

SURVIVAL & GROWTH OF LARVAL MUSKELLUNGE (Esox masquinongy)
INITIALLY FED FORMULATED DIETS

1986

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Survival & Growth of Larval Muskellunge (Esox masquinongy)
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Abstract:

Several formulated diets were tested to initiate feeding of larval muskellunge (Esox masquinongy). After 27 days, one group had significantly ($P < 0.05$) greater survival and size. Tanks of larvae initially fed Fry Feed Kyowa B (FFKB) averaged 68.7% survival and 256 mg per fish. Tanks of larvae fed the other diets averaged from 23.4% to 47.7% survival and from < 183 to 205 mg per fish. Surviving fish initially fed FFKB were converted to W16 diet by day 20. By day 60, these fish averaged 2.6 gm per fish and were approximately 7.6 cm (3.0") in length. Survival from swimup fry to this size was 59.7%.

Introduction:

Intensive rearing of muskellunge (Esox masquinongy) with formulated diets has been limited by poor survival during early larval development (up to 38 mm/1.5 in) at our facility. Fish culturists in Pennsylvania (Bender et. al. 1983) and Wisconsin (anonymous 1985) have reported similar results. Pennsylvania Fish Commission biologists have intensively reared muskellunge successfully ($> 92\%$ survival of fry to 76-178 mm [3-7 in] fingerlings) using live Daphnia and fathead minnow (Pimephales promelas) larvae (Sorenson et. al. 1966), but availability and costs for live forage limits production by this method at our facility and most others. Graff (1970) reported that tests to convert muskellunge, reared to 25 mm (1 in) in length with Daphnia, to dry feed were unsuccessful.

We have tried numerous dry diets to initiate feeding muskellunge fry in the past, including U.S. Fish and Wildlife Service open-formula diets (i.e. W14, W15, W16, X411, Abernathy) and commercial closed-formula diets (i.e. Silvercup¹, Biopellet¹, Red Prime Flake¹), none of which have given good results. Feeding Artemia in combination with W16 provided good survival through early development (65% survival up to 20 mm [.75 in], but effects of cannibalism greatly reduced survival after fry were weaned from Artemia.

Past observations of muskellunge fry found that many accepted the dry diets but did not appear to be in good shape. Examination of dead and dying fry found feed particles in the digestive tract, but gas bubbles were also found along with bone

¹ References to trade names or manufacturers does not imply U.S. Government endorsement of commercial products.

particles from feed, which appeared to block the tract. Gill fungus (Saprolegnia sp.) was observed in some fish and was thought to be caused by fine feed particles lodging amongst gill filaments. Abnormalities (i.e. scoliosis, lordosis, cataracts and stunted fish) were common among surviving fingerlings fed exclusively dry feed, indicating nutrient deficiencies.

A formulated diet that is readily accepted by larval muskellunge, does not cause complications and contains the nutrients for proper development is needed to improve the success of our intensive culture program for this specie. We recently experienced high survival and good growth of larval lake whitefish (Coregonus clupeaformis) exclusively fed two commercial diets. We tested these and other diets for initial feeding to larval muskellunge and compared survival and growth.

Materials and Methods:

Triplicate tanks of muskellunge fry were assigned one of six test diets for initial feeding. These six diets are:

- 1) W16 - Manufactured by Zeigler Bros. Inc., Gardners, PA.
- 2) CW2 - Modified W16 with 10% propylene glycol and 1% betaine, Beulah Fish Technology Center (BFTC), Beulah, WY.
- 3) CW3 - Modified W16 with 10% propylene glycol and krill meal substituted for shrimp meal, BFTC.
- 4) Artemia - Live
- 5) Larval AP100 - Zeigler Bros. Inc.
- 6) Fry Feed Kyowa B¹ (FFKB) - Kyowa Hakko Kogyo Co. Ltd., Tokyo, Japan.

