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Enrichment of Live Food for Use in Larviculture of Fish and Crustacean Species

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Abstract:

In the late 1960s and early 1970s, several authors reported problems in larviculture success with marine fish and crustacean species when using *Artemia* sources other than San Francisco Bay (California, USA; SFB) . High doses of toxic compounds, e.g., chlorinated hydrocarbons and heavy metals, were initially suspected to be the cause of the poor nutritional value of *Artemia* from Great Salt Lake (Utah, USA; GSL) and the People's Republic of China (review in Leger et al., 1986).
A comparative study with eight strains of *Artemia* spp. using the flounder *Pseudopleuronectes* americanus as predator test species confirmed the nutritional variation among *Artemia* sources (Klein-MacPhee et al., 1982). Soon thereafter scientists at Ghent University in Belgium documented the nutritional variability in 11 batches of SFB *Artemia* nauplii for the mysid shrimp *Mysidopsis bahia* .

Similar to earlier findings of Watanabe et al. (1978) and Kanazawa et al. (1979), Leger et al. (1985) could conclude that the main factor affecting the nutritional value of *Artemia* was the content of the highly unsaturated fatty acid HUFA eicosapentaenoic acid, 20:5n-3 EPA.

Taking advantage of the primitive feeding characteristics of *Artemia* nauplii, it is possible to manipulate the nutritional value of HUFA-deficient *Artemia, e.g.,* the GSL strain. Since brine shrimp nauplii that have molted into the second instar stage i.e., about 8 h following hatching are non-selective particle feeders, simple methods have been developed to incorporate different kinds of products into the *Artemia* prior to feeding to predator larvae. This method of "bioencapsulation", also called Artemia enrichment or boosting, is widely applied in marine fish and crustacean hatcheries for enhancing the nutritional value of *Artemia* with essential fatty acids and/or other components.

British, Japanese, and Belgian researchers developed enrichment products and procedures using selected microalgae, micro-encapsulated products, yeast, emulsified preparations, self-emulsifying concentrates, and micro-particulate products, either singly or in various combinations.

In *Artemia,* the most commonly applied boosting technique is a 24-h enrichment period after hatching. However, the variability of enrichment studied in GSL *Artemia* by the ICES Working Group on Mass Rearing of Juvenile Fish, showed a high variability in fatty acid bioaccumulation under laboratory or commercial

conditions. To avoid the variation originating from differences in commercial preparations, standardized ICES emulsions with different HUFA and DHA/EPA ratios have been formulated and are available for research purposes. It should be emphasized, however, that the enrichment technique has limitations as *Artemia* are selectively catabolizing some of the nutrients such as DHA and phospholipids. Research on the kinetics of DHA catabolism in various *Artemia* strains has shown that DHA catabolism is strain-dependent and could partially be overcome by the use of strains of different geographical origin.

Since rotifers are not selective for the uptake or catabolism of highly unsaturated fatty acids, high HUFA levels can be accumulated through the same bioencapsulation process as in Artemia.

Nowadays, various enrichment emulsions have been formulated differing in the fatty acid composition of their triglycerides. In this respect, the traditional formulations rich in EPA have been replaced by new products rich in DHA and arachidonic acid. To reduce the risks for oxidation of these fatty acids, higher concentrations of vitamin E are incorporated into the emulsions. Also, vitamin C has been incorporated in booster formulations that increase the level of ascorbic acid in *Artemia* or *Brachionus up* to 2000 ppm.

All these changes in the formulation of the enrichment diets offer more possibilities to cover the larval needs of different aquaculture species

and to reduce problems related to diseases, stress resistance, malformation, and pigmentation.

Kanazawa, A., Teshima, S.I., Ono, K., 1979. Relationship between fatty acid requirements of aquatic animals and the capacity for bioconversion of linolenic acid to highly unsaturated fatty acids. Comp. Biochem. Physiol. 63B, 295-298.

Klein-MacPhee, G., Howell, W., Beck, A.D., 1982. Comparison of a reference strain and four geographical strains of *Artemia* as food for winter flounder *Pseudopleuronectes americanus* larvae. Aquaculture 29, 279-288.

Leger, P., Sorgeloos, P., Millamena, O.M., Simpson, K.L., 1985. 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 Bay and San Pablo Bay *Artemia. J.* Exp. Mar. Biol. Ecol. 93, 71-82.

Leger, P., Bengtson, D.A., Simpson, K.L., Sorgeloos, P., 1986. The use and nutritional value of *Artemia* as a food source. Oceanogr. Mar. Biol. 24, 521-623.

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. Bull. Jpn. Soc. Sci. Fish. 44,1115-1121.

How to improve the health and quality of finfish larvae?

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While stock enhancement and aquaculture have their origins in antiquity, development of modern techniques began in the late 1800s and the beginning of the 1900s. In 1887, the first rearing trial of red sea bream Pagrus major was carried out in Okayama Prefecture, and Kajiyama and Nishioka attained successful larviculture in 1930. In 1961, the Japan Fisheries Agency (JFA) established a plan to promote coastal fisheries by stock enhancement. This was the first step of modern research and development of hatchery technology for intensive larviculture Japan. The Seto Inland Sea Fish Farming Association (SISFA) was established in 1963 and merged with Japan Sea Farming Association (JASFA) in 1978, with the goal of developing the needed technology for stock enhancement. Since the establishment of SISFA, the goal of development of marine finfish and shellfish hatchery technology in Japan is developing intensive mass production system.
In the late 1970s, the technology of artificial seed production of red sea bream *Pagrus major* had been

established, and intensive mass production system of red sea bream developed until the middle of 1980s. The intensive mass production technology of red sea bream supported the development of hatchery technology for other finfish species.

The first runner of development of hatchery technology of Crustacea was Kuruma prawn *Penaeus japonicus*, and second one was swimming crab *Portunus trituberculatus*.

The hatchery-raised red sea bream have dominated the finfish aquaculture trade. Hatchery-raised red sea bream juveniles for stock enhancement and aquaculture numbered 63 million individuals in 1988. A successfully average of 96 million red sea bream juveniles was successfully raised in hatcheries, both of belonging to official and private bodies, each year from 1993 to 1998. Of these 30 million individuals were used for stock enhancement and 67 million for aquaculture.

