the field of *Artemia* production and utilization.

The present article summarizes the most recent techniques for optimization in production and use of different stages of *Artemia* in aquaculture :

. cyst selection in function of hatching quality, purity, biometric characteristics, etc. ;

. nauplii production : techniques for hatching, disinfection and decapsulation ;

. nutritional value of nauplii : quantitative and qualitative aspects with regard to essential components and contaminants ;

. bioencapsulation of nauplii with different enrichment components : methodology and results in fish and shrimp culturing ;

. utilization of (pre-) adult biomass for juvenile or postlarval feeding and as maturation trigger in shrimp ;

. extensive production of *Artemia* in salt pans or integrated in solar salt operations ;

. intensive production of *Artemia* using waste products from agricultural crops as feed ;

. bioencapsulation of adults with different enrichment products ;

. perspectives with regard to future use of *Artemia* in aquaculture.

Résumé

Production, utilisation et manipulation de l'*Artemia* comme source de nourriture pour larves de poissons et de crevettes

La larviculture de poissons et de crevettes marins s'est améliorée grâce aux résultats des recherches et aux développements dans le domaine de la production et de l'utilisation d'*Artemia* à ses différents stades.

Le présent article donne un aperçu des techniques les plus récentes de production et d'utilisation d'*Artemia* en aquaculture :

. sélection de cystes en fonction de leur qualité d'éclosion, de leur pureté, de leurs caractéristiques de biométrie, etc. ;

. production de nauplii : techniques d'éclosion, de désinfection et de décapsulation ;

. valeur nutritive des nauplii : taux de composés essentiels, niveau de contamination ;

. possibilités de bioencapsulation de différents produits d'enrichissement dans les nauplii : méthodologie et résultats avec poissons et crevettes;

. emploi d'adultes comme nourriture pour juvéniles et postlarves et comme stimulant de la reproduction chez les crevettes ;

. production extensive d'*Artemia* dans les marais salants, éventuellement intégrée avec la production de sel ;

. production intensive d'*Artemia* avec des sous-produits de l'agriculture comme source de nourriture ;

. possibilités de bioencapsulation de différents produits d'enrichissement chez les adultes ;

. perspectives futures.

INTRODUCTION

Intensive production of most juvenile fishes and crustaceans is still hampered by the essential requirement for live food, at least during their early stages (Girin & Person-Le Ruyet, 1977; Goodwin & Hanson, 1977). Since the techniques for collecting and feeding their natural diet (characterized by a wide diversity of plankton) have been designated as hardly applicable at commercial scale, a readily available diet is to be selected being easily accepted and digested, and having a reproducibly nutritional quality.

One of the most used live foods in the successful larval rearing of fishes and crustaceans are the small nauplii of the brine shrimp, *Artemia*. These larvae have a good nutritional value with regard to protein, amino acid and fatty acid levels and, moreover, they are readily available over the total water column. Since *Artemia* is available as an "off the shelf food" under the form of dry cysts, it is a more attractive live food as compared to rotifer, cladocerans and copepods. Ready to feed nauplii are obtained through simple hatching procedures from the dormant embryo making their utilization in hatcheries very practical.

Not only the freshly hatched *Artemia* can be used successfully in aquaculture productions, also brine shrimp biomass, commercially available as live, frozen, dried or freeze-dried product (Lai & Lavens, 1986), is attractive as food source for several stages of specific predators (Mock *et al.*, 1980; Palmegiano & Trotta, 1981; Sorgeloos, 1981b).

However, the popularity of *Artemia* as "ideal" larval food source in fish, shrimp, crab and lobster farming has also created its particular problems. Back in the 70's increasing demands for cysts exceeded the commercial provisions from classical *Artemia* sites (e.g. Great Salt Lake and San Francisco Bay) and resulted in a temporary shortage. Initially cyst prices skyrocketed ; later other natural strains were tapped. Nowadays *Artemia* products are available from various origin and with varying hatching and nutritional quality.

We present here an overview of recently developed techniques which deal with these difficulties and aim to optimize the production and use of this precious live food *Artemia* in aquaculture.

A. PRODUCTION AND USE OF FRESHLY HATCHED NAUPLII

The production of brine shrimp nauplii by incubation of cysts in seawater is a very simple procedure. Today, however, most of the aquaculture producers are still unaware of the tremendous cost savings that are possible when appropriate techniques for large scale hatching are applied. Indeed, various abiotic parameters seriously affect hatching efficiency, hatching rate and/or hatching output ; their qualitative effect being similar for the

various *Artemia* strains, their quantitative effect being different (Sorgeloos, 1980). Proper application of these hatching techniques will result in maximal hatching efficiency and, in this way, minimize the quantity of cysts needed.

The fastest hatching rate and maximal hatching efficiency are attained at a temperature of 28 to 30°C (Vanhaecke & Sorgeloos, 1983). A salinity above 5 ppt and lower than that of normal seawater may increase the hatching percentage, and result in a higher energy content of the freshly hatched nauplii (Vanhaecke *et al.*, 1980). It is furthermore proven that pH levels above 8 are essential for a complete hatching (Sato, 1967). In this regard it is advised, when using diluted seawater (5 ppt) and especially at high cyst densities, to increase the buffer capacity of the hatching medium by supplementing up to 1 g $NaHCO_3$ per liter. Also illumination of the incubated cysts, at least during the first hours of their hatching incubation, is critical to obtain maximal hatching outputs and to optimize hatching rates (Fig. 1). Considering the differences observed among strains (Vanhaecke *et al.*, 1981), a continuous illumination with a light intensity of about 2,000 lux will assure maximal production of brine shrimp nauplii.

Aside from these essential conditions for optimal hatching, but also very important in the optimization process, is the moment of harvesting. Indeed, when long hatching incubation periods are applied, freshly hatched