Diets CW2 and CW3 have propylene glycol to attempt to improve growth and feed efficiency. Krill meal was substituted for shrimp meal or betaine (a quaternary amine) was added to possibly improve food attractiveness and stimulate feeding. Starter granule size particles of W16, CW2 and CW3 were used at the start of the trial. Particle size of Larval AP100 and FFKB used were 500 and 400 microns, respectively. W16 number one granule feed was used to wean fry from Artemia, Larval AP100 and FFKB diets when fry developed enough to accept larger feed. Feed was dispensed once every 15 minutes for 24 hours each day from Loudon II¹ automatic feeders. Approximately 5 to 6 grams of dry feed was dispensed daily to each tank. A brine shrimp feeder, constructed from a 57 L (15 gal) container with an electric solenoid valve and air stone, dispensed live Artemia once every 10 minutes for 24 hours each day. From densities in the feeder, we estimated approximately 5000 Artemia were offered to each fish throughout a 24 hour period.

Muskellunge sac fry, hatched from eyed eggs obtained from Spooner Fish Hatchery in Wisconsin, were placed into a floating screen basket until swimup. Three-hundred swimup fry were counted into each tank, providing a stocking density of 22 fry/L (86 fry/gal). Tanks used were constructed from 19 L (5 gal) plastic buckets. The bottom was removed and nylon window screen was stretched across it. These containers were placed into rectangular aluminum tanks, which have a standpipe at one end to maintain a water column in the bucket. Rearing volume of each bucket was 13.4 L (3.5 gal) with a water column of 23 cm (9 in). Water was introduced in a horizontal flow above the water column in each bucket at a rate of 1.4 L (0.37 gal) per minute giving an

exchange rate of 6.3 rearing volumes per hour. Tank design allowed most waste to pass through the tank bottom, thereby, allowing cleaning every other day. Overhead light from four, 122 cm (48 in), 40 watt fluorescent bulbs illuminated tanks 24 hours each day. Rearing temperatures ranged from 16.6 to 18.4°C (62-65°F) up to day 12 and from 21.1 to 23.9°C (70 to 75°F) from day 12 to 27. Dissolved oxygen levels in the rearing units were \approx 7 mg/L throughout the trial.

Percent survival and size in mg per fish (total weight of fish measured on a triple beam balance divided by the number of surviving fish) was determined for each tank. Average length was not determined for each tank because it would have required handling more fish, which could result in stress, disease and possible heavy mortality. Length was measured on only a few fish in each diet group. Analysis of variance and Duncan's new multiple range test (Steele and Torrie 1960) were applied to compare mean values of percent survival and average weight per fish. The level of significance was accepted as being $P < 0.05$.

Results and Discussion:

Fry that appeared to not accept feed died between days 10 and 12. Tanks of fry fed Artemia, W16 and FFKB had low mortality through these dates, ranging from 6% to 8%. Tanks of fry fed the other diets ranged from 29% to 39% mortality. Much cannibalism was observed in tanks fed Artemia and W16 starting day 10. On day 15, we started to wean larvae from Artemia, Larval AP100 and FFKB diets. W16 diet was fed along with Artemia through day 20, after which only W16 was offered to those fish. W16 was mixed with the other diets at a ratio of 1:1 until day 20 when only W16 was offered.

After 27 days, tanks of larval muskellunge fed FFKB and Larval AP100 had significantly higher survival and were significantly larger than larvae fed the other diets (Table 1). Larvae also appeared more even in length in these two groups than in the other groups. Survival of fry fed CW2 and CW3 was fair, though fry were quite small at the end of the trial. Most of the losses which occurred after day 12 in groups fed W16 and Artemia were believed due to effects of cannibalism.

A closer examination of the dry diets we used revealed that feed particles of the Larval AP100 and FFKB diets were uniform in color and size. Each individual particle looked as though it was a representative sample of the diet. Feed particles of the other dry diets were very dissimilar in size and color, indicating that a combination of the various different particles would be required to represent the diet. Because the feed particles are similar in the Larval AP100 and FFKB diets, larvae are probably obtaining all the nutrients these diets have to offer since they have only one kind of particle to select. Whereas, they may be missing some of the nutrients the other diets contain if they did not select all of the different particles that compose those diets.

Applegate (1981) reported food organisms found in the digestive tracts of muskellunge fry, beginning to feed, had a mean length of 520 microns and width of 280 microns. The

Table 1. Survival and size of muskellunge fry fed different starter diets after 27 days. Values given are means and within each column values followed by the same letter (or no letter) are not significantly different ($P < 0.05$).