Hatchery-raised juveniles are used not only for stock enhancement but also aquaculture. The number of hatchery-raised seed for stock enhancement and aquaculture were 726 million and 418 million, respectively.

The aquaculture of many species relies upon a supply of hatchery-raised finfish juveniles. The capture of natural seedlings and young red sea bream for aquaculture has drastically decreased due to the progress of artificial seed production. Hatchery-raised finfish seedlings, ocellate puffer, striped jack, and Japanese flounder, supply the demands of aquaculture.

The development of hatchery-technology was assisted by progress made in early developmental biology, physiological studies of fish larvae, nutrition requirement, and rotifer production techniques. Remarkable progress has been made in developing labor-saving mechanizations such as the automation of feeding and bottom sweeping equipment, seedling transfer and counting pump systems, and apparatus for continuous culturing of rotifers and water quality monitoring.

The development of enriched live food and micro-formulated diets has resulted in improved survival rates and fish condition for hatchery-raised juveniles. Nutritional studies on finfish and shellfish larvae have contributed to recent progress in improving health and quality of hatchery-raised juveniles.

To a greater or less, there are deformities in hatchery-raised juveniles. It is the key factor for survival in the field after release and for stocking effectiveness. It is also key issue of severe mortality and low market price in aquaculture industry. Fish quality, or ecological robustness, is defined as the ability to adapt to natural conditions at release site. The objective of the production of the hatchery-raised seed is to supply high quality seeds for stock enhancement and aquaculture. The key concept is improving fish quality, i.e., increasing the aptitude of fish for release, so that fewer fish needed to be produced in the hatchery, and thus, decreasing the cost for stock enhancement and aquaculture.

One of the main causes of deformity should be nutritional condition, such as Vitamin A, D, and DHA

concentration. We are now conducting the research and development on determination of suitable nutritional condition of live food, such as rotifer and Artemia, for finfish larviculture. In first, we decided suitable stocking density of newly hatched embryo. Second, we decided and characterized developmental stages of larvae under laboratory condition using the combination of allometric growth and morphogenesis. Finally, we conducted experimental larval rearing of using live food with different Vitamin A and HUFA concentration. Safety level of Vitamin A concentration for Japanese flounder should be higher than reported level. Nutritional condition of rotifer should be more important than that of *Artemia* for improvement of health and quality of hatchery-raised finfish juvenile.

Enriching Artemia with yeast strains: possible consequences for larviculture

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Using axenically-grown *Artemia*, a model system was developed to evaluate the effect of feed composition on the survival and development of this crustacean. Two strains of baker's yeast (*Saccharomyces cerevisiae*) were used in all experiments as feed for *Artemia*: a wild type strain and its mnn9 mutant, defective in the synthesis of mannoproteins in the outer cell wall. The genetic background, yeast growth phase and growth medium appeared to be important parameters determining the quality of yeast cells as feed for *Artemia*. A very strong positive correlation between *Artemia* performance and yeast cell wall chitin and glucan content was obtained, while mannoprotein content was negatively correlated. Mnn9 yeast cells grown till exponential phase in minimal medium proved to be excellent feed for *Artemia*, yielding an average 95% survival and 4mm growth after 6 days at 28°C, which is comparable to best results obtained with algal feed. Building on these results, the nutritional value of isogenic yeast strains and two algal species for gnotobiotically grown *Artemia* was examined. Yeast cell wall mutants were always better feed for

Artemia than their respective wild type. Yeast cells harbouring null mutants for enzymes involved early in the biochemical pathway for cell wall mannoproteins synthesis performed best as feed for *Artemia*. Yeast cells defective in chitin or β-glucan production were scored in second order, and the lowest performance, although still performing good growth, was with the mnn6 isogenic yeast mutant, harbouring a null mutation for mannoprotein phosphorylation. These results suggest that any mutation affecting the yeast cell wall scaffolding, by reducing the amount of covalent links between the major components of yeast cell wall, namely mannoproteins, β-glucans and chitin, is sufficient to improve the digestibility for *Artemia*. The results with algae indicated that within a species, strains can have different nutritional value under gnotobiotic conditions. The growth phase was another parameter influencing feed quality, although here it was not possible to reveal the exact cause.

The mode of action of micro-organisms (separating the nutritional effects from the probiotic/pathogenic/neutral effects of a bacterial strain) in *Artemia* cultures was examined with this gnotobiotic model system. Ten different bacterial strains were added dead or alive to a model system where *Artemia* is cultured under axenic conditions. In addition, four different axenic live feeds (two different strains of *Sacharomyces cerevisiae* and two different strains of *Dunaliella tertiolecta*) were added to *Artemia*, according to their nutritional value. It was

found that bacteria, only in combination with certain live feeds, had a very strong effect on *Artemia* survival and a rather weak effect on growth when added in small quantities (10^6 per ml). Also, direct effects of a bacterial strain are independent from the nutritional value of the same bacterium. Moreover, the quality of the feed used in *Artemia* cultures has a strong influence in both direct (probiotic/neutral/pathogenic) and indirect (nutritional) effects of a bacterial strain. These effects can be smaller or even disappear when medium/good quality feeds are used probably due to improvements in *Artemia* status (health condition) caused by better nutritional quality of the feeds or as a result of nonspecific stimulation of the brine shrimp immune response against the bacteria.

The present approach can be an excellent tool to study the exact mode of action of probiotic and pathogenic bacteria and more in general food composition, especially if it can be combined with challenge tests and other research areas. Enriching live food with tailor-made food particles could constitute a technology for improved production of larvae.

Probiotics and Prebiotics in Aquaculture: Review

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Abstract

Probiotics are viable microrganisms that beneficially affect the host physiology by modulating mucosal and systemic immunity, as well as improve the nutritional and microbial balance in the intestinal tract. The probiotic concept was first introduced by the Nobel Prize Elie Metchnikoff at the beginning of the 20th century. He believed that bacteria in milk products consumed by Bulgarian peasants were responsible for their good health and longevity. Metchnikoff isolated the *Bulgarian bacillus* from soured milk. This organism is presently known as *Lactobacillus delbrueckii* subsp *bulgaricus*. Probiotics used in human and terrestrial animals (poultry, cattle...) belong mainly to lactic acid bacteria, particularly strains from *Bifidobacterium*, *Lactobacillus* and *Streptococcus*. Lactic

acid bacteria are in fact able to survive in the digestive tract because of their tolerance to acidity and bile salts, which is an important criterion in selecting potential probiotics. It is know well established that probiotics are important in human and animal nutrition.

