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Project Summary

Nutritional Requirements of Marine Larval and Juvenile Fish

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This project summary describes and assesses the results of a cooperative research effort to evaluate a variety of diets and diet regimes for the laboratory culture of a marine larval fish. The Atlantic silverside (*Menidia menidia*) was chosen for this study on the basis of its regional availability, ease of handling in the laboratory, and its use as a bioassay organism. A major aim of the study was to develop a diet that would provide good growth and survival and provide a test fish similar to its wild counterpart.

Initially, data were gathered on the composition of the silverside egg and wild fish, plankton, brine shrimp and various commercial and modified diets. Following an analysis of the diet components, some improvements were made in the artificial diets. Later studies concentrated on the effect of modified diets in relation to the feeding of brine shrimp nauplii.

Silversides cultured on artificial diets generally had poor growth and survival rates. Freeze-dried brine shrimp (*Artemia*) nauplii and ground-up adult silversides also failed to promote good growth and survival. A superior diet was provided by live, 3-day-old brine shrimp nauplii. However, a combination diet of brine shrimp and artificial diets provided growth and survival more similar to the "all live" brine shrimp diet, even when the live diet was fed only every eighth day.

Biochemical data were compared to reported requirement levels for essential amino acids and essential fatty acids in marine fish, and it was concluded that all diets seemed adequate in these two components. These data also indicated that fish accepted both artificial and live diets equally. The characteristics of the storage of dietary amino acids and dietary fatty acids in silversides was assessed. Although the reason for the live diet's nutritive superiority remains unclear, several recommendations are presented.

This Project Summary was developed by EPA's Environmental Research Laboratory, Narragansett, RI, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

The purpose of this study was to formulate an artificial diet for Atlantic silversides (*Menidia menidia*) such that this fish could be cultured as a representative test organism. Toward this end, a comprehensive project was undertaken with the close cooperation of the Tunison[1], URI[2], and EPA laboratories[3].

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Atlantic silversides are euryhaline fish of importance as forage for piscivorous fish including such valuable commercial species as striped bass, bluefish, cod, haddock, mackerel, and summer flounder. All life history stages of silversides are found in the estuaries along the Atlantic coast. The natural diet of silversides includes algae, detritus and zooplankton, such as megalopa larvae, copepods, and mysids.

The purpose of the University of Rhode Island's part of the study was to provide biochemical evaluations of the diets and the cultured organisms. The diets were formulated to specifications worked out by URI, EPA and Tunison laboratory personnel. The diets came from feeds formulated by the research group and by others (Tables 1A and 1B). Composition of the artificial diets and the artificial semi-purified diets are shown in Tables 2 and 3.

Initial studies concentrated on analysis of the diet components and the wild silverside eggs and adults. Later studies concentrated on the effect of modified diets in relation to the feeding of brine shrimp nauplii.

An additional diet, the Conklin modified lobster diet (CMLD) was included in only experiment 1 and was only evaluated from a fatty acid point of view (Schauer and Simpson, 1978). Therefore, it was not included in the general diet treatment, although the performance of this diet will be included in the fatty acid results of this year's feeding trials. The CMLD diet has been proposed as a reference standard for nutritional studies of invertebrates.

Test fish in experiment 1 were 23 days old at the beginning of the experiment (17.6 ± 0.59 mg; 15.5 mm) and they were fed the various diets for 24 days. In experiment 2, fish were 59 days old (92.8 ± 0.85 mg; 25.8 mm) at the onset of the study and were fed the various diets for 50 days.

Discussion and Results

The joint research project between the URI and the EPA to determine some nutritional requirements of the Atlantic silverside was begun in July 1975 and concluded in January 1979. Experiments were conducted in the summer of each research year and the samples were analyzed during the off-season.

Pre-Diet Testing Analyses

Much of the first year's work consisted of the amino acid analyses of various life stages of winter flounder (*Pseudopleuronectes americanus*) and a number of natural food organisms known to be consumed during the early life stages of wild marine fish. The basic reason for the initial sample analyses was to elucidate the amino acid pattern of a number of marine organisms so that an artificial diet of suitable quality could be formulated.