STARTER DIET	SURVIVAL %	WEIGHT (MG/FISH)	LENGTH ^a (MM)
W16	23.4 z	183 z	28
Live <u>Artemia</u>	28.3 yz	204 y	30
CW2	37.7 xy	No data ^b	17
CW3	47.4 wx	No data ^b	19
AP100-500 micron	47.7 w	205 y	30
FFKB-400 micron	68.7 v	256 x	35

^a Statistical analysis was not conducted on this data as lengths for individual tanks were not determined.

^b Because these fish were very small we believed the equipment we had would not provide an accurate measurement.

manufacturer of Larval AP100 has indicated to us that the particles of the diet we used could range in size from 425 to 575 microns. If we were to use the dimensions given by Applegate (1981) as particle size limits for feeding muskellunge fry, the Larval AP100 we used may have been too large, which could explain the large number of nonfeeders lost in those tanks early in the trial. Manufacturer specifications for the FFKB diet we used state particle sizes range from 250 to 400 microns, which covers the majority of these particle size limits. Particles of the other dry diets we started with ranged from \approx 10 up to approximately 520 microns, which also covers these size limits. However, those particles less than 280 microns may be ignored by the larvae and could possibly contain a nutrient or nutrients required for proper development.

After day 27, surviving fingerlings from FFKB and Larval AP100 groups were sorted, combined and placed into rectangular rearing tanks and fed W16 feed. After 33 days in these tanks fingerlings had grown to 2.6 gm/fish and 7.6 cm (3 in) in length with no observable deformities. Survival through the 33 days in these rectangular tanks was 86.9%. Using the average survival we experienced for larvae fed FFKB diet, the overall survival from swimup fry to this size calculates to 59.7% over the entire 60 day period.

Conclusion:

Larval AP100 and FFKB diets appear to contain the required nutrients needed for proper development of larval muskellunge reared under our test conditions. Although additional testing is required, it appears the more uniform feed particles and size of these diets used may be responsible for the success we observed. Although FFKB and Larval AP100 are expensive, our results indicate these diets may only be required as an initial feed through an early stage of development. More research is needed to determine when larvae can be switched to a more economical feed. From our results, it appears that once muskellunge larvae are approximately 25 to 38 mm (1.0-1.5 in) in length, W16 may be a suitable diet to continue their growth.

The rearing containers used may have assisted in the results we experienced. By providing a more suitable environment for rearing larval fishes and requiring little maintenance, we lower the chances for stress or introduction of diseases. Additional testing with these diets and larger rearing units is required to determine the feasibility of these methods on a production level.

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ABSTRACT

John M. Johnson, Jr., A STUDY OF CHANGES IN BODY COMPOSITION OF FINGERLING STRIPED BASS (*MORONE SAXATILIS*) FED PURIFIED DIETS. (Under the direction of Dr. Charles W. O'Rear, Jr.) Department of Biology, May, 1978.

The purpose of this study was to determine changes in the body composition of fingerling striped bass when fed purified diets containing various ratios of plant and animal proteins and to determine if plant protein can efficiently replace animal protein as a protein source in fish diets.

Fingerling striped bass were maintained at a temperature of 20°C, and fed purified diets for a period of six weeks. The basic diet composition was modified from a channel catfish purified diet (Hastings and Dupree, 1969). The diets differed in the concentrations of casein and soy protein; the control diet consisted of commercial trout chow.

The greatest weight gain, 84% was obtained from fish fed the 75% casein diet. This gain was due primarily to an increase in fat and moisture content of the fish, with no increase in protein. Fish fed diets containing less than 50% casein showed increase in fat concentration accompanied by a decrease in body moisture. The fish fed the 50% casein diet were the only fish which had a gain in protein concentration (18%), with a slight increase in fat and little or no change in moisture content. Results also indicated lack of acceptance by the fish of the 100% soy protein diet. Fingerling striped bass can be maintained on a diet of plant and animal protein, but a careful balance of plant and animal protein is necessary to achieve proper growth at a minimal cost. (This study was supported by the Sports Fishing Institute.)

A STUDY OF CHANGES IN BODY COMPOSITION
OF FINGERLING STRIPED BASS (MORONE SAXATILIS)
FED PURIFIED DIETS

A Thesis

Presented to

The Faculty of the Department of Biology
East Carolina University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science in Biology

by

John Marshall Johnson, Jr.

May, 1978

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Laboratory facilities and equipment were provided by the Department of Biology at East Carolina University and funded by the Sports Fishing Institute. Fingerling striped bass were obtained from the Edenton National Fish Hatchery at Edenton, North Carolina.