The introduction of probiotics in aquaculture is comparatively new, but probiotics are currently recognized as important biological control agents against diseases. In contrast to human and animal nutrition, in aquaculture a wide range of microorganisms including microalgae (*Tetraselmis*), yeasts (*Debaryomyces, Phaffia, Saccharomyces*), Gram-positive bacteria (*Bacillus, Carnobacterium, Lactobacillus*) and Gram-negative bacteria (*Aeromonas, Vibrio, Pseudomona*) have been evaluated for use as probiotics.
Probiotics have been reported to be successful with crustaceans, bivalve molluscs as well as with fish at larval, juvenile and adult stages (for review see Gatesoupe, 1999; Verschuere et al., 2000; Irianto & Austin, 2002).

The exact modes of action of the probitoics are still not fully understood in these aquatic animals, but it is accepted that competitive exclusion is a main action of

probiotic bacteria. This includes production of inhibitory substances against potential pathogen, competition for nutrient and adhesion sites in the gut and stimulation of host immune system. Probiotics may also produce specific substances (vitamins, enzymes), which stimulate the growth of the host.
Many criteria have to be considered when selecting potential probiotics: phenotypic and genotypic stability, acid and bile tolerance, adhesion to intestinal epithelium, production of antimicrobial substances against known pathogens, carbohydrate utilisation pattern and viability during processing and storage.

Probiotics can be used in three different ways: (1) by introducing selected strains into the digestive system via live (rotifers, *Artemia*) and inert (dried pellets) feed; (2) by adding the beneficial probiotics to the rearing water (microbially matured water); or (3) by adding natural compounds, mainly indigestible carbohydrates to the inert diet which might selectively stimulate the growth of beneficial gut microflora. This is the so-called prebiotic concept.
The two first methods are facing many problems such as species specificity, resistance to acids and bile salts in the digestive tract, adherence to intestinal

epithelium and survival in the gut. It is indeed not very probable that exogenous addition of a selected probiotic will result in long-term dominant gut colonisation, especially when the strains used do not belong to the normal dominant intestinal flora. On the other hand the stimulation of indigenous microflora by supplementing fish feed with indigestible carbohydrates that act, as a prebiotic could be an interesting approach to increase the proportion of health-promoting bacteria in the gut.

Prebiotics are a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health (Gibson & Roberfroid, 1995). To be classified as a prebiotic, a food ingredient should (1) resist gastric acidity, hydrolysis by digestive enzymes and gastrointestinal absorption; (2) be fermented by the intestinal microflora and (3) be able to selectively stimulate the growth and activity of intestinal bacteria associated with health and wellbeing (Gibson et al., 2004)

Among the food ingredients, carbohydrates are the most widely evaluated compounds

for use, as prebiotics, in human and animal nutrition. They include resistant inulin and oligofructose, transgalacto-oligosaccharides (TOS), lactulose, isomalto-oligosaccharides (IMO), lactosucrose, xylo-oligosaccharides (XOS), soyabean oligosaccharides and gluco-oligosaccharides. From *in vivo* and *in vitro* studies, inulin and oligofructose, TOS and lactulose are presently classified as prebiotics. IMO, lactosucrose, XOS, soyabean oligosaccharides and gluco-oligosaccharides are not considered as functional ingredients since they do not fulfil all criteria for classification as prebiotics (Gibson et al., 2004).

To date, mainly inulin and oligofructose are used as prebiotics in human and animal nutrition. They are fructans with a degree of polymerisation of 2 to 60 and 2 to 20, respectively. Inulin is extracted from chicory roots and oligofructose is obtained by enzymatic hydrolysis of inulin. Inulin and oligofructose are also present in a number of common foods such as, garlic, onion, artichoke, asparagus (Van Loo et al. 1995).

Inulin and oligofructose are selectively fermented by *Bifidobacteria*, *Lactobacillus* and *Bacteroides* to produce short chain

fatty acids (acetate, butyrate, propionate) and lactate. It has been demonstrated that short chain fatty acids are absorbed through the intestinal epithelium, thus becoming an energy source for the host, whereas lactate enters the liver and is used as precursor for gluconeogenesis (Gibson et al., 1995).

Bifidobacteria and *Lactobacillus* also inhibit the growth of bacteria that are harmful to the host. They produce substances that stimulate the immune system, thus, enhancing the host's protection against infections.

Besides being prebiotics and bifidogenics, inulin and oligofructose have many nutritional properties. They improve lipid metabolism by decreasing cholesterol, triglycerides and phospholipides in serum (Van Loo et al., 1999).

Inulin and oligofructose significantly increase the intestinal absorption of mineral, mainly calcium, which results in increased bone-mineral density.

In aquaculture, few reports are available on the influence of prebiotics on growth and intestinal microflora in fish. Lactosucrose has been shown to increase the thickness of intestinal tunica muscularis of red sea bream, while this dietary supplement was used as substrate by the intestinal

microflora (Kihara et al., 1995). However, lactosucrose was poorly used by trout (Kihara & Sakata, 2001) and carp (Kahara & Sakata, 2001) microbiota. Olsen et al., 2001, have observed a damaging effect of inulin on enterocytes of Arctic charr, but the amount of the prebiotic in the diet was extremely high (15% of the diet).

Currently inulin and oligofructose are investigated for use as prebiotics in fish nutrition. The species studied are the turbot, *Psetta maxima*, the Siberian sturgeon, *Acipencer baerii*, and the African catfish, *Clarias gariepinus*. It has been demonstrated that oligofructose improves growth of turbot larvae at the weaning. 14% of the isolated intestinal flora was identified as *Bacillus subtilis* in turbot fed oligofructose (Mahious et al., in press). Same authors (unpublished data) have observeded a better growth of Siberian sturgeon (*Acipencer baerii*) and African catfish (*Clarias gariepinus*) fed diets supplemented with inulin and oligofructose (unpublished data). In an vitro study with Siberian sturgeon, it was established that inulin and oligofructose improve short chain fatty acids, lactate and gases production in the the spirale intestine (Mahious et al., unpublished)

Litterature

Gatesoupe, F.J. (1999). The use of probiotics in aquaculture : Review. Aquaculture, 180, 147-165.

