PROJECT COMPLETION REPORT

96-2 "Development of a Microparticulate Diet for Striped Bass Larvae"

Termination Report Period: June 1, 1996 - November 30, 1997

NRAC Total Funding: $73,500 (June 1, 1996 - November 30, 1997)

Principal Investigator: Allen Place, Center of Marine Biotechnology

Participating Investigator/ Cooperative Agencies:

Center of Marine Biotechnology	Maryland
AquaFuture	Massachusetts
Martek BioSciences, Ink.	Maryland

Reason For Termination:

The project objectives have been successfully completed and allocated funds have been spent.

Project Objectives:

This study investigated the development and application of a complex microencapsulated diet as full or partial replacement of conventional live food used in the culture of striped bass and hybrid striped bass larvae.

The specific objectives were to:

1. develop a complex microencapsulated diet by incorporating water soluble nutrient loaded liposomes into a protein matrix.
2. determine the acceptability and digestibility of complex-protein microspheres (CPM) by striped bass larvae and sea bream at first feeding and pre-metamorphic stages.
3. assess the potential use of CPM containing high amounts of PUFA as a supplement to low quality (PUFA deficient) *Artemia*/rotifers.
4. determine the effects of encapsulated dietary enzymes on the growth and development.

Anticipated Benefits:

One of the major costs in the intensive production of marine fish larvae is the enrichment of low quality strain *Artemia* with essential polyunsaturated fatty acids, especially eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids. We strongly believe that these essential lipids can be delivered to live food and larval fishes by microspheres. We have the capability to design microencapsulated diets with >25 % lipid content and an DHA/EPA ratio of whatever value, using microbally derived oils (Martek Inc., Columbia, MD). These oils (largely triglycerides) can contain DHA to nearly 50% of the total fatty acid content.

Progress and Principal Accomplishments:

In November of 1996, Dr. Ozkizilcik left the project to establish his aquaculture company, **SisteMar A. S**. in Alsanacak-Izmir, Turkey. To replace Dr. Ozkizilcik, we have hired Dr. Mordechai (Moti) Harel (C.V. enclosed). Unfortunately, Dr. Harel could not join the project until Jan., so we will be asking for a 6 month extension on the project. Before Dr. Ozkizilcik left the project, he completed construction of our larval rearing facility. Dr. Harel since his arrival has performed the enrichment experiments with Artemia and rotifers and the performed the feeding studies with hybrid striped bass larvae.

Fig. 1 Larval Rearing Facility. The tanks are 90 liters and can have the salinity and temperature controlled. *

Patents issued and applied for:

The original method for producing the microparticle designed by Dr. Ozkizilcik as a graduate student with Dr. Chu has been issued a US Patent ("Complex Protein-Walled Microcapsules Containing Lipid-Walled Microcapsules and Method for Producing Same). In addition, we have applied for a US Patent ("PUFA Enrichment of Aquaculture Feed Stocks") based on the findings from our NRAC-sponsored research.

Enrichment of Live Food Items:

Despite our major of objective of replacing live food with microdiets we thought it prudent to examine the ability of our liposome and sodium soap based material for enrichment procedures. Byproduct material resulting from winterization and alkali washing of DHAsco and ARAsco oils (Martek, Designer Oils) were evaluated as fatty acid enrichment materials of Artemia and other larval fish dietary items. Most strains of Artemia have no DHA, so typically fish oil emulsions are used as an enrichment. The goal was to attain DHA:EPA ratios of 2 or above, which is similar to that found in fish eggs and the polar lipids of copepodites, the major feed item of larval fish.

As evident in Figures 2-4, the sprayed dried material is extremely effective in enriching *Artemia* nauplii. In fact, this level of enrichment exceeds all the current commercial products available (AlgaMAC 2000, SuperSelco, High DHA, etc.).

Fig. 2 The effect of the enrichment level with DHA phospholipids on length increase of *Artemia* nauplii. *

Artemia cysts were decapsulated and hatched over night at 28°C, The newly hatched nauplii were kept at room temperature (10-15°C) until they reached Instar II stage (complete development of the digestion system), then they were stocked in 27 cones of 1 liter each at density of 140,000 nauplii/liter. Different rations of 0.1g , 0.2g and 0.3g of spray dried DHA phospholipids were homogenized with fresh water and given to the *Artemia* nauplii every eight hours. Water salinity was 30ppt, temperature were kept at 26-28°C during the 24 hours of the enrichment. The cones were strongly aerated to keep the oxygen level above 4 ppm. The system was illuminated continuously with a wide range spectrum fluorescent light. Each data point represents the mean values with their standard errors (n=3).

Fig. 3 Lipid content of the artemia nauplii at different enrichment levels and times. *

Fig. 4 The DHA/EPA ratio of artemia nauplii in relation to the enrichment levels with DHA phospholipids and time periods. *

Fig, 5 Comparison of enrichment materials for rotifers and *Artemia*. *

Impacts:

As a result of this study we have developed an improved live food enrichment product to be used in the production of high performance live larval feeds for cultured cold water and warm water fish. The product has been tested with halibut, sea bream, winter flounder, and striped bass larvae. The commercial availability of this product is expected to be in mid-1998.