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INTRODUCTION

The striped bass, (Morone saxatilis) is an important species to the sports and commercial fisherman and possibly as a new source of protein for human use. The increasing population of the world will require new and additional protein sources, and aquaculture of fish for sources of protein is relatively unexploited. Striped bass have been kept in fish hatcheries four to five years for breeding purposes and fingerlings have been raised for the purpose of stocking streams and rivers. In the 1890's fingerling striped bass were transported to the pacific coast. They have since become established and provide millions of dollars to state revenue and fisheries each year. Striped bass have been transferred to the U.S.S.R. and other places around the world for the purpose of establishing fisheries and to provide an exotic species of fish for the sports fisherman.

In early April, four-to six-day-old fry are stocked in the hatchery ponds at the National Fish Hatchery at Edenton, North Carolina. These fry are fed a supplementary diet of commercial trout chow until they grow to suitable stocking size. Trout chow is a high protein diet which is being used by most hatcheries in the United States to raise trout and salmon. No specialized diet has been formulated for the rearing of striped bass. Different species of fish have different metabolic requirements, the requirements of one species might not be adequate for another species (Halver, 1972). To the fish culturist raising striped bass, a diet which is efficient for the fish yet practical and relatively inexpensive is highly desirable. One of the basic problems confronting the formulation of such specialized diets for any species of fish is the limited knowledge

of the metabolism, enzyme systems and nutritional requirements.

The objectives of this study were to determine changes in the levels of protein, fat and moisture of fingerling striped bass when fed purified diets, and to determine if the animal protein used in fish diets can be replaced completely or partially by plant protein.

LITERATURE REVIEW

In recent years, the trend in aquaculture has been to establish breeding stocks of selected species of fish for commercial and restocking purposes. To fulfill these needs, research was undertaken to establish the nutritional requirements of these fish for growth and development. Feeding studies were conducted with different species of fish, primarily salmonids and catfish, to determine the necessary requirements of fats, carbohydrates and proteins to maintain growth.

In nature, fish normally consume an array of organisms which vary with the seasons and the size of the fish. This natural food usually provides the fish a balanced diet for proper growth and reproduction. (Keast, 1965; Keast and Webb, 1966). Fish hatcheries were unable to keep up with the collection of natural foods for fish as the hatcheries increased in size; therefore, studies were begun to formulate commercial diets to substitute for natural foods. Studies revealed that both the levels and ratios of fats, carbohydrates and proteins in fish diets alter the energy requirement of the fish, and show varying growth efficiencies.

Phillips et al (1966) were able to demonstrate that carbohydrates (maltoses) have a sparing action on protein used by trout. Fats used in diets were also shown to have a sparing action on protein for brook and brown trout by Phillips et al (1964, 1965) and for salmon by Combs et al (1962). Smith (1967) found that rainbow trout fed a high protein diet showed an increase in the level of the products of protein metabolism. Other factors such as the sparing actions of other nutrients on protein play an important role in the utilization and metabolism of protein by the fish.

With the recognition of the importance of vitamins to human consumption, fishery scientists began to realize the possible importance of essential vitamins to the proper growth of fish. Diet studies conducted by Jewell, Schneberge, and Ross (1933) on fingerling channel catfish determined not only the importance of essential vitamins for proper growth but also that deficiencies in these vitamins caused many fish diseases. Dupree and Sneed (1966a) were able to correlate the disease symptom with the corresponding vitamin deficiency in the diets of fingerling channel catfish.

Specific protein studies were conducted in an attempt to achieve a more economical and feasible type of protein source other than meat protein for the rearing of commercial fish. Studies revealed that both the level and the source of protein affected the growth of the fish. Dupree and Sneed (1966b) were able to show that a protein diet of casein was superior for the growth of fingerling channel catfish when compared to protein diets of cottonseed oil and diets of soymeal oil. Similar feeding studies conducted by Fowler, McCormick, and Thomas (1966) pointed out that soymeal oil was an inferior protein substitute when compared to cottonseed oil used in purified diets for fingerling chinook salmon. The soymeal oil diet when fed to the salmon showed a reduction in deposition and utilization of protein and a loss in fat deposition by the salmon. The evidence indicates that different species of fish require different quantities and qualities of protein for the maintenance of proper growth and development.