Gibson, G.R. & Roberfroid, M.B. (1995) Dietary modulation of the colonic microbiota: introducing the concept of prebiotics. Journal of Nutrition 125, 1401-1412.

Gibson, G.R., Beatty, E.R., Wang, X., Cumming, J.H. (1995) Selective stimulation of bifidobacteria in the human colon by oligofructose and inulin. Gastroenterology, 108, 975-982.

Gibsoon, G.R., Probert, H.M., Van Loo, J., Rastall, R., Roberfroid, M.B. (2004) Dietary modulation of the human colonic microbiota: updating the concept of prebitoics. Nutrition Research Reviews, 17, 259-275.

Irianto, A. & Austin, B. (2002) Probiotics in Aquaculture: Review. Journal of Fish Diseases, 25, 633-642.

Kihara, M., Ohba, K. & Sakata T. (1995) Trophic effect of dietary lactosucrose on the intestinal tunica muscularis and

utilization of this sugar by gut microbes in red seabream, *Pagrus major*, a marine carnivorous teleost, under artificial rearing. Comp. Biochem. Physiol. 112A, 629-634.

Kihara, M. & Sakata, T. (2001) Influence of incubation temperature and various saccharides on the production of organic acids and gases by gut microbes of rainbow trout, *Onchorhynchus mykiss* in a micro-scale batch culture. Journal of Comprehensive Physiology B 171, 441-447.

Kihara, M. & Sakata, T. (2001) Effects of rearing temperature and dietary on the production of gases and organic acids by gut microbes of an omnivorous Teleost, carp, *Cyprinus carpio*, in micro-scale batch cultures. Suisanzoshoku 49 (3), 329-338.

Olsen, R.E., Myklebust, R., Kryvi, H., Mayhew, T.M., & Ringø, E. (2001) Damaging effect of dietary inulin on intestinal enterocytes in Arctic charr (*Salvelinus alpinus* L.). Short Communication in Aquaculture Research, 2001, 32, 931-934.

Van Loo, J. (1995) De stand van zaken in het nutritioneel onderzoek van inuline

(Raftiline) en oligofructose (Raftilose). Voeding, vol. 56 (4), 6-9.

Van Loo, J. Cummings, J.H., Delzenne, N., Franck, A., Hopkins, M.J., MacFarlane, G.T., Newton, D.F., Quigely, M.E., Roberfroid, M.R., Van Vliet, T. & Van den Heuvel, E.G.H.B. (1999) Functionnal food properties of non-digestible oligosaccharide: a consensus report from the ENDO project (DGXII AIRII-CT94-1095). Brit. J. Nutr. 81, 121-132.

Verschuere, L., Rombaut, G., Sorgeloos, P & Verstraete, W. (2000) Probiotic Bacteria as Biological Control Agents in Aquaculture. Microbiology and Molecular Biology Reviews, Dec. 2000, p. 655-671.

Evaluation of Free-Living Nematode *Panagrellus Redivivus as* a Live Food Organism for Silver Barb *Barbodes Gonionotus Larvae*

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Abstract

A series of experiments were conducted to develop mass production and improve nutritional quality of free-living nematode *Panagrellus redivivus*. The performance of nematodes *P. redivivus* produced on different culture media on growth and survival of silver barb *Barbodes gonionotus* larvae were also studied.

Prior to evaluation of *P. redivivus* as a larval live food organism, a study was conducted on the optimal stocking density of *B. gonionotus* larvae. Best larval growth and survival were obtained at 10 larvae L^{-1} for a 16-days rearing period. However, a range of 34-65 larvae L^{-1} was recommended for its commercial hatchery production.

A comparative study on performance of nematode, rotifer, *Moina* and *Artemia* was carried out. *B. gonionotus* larvae fed with *Artemia* and nematode exhibited significantly ($P<0.05$) highest growth followed by those fed with rotifer and *Moina* respectively. *P. redivivus* was found to be a suitable food for *B. gonionotus*. A following study indicated that 20 nematodes mL^{-1} was the optimal feeding density for silver barb *B. gonionotus* stocked at 10 larvae L^{-1} for a 16-days culture period.

The study also revealed that 8% starch is the optimal level for the maximum production of *P. redivivus*. Nematodes produced at 8% starch also gave the best growth and survival of silver barb larvae. A following study showed that the total production of free-living nematode *P. redivivus* was significantly affected by the source of starch in culture medium. Potato starch was the best starch for culture of *P. redivivus.* Starch sources, however, did not have any significant ($P>0.05$) effect on the biochemical composition and nutritional value of *P. redivivus* for silver barb *P. gonionotus* larvae. Another study was conducted to determine the effect of lipid enrichment on the production of *P. redivivus*.

The effects of different levels of lipid (0, 0.75, 1.5, 3, 4.5 and 6% cod liver oil) on the mass production and biochemical composition of free-living nematode *Panagrellus redivivus* were evaluated. Lipid enrichment of 3% level resulted in the highest total nematode production followed by 1.5, 4.5, 6, 0.75 and 0% level, respectively. The polynomial regression (second order)

analysis showed that the maximum number of nematodes could be produced at 3.43% lipid enrichment level. The extremely low production of *P. redivivus* in unenriched medium suggested that lipid has an important role in reproduction, metabolism and as the main energy source. The results of a feeding trial showed that nematodes grown in media enriched with >2.6% oil level were unsuitable for silver barb *B. gonionotus* larvae.

The effects of different lipid sources (cod liver, corn, linseed, pure bleached palm, pure bleached palm kernel and sunflower oils) used in the lipid enrichment of free-living nematode *Panagrellus redivivus* on its mass production and biochemical composition were studied. This study demonstrated that lipid source has an enormous effect on production and fatty acid composition of *P. redivivus*. The highest production was achieved when nematodes were grown on sunflower oil enriched medium, followed by those grown in corn, linseed, fish, bleached palm kernel and bleached palm oil enriched respectively. The multiple regression analysis revealed that palmetic acid (16:0), saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and iodine value (IV) of oil sources had significant effects ($P<0.001$) on the nematode production. Nematodes enriched with cod liver and linseed oil had the highest *n*-3 PUFAs while sunflower and corn oil enriched nematodes contained significantly higher *n*-6 PUFAs.