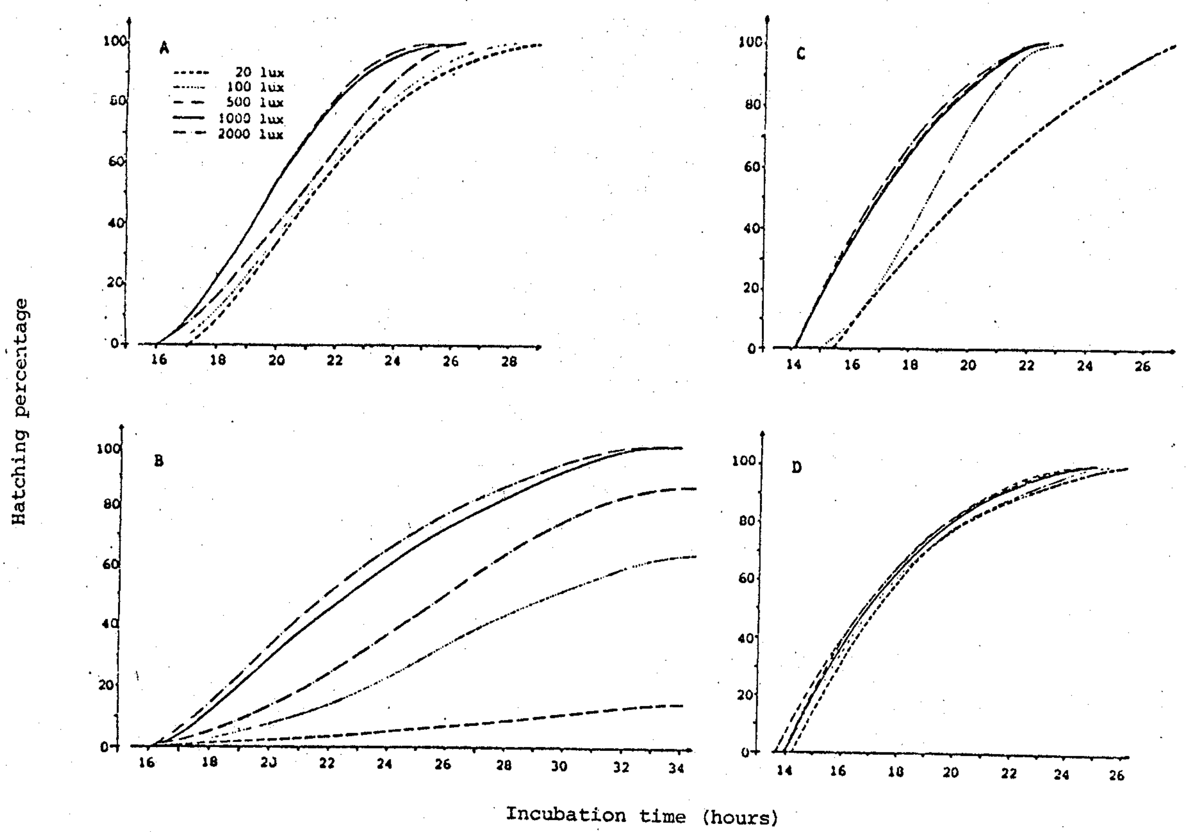

Fig. 1 - Effect of light intensity on hatching speed and ratio of *Artemia* cysts from (**A**) Buenos Aires, (**B**) Chaplin Lake, (**C**) San Pablo Bay, and (**D**) Great Salt Lake (from Vanhaecke & Sorgeloos,1981).
- *Effet de l'intensité de la lumière sur le taux et la vitesse d'éclosion de cystes d'*Artemia *en provenance de (**A**) Buenos Aires, (**B**) Chaplin Lake, (**C**) San Pablo Bay, et (**D**) Great Salt Lake (d'après Vanhaecke & Sorgeloos, 1981).*

instar I nauplii will molt into second and following instar stages (Hentschel, 1968). The same occurs when instar I nauplii are being fed to predator larvae under suboptimal feeding conditions. For example, when feeding is done ad libitum, the retention time of part of the nauplii may exceed 24 hours and molting into instar II stages will occur before their ingestion by the predator. Several authors have demonstrated the nutritional inferiority of starved metanauplii. A first reason is their larger size (Smith, 1976; Claus *et al.*, 1979) which may hamper or even prevent their ingestion resulting in starvation of the predator larvae. Moreover, instar II are translucent and swim faster than instar I nauplii (Miller *et al.*, 1979), as a result they are more difficult to catch. The main reason for their inferior value is however, undoubtedly, their poorer nutritional composition. In 1956 already Morris attributed the lower nutritional performance of 2 to 3 days old metanauplii for fish larvae to their exhausted yolk reserves. This consumption is expressed as a drastic decrease of their individual dry weight and energy content (see Fig. 2; Paffenhöfer, 1967; Benijts *et al.*, 1976; Vanhaecke *et al.*, 1983) which accounts for poorer growth in the predator larvae.

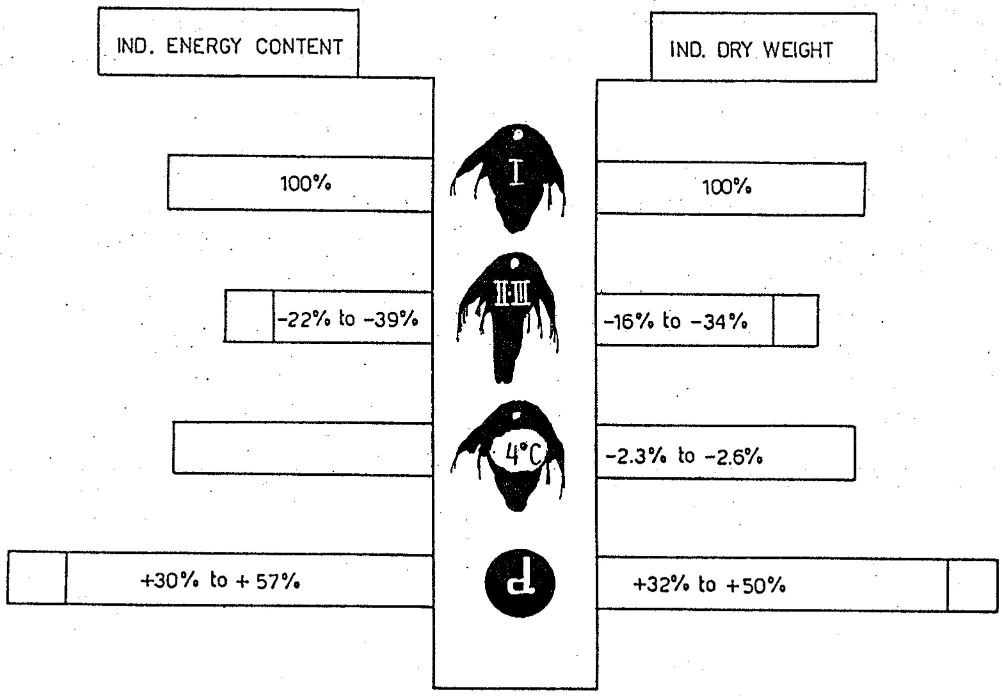

Fig. 2 -Schematic drawing of decrease of individual energy content and dry weight in instar II-III metanauplii, 24 h cold stored nauplii, and decapsulated cysts as compared to freshly hatched instar I *Artemia* nauplii (modified from Léger *et al.*, 1986b).

- *Pertes individuelles en poids sec et en contenu énergétique des métanauplii instar II-III, des larves stockées pendant 24 heures à 4°C et des cystes décapsulés, en comparaison avec des nauplii fraîchement éclos (Léger* et al.*, 1986b).*

This decrease in energetic content in brine shrimp larvae may be prevented by applying optimized feeding regimes, by improving hatching procedures or by automatically incubating the cysts at the desired moment (Léger & Sorgeloos, 1982). Another solution consists in storing high densities of freshly hatched nauplii in a refrigerator at 2-4°C (Léger *et al.*, 1983; Sleet & Brendel, 1983). During this cold storage the larvae remain in the first stage for more than 48 hours with a maximal loss in energetic content of only 8 % (Léger *et al.*, 1983). Moreover, when fed to the fish and crustacean culture tanks, these cooled larvae move slowlier in the water column, and thus can be taken up more easily by the predator larvae.

In spite of the various methods described in literature (see Sorgeloos, 1980), complete separation of the nauplii from their cyst shells is not always possible. The cyst shells are indigestible and may be harmful when ingested by the predator (Morris, 1956). Moreover, their high bacterial load may cause contamination of the cultures (Wheeler *et al.*, 1979). In this regard it is recommended to disinfect the *Artemia* cysts prior to their use. This can be done by simple soaking of the cysts in a 20 ppm hypochlorite solution during approximately 1 hour preceeding the hatching incubation (Sorgeloos *et al.*, 1986).

An extreme form of disinfection is obtained by decapsulation of the cysts, i.e. the complete dissolution of the outer shell in a concentrated hypochlorite solution, without affecting the viability of the embryo (Bruggeman *et al.*, 1980). The decapsulation technique not only eliminates all separation and disinfection problems but offers also several advantages, the most important ones being both an increased hatchability up to 20 % for some *Artemia* strains (Bruggeman *et al.*, 1980; see Table 1) and the fact that these decapsulated cysts can be used as a direct food source for many predators (see review by Léger *et al.*, 1986a). In this way (cheap) cyst material which does not hatch anymore can be valorized and, moreover, cyst amounts can be used more economically (Vanhaecke *et al.*, 1983; see Table 1 and Fig. 2). De Vrieze (1984) indeed demonstrated that for the production of the same carp biomass 10 to 23 % cysts could be saved during the first week, and 32 to 36 % during the second week by using decapsulated cysts instead of instar I nauplii. The same author obtained also significantly better growth when offering dried instead of freshly decapsulated cysts; this is due to a better buoyancy which improves the availability of these dehydrated, decapsulated cysts in the total water column.