The increased knowledge of general fish nutrition and metabolism has brought about changes in aquaculture. Hatchery fish once fed natural

foods, or meat protein diets, are now being raised on meal protein diets fortified with vitamins. Specific diets such as the Abernathy Salmon diet have been developed specifically for the rearing of salmon. Each species of fish raised in hatcheries should have a specialized diet to meet the demands of that fish for proper growth and reproduction.

With the increasing interest in the striped bass as an important commercial fish and for restocking purposes, fish hatcheries have begun propagation and production studies, but no known nutritional studies with the use of purified diets have been conducted (Striped Bass- Report on the Development of Essential Requirements for Production, 1968-1970, U. S. Department of Interior).

MATERIALS AND METHODS

Experimental Animals

In July, October, and December of 1972, fingerling striped bass were obtained from the Edenton National Fish Hatchery, Edenton, North Carolina, and transported to the Department of Biology, East Carolina University in Greenville, North Carolina. A closed water system (Figure 1) consisting of a fiberglass reserve tank with a one-horsepower submersible pump and a series of six 80 liter tanks was incorporated for feeding studies. The fingerlings were acclimated in the lab for a period of 48 hours in a 520 liter fiberglass holding tank. The fish were then divided into groups of 25 fish each and transferred to six feeding tanks. A 12-hour day was simulated in the lab by placing 60-watt lights above each feeding tank. The lights were controlled by a timer which automatically switched the current off and on every 12 hours. The fish in the feeding tanks were fed commercial trout chow five times daily and were maintained on this diet until they no longer showed signs of stress. This period lasted from one to two weeks.

Diets and Feeding Trials

The training diets fed the fish consisted of the purified diet formulations for each group with the addition of decreasing amounts of commercial trout chow, 50%, 25%, and 10%. This allowed the fish to adjust gradually to the color difference of the purified diets.

The control diet was commercial trout chow; the other five purified diets were 100% casein, 75% casein-25% soy protein, 50% casein-50% soy protein, 25% casein-75% soy protein, and 100% soy protein. Table I shows