Young winter flounder adults (16-18 cm) varied little in amino acid content from the juveniles (4-5 cm). Plankton collected from Narragansett Bay showed marked variations in amino acid profiles. It was assumed that the levels of amino acids in the winter flounder eggs were optimum for growth. Based upon the resemblance of the plankton amino acid profile to that of the winter

Table 1A. *Treatments in 1977**

Treatment/Diet	*Source and Description*
1. Artificial, Conklin modified lobster diet	*University of California Bodega Marine Lab diet 76C. Ground to coarse powder passing through a 600-µm mesh sieve.*
2. Artemia salina *nauplii, live*	*Nauplii hatched from San Francisco Bay brine shrimp,* Artemia salina, *eggs and harvested 72 hours after immersion of eggs in filtered seawater of 20-22°C and 31% salinity. Size approximately 140-µm x 400 µm.*
3. Starved	*Unfed.*
4. Artificial, HPM-1 (pellet form)	*Cortland modified practical diet with soy oil and fish oil. Ground to coarse powder passing through a 600-µm mesh sieve.*
5. Artificial, MF-1 (flake form)	*Closed formula prawn diet, Dr. S. Meyers, Louisiana State University. Ground to a coarse powder passing through a 600-µm mesh sieve.*

**For further description see Bengtson et al., 1978.*

Table 1B. *Description of Diets Used in 1977*

Treatment/Diet	*Description*
1	*Combination diet. Artificial prawn flake diet MF-1 given for 3 consecutive days, live* Artemia salina *3-day nauplii substituted on the fourth day.*
2	*Live* Artemia salina *3-day nauplii given every day.*
3	*Unfed.*
4	*Combination diet. Artificial salmonid flake diet HPM-1 and live* Artemia salina *3-day nauplii given on alternating days.*
5	*Combination diet. Artificial salmonid flake diet HPM-1 given for 3 consecutive days, live* Artemia salina *3-day nauplii substituted on the fourth day.*
6	*Combination diet. Artificial salmonid flake diet HPM-1 given for 7 consecutive days, live* Artemia salina *3-day nauplii substituted on the eighth day.*

flounder egg, the plankton appeared to be an excellent diet and was included as a diet in study year 1976.

Feeding Studies

The initial study year was comprised of two separate investigations. One study was undertaken to analyze the effect of a continuous live brine shrimp diet on larval 60+ day-old juveniles. The second study was initiated to evaluate a practical commercial hatchery diet, Cortland 1 (Tunison), which was formulated with respect to the nutritional requirements of a freshwater fish, the Atlantic salmon fry.

The second year of the project (1976) centered mainly on the chemical and biochemical aspects of the diets fed and the analysis of the fish cultured on these diets. The diets consisted of wild plankton, brine shrimp nauplii (fresh and freeze-dried), freeze-dried silversides, Tetra Marin, and four other artificial diets. The latter four diets incorporated changes suggested by the first year's trials (e.g., use of a marine oil and the incorporation of a semi-purified diet with and without an amino acid supplement).

The diets fed in the two experiments for the 1977 feeding trials are listed in Tables 1A and 1B, and included the live, unfed 3-day-old brine shrimp nauplii, the Tunison flake diet (Table 2) and the Meyers prawn diet 1023-77-1 (Table 3). The other treatments in the two experiments consisted of feeding regimes of artificial diets with brine shrimp nauplii at variable intervals. An additional diet, the CMLD (Table 4) was included in only experiment 1 (Table 1A).

The growth and survival data of silversides in 1977 experiments 1 and 2 are presented in Table 5 and Figure 1, respectively. In experiment 1, survival was best on the live brine shrimp diet, slightly less on the combination diet and poor on all artificial diets. Growth of the brine shrimp (only) fed fish was significantly better than in the combination diet group, which in turn was significantly better than any of the artificial diets.