The use of freshly hatched nauplii or decapsulated cysts in hatcheries can further be optimized by selecting the most suitable *Artemia* strain or cyst batch. Indeed, hatching characteristics, e.g. hatching efficiency, rate and output, differ tremendously between commercial cyst sources from different geographical origin or even between batches from the same strain (Vanhaecke & Sorgeloos, 1983; see Table 1). Moreover, some strain specific biometrics may interfere with the physical requirements that a prey organism has to meet for the predator larvae, and are thus of great importance to the aquaculturist. Especially variability in size of freshly hatched nauplii may affect their efficiency as food organisms since the latter is in the first place determined by their ingestibility; e.g a high correlation has been detected by Beck and Bengtson (1982) between *Artemia*-prey and mortality rates for *Menidia menidia*. This has also been confirmed by Smith (1976) who observed higher survival in fish larvae fed on the smaller San Francisco Bay nauplii instead of the bigger Great Salt Lake nauplii. On the other hand, when prey size is not limiting for ingestion, selection of larger strains is recommended since their energetic content is higher and, consequently, less hunting effort will be required to meet the energetic needs, as it is evidenced for carp larvae (De Vrieze, 1984).

Cyst source	Hatching Percentage (%)		Hatching Efficiency (nauplii/g cysts)	Hatching Output (mg/g cysts)	Hatching Rate (hours)			Nauplius Dry weight (µg)	Nauplius Length (µm)
	cysts	decaps. cysts			T_o	T_{90}	Δ		
San Francisco Bay (2596)	71.4	82	267,200	435.5	15.0	20.5	5.5	1.63	428
San Francisco Bay (2016)	-	-	249,600	-	25.8	37.8	12.0	-	-
San Pablo Bay (1628)	84.3	91	259,200	497.7	13.9	20.1	6.2	1.92	433
Great Salt Lake	43.9	55	106,000	256.5	14.1	21.7	7.6	2.42	486
Reference *Artemia* Cysts	45.7	-	211,000	375.6	18.0	32.2	14.2	1.78	448
Lavalduc	75.8	77	182,400	561.8	19.5	30.5	11.0	3.08	509
Tientsin	73.5	76	129,600	400.5	16.0	27.2	11.2	3.09	515

Table 1 - Hatching characteristics of cysts and biometrical data of instar I stages of *Artemia* from various geographical origin (from Vanhaecke, 1983).
- *Caractéristiques d'éclosion des cystes et données biométriques des* Artemia *instar I de diverses origines (d'après Vanhaecke, 1983).*

B. THE NUTRITIONAL VALUE OF *Artemia* NAUPLII

Artemia nauplii have been used as a suitable live food source for the culture of a wide variety of aquatic organisms (review by Léger *et al* ., 1986a). It now appears that the suitability of *Artemia* has more to do with practical reasons than nutritional ones. Indeed, it has been shown on several occasions that particular strains or batches of *Artemia* perform significantly less as a larval food than natural zooplankton. This observation has resulted in depth research, e.g. the "International Study on *Artemia*", aiming to explain nutritional deficiencies and differences between and within brine shrimp strains. Chemical and biochemical analyses on nine strains (San Francisco Bay, San Pablo Bay, Great Salt Lake, U.S.A. ; Macau, Brazil ; Chaplin Lake, Canada; Margherita di Savoia, Italy ; Lavalduc, France ; Shark Bay, Australia ; Tientsin, People's Republic of China ; and reference *Artemia* cysts (Sorgeloos, 1981a) were correlated with the results of culture tests using several fish and crustacean species (Table 2). This study revealed that culture success can be unequivocally correlated with the highly unsaturated fatty acid (H.U.F.A.) content in the *Artemia* nauplii (Léger *et al*., 1985b; cf. earlier findings of Watanabe *et al*., 1980).

Tests with commercial predators, e.g. *Dicentrarchus* or *Penaeus* also resulted in higher survival rates and biomass production when *Artemia* lots were used with high H.U.F.A. levels (Van Ballaer *et al*., 1985; and Léger *et al*., 1985a, respectively).

The level of contaminants (heavy metals and chlorinated hydrocarbons) as found in the brine shrimp sources studied (Olney *et al*., 1980 ; Seidel *et al*., 1982) could not account for the mortality observed in larvae fed particular strains. However, this may probably not be extrapolated for *Artemia* cysts produced on smaller scale in extensive systems or saltworks in Third World countries, where environmental standards (e.g. use of pesticides) are much more permissive. Furthermore, its effect may not necessarily be observed in the bioassay-tests : i.e. it might only be during stress periods in larval culturing (e.g. weaning) that an increased sensitivity resulting in larval mortality may be an expression of toxicity of contaminated live food.

		ARTEMIA STRAINS									
		AUSTRALIA	BRAZIL	CANADA	CHINA	FRANCE	ITALY	UTAH	SAN PABLO BAY	SAN FRANCISCO BAY	R.A.C.
FATTY ACID (HUFA) CONTENT	18 : 3w3 (% of total fat content)	14.8	4.9	19.9	7.4	20.9	6.4	31.5	33.6	5.2	2.6
	20 : 5w3 (% of total fat content)	10.5	9.0	9.5	15.4	8.0	13.6	3.6	1.7	12.4	8.5
CULTURE TESTS: MARINE – CRUSTACEANS	METAMORPHOSIS	+	+	±	+	+	+	–	–	+	+
	----------	+	+	±	+	+	+	+	–	+	+
MARINE – FISH	METAMORPHOSIS	+	+	+	+	+	+	–	–		+
	----------	±	+	±	±	±	–	±	–	+	+
FRESHWATER FISH	----------	+	+	+	+	+	+	+	+	+	

Table 2 - Synopsis of the results of fatty acid content and I.S.A. culture tests with larvae of marine crustaceans *(Rhithropanopeus harrissii, Cancer viroratus, Mysidopsis bahia)* and fish (*Pseudopleuronectes americanus, Manidia menidia*), and freshwater fish (*Cyprinus carpio, Cyprinodon variegatus)* fed *Artemia* nauplii from different geographical strains. Culture results are expressed as good (+), medium (±) or bad (-) survival and growth (after Léger *et al.*, 1986a).

*- Résultats des tests de culture d'acides gras et d'I.S.A. sur des larves de crustacés marins (*Rhithropanopeus harrissii, Cancer viroratus, Mysidopsis bahia*), de poissons (*Pseudopleuronectes americanus, Manidia manidia*) et de poissons d'eau douce ((*Cyprinus carpio, Cyprinodon variegatus) *nourris de nauplii d'*Artemia *venant de souches d'origine géographique diverse. Résultats concernant la survie et la croissance : (+) = bons ; (±) = moyens ; (-) = mauvais (d'après Léger* et al.*, 1986a).*

Differences in content of essential fatty acids (e.g. H.U.F.A. 20:5ω3) may not only be considerable between strains (Watanabe *et al.*, 1978b, 1980, 1982 ; Schauer *et al.*, 1980 ; Vos *et al.*, 1984), but also within strains (Watanabe *et al.*, Léger *et al.*, 1985b). These differences in fatty acid profile are considered to be a reflection of the varying fatty acid profile in their natural diet (Vos *et al.*, 1984 ; Lavens *et al* ., 1986b). Except for small systems where feed composition may be controlled, no a priori solution to this type of nutritional variation in *Artemia* is available. The only means at the disposition of the culturist are the selection of H.U.F.A. rich *Artemia* batches or the use of H.U.F.A. enrichment techniques.