Fish fed on nematodes grown in palm kernel oil-enriched media showed relatively a lower SFAs content compared with others. The levels of *n*-3

PUFAs in the fish body were considerably higher than those of nematodes. The fish feeding trial, however, demonstrated that silver barb *B. gonionotus* larvae had a high capability to utilize a wide range of lipid source and dietary fatty acids without any negative effect on its growth and survival.

Keywords: Free living nematode, *Panagrellus redivivus*, Culture, Starch, Oil, Silver barb, *Barbodes gonionotus*

Enrichment of *Artemia urmiana* with Highly Unsaturated Fatty Acids (HUFA) Emulsions, Fish Oils, Vitamin C and Antibiotics: Applications in Larviculture

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Abstract :

Enrichment of live food and in particular enrichment of *Artemia* nauplii and rotifer has brought about a great change and advancement in larviculture and aquaculture industry during last two decades. Aquaculture industry is growing day by day; the production has increased by about 10 million tones just in five years period from 1996 to 2001, exceeding 37.5 million tones in 2001. Development of aquaculture industry is directly dependent to the production of good quality larvae. Enrichment of *Artemia* nauplii and rotifer with HUFAs became very popular since scientists understood about the crucial importance of these fatty acids in survival of fish and shellfish larvae. Later this procedure were used to transfer many other essential requirements of the fish larvae such as amino acids, vitamins, pigments, drugs, vaccines and in recent years probiotics and probiotics.

Artemia urmiana is deprived of DHA and contains very little amount of EPA (Table 1) therefore it should be enriched with these essential fatty acids before its nauplii are used as starter food for a number of

different species of fish and shell fish. A number of experiments were carried out at Artemia and Aquatic Animals Research Center to enrich *Artemia urmiana* nauplii with HUFAs, vitamin C and antibiotics and their use in larviculture of sturgeon fishes and rainbow trout. Enrichment with fish oils showed be as good as commercial fatty acid emulsion for improving EPA level and cold incubation proved to be very effective in maintaining small size of nauplii and fatty acid level high (Tables 4-6).

Table 1: Fatty Acid composition of newly hatched *Artemia urmiana* nauplii in compare to other species (mg/g DW)

	18:3n-3	20:4n-3	20:5n-3	22:6n-3
A.franciscana	37.4	1.7	8.5	Tr
A. sinica	29.3	2.3	4.6	Tr
A. persimilis	16.7	1	7.8	0.3
A. tibetiana	Tr	1.8	44.7	0.2
A. urmiana	38.9	0.8	1.2	Tr

Enrichment Diets for *Artemia*

- Unicellular Algae
- Ω yeast
- Microcapsules

- Lipid emulsions
  - Easy Selco
  - Easy Super Selco
  - DHA Super Selco
  - Fish Oils

Table 2: Effects of enrichment with different fatty acid sources on improving HUFA levels in *A. urmiana*

fatty Acids	unenriched	emulsion	cod liver oil	sturgeon Oil
C20:5n3	3.14	3.52	3.41	4.23
C22:6n3	0	4.7	0.62	1

Table 3: Biometry of non-enriched, enriched with fatty acid emulsions, and fed with *Dunalliela tertiolecta* 0-72 hours after enrichment at cold storage (size in mm)

hour after enrichment	Non-enriched nauplii	Enriched with FA emulsion	Enriched with *D. tertiolecta*

0	**0.517**	**0.715**	**0.827**
12	**0.534**	**0.743**	**0.848**
24	**0.539**	**0.745**	**0.840**
36	**0.530**	**0.773**	**0.843**
48	**0.527**	**0.763**	**0.834**
60	**0.522**	**0.762**	**0.817**
72	**0.521**	**0.728**	**0.813**

Table 4: Change in EPA level in enriched and non-enriched nauplii preserved in cold, 0-72 hours after enrichment

hour after enrichment	*Non-enriched nauplii*	Enriched with FA emulsion	Enriched with D. tertiolecta
0	*1.47*	*14*	*3.3*
12	*1.35*	*10.2*	*2.9*
24	*1.38*	*13.1*	*3.2*
36	*1.4*	*12.4*	*4.1*
48	*1.2*	*14*	*3.5*
60	*1.1*	*12.3*	*3.6*
72	*1.15*	*11.3*	*3.4*

Table 5: Change in DHA level in enriched and non-enriched nauplii preserved in cold, 0-72 hours after enrichment

hour after enrichment	Non-enriched nauplii	Enriched with FA emulsion	Enriched with *D. tertiolecta*
0	*0*	*17.5*	*0*
12	*0*	*14.4*	*0*
24	*0*	*12.1*	*0*
36	*0*	*10.1*	*0*
48	*0*	*9.3*	*0*
60	*0*	*3.11*	*0*
72	*0*	*0.9*	*0*

Table 6: Change in 18:3n3 level in enriched and non-enriched nauplii preserved in cold, 0-72 hours after enrichment

hour after enrichment	Non-enriched nauplii	Enriched with FA emulsion	Enriched with *D. tertiolecta*
0	25.2	27	27.5
12	28.5	25	25.2
24	24.3	28.1	24.2
36	23.6	12.3	13.5
48	21.5	11.5	12.1
60	18.7	10.2	11.5
72	16.7	6.1	10.7

Freshwater fish larvae culture: experimental set-up to investigate quantitative feed requirements

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In spite of many efforts to develop artificial diets, the culture of fish larvae
during the primary nursery phase still depends heavily on natural food, especially zooplankton (Dabrowski 1984, Nellen 1986…) Reasons for the poor success with artificial diets may be physiological: simple alimentary canal of fish larvae and hence poor efficiency in digestion and assimilation (Govoni et al 1886; Verreth 1987…), and behavioural: very selective food preferences determined by behavioural mechanisms (Meyer 1987; Pascual and Yufera 1987; Verreth 1987…).