The Conklin diet produced a survival rate of 78.7%, although growth was the poorest of all diet fed groups (22.8 ± 1.8; 17.3 mm).

In experiment 2, Figure 1, survival was equally high in three of the diet groups. These were brine shrimp alone, Meyers diet + brine shrimp every 4th day, and Tunison diet + brine shrimp every 4th day. Statistically, growth was best, and similar, on brine shrimp as a single diet source and Tunison diet + brine shrimp every 2nd day.

In the diet studies and in the combination live and artificial diet studies, silversides actively consumed all diets which seems to eliminate leaching as a major cause of nutritional incompleteness of artificial diets or freeze-dried brine shrimp. If a critical nutrient was leached out, feeding brine shrimp every 8th day would give results similar to feeding an artificial diet alone, which was not the case. The reasonable growth and survival which resulted from feeding brine shrimp every 8th day also tends to eliminate a digestive enzyme as the key growth factor since an enzyme would not likely remain active over an 8-day

Table 2. *Composition of Cortland (Tunison) Diet (HPM-1F)*

Ingredient	*Percent Composition*
Herring Meal	*37.14*
Soybean meal, 49% protein	*9.28*
Corn gluten meal, 60%	*9.28*
Wheat middlings, standard	*8.36*
Brewer's dried yeast	*4.64*
Dried whey	*4.64*
Dried condensed fish solubles	*4.64*
Meat and bone meal	*4.64*
*Soybean oil, table grade**	*4.64*
Fish oil	*4.64*
Mineral mixture†	*0.37*
Vitamin mixture§	*0.56*
Choline chloride, 70%	*0.002*
Niacinamide	*0.02*
Starch, Instant Clear Jel, national	*1.86*
Lecithin	*1.86*
Cholesterol	*0.19*
Kelgin (HV) alginate	*2.32*
Sodium hexametaphosphate	*0.93*

**The soybean oil, fish oil, lecithin and cholesterol were blended and added to premix simultaneously.*

†Mixture provided the following minerals (in gm/kg of diet): magnesium sulfate, 1.78; potassium iodate,0.0076; sodium selenite, 0.00027; zinc sulfate, 0.4; ferrous sulfate, 0.3; cupric sulfate, 0 33; and manganous sulfate, 0.889.

§Mixture provided 13,800 IU Vitamin A palmitate; 5,500 IU Vitamin D_3; 100 IU Vitamin E, as dl-α-tocopheryl acetate, and the following amounts (mg) of other vitamins per kg of diet: Vitamin K as Menadione dimethylpyriminidol bisulfite, 30; thiamin·HCL, 28; d-biotin, 8; folic acid, 20; Vitamin B_{12}, 0.003, inositol, 691; and ethoxyquin, 207.

Table 3. *Composition of the Meyers Prawn Diet*†*

Ingredient	*Percent Composition*
Shrimp meal (South)	*15*
Fish meal	*14*
Fish protein concentrate	*15*
Soy protein	*15*
Yeast protein	*14*
Rice gel	*5*
Whey	*2*
Linolenic acid	*0.5*
Fish oil (menhaden)	*4*
Cod liver oil	*1*
Soy oil	*2*
Cholesterol	*0.1*
Vitamin mix	*2*
Fish solubles	*5*
Lecithin	*2*
Kelgin	*2.5*
Sodium hexametaphosphate	*1*

**cf. I.B. Tarshis (1978).*

†Proximate analysis: protein, 53.1%; fat, 11 7%; fiber, 4.7%; ash, 14.0%; carbohydrate, 16.5% (dry weight basis).