Several techniques have recently been elaborated for the enhancement of the nutritional value of *Artemia* through bioencapsulation with enrichment

diets containing essential components, especially H.U.F.A. (Watanabe *et al.*, 1978a, 1982, 1983 ; Robin *et al.*, 1981, 1984 ; Gatesoupe, 1982 ; Robin, 1982, 1985 ; Léger *et al.*, 1985a, 1986b). Marine algae, compound and microencapsulated diets, ω-yeast and emulsions have been used as H.U.F.A. sources by British, French, Japanese and Belgian researchers (review in Léger *et al.*, 1986a). As a rule the enrichment techniques consist in hatching *Artemia* cysts, separating the nauplii from hatching debris, and incubating them in the enrichment medium. In this medium nutrient-rich particles are ingested by these non-selective filter feeders and packed in their gut. Through adjusting hatching and enrichment procedures smaller or larger enriched metanauplii may be produced and offered according to larval growth.

The Belgian technique may have some important advantages over the others. The use of the self-emulsifying enrichment concentrate is not only very practical (off the shelf product; simple technique) but also enables to obtain very high enrichment levels, e.g. H.U.F.A. contents up to 50 $mg.g^{-1}$ dry weight (Table 3). Moreover, these enriched nauplii are already available 36 to 48 hours after incubation for hatching and thus can be fed to earlier larva stages.

The enhanced nutritional composition of enriched brine shrimp is attributed to the increased levels of 20:5ω3 and 22:6ω3 fatty acids essential for marine predators. Especially the incorporation of the latter fatty acid is important when it is lacking in freshly-hatched *Artemia* nauplii from whatever geographical source. Other components (nutrients, prophylactics, therapeutics, etc.) may also be incorporated in *Artemia* by similar techniques and transferred in this way to the predator. The improved nutritional value of this enriched live food may not only be reflected in improved culture success at the hatchery stage but, as a result of the better physiological condition of the predator larvae, improved survival and growth may be noted in the nursery and grow-out stages as well (Léger *et al.*, 1986b).

C. THE USE OF ONGROWN *Artemia* AS FOOD SOURCE

It is known from literature that juvenile or adult *Artemia* have in fact a higher nutritive value than freshly hatched nauplii : i.e. adults contain 60 % protein, with all essential amino acids, are rich in highly unsaturated fatty acids and have an ash content of 10 %. In contrast with other food organisms, the exoskeleton of adult *Artemia* is extremely thin, which facilitates digestion of the whole animal by the predator.

Furthermore, these brine shrimp can also be packed with other nutrients or components as to fulfil the nutritional requirements of postlarval shrimp and juvenile fish. Indeed, similar enrichment diets as for nauplii can be used with (pre-)adult brine shrimp : as a result of their faster and more efficient filter rates the digestive tract of ongrown *Artemia* is filled with the enrichment product in less than one hour.

Despite the many advantages mentioned above, the use of ongrown *Artemia* for aquacultural purposes is still in a developmental phase, mainly due to the restricted commercial availability of a highly prized product. However, recent findings and developments indicate that this might change soon; it was predicted at the 2nd *Artemia* Symposium (Antwerp, 1985) that the demand for live, as well as frozen or (freeze)-dried biomass will increase significantly in the future (Lai & Lavens, 1986). Improved outputs have been obtained e.g. in penaeid hatchery-nursery (Guimares & De Haas, 1986; Millamena *et al*, 1986; Trotta *et al.*, 1986), and in milkfish fry rearing and weaning (de los Santos *et al.*, 1980) through extra feeding with ongrown *Artemia* (see review by Léger *et al.*, 1986a). Furthermore, a diet of reproductively active

	20 : 5w3		22 : 6w3		Σ w3 - HUFA	
	area %	mg.g^{-1}	area %	mg.g^{-1}	area %	mg.g^{-1}
Instar I - non enriched						
24h + 0h	0.3	0.5	-	-	1.2	1.9
Nauplii - enriched						
24h + 12h (incubation without separation of the nauplii)	4.5	6.4	2.4	3.3	8.2	11.2
24h + 24h (after separation)	9.9	21.3	5.9	12.7	17.8	37.4
24h + 48h (after separation)	13.5	35.2	7.0	18.1	23.0	58.6

mg.g^{-1} : dry weight basis.

Table 3 - Effect of different enrichment periods, following the Belgian bioencapsulation procedure with a selfemulsifying concentrate, on the HUFA levels of Great Salt Lake *Artemia* (after Léger *et al.*, 1986a).

- Effet des diverses périodes d'enrichissement, suivant le procédé belge de bio-encapsulation avec un concentré auto-émulsifiant, sur le niveau des HUFA des Artemia *de Great Salt Lake (d'après Léger* et al.*, 1986a).*

Artemia adults may induce maturation in shrimp without application of the eyestalk ablation technique (Camara & De Medeiros Rocha, 1986; Flores Tom, 1986). Brine shrimp biomass can also be used as dietary ingredient or gustatory attractant in artificial diets for predator larvae (Sick, 1975; Metailler *et al.*, 1977; Gatesoupe & Luquet, 1984), and even as a complete substitution in micronized form of freshly hatched nauplii in the hatchery production of *Penaeus* (Guimares & De Haas, 1986).

D. MASS PRODUCTION OF *Artemia*

Most commercial *Artemia* products are obtained by simple exploitation of natural resources, in most cases solar salt operations. Since it has been proven that proper *Artemia* management can lead to a beneficial impact on quality and quantity of the solar salt production (Persoone & Sorgeloos, 1982), it is expected that transplantation of *Artemia* for improved salt production purposes might yield increased harvest of brine shrimp cysts and biomass.

Artemia production, however, is not only limited to salinas. The know-how acquired during recent years in Southeast Asian *Artemia* projects proves the large potential of brine shrimp inoculation and cultivation in extensive pond systems (de los Santos *et al.*, 1980; Jumalon *et al.*, 1986; Tarnachalanukit & Wongrat, 1986). Provided that salinities in the culture ponds can be kept above 90 ppt, monthly yields of cysts and biomass of 15 to 30 kg per hectare, respectively 500 to 1000 kg per hectare can easily be maintained by weekly fertilizations with chicken dung, cow manure or inorganic fertilizer.

Although the cheapest ways for brine shrimp biomass exploitation are the natural and extensive pond production systems, *Artemia* produced in intensive culture systems may become more attractive, especially in climates unsuitable for outdoor production, or when nutritional quality control is critical (Sorgeloos, 1983 ; Lavens *et al.*, 1985).

Intensive culture systems can be operated at very high densities of more than 10,000 animals per liter (in outdoor ponds densities of 100/l are considered very high). Instead of live algae, different byproducts from agricultural crops (e.g. rice bran corn husks, etc.) or Single Cell Protein can be successfully used as food source (Dobbeleir *et al.*, 1980 ; Lavens *et al.*, 1986a); however, their particle size should be less than 50 micron (Dobbeleir *et al.*, 1980).

Batch production of brine shrimp in air-water lift operated raceways yields up to 5 kg wet weight biomass per m^3 culture water within 14 days (Bossuyt & Sorgeloos, 1980). The construction and operation of the culture systems is rather simple, and since almost no water is renewed, this technique can be operated independently at most sites. The main disadvantage for commercial application, however, is the fact that in proportion to the amount of of labour input biomass outputs are rather small, mainly as a result of the low stocking densities (5,000 l) commanded by water quality restrictions.