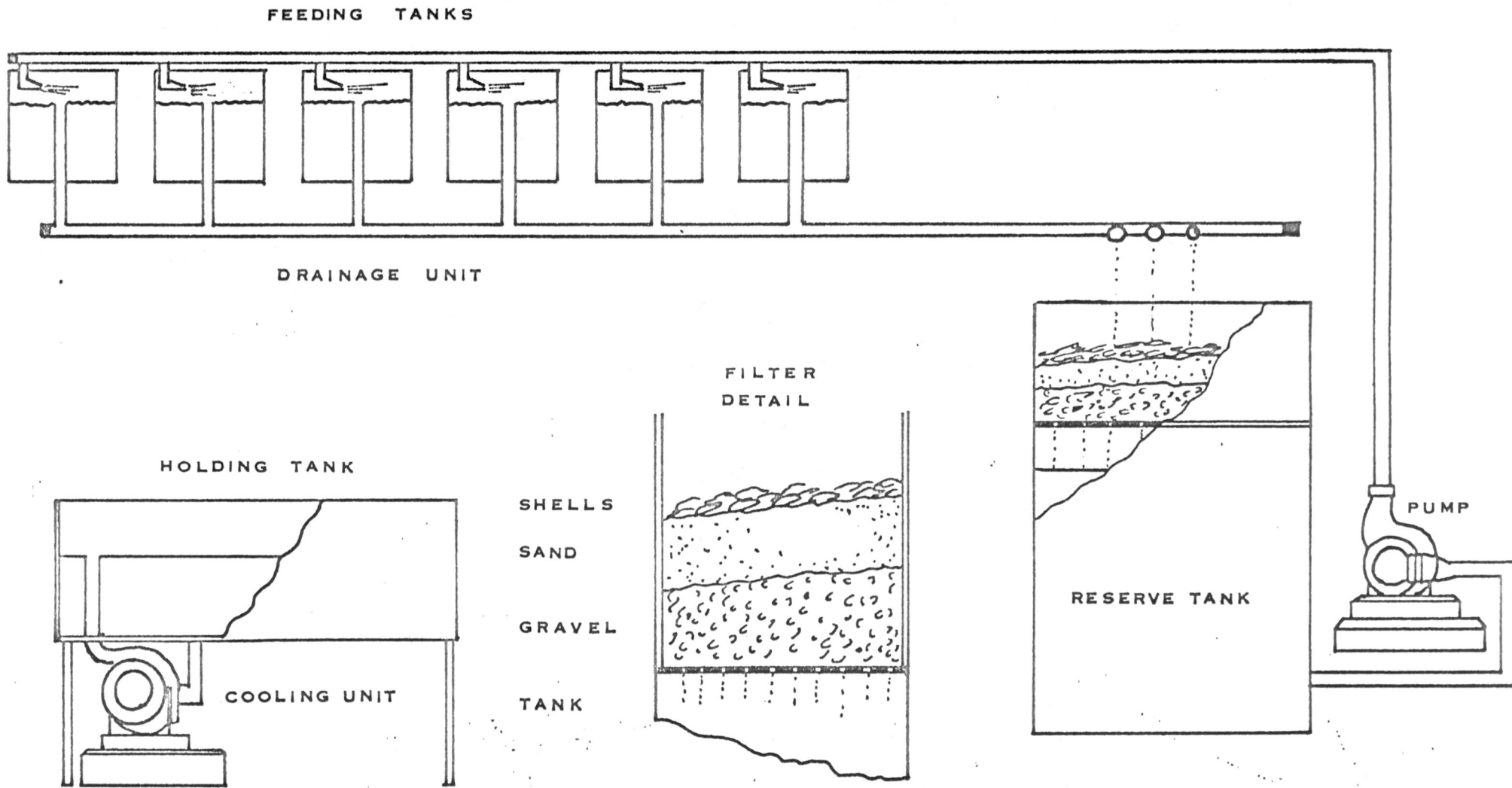


FIGURE 1. CLOSED WATER SYSTEM USED IN DIET STUDY.

the basic formula for the purified diets. The procedure for the preparation of the diets is given in Appendix II. The purified diets varied only in the quality of protein and were modification of diets used by Dupree and Sneed (1966c) and the Abernathy Salmon diet (Fowler & Burrows, 1971).

Once the fish were feeding on the 10% commercial trout chow training diets, they were then started on the purified diets. The initial day of the study was the first day that the fish began feeding on the purified diets. The fish in each group were fed a weighted amount of food five times daily. They were observed for fifteen minutes after each feeding to estimate the amount of food consumption by each group.

Nutritional Analysis

Diet Analysis: The caloric value of each diet was determined with the microbomb calorimeter. Samples from each diet were oven dried at 70°C for 24 hours and the mean caloric content was determined for each diet (Appendix III).

Fish Analysis:

(A) Weight: At the beginning and end of the study, every fish in each group was anesthetized with .02% quinaldine, wrapped in dampened cheese cloth, weighed to the nearest tenth of a gram, and replaced into the feeding tanks. Subsamples of 5 fish were taken at the beginning and at the end of the six week feeding period from each group, weighed, homogenized, and analyzed for protein, fat, and moisture content. Moisture content was determined by subtracting the dry weight of the fish from the initial wet weight. Total weight gain for each group was determined by comparison of initial weights with weights of fish from the same diet group at the completion of the feeding study.

TABLE I - Diet Composition and Type of Protein Used in Purified Diets

Diet Composition			
NUTRIENT	INGREDIENT	PERCENT (WET)	PERCENT (DRY)
Protein	Casein or Soy Protein	30	69.8
Lipid	Vegetable Oil	2	4.7
	Cod Liver Oil	1	2.3
Carbohydrates	Sucrose	3	7.0
Vitamins	Vitamin Mixture	2	4.7
Minerals	Salts	3.7	8.6
Non-nutrient bulk	Cellulose Flour	1.1	2.6
Binder	Agar	0.2	0.5
Water	Distilled Water	57	---

Type of Protein in Purified Diets

1. 100% Casein
 2. 75% Casein - 25% Soy Protein
 3. 50% Casein - 50% Soy Protein
 4. 25% Casein - 75% Soy Protein
 5. 100% Soy Protein
-

(B) Protein, Fat, and Moisture: Each fish to be analyzed for body composition was homogenized into a fine paste and each fish divided into three portions for protein, fat, and moisture analysis. Protein determination of fish samples were by micro-kiel-dahl digestion procedures; fat by acetic-perchloric acid digestion (AOAC Handbook, 1969). The percent moisture of the fish was determined by drying a weighed fish sample in a drying oven at 105°C for 25 hours and weighed to determine loss of weight due to moisture content.