Table 4. *Composition of the Conklin Modified Lobster Diet*

Ingredient	*Percent Composition*
Vitamin free casein	*30.00*
Cellulose	*16.10*
Wheat gluten	*15.00*
Brewer's Yeast	*15.00*
*Lipid mix S**	*6.00*
Corn starch	*5.00*
Albumin	*4.00*
Salt mix, BTM†	*3.00*
Vitamin mix D§	*2.00*
Choline chloride	*1.00*
Thiamin	*2.00*
Cholesterol	*1.00*
Rovimix E	*0.20*
Vitamin C	*0.20*

**Corn oil, 2 03%; cod liver oil, 3.96%; and ethoxyquin, 0.01%.*

Salt mixture in % of mix: $CaCO_3$, 2.10%; $Ca(PO_4)_2$, 73.5%, citric acid, 0.21%; $2Cu_2C\ H_5O_7\text{-}5H_2O$, 0.05%; $FeC_6H5O_7\text{-}5H_2O$, 0.56%; MgO, 2.50%; KI, 0.001%, K_2HPO_4, 8.10%; K_2SO_4, 6.80%; NaCl, 3.06%; $Na_2HPO_4\text{-}2H_2O$, 21.4%; $7N_3\ (C_6H_5O_7)_2\text{-}2H_2O$, 0.13%; NaF, 0.02%; and $CoCl_2$, 0.02%.

§Vitamin mixture in % of mix: thiamin hydrochloride, 0.32%; riboflavin, 0.72%; niacinamide, 2 56%, biotin, 0.008%, Ca-Pantothenate, 1.44%.

Table 5. *Summary of Results From Three Experiments to Evaluate Effects of Diet on Survival and Growth of the Atlantic Silverside*

Experiment Diet	*Treatment Diet*[1]	*Amt. Fed. % Wet Wt. of Fish*	*Duration (Days)*	*Age of Fish*	*Survival (%)*	*Initial Wt. (mg)*	*Final Wt. (mg)*	*Gain % Initial Wt.*
1977-#1	*Conklin modified lobster diet*	*10*	*23*	*24*	*79*	*17.8*	*22.6*	*27.0*
	Artemia *nauplii*	*10*	*23*	*24*	*93*	*17.8*	*103.6*	*482.0*
	HPM-1 pellet	*10*	*23*	*24*	*47*	*17.8*	*43.3*	*143.3*
	HPM-1 flake	*10*	*23*	*24*	*43*	*17.8*	*40.5*	*127.5*
	MF-1	*10*	*23*	*24*	*45*	*17.5*	*34.4*	*92.7*
	Combination Artemia *& MF-1*	*10*	*23*	*24*	*88*	*17.8*	*91.0*	*411.2*

[1]Three replicates per treatment were provided Statistical analyses given in individual references noted elsewhere