The development of flow-through culture systems may have been the major break-through in the application of intensive biomass production systems for aquaculture purposes (Lavens *et al.*, 1985). In general, these techniques allow higher stocking densities (more than 15,000 animals per liter), a greater degree of control over environmental parameters, and last but not least an increased automation (e.g. food distribution and control). In this way much higher production yields, up to 25 kg live biomass per m^3 culture tank per two weeks, can be achieved (Brisset *et al.*, 1982 ; Lavens *et al.*, 1985, 1986a). In view also of its large tolerance for the ionic composition of the culture water and its total contents, brine shrimp can be cultured in a wide variety of thermal effluents, e.g. from power or desalination plants, or from geothermal wells, etc., which will further reduce production costs. Indeed, a basic requirement for open flow-through culturing is the availability of large volumes of warm seawater or brine. The same production technique can, however, be adapted for operation in closed flow-through conditions (Fig. 3a). Using as

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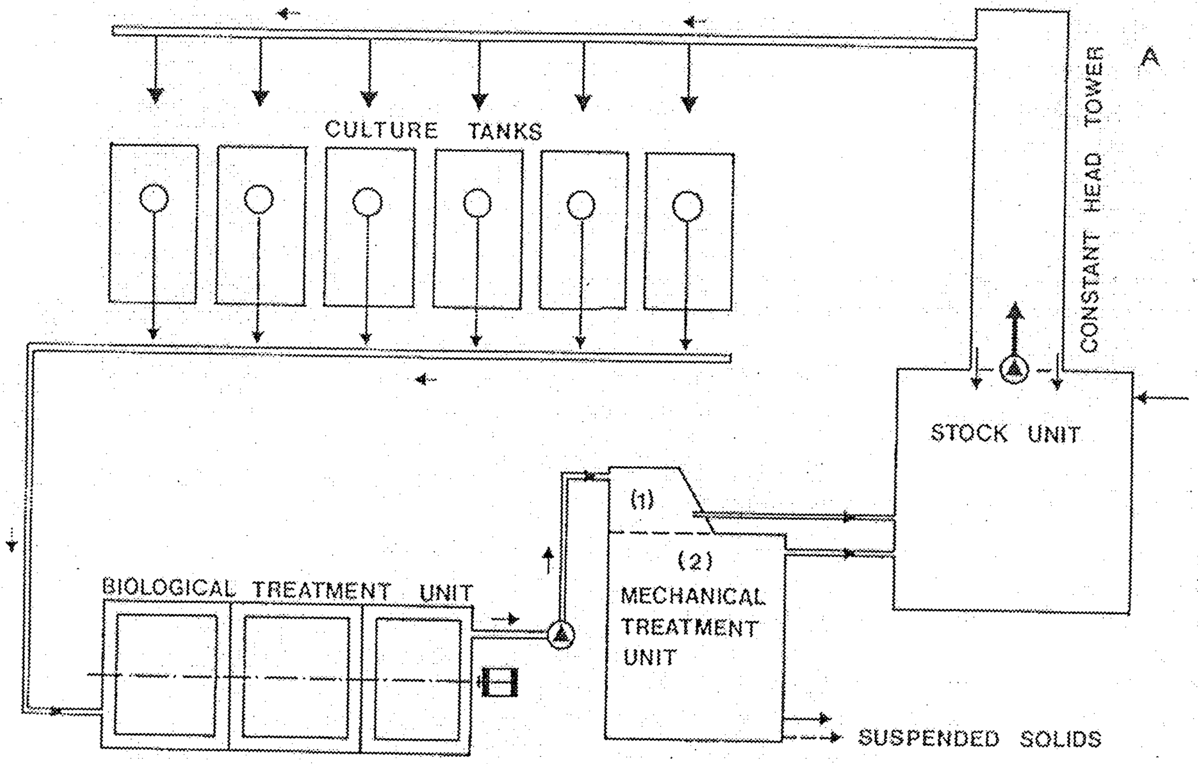
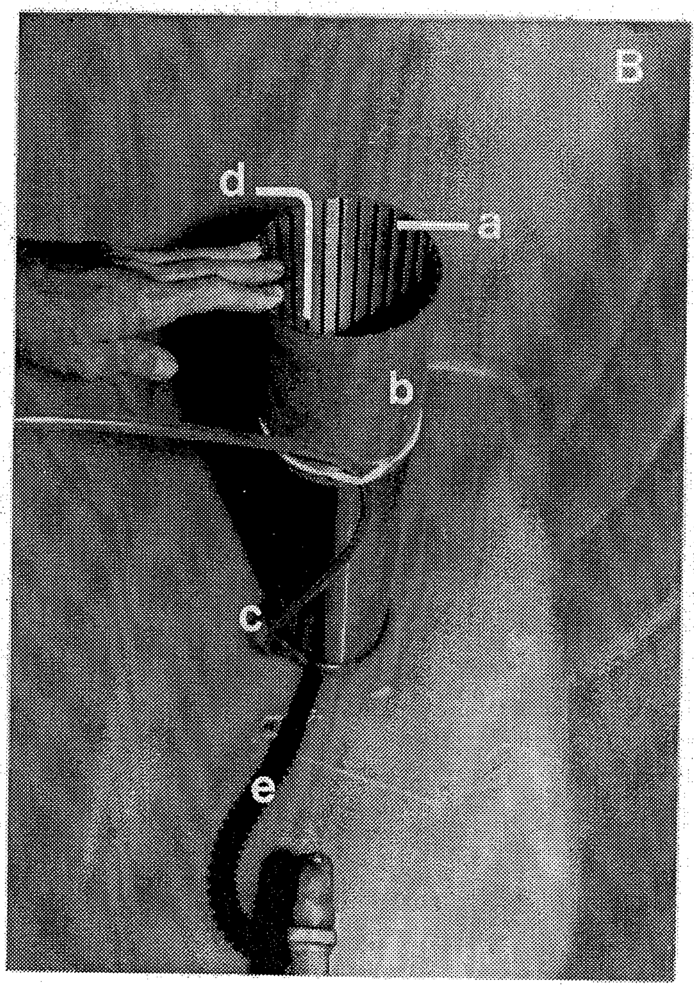
Fig. 3A - Schematic view of a flow-through recirculation system for culturing. Arrows denote water flow direction. (**1**) = cross-flow sieve ; (**2**) = séparateur à sédimentation.

- *Schéma du système "flow-through" en recirculation pour la culture des* Artemia. *Les flèches indiquent la direction du courant d'eau. (1) = séparateur à lames "cross-flow" ; (2) = séparateur à sédimentation.*

Fig. 3B - View of the new filter system for selective draining of water and waste particles, consisting of (**a**) a stainless steel welded wedge screen cylinder with (**b**) upper PVC ring, (**c**) aeration collar, (**d**) inner PVC cylinder, and (**e**) drain tube (after Lavens *et al.*, 1985).

- *Photo du nouveau système de filtre pour l'enlèvement sélectif de l'eau usée : cylindre en acier inoxydable avec fentes (**a**), pourvu d'un anneau de PVC (**b**), d'un anneau d'aération (**c**), d'un cylindre en PVC à l'intérieur (**d**) et d'un tube de drainage (**e**) (d'après Lavens* et al.*, 1985).*


A
CULTURE TANKS
CONSTANT HEAD TOWER
STOCK UNIT
(1)
(2)
MECHANICAL
TREATMENT
UNIT
BIOLOGICAL TREATMENT UNIT
SUSPENDED SOLIDS


B
d
a
b
c
e

water treatment system a rotating biological contactor in conjunction with a cross-flow sieve and plate separator, biomass yields comparable to those from open flow-through conditions can be achieved (Lavens *et al.*, 1985, 1986b).

Another key element in the successful high density flow-through culturing is an interchangeable and self-cleaning screen system that retains all brine shrimp in the culture tank but allows drainage of the effluent waters with the dissolved and particulate waste matter. A new filter concept has been recently developed by Lavens *et al.* (1985) which may be of great importance in the optimization and upscaling of flow-through culturing (Fig. 3b). These stainless steel welded wedge screen cylinders, as compared to the conventional mesh-screen filters (Tobias *et al.*, 1980 ; Brisset *et al.*, 1982), are much more efficient in particle removal and in the reduction of clogging rates. This also implies that higher flow rates can be maintained and, furthermore, that the frequency of manual cleaning of the filters can be reduced, i.e. once a day for the smallest screen filters, but only every second or third day for the filters with slits larger than 250 microns.

Considering the beneficial role of the integrated use of various *Artemia* products (e.g. with sizes ranging from 0.5 mm up to 10 mm) in shrimp and fish farming, the latter culture technique may offer potentialities for world-wide application in the field of aquaculture. When applied as an integral part of hatchery, nursery and maturation operations, its cost effectiveness might greatly improve, not only as a result of the reduced labour costs but especially in view of the significant increase in fish and crustacean outputs (Lavens *et al.*, 1985 - Fig. 4).