Research need to be done on the effect of temperature and feeding level on fish growth. In fish nutrition studies, growth and feeding level are mostly expressed as a percentage of the live body weight per day (%BW.d^{-1}). For short culture periods the specific growth rate remains rather constant and as a consequence feeding level (expressed as a % BW.d^{-1}) can be kept during those periods as well. The resulting

growth performances can be compared by the specific growth rates.

In larval fishes this procedure cannot be applied reasonably. Although the larval growth period is very short, in a few days the larval weight increases twenty to fifty fold, its dry matter changes considerably and the specific growth rate decreases continuously.
Hogendoorn (1980) reported a decreasing specific growth rate in larvae of *C.gariepinus*
From 85% to less than 20% of the bodyweight .day^{-1} during the first 28 days of feeding.
These observations imply that the daily feed rations should be adjusted accordingly and cannot be kept constant without changing its physiological value. In studying the effect of feeding level on growth and feed utilization in larval fishes, fixing the feeding levels as a % of BW.d^{-1} should be meaningless.

For larval rearing it is necessary to asses growth and feeding levels in another way. After a cube root transformation on the weight data, a linear relation (1) exists between the body weights and the length of the entire larval culture period (Hogendoorn 1980)

$Y_t^{1/3} = Y_0^{1/3} + g\,t$ equation (1)
where Y_t= weight at time t, Y_0= weight at start, g= regression coefficient , t= time or length of culture period in days.
The slope of this regression line remains constant for the entire larval culture period and is a useful measure of the larval growth rate. The feedinglevels are chosen

according to growth rates predicted by g= 0.1, 0.2, 0.3 ….

Daily feed ratios are calculated on the basis of the expected growth for every particular day of culturing. Because of the rapidly changing of dry matter content in fish larvae, all calculations are based on dry weight of the larvae and the food.

$$R_t = \Delta y_t \cdot \frac{DM_f}{DM_a} \cdot FCR \qquad \text{equation (2)}$$

With R_t = the food requirements/fish on day t (mg wet weight)
Δy_t = the average individual growth on day t, or = $y_{t+1} - y_t$ (mg wet weight)
DM_f = dry matter content of fish larvae
DM_a= dry matter content of *Artemia*
FCR estimated food conversion ratio on dry weight basis.

In order to adjust the food quantities for eventual deviations of the actual growth rate from the predicted one, samples of the larvae are taken regularly and their weights are used accordingly in equation (2)

Fish farms are mainly interested in data on growth performance and food conversion ratios. In order to evaluate the feed as a reference to other diets, the performance of the fish larvae should be investigated under different environmental and husbandry conditions. The effect of temperature and feeding level

on larval fish growth can be investigated by experimental set-ups.

Chromatographic methods for analysis of biological materials

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Chromatography was invented by the Russian botanist, Mikhail Tswett in 1906 [1]. He employed the technique to separate various plant pigments such as chlorophylls and xanthophylls, by passing solutions of them through glass columns packed with finely powder divided Calcium Carbonate. The separated species appeared as colored bands on the column, which accounts for the name he chose for the method (Greek chroma meaning color and graphein meaning to write).

Important advance in chromatography was made by Martin and Synge [2]:

a. First, in 1941 they reported the basic concepts of partition chromatography.

b. Second, development of gas-liquid chromatography in 1952.

In the early 1960s, Giddings introduced the theoretical aspects of gas chromatography and applied equally well to liquid chromatography and between 1967-1969, Kirkland, Huber and Horvath described the first high performance liquid chromatograph. High performance liquid chromatography (HPLC) is one of the fastest developing analytical techniques which is rapidly becoming and integral part of many research in chemistry and bioscience.

Most biological materials are complex matrices with thousands of constituents, many of which may not be

known. So accurate and reproducible assay of very small quantities of biological important analytes have a great importance in analytical chemistry (i.e. analysis of fatty acids, vitamins, drugs, minerals and … in aquaculture investigations).

Now day chromatography is an analytical method thus is widely used for the separation, identification and determination of the chemical and biological components in complex mixture. No other separation method is as powerful and generally applicable as is chromatography.

Finally it is important to note that selection of the most suitable chromatographic method for a particular sample not only depend on the properties and structure of the analyte but also the nature of the matrix of the sample, the reasons for the analysis and the availability of suitable equipment are also important [3].

References:

1. H. H. Strain and J. Sherma, "Micheal Tswetts contributions to sixty years of chromatography" J. *Chem. Educ*. **1967**, *44*, 235.
2. A. J. P. Martin, R. I. M. Synge, *Biochem. J.* **1941,** *35***,** 1358.
3. R. P. W. Scott, "Techniques and practices of chromatography" Marcel Dekker, **1995**.

Evaluation of *Artemia* energetic Vakue using Microcalorimetric method

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Introduction

Artemia is applied in aquaculture as a live start prey in all of its ontogenic stages, but the new hatched nauplia are the most preferable food for crustacean and fish larvae. It's connected with the small size and higher energy contents (Navarro et al., 1991). Thus it is very important to determine the energy content of *Artemia* in different stages of its life cycle. Energetic content of aquatic animals are determined by various analytical techniques, including biochemical and physiological analysis. Direct calorimetry is preferable technique for a non-destructive method of measuring the metabolism, independent of oxygen consumption (Baker & Mann, 1991). Microcalorimetry is used for study metabolic rate of the animals i*n vivo* and the changes of its dynamics during development, that is the great advantage as compared with the other biochemical and physiological methods. Besides that the technique could be used for the evaluation of other

living food (microalgae) heat production and to select the most preferable for *Artemia*.

The determination of heat production of the organisms by the microcalorimetry is based on the fundamental relationship between changes of the Gibbs energy (G), enthalpy (H) and entropy (S), which are depended according the following equation $\Delta H = \Delta G + \Delta S$, where $\Delta H + \Delta E = \Delta PV$. ΔE is the change of the total system energy, P - pressure and V - volume. In biological systems parameters T,V and P are the constant and $PV = 0$. Thus $\Delta H = \Delta E$ and $\Delta E = \Delta G + T\Delta S$. ΔG is the characteristic of the biological system and it reflects the catabolic/anabolic processes in the organism.

Thus the goal of the present study was to determine the heat production of some geographical *Artemia* strains in various developmental stages by microcalorimetry method and to evaluate their energetic level.