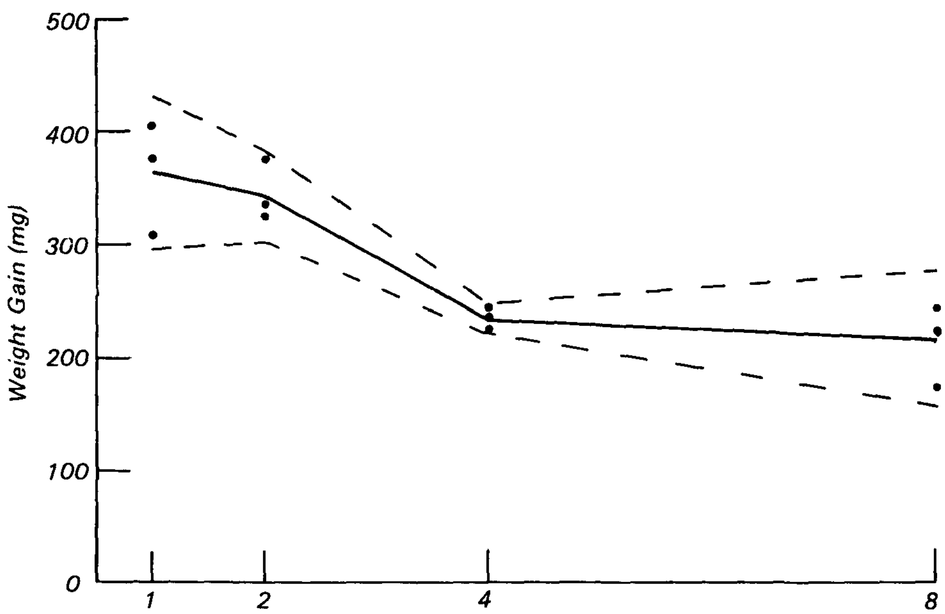

Figure 1. *Interval between* Artemia *feedings (days).*

period. Freeze drying would not substantially destroy digestive enzymes, amino acids or fatty acid components of the brine shrimp, yet feeding freeze-dried brine shrimp gave results equally as poor as feeding an artificial diet alone. It appears unlikely from our present knowledge, therefore, that the live brine shrimp is providing a digestive enzyme, amino acid or fatty acid to silversides that is not being provided by the artificial diets or freeze-dried brine shrimp.

Biochemical analyses demonstrated that the various test diets provided the majority of the 10 essential amino acids in quantities at, or above those noted as adequate. The whole body amino acid profiles for every essential amino acid of all cultured fish were in excess of the minimum requirement levels. The differences observed in growth and survival of silversides cannot be attributed directly to amino acid deficiencies of the diet.

It was shown that a combination of the Meyers + brine shrimp diet produced a fatty acid spectrum in silversides which was nearly identical to the average spectra of each single source diet (Meyers alone; brine shrimp alone). Two important differences were evident between the cultured and wild fish lipids. The first was that the cultured fish contained a greater amount of the 16 and 18 carbon fatty acids, whereas the wild fish contained more of the 20-22 carbon polyunsaturated faty acids (PUFA). The second major difference was that total lipid levels were substantially higher in the cultured group than in the wild fish group.

Live brine shrimp have provided some added requirement for maintaining good survival. With this increased survival, the nutritional requirements of marine larval fish can now be more accurately evaluated by feeding modified artificial diets. It would appear that brine shrimp cannot be totally eliminated from the dietary regime of the larval fish, but they can be more conservatively utilized.

It is thus possible to supplement the fatty acid content of the brine shrimp so that the resulting fish does survive, grow and more closely resemble the natural (wild) fish than do fish grown on only the live brine shrimp nauplii. This can be done without having to maintain the fish on a total live diet.

Conclusions

The intent of this project, started in 1975, was to provide an artificial diet that would give good growth and survival of test fish. Generally speaking, all of the artificial diets—commercial or prepared, flaked or pelleted, and freeze-dried silversides or brine shrimp—gave poor survival and/or growth. Only live brine shrimp nauplii, or a combination of an artificial diet with brine shrimp nauplii, gave acceptable survival and growth. Nevertheless, brine shrimp feedings resulted in a test organism that did not resemble its wild counterpart in biochemical composition. However, a combination feeding of an artificial diet and brine shrimp gave good survival and growth and appeared to correct some of the deficiencies of brine shrimp alone. While the silversides were not completely "weaned" from the expensive (time and cost) brine shrimp, at least it was minimized.

Because of the importance of *Artemia* nauplii in feeding laboratory fish the authors and the EPA group embarked on a study of brine shrimp cysts. At a conference at Szymbark, Poland, in September 1977, where some of these artificial diet data were presented, an International Study of *Artemia* (ISA) group was formed. This group was formed in response to the obvious need to study the properties of brine shrimp, in general, and some commercial strains, in particular. The same close cooperative effort between the EPA and URI laboratories in the first project continued into the second. The comparative studies on the five geographical strains of brine shrimp were presented at the 1st International Symposium on *Artemia* (1979, Corpus Christi, TX) in seven papers from the two laboratories. The results showed clearly that there is wide variation in the brine shrimps' ability to support the life and growth of test organisms and also a variation in their chemical composition. The cause of the variability of biological value of brine shrimp is the subject of further investigation between the two laboratories.

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The complete report, entitled "Nutritional Requirements of Marine Larval and Juvenile Fish," (Order No. PB 81-248 130; Cost: $8.00, subject to change) will be available only from:
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