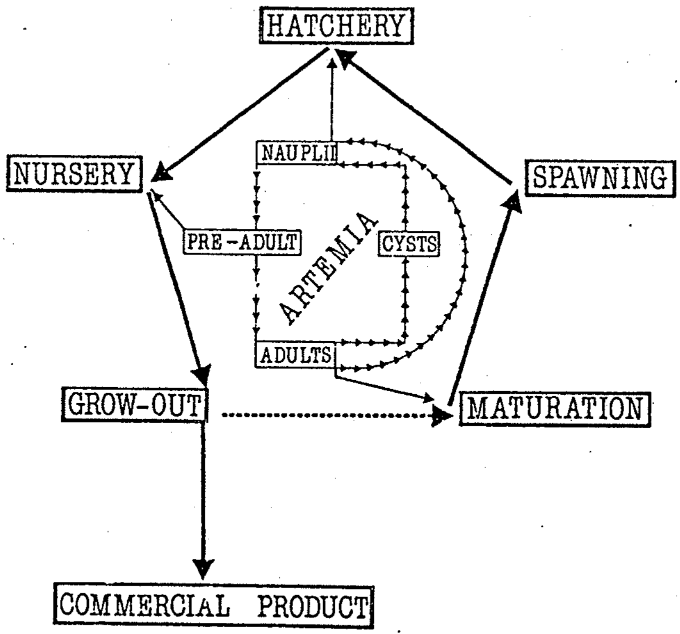


Fig. 4 - Outline of the integrated use of various *Artemia* products in fish or shrimp production (after Lavens *et al.*, 1985).
- *Aperçu de l'utilisation intégrée de différents produits d'*Artemia *dans la production de crevettes ou de poissons (Lavens* et al., *1985).*

In addition, *Artemia* biomass produced in intensive culture systems might be preferred to wild harvests for other reasons ; e.g. wild brine shrimp can be carrier of infectious organisms (see review by Léger *et al.*, 1986a), while *Artemia* cultured on various agricultural byproducts have shown to be relatively clean in terms of microbial contamination (Dobbeni, 1983). Brine shrimp harvested from outdoor ponds may also not survive equally long when transferred from their high salinity ponds into the predator tanks with natural seawater salinities.

CONCLUSION AND PERSPECTIVES

The practical use of *Artemia* as food source for the larval rearing of fish and crustaceans can be significantly improved by applying the recently developed know-how. Savings in quantities of brine shrimp cysts needed for the raising of the aquaculture species as well as improved outputs of shrimp or fish production may be the result of e.g. the use of efficient hatching techniques, different lifestages of *Artemia*, decapsulated cysts, cold stored nauplii, ongrown juveniles and adults, etc., and not least the selection of appropriate strains.

The characterization study of the more than 300 natural strains of *Artemia* (Vanhaecke *et al.*, 1986) needs to be continued in order to allow selection of the best ones for use in aquaculture. Heritability studies and crossbreeding tests might be substantial in the production of strains with a combination of essential or 'supra-natural' characteristics for specific use in aquaculture productions.

It is to be expected that further research and development on extensive and intensive culturing of *Artemia* will lead to mass scale use of biomass in the maturation, hatchery, nursery and grow-out facilities. In view of the fast expanding aquaculture industry, we are convinced that biomass consumption will increase exponentially in the years to come, and that improved production systems will be able to meet future demands for *Artemia* products. In this regard upscaling of the promising laboratory technique for controlled nauplii production (Lavens & Sorgeloos, 1986) might create possibilities for a vertical integration of the brine shrimp culture system in the aquaculture operations (see Fig. 4). As a consequence, this may create a complete independence of the fish and shrimp culturing industry for this very valuable live food *Artemia*.

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REFERENCES BIBLIOGRAPHIQUES

Beck A.D. & Bengtson D.A., 1982. - International Study on *Artemia*. XXII. Nutrition in aquatic toxicology : Diet quality of geographical strains of the brine shrimp *Artemia*. *In : Aquatic Toxicology and Hazard Assessment*. ASTM Special Technical Publication 766 (J.G. Pearson, R.B. Foster & W.E. Bishop eds) : 161-169. - A.S.T.M., Philadelphia, Pennsylvania, U.S.A.

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

Bossuyt E. & Sorgeloos P., 1980. - Technological aspects of the batch culturing of *Artemia* in high densities. *In : The brine shrimp Artemia*. Vol. 3. *Ecology, Culturing, Use in Aquaculture* (G. Persoone, P. Sorgeloos, O. Roels & E. Jaspers eds) : 133-152. - Universa Press, Wetteren, Belgium.

Brisset P. , Versichele D., Bossuyt E., De Ruyck L. & Sorgeloos P., 1982. - High density flow-through culturing of brine shrimp *Artemia* on inert feeds. Preliminary results with a modified culture system. - *Aquacultural Engineering*, 1 (2) : 115-119.

Bruggeman E., Sorgeloos P. & Vanhaecke P., 1980. - Improvements in the decapsulation technique of *Artemia* cysts. *In : The brine shrimp Artemia*. Vol. 3. *Ecology, Culturing, Use in Aquaculture* (G. Persoone, P. Sorgeloos, O. Roels & E. Jaspers eds) : 261-269. - Universa Press, Wetteren, Belgium.

Camara M.R. & De Medeiros Rocha R., 1986. - *Artemia* culture in Brazil : an overview. *In : Proceedings 2nd International Artemia Symposium*, Antwerp, Belgium, in press.

Claus C., Benijts F., Vandeputte, G. & Gardner W., 1979. - The biochemical composition of the larvae of two strains of *Artemia salina* (L.) reared on two different algal foods. - *Journal experimental marine Biology and Ecology*, 36 : 171-183.

De Los Santos jr. C., Sorgeloos P., Lavina E. & Berardino A., 1980. - Successful inoculation of *Artemia* and production of cysts in man-made salterns in the Philippines. *In : The brine shrimp* Artemia. *Vol. 3. Ecology, Culturing, Use in Aquaculture* (G. Persoone, P. Sorgeloos, O. Roels & E. Jaspers eds.) : 159-163. -Wetteren (Belgium) : Universa Press.

De Vrieze L., 1984. - Evaluatie van de nutritionele geschiktheid van gedecapsuleerde *Artemia*-cysten als rechtstreekse voedselbron voor karperlarven *(Cyprinus carpio* L.). - Thesis, State University of Ghent, Belgium, 105 p.

Dobbeleir J., Adam N., Bossuyt E., Bruggeman E. & Sorgeloos P., 1985. - New aspects of the use of inert diets for high density culturing of brine shrimp. *In : The brine shrimp* Artemia. *Vol. 3. Ecology, Culturing, Use in Aquaculture* (Persoone G., Sorgeloos P., Roels O. & Jaspers E. eds.) : 165-174. - Wetteren (Belgium) : Universa Press.

Dobbeni A., 1983. - Resultaten bekomen op het PHITS Coovi in verband met het screening onderzoek op *Artemia salina*. - PHITS Coovi Report, Anderlecht (Belgium).

Flores Tom A., 1986. - Preliminary production results of *Artemia* to be used in local shrimp farming in La Paz (Mexico). - *In : Proceedings 2nd International Artemia Symposium,* Antwerp, Belgium, in press.

Gatesoupe J., 1982. - Nutritional and antibacterial treatments of live food organisms : the influence on survival, growth rate and weaning success of Turbot (*Scopthalmus m.*). - *Annals of Zootechnology,* 31 (4) : 353-368.

Gatesoupe F.J. & Luquet P., 1982. - Weaning of sole (*Solea s.*) before metamorphosis. - *Aquaculture,* 28 : 359-368.