Materials and methods

The rate of heat production of *Artemia* samples of the commercial strain Great Salt Lake and the local strain from Kuaylnik Bay and Sasyk Syvash Lake (Black Sea, Ukraine) was studied. The metabolic rate was measured with a multichannel heat conduction type microcalorimeter (The Thermometric 2277 Thermal Activity Monitor, TAM, Sweden). The TAM was fitted with four channels both containing a

measuring and reference cell (twin calorimeter). The heat output signal was recorded by Digitam Data program on PC and later analyzed by Digitam Data Analysis program (Thermometric, Sweden).

Eggs (100-200) were transferred in a 3 -ml glass measuring ampoule containing 2 ml of sterile marine water (salinity 35‰). The reference ampoule contained 2 ml sterile marine water to avoid output cause by microorganisms. The baseline was measured both before and after the metabolic rate determinations. Measurements took place at the temperature + 20^0 C. Steady rates of heat dissipation were reached within 1 h after the eggs were placed in the calorimeter cells. Heat output was continuously monitored over 50 h. Four individual measurements could be done simultaneously. The durations were identical for all examined *Artemia* cysts. After the end of the experiment the number of hatching nauplia was detected and heat production was estimated as μWt per individual nauplia.

Furthermore hatching nauplia were transferred in the tanks for growing adult brine shrimps. Nauplia cultivated during two weeks in the medium containing mixing cultures of microalgae. Heat production of adults was measured. In this case the individual shrimp was transferred and sealed alive in ampoule containing 2 ml of air-saturated sterile marine water and the procedure was identical as in the previous determinations of nauplia metabolic rate.

Results

The results demonstrated the differences between heat production values of examined *Artemia* strains and their developmental stages (Tabl. 1).

Table 1. Heat production of two *Artemia* strains (μWt per individuals, M ± SD, n=5)

Developmental stages	Great Salt Lake	Kuaylnik Bay	Sasyk Syvash Lake
Developing embryo	0.084 ± 0.002	0.312 ± 0.080	0.074 ± 0.001
Hatching nauplia	0.170 ± 0.005	0.940 ± 0.095	0.246 ± 0.017
Adults	4.650 ± 0.420	5.300 ± 0.240	-

The obtained results show that during life cycle *Artemia* heat production changes. Metabolic rate of the hatching nauplia increased in 2-3 - fold as compared with the developing embryos in all cases. Heat production of the adults are higher in 17-53-fold than in nauplia. Additionally the metabolic rate was differed in examined *Artemia* strains.

Heat production of early developmental stages of the local Kuyalnik strain (Black Sea) was significant higher with the comparison of the commercial Great Salt Lake and local Sasyk Syvash Lake *Artemia.* This parameter of adults of two strains was similar.

Discussion

Metabolic rate of the living organism changes during its life cycle that connects with the levels of chemical substances and their involving in the metabolic pathways. According our data the highest level of heat production during *Artemia* early life was noted in hatching nauplia, then this parameter was decreased and stabilized in larvae during the next 20 h of the measuring period. It could be suggested that the decline of heat production in this case is connected with the expense of the main energetic substrates (lipids and carbohydrates) on the hatching process and further larvae living needs. This fact is agreed with the decrease of lipids (triacylglycerids) content in nauplia as compared with the developing embryos (Rudneva, 1991). Additionally at the same period the glycogen concentration declines also and due 24 h after hatching its level decreases in 65% (Boulton & Huggins, 1975). Examined strains of *Artemia* demonstrated the general tendency of the heat production dynamics during early development, but the significant differences in the values of this parameter was noted that it is connected with the different biochemical composition of

geographical strains (Vanhaecke et all., 1985). At the same time the metabolic rate of the adults was not differed in both strains. It could be proposed that in this case the heat production value depended on the food peculiarities but not on strains. It is very important to take into account for selecting the corresponding food and the methods of its enrichment. In this case the microcalorimetry technique could be used for the evaluation of the nutritional quality of the living food for *Artemia* (microalgae). Additionally, the consumption and quality levels of different food including enrichment *Artemia* can be analyzed by microcalorimetry. Thus the measuring of metabolic rate using microcaloimetric method can be applied for the evaluation of energetic status of *Artemia* and other living food used in aquaculture.

References

Baker S.M.,Mann R. 1991. Metabolic rates of metamorphosis oysters (*Crossostrea virginica*) determined by microcalorimetry. Am. Zool. 31: 134 A.

Boulton A.P., Huggins A.K. , 1978. Biochemical changes during morphogenesis of the brine shrimp *Artemia salina* and the effect of alterations in salinity. Comp. Biochem. Physiol. 61B: 593-595.

Navarro J.C., Amat F., Sargent J.R., 1991. A study of the variations in lipid levels, lipid class

composition and fatty acids composition in the first stages of *Artemia sp*. Marine Biology 111: 461-465.

Rudneva I.I., 1991. *Artemia*: perspectives of application in common wealth. Kiev, Naukova Dumka. 142.

Vanhaecke P., Tackaert W., Sorgeloos P. , 1985. The biogeography of *Artemia*: updated review. II Int. Symp. on the Brine shrimp *Artemia,* Antwerpen: 133.

Larviculture of Shrimp and brood stock maturation, with a hatchery exercise

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The recent history of crustacean farming is beset with failures as well as successes. A major research effort has gone into identification, prevention and treatment of crustacean diseases during the past 10 years.
Shrimp already stressed by high-density culture practices, environmental degradation or inadequate diet, succumbed to outbreaks of infectious diseases. Most infections were caused by viruses or bacteria.
Specific pathogen free (SPF) and specific pathogen resistant (SPR) broodstock of shrimp species are now available. Immunostimulants and probiotics are potentially useful tools in disease prevention and control. Transplantation of wild-caught or cultured species around the world continues to spread diseases despite international recommendations on control of movements.

Also considerable advances have been made in understanding nutritional requirements for broodstock, larvae and juveniles stages of shrimp with the formulation of specialised compounded diets. The value en limitations of live prey as vector for essential nutrients and medicines for larvae have also been established.