The Effect of Enriched Live Feed on Larval Growth:

Poor performance and high mortality during early developmental stages in captive fish species are major obstacles in the development and intensification of aquaculture. Nutritional deficiencies are suggested as one of the main causes for these problems. It is well known that marine fish, including larval stages, must be provided with sufficient levels of highly unsaturated fatty acids (HUFA) to meet their requirements for optimal growth and development. Most nutritional studies on essential fatty acids (EFA) in fish have focused on the (n-3) series of fatty acids especially the long chain polyunsaturated fatty acids EPA (20:5) and DHA (22:6) while the (n-6) series have received little attention until recently. It has been shown that the ratio of (n-3) to (n-6) fatty acids can be extremely important because EPA (20:5n-3) can modulate the production of eicosanoids from 20:4n-6 (ARA) in all vertebrates, including marine fish.

Given the lack or low activity of D-5 desaturase activity in marine fish ARA must be present in the diet. An absolute requirement for ARA has been recently established for juvenile turbot, *Scophthalmus maximus* and Japanese flounder, *Paralichthys olivaceus*. Recent work in our laboratory have shown that hybrid striped bass larvae grow up to 50% better when fed with a combination of both DHA and ARA enriched *Artemia* compared to only DHA or ARA enriched *Artemia*. The combined requirements for these two fatty acids in absolute amounts or, more importantly in relative amounts, are not known for any *Morone* species.

The objective of the present research was to establish the combined nutritional requirements for both DHA and ARA, during striped bass early developmental stages. Experiments were focused on larval and post metamorphosis juveniles, which are the two extreme stages of development in fish when high mortalities occur.

A fully defined EFA requirement for a cultured fish larvae is essential for formulating nutritionally complete feeds. Once the dietary EFA requirements are determined, appropriate lipid sources can be used in feed formulations to meet the specific EFA

requirements of fish. During fingerling and grow-out phases, determination and meeting the EFA requirements of a fish species can be accomplished relatively easily by methods of feed formulation. However, the larval stages require extensive use of live feeds. The most widely used larval food rotifers and Artemia nauplii, however, do not meet the EFA requirements of most larval fish. The EFA content of live feed, therefore, must be altered to meet the EFA requirements of the larvae. For this purpose, several enrichment products and protocols have been developed to boost the n-3 HUFA content of live feeds.

Recent work in our laboratory has shown that hybrid striped bass (Striped bass X white bass [*M. chrysops*]) are unable to bioconvert sufficiently linolenic acid to n-3 HUFA for growth and survival, thus dietary supplements of n-3 HUFA were necessary through *Artemia* enrichment. Further growth results of enriched Artemia fed larvae showed that best larval performance were achieved only when fed with a combination of DHA and ARA enriched *Artemia*. However, additional studies are required to evaluate optimal levels and ratios of both EFA, as well as basic understanding of their role in larvae metabolism.

Fig. 6. Mean larval wet weights of hybrid striped bass fed equivalent live feed rations (*Artemia*) enriched with different lipid emulsions for 21 days. Survivorship was identical in all treatments.

Impacts:

As a result of this study we developed an improved live food enrichment product (varying both DHA and ARA) to be used in the production of high performance live larval feeds for cultured cold water and warm water fish. We hope to test the material with halibut, sea bream, winter flounder, white bass and striped bass larvae. The commercially availability of this product is expected to be in late-1998.

Support:

Based on the use of the Martek byproducts in our microdiets, we applied for a Maryland Industrial Partnerships grant and received an award of $53,851 to assist in the development of our microdiets.

Publications, Manuscripts, Or Papers Presented:

S. Ozkizilcik, F-L. Chu and A. R. Place (1995) Complex microencapsulated diets as partial replacement of live food in larval fish culture. Larvi'95.

Lein, I., Barr, Y., Harel, M., Behrens, P., Place, A. and Berge, G.M. 1997. Improved enrichment of *Artemia* using a novel algal derived material. International Conference "Aquaculture Trondheim 97". Cultivation of cold water species: production, technology and diversification. Aug. 10-12, Trondheim, Norway.

Lund, E.D. and A. R. Place (1996) Structure/Function Correlates of Polyunsaturated Fatty Acids: Have you had your docosahexaenoic acid (DHA) today? The Biology of Lipids: Integration of Structure and Functions, Society of Integrative and Comparative Biology.

Harel, M. and Place, A. 1997. Quantitative dietary requirements of marine fish larvae. Live Feed Workshop (invited speaker). Malaspina University-College, Dec. 4-5, Nanaimo, BC, Canada.

Harel, M. and Place, A. 1997. Nutrition of marine fish larvae. Live Feed Workshop (invited speaker). Malaspina University-College, Dec. 4-5, Nanaimo, British Columbia.

Harel M., Koven, W., Lund, E.D., Behrens, P. and Place A.R. 1998. High DHA (Docosahexanoic acid) enrichment of live food organisms using novel DHA-rich phospholipids. The International Triennial Conference & exposition "Aquaculture 98". Feb. 15-19, Las Vegas. USA. Accepted for an oral presentation.

Harel, M., Behrens, P., Herbert, R. and Place, A.R. 1998. Effect of DHA (Docosahexanoic acid) sodium salts on *Artemia nauplii* enrichment. The International Triennial Conference & exposition "Aquaculture 98". Feb. 15-19, Las Vegas. Poster presentation.

Harel, M., A. Tandler, G.W. Kissil, and S.W. Applebaum. 1997. Nutritional effects on seabream (*Sparus aurata*) reproduction performance, egg quality and larval growth and survival. Fish nutrition workshop. Sept. 22-23. Frankfort. USA.

Harel, M., Ben-Atia, S., Zlotkin, V. and Tandler, A. 1998. Mass production of grey mullet, *Mugil. cephalus*: Effect of environmental and nutritional factors on larval performances. J. Bamidgeh. In press.

*** The Full report with all the data, tables and graphs is available at the NRAC office upon request.**