Girin M. & Person-Le Ruyet J., 1977. - L'élevage larvaire des poissons marins : chaînes alimentaires et aliments composés. - *Bulletin Français de Pisciculture,* 264 : 88-101.

Goodwin H.L. & Hanson J.A., 1977. - Freshwater prawn farming (genus *Macrobrachium*) in the western hemisphere : A state-of-the-art review and assessment. *In. : Shrimp and prawn farming in the western hemisphere* (J.A. Hanson & H.L. Goodwin eds) : 193-291. - Dowden, Hutchingson & Ross Inc., Stroudsburg, U.S.A.

Guimares J.I. & De Haas M.A.F., 1986. - The use of freeze-dried brine shrimp as food source in a *Penaeus japonicus* shrimp hatchery. *In : Proceedings 2nd International Artemia Symposium,* Antwerp, Belgium, in press.

Hentschel E., 1968. - Die postembryonalen Entwicklungsstadien von *Artemia salina* Leach bei verschiedenen Temperaturen (Anostraca, Crustacea). - *Zooloogischen Anzeigungen* 180 : 372-384.

Jumalon N.A., Estenor D.G., Bombeo R.F. & Dadole A.M., 1983. - Studies on *Artemia* production in earthen ponds in the Philippines. Paper presented during the First International Biennial Conference on Warm-Water Aquaculture - Crustacea in BYTU - Hawaii Campus, February 9-11, 1983. SEAFDEC Contr. No. 1257, 18 pp.

Lai L. & Lavens P., 1986. - Workshop Report. *Artemia* as a business perspective. *In : Proceedings 2nd International Artemia Symposium,* Antwerp, Belgium, in press.

Lavens P., Baert P., De Meulemeester A., Van Ballaer E.& Sorgeloos P. 1985. - News developments in the high density flow-through culturing of brine shrimp *Artemia.* - *Journal of the World Mariculture Society,* in press.

Lavens P., De Meulemeester A. & Sorgeloos P., 1986a. - Evaluation of mono- and mixed diets as food for intensive *Artemia* culture. *In : Proceedings 2nd International Artemia Symposium,* Antwerp, Belgium, in press.

Lavens P., Léger Ph. & Sorgeloos P., 1986b. - Manipulation of the fatty acid profile in *Artemia* offspring in a controlled production unit. In preparation.

LavensP. & Sorgeloos P., 1986. - Design, operation, and potential of a culture system for the continuous production of *Artemia* nauplii. *In : Proceedings 2nd International Artemia Symposium,* Antwerp, Belgium, in press.

Léger Ph., Bengtson D.A., Simpson K.L. & Sorgeloos P., 1986a. - The use and nutritional value of *Artemia* as a food source. *In : Marine Biology and Oceanography : An Annual Review* (M. Barnes ed.). In press.

Léger Ph., Bengtson D.A., Simpson K.L. Sorgeloos P. & Beck A.D., 1986b. Review on the nutritional value of *Artemia*. - *In : Proceedings 2nd International Artemia Symposium*, Antwerp, Belgium, 24 : 521-623.

Léger Ph., Bieber G.F. & Sorgeloos P., 1985a. - International Study on *Artemia*. XXXIII. Promising results in larval rearing of *Penaeus stylirostris* using a prepared diet as algal substitute and for *Artemia* enrichment. - *Journal of the World Aquaculture Society*, in press.

Léger Ph., Candreva P., Naessens-Foucquaert E. & Sorgeloos P., 1986c. - International Study on *Artemia*. XXXV. Techniques to manipulate the fatty-acid profile in *Artemia* nauplii and the effect on its nutritional effectiveness for the marine crustacean *Mysidopsis bahia* (M.). *In : Proceedings 2nd International Artemia Symposium*, Antwerp, Belgium, in press.

Léger Ph. & Sorgeloos P., 1982. - Automation in stock-culture maintenance and juvenile separation of the mysid *Mysidopsis bahia* (Molenock). - *Aquacultural Engineering*, 1 : 45-53.

Léger Ph., Vanhaecke P. & Sorgeloos P., 1983. - International study on *Artemia*. XXIV. Cold storage of live *Artemia* nauplii from various geographical sources : Potentials and limits in aquaculture. - *Aquacultural Engineering :* 2(1) : 69-78.

Léger Ph., Sorgeloos P., Millamena O.M. & Simpson K.L., 1985b. - International Study on Artemia, XXV. Factors determining the nutritional effectiveness of *Artemia*; the relative impact of chlorinated hydrocarbons and essential fatty acids in San Francisco and San Pablo Bays *Artemia*.- *Journal of Experimental Marine Biology and Ecology*, 93 : 71-82.

Métailler R., Méry C., Depois M. & Nédelec J., 1977. - Influences de divers aliments composés sur la croissance et la survie d'alevins de bars *(Dicentrarchus l.)* 3rd meeting of the ICES. Working group on Mariculture Brest, France, May 10-13, 1977. *Actes de colloques du CNEXO*, 4 : 93-109.

Millamena O.M., Bombeo R.F., Jumalon N.A. & Simpson K.L., 1985. - The effects of various diets on the nutritional value of *Artemia* as feed for *Penaeus monodon* larvae. *In : Book of Abstracts*. World Mariculture Society, 16th Annual Meeting and Florida Aquaculture Association, Orlando, Florida, Jan. 13-17, 1985 : p 21.

Miller D.C., Lang W.H., Marcy M., Clem P. & Pechenik J., 1979. - International study on *Artemia*. Naupliar locomotory rates, patterns and photoresponses : A comparative study of various strains of *Artemia salina*. *In : Book of Abstracts*. International Symposium on the brine shrimp *Artemia salina*, Corpus Christi (Texas), Aug. 20-23, 1979 : 91. - Artemia Reference Center, Ghent, Belgium.

Mock C.R., Revera D.B. & Fontaine C.T., 1980. - The larval culture of *Penaeus stylirostris* using modifications of the Galveston Laboratory technique. - *Proceedings World Mariculture Society*, 11 : 102-117.

Morris R.W., 1956. - Some aspects of the problem of rearing marine fishes. - *Bulletin de l'Institut Oceanographique*, 1082 : 61.

Olney C.E., Schauer P.S., McLean S., You Lu & 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 : The brine shrimp Artemia.* Vol. 3. *Ecology, Culturing, Use in Aquaculture* (G. Persoone, P. Sorgeloos, O. Roels & E. Jaspers eds) : 343-352. - Universa Press, Wetteren, Belgium.

Paffenhofer G.A., 1967. - Caloric content of larvae of the brine shrimp *Artemia salina*. - *Helgolander Wissenschaftlichen Meeresuntersuchungen*, 16 : 130-135.

Palmegiano G.B. & Trotta P., 1981. - *Artemia salina* as pabulum for growing penaeids under laboratory conditions. *In :* Contributed papers (Poster Session), World Conference on Aquaculture (Venice-Italy, September 21-25, 1981), poster No. 121.

Persoone G. & Sorgeloos P., 1982. - Perspectives in mariculture technologies.- *Philosophical Transactions of the Royal Society, London*, A307 : 363-375.

Robin J.H., 1982. - Comparaison de deux méthodes d'amélioration de la valeur alimentaire d'*Artemia salina* pour nourrir des larves de bar (*Dicentrarchus. l.*). - *I.C.E.S. Mariculture Committee*, F13 : 11.

Robin J.H., 1985. - Production of *Artemia* using mixed diets - Importance for marine fish larvae culture. *In : Proceedings of the 2nd International Artemia Symposium*.- Antwerp (Belgium), in press.

Robin J.H., Gatesoupe F.J. & Ricardez R., 1981. - Production of brine shrimp (*Artemia salina*) using mixed diets : consequences on rearing of sea bass larvae (*Dicentrarchus labrax*). - *Journal of the World Mariculture Society*, 12(2) : 119-120.