Many hatcheries opt for the induction of gonad development in captivity. The facility with which broodstock will mature, copulate and spawn in captivity varies greatly between different species. The technique of unilateral eyestalk ablation is usually applied.
Indoor circular tanks (3-5m diameter) are favoured for maturation since they permit greater control of temperature, light intensity and photoperiod, all of which influence ovarian development.
Females are placed in individual or mass spawning tanks. Penaeid eggs are released directly into the water. Eggs are transferred to incubators, and nauplii hatch 12-18hours later. The nauplii are separated from the empty shells, counted and dipped in dilute iodine-based disinfectant, before being stocked into larvae rearing tanks.

Different operations of Penaeid larvae culture can be broadly characterised depending on three factors: overall size of the operation, the size of the larval rearing tanks, and the use of western or oriental culture techniques.
Hatcheries range in size from large operations producing more than 100x 10^6 post-larvae
(PL) yr^{-1}, with a large often highly trained workforce (year round production with high investments costs), down to small 'backyard' family enterprise producing less than 10x10^6 post-larvae per year, staffed only by a handful of workers of family members (very flexible production schedules).

During the natural planktonic existence of penaeid larvae, live phytoplankton and zooplankton are the most important components of the diet. Correspondingly, in culture, the best results are obtained with live feeds such as microalgae and *Artemia* nauplii.

A variety of compounded microparticulate and microencapsulated diets, and liquid suspensions containing nutrient droplets and probiotic bacteria, have been developed to reduce dependence on live feeds. Although some are claimed to be suitable replacements, their role in commercial units has been mostly limited to partial replacement of live feeds, or as supplements.

To achieve a relatively stable community of bacteria (autotrophic and heterotrophic) and algae, no water is exchanged in the rearing tanks until the larvae have completed the mysis stage. The tanks are only filled to 60-70% of their capacity when stocked with nauplii and were then progressively topped up each day.

After metamorphosis food consumption, particulary of *Artemia*, increases substantially. In an attempt to reduce reliance on such expensive feeds, hatcheries try to promote the development of benthic algae and periphyton attached to artificial seagrass.

The stage at which post-larvae leave a hatchery for transfer to the nursery or ongrowing site varies considerably between different species and countries. In Taiwan, *Penaeus monodon* hatcheries usually produce PL_{10-15}, and in Ecuador *Litopenaeus vannamei*

Hatcheries typically rear post-arvae to $PL_{4-15.}$

For biosecurity it is advisable to design and build the different areas of a hatchery apart from eachother (maturation and spawning- eclosion and nauplii collection and desinfection–larvae rearing tanks- algae room- (rotifer room)- *Artemia* room- raceways with the PL- foodstorage)

Protocols for enrichments with HUFAs, Fish Oils, Vitamin C and Antibiotics

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Abstract

This paper explains the standard methods for enrichments methods for enrichment of *Artemia* nauplii with Highly Unsaturated Fatty Acids (HUFA), Local Fish Oils, Vitamin C and Antibiotics.

1. Standard Method for enrichment of *Artemia* nauplii with HUFA emulsions:

· Seawater disinfection

· add 1 mg. l^{-1} NaOCl (100 µl bleach solution 10 l^{-1} of 0.45 µm filtered seawater)
· incubate 1 h
· aerate strongly overnight
· add 0.5 g.l^{-1} $NaHCO_3$ (dissolved in deionized water and GF filtered)

· Cyst disinfection

· use cylindroconical cintainer
· 4 g cysts. l^{-1} tapwater
· 20 min at 200 mg.l^{-1} NaOCl (±2.0 ml bleach solution.l^{-1})
· harvest and rinse well, weigh out 2 × 50%

· Hatching

· 2 cylindroconical containers
· add 1/2 of the cysts per litre disinfected natural/artificial seawater
· incubate for 24 h, at 28°C, 2000 lux light, strong aeration
· separate nauplii from debris if needed in an aquarium in seawater
· make nauplii suspension of about 300 N/ml, count accurately (3 × 250 µl samples).

· Enrichment (triplicate)

· transfer volume containing 200,000 nauplii to a sieve
· rinse them well with filtered seawater
· stock in 1 l cone with point aeration at 200 nauplii. ml^{-1}
· count initial density (3 × 250 $N.ml^{-1}$)
. Prepare HUFA emulsion:
- Weight 2 g commercial emulsion in a clean Falcon tube
- Add 20 ml filtered sea water
- Mix thoroughly using a tube shaker

. Flush the emulsion with N_2 if you are not using it immediately and preserve it in refrigerator
. Add 2 × 0.2 g of emulsion (2 × 2 ml of diluted emulsion) over 24 h (t = 0 h and t = 10-12 h)
. Determine the size of droplets using a light microscope
. Flush the emulsion stock with N_2 each time after use

· incubate for 24 h at 28°C, strond aeration, monitor O_2 and pH regularly, especially after addition of 2nd dose of emulsion.

· Harvesting

· count survival, *i.e.* count dead nauplii (no lugol) and total nauplii (+lugol) from 3 × 250 µl sample per cone
· remove all aeration
· concentrate nauplii using light
· siphon nauplii on sieve
· rinse well with tapwater
. Transfer the nauplii to cold incubator

· Addition Vitamin C to fatty acid emulsion

· Add 20% w/w Ascorbyl palmitate to the emulsion
. Dilute the solution by 10 times in order to facilitate the mixing process
. Continue mixing using an electric mixer as long as palmitate is dissolved in the emulsion
. Determine the size of droplets using a light microscope

· Addition Antibiotic to fatty acid emulsion

. Find out the required dose of antibiotic as the dose required varies for different antibiotics and it may also vary for different species of fish or shrimp larvae
. Add the required amount of antibiotic (e.g 10% w/w) to the emulsion
. Mix thoroughly by a tube shaker
. Flush with N_2

. Preserve in refrigerator before use
. Feed the fish/shrimp larvae with medicated nauplii according to the dose requirement and half life of the antibiotic

2. Enrichment with Fish Oils:

Precedure:
. Weight 2.5 grams of fish oil (Cod liver oil or local fish oil, e.g. oil extracted from sturgeon ovary) in a clean 50 ml Falcon tube
. Add 25 ml sea water
. Add 0.25 g Lecithin
. Mix thoroughly using a tube shaker (at least for 15 minutes)
. Determine the size of droplets using a light microscope
. Flush with N_2 and preserve in refrigerator

- For addition of Vitamin C or antibiotics follow the procedure explained above

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