Robin J.H., Gatesoupe F.J., Stephan G., Le Delliou H. & Salaun G., 1984. - Méthodes de production de filtreurs-proies et amélioration de leur qualité alimentaire. - Journées Aquariologiques de l'Institut Océanographique, *Océanis*, 10(5) : 497-504.

Sato N.L., 1967. - Enzymatic contribution to the excystment of *Artemia salina*. - *Scientific Reports of the Tohoku University*, 33(3-4) : 319-327.

Schauer P.S., Johns D.M., Olney C.E. & 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 : The brine shrimp Artemia.* Vol. 3. *Ecology, Culturing, Use in Aquaculture* (G. Persoone, P. Sorgeloos, O.A. Roels & E. Jaspers eds) : 365-373. - Universa Press, Wetteren, Belgium.

Seidel C.R., Johns D.M., Schauer P.S. & Olney C.E., 1982. - International study on *Artemia*. XXVI. Food value of nauplii from reference *Artemia* cysts and four geographic collections of *Artemia* for mud crab larvae. - *Marine Ecology Progress Series*, 8(3) : 309-312.

Sick L.V., 1975. - Selected studies of protein and amino acid requirements for *M. rosenberghii* larvae fed neutral density formula diets. - *Proceedings 1st. International Congress on Aquaculture Nutrition*, Oct. '75, Lewes, Delaware : 215-228.

Sleet R.B. & Brendel K., 1983. - A flow-through hatching and cold storage system for continuous collection of freshly hatched Artemia nauplii. - *Journal of Aquariculture and Aquatic Sciences*, 3(4) : 76-83.

Smith W.E., 1976. - Larval feeding and rapid maturation of bluegills in the laboratory. - *Progressive Fish Culturist,* 38(2) : 95-97.

Sorgeloos P., 1980. - The use of the brine shrimp *Artemia* in aquaculture. *In : The brine shrimp Artemia.* Vol. 3. *Ecology, Culturing, Use in Aquaculture* (G. Persoone, P. Sorgeloos, O. Roels & E. Jaspers eds) : 25-46. - Universa Press, Wetteren, Belgium.

Sorgeloos P., 1981a. - Availability of reference *Artemia* cysts. - *Aquaculture,* 23 : 381-382.

Sorgeloos P., 1981b. - Live animal food for larval rearing in aquaculture : The brine shrimp *Artemia. In : World Conference on Aquaculture. Book of Abstracts.* Venice, Italy.

Sorgeloos P., 1983. - Potentials of the mass production of brine shrimp *Artemia.* - *Journal of the Society for Underwater Technology :* 27-30.

Sorgeloos P., Lavens P., Léger Ph., Tackaert W. & Versichele D., 1986. - Manual for the culture and use of brine shrimp *Artemia* in aquaculture. - Manual prepared for the Belgian Administration for Development Cooperation and for the FAO. State University of Ghent, Belgium.319 p.

Tarnchalanukit W. & Wongrat L., 1986. - *Artemia* culture in Thailand. *In : Proceedings 2nd International Artemia Symposium,* Antwerp, Belgium, in press.

Tobias W.J., Sorgeloos P., Roels O.A. & Sharfstein B.A., 1980. - International study on *Artemia.* XIII. A comparison of production data of 17 geographical strains of *Artemia* in the St. Croix Artificial Upwelling-Mariculture system. *In : The brine shrimp Artemia.* Vol. 3. *Ecology, Culturing, Use in Aquaculture* (G. Persoone, P. Sorgeloos, O.A. Roels & E. Jaspers eds) : 383-392. - Universa Press, Wetteren, Belgium.

Trotta P., Villani P. & Palmegiano G.B., 1986. - Laboratory-grown *Artemia* as reference food for weaning of fish and shrimp postlarvae. *In : Proceedings 2nd International Artemia Symposium,* Antwerp, Belgium, in press.

Van Ballaer E., Amat F., Hontaria F., Léger Ph. & Sorgeloos P., 1985. - Preliminary results on the nutritional evaluation of ω3-HUFA enriched *Artemia* nauplii for larvae of the seabass *Dicentrarchus labrax.* - *Aquaculture,* 49 : 223-229.

Vanhaecke P., 1983. - Vergelijkende studie van diverse geografische rassen van het pekelkreeftje, *Artemia,* ter verbetering van zijn gebruik in de aquakultuur. - Ph. D. Thesis. State University of Ghent, Belgium, 420 pp.

Vanhaecke P., Cooreman A. & Lavens P., 1980. - Vergelijkende studie van de geografische rassen van het pekelkreeftje, *Artemia.* - Jaarverslag F.K.F.O. 2.0010.78, 42 pp.

Vanhaecke P., Cooreman A. & Sorgeloos P., 1981. - International study on *Artemia.* XV. Effect of light intensity on hatching rate of *Artemia* cysts from different geographic origin. - *Marine Ecology Progress Series,* 5(1) : 111-114.

Vanhaecke P., Lavens P. & Sorgeloos P., 1983. - International study on *Artemia.* XVII. Energy consumption in cysts and early larval stages of various geographical origin. - *Annales de la Société zoologique de Belgique,* 113(2) : 155-164.

Vanhaecke P. & Sorgeloos P., 1980. - International study on *Artemia*. IV. The biometrics of *Artemia* strains from different geographical origin. *In : The brine shrimp Artemia*. Vol. 3. *Ecology, Culturing, Use in Aquaculture* (G. Persoone, P. Sorgeloos, O. Roels & E. Jaspers eds) : 393-405. - Universa Press, Wetteren, Belgium.

Vanhaecke P. & Sorgeloos P., 1983. - International study on *Artemia*. XIX. Hatching data on 10 commercial sources of brine shrimp cysts and re-evaluation of the "hatching efficiency" concept. - *Aquaculture*, 30(1/4) : 43-52.

Vanhaecke P., Tackaert W. & Sorgeloos P., 1986. - The biogeography of *Artemia* : an updated review. *In : Proceedings Second Artemia Symposium* , Antwerp, Belgium, in press.

Vos J., Léger Ph., Vanhaecke P. & Sorgeloos P., 1984. - Quality evaluation of brine shrimp *Artemia* cysts produced in Asian salt ponds. - *Hydrobiologia*, 108(1) : 17-23.

Watanabe T., Aradawa T., Kitajima C., Fukusho K. & Fujita S., 1978a. - Nutritional quality of living feed from the viewpoint of essential fatty acids for fish. - *Bulletin of the Japanese Society for Scientific Fisheries*, 44(11) : 1223-1227.

Watanabe T., Ohta M., Kitajima C. & Fujita S., 1982. - Improvements of dietary value of brine shrimp *Artemia salina* for fish larvae by feeding them ω3 highly unsaturated fatty acids. - *Bulletin of the Japanese Society for Scientific Fisheries*, 48(12) : 1775-1782.

Watanabe T., Oowa F., Kitajima C. & Fujita S., 1978. - Nutritional quality of brine shrimp, *Artemia salina*, as a living feed from the viewpoint of essential fatty acids for fish. - *Bulletin of the Japanese Society for Scientific Fisheries*, 4(10) : 1115-1121.

Watanabe T., Oowa F., Kitajima C. & Fujita S., 1980. - Nutritional studies in the seed production of fish. IX. Relationship between dietary value of brine shrimp *Artemia salina* and their content of ω3 highly unsaturated fatty acids. - *Bulletin of the Japanese Society for Scientific Fisheries*, 46 : 34-41.

Watanabe T., Tamiya T., Oka A., Hirata M., KitajimaC. & Fujita S., 1983. - Improvements of dietary value of live foods for fish larvae by feeding them on ω3 highly unsaturated fatty acids and fatsoluble vitamins. - *Bulletin of the Japanese Society for Scientific Fisheries*, 49(3) : 471-479.

Wheeler R., Yudin A.I. & Clark W.H.jr., 1979. - Hatching events in the cysts of *Artemia salina*. - *Aquaculture*, 18 : 59-67.