Analysis of Data

Log transformation of data gathered before and after feeding studies was analyzed by multiple regression with the use of SPSS computer programs at East Carolina University.

RESULTS

The highest weight gains, 3.1 grams and 3.6 grams (Tables II and III) were from fingerlings fed 75% and 25% casein, respectively (Figure 4). This weight gain is primarily due to increases in fat and moisture content of the fish in these groups, as shown in Figures 2 and 3.

Fingerlings fed 50% casein-50% soy protein diets were the only group of fish which showed a gain in body protein concentration (18%) (Figure 5), with a slight increase in fat and no significant change in moisture content. Studies conducted by Slaughter (unpublished), using a combination of 50% casein-50% soy protein purified diet for fingerling striped bass, showed more protein deposition, less adiposity, and higher energy efficiency when compared to diets of common trout chow and 100% animal protein (casein). Fish fed diets of less than 50% casein showed a gradual increase in fat concentration accompanied by decreases in body moisture.

Factors of stress and handling limited the study to one, six-week feeding trial with fingerlings collected in July. Columnaris disease and parasitic infections of Ichthyophthirius multifiliis were identified on the body and gill rakers of fingerlings collected at other times during the year. The fingerlings collected in July showed no outward signs of disease during the duration of the study.

TABLE II. CHANGES IN BODY COMPOSITION OF FINGERLINGS BEFORE FEEDING TRIAL

Mean and Standard Error

DIET	N	WEIGHT	N	PERCENT	PERCENT	PROTEIN
		(grams)		FAT	MOISTURE	(Mg/gm)
		$\bar{X} \pm$ S.E.		$\bar{X} \pm$ S.E.	$\bar{X} \pm$ S.E.	$\bar{X} \pm$ S.E.
Common Trout Chow	25	5.83 \pm 0.26	5	6.42 \pm 0.92	69.10 \pm 1.64	18.32 \pm 2.02
100% Casein	25	5.32 \pm 0.48	5	7.13 \pm 0.56	72.14 \pm 0.68	18.16 \pm 1.27
75% Casein-25% Soy Protein	25	4.34 \pm 0.20	5	5.78 \pm 0.95	72.94 \pm 0.85	19.93 \pm 2.12
50% Casein-50% Soy Protein	25	5.66 \pm 0.28	5	5.94 \pm 1.06	71.81 \pm 0.38	16.28 \pm 1.59
25% Casein-75% Soy Protein	25	4.50 \pm 0.33	5	6.49 \pm 0.39	73.77 \pm 0.29	19.45 \pm 0.64
100% Soy Protein	25	4.41 \pm 0.24	5	5.88 \pm 0.85	72.58 \pm 0.62	18.34 \pm 0.06

TABLE III. CHANGES IN BODY COMPOSITION OF FINGERLINGS AFTER FEEDING TRIAL

Mean and Standard Error

DIET	N	WEIGHT	N	PERCENT	PERCENT	PROTEIN
		(grams)		FAT	MOISTURE	(Mg/gm)
		$\bar{X} \pm$ S.E.		$\bar{X} \pm$ S.E.	$\bar{X} \pm$ S. E.	$\bar{X} \pm$ S.E.
Common Trout Chow	20	11.16 \pm 0.64	5	4.05 \pm 0.33	74.16 \pm 0.79	16.19 \pm 2.14
100% Casein	20	8.22 \pm 0.76	5	6.14 \pm 1.07	72.52 \pm 2.74	17.34 \pm 2.44
75% Casein-25% Soy Protein	20	*7.97 \pm 0.56	5	6.06 \pm 0.44	75.05 \pm 0.60	18.84 \pm 0.53
50% Casein-50% Soy Protein	20	8.70 \pm 0.60	5	6.72 \pm 0.65	72.06 \pm 0.56	19.18 \pm 0.65
25% Casein-75% Soy Protein	20	7.58 \pm 0.79	5	8.88 \pm 0.52	71.18 \pm 0.33	18.35 \pm 1.22
100% Soy Protein	14	5.00 \pm 0.50	5	7.61 \pm 1.09	72.39 \pm 0.87	17.78 \pm 1.64

*Significant correlation between weight and variables; protein, fat, and moisture. (P > 0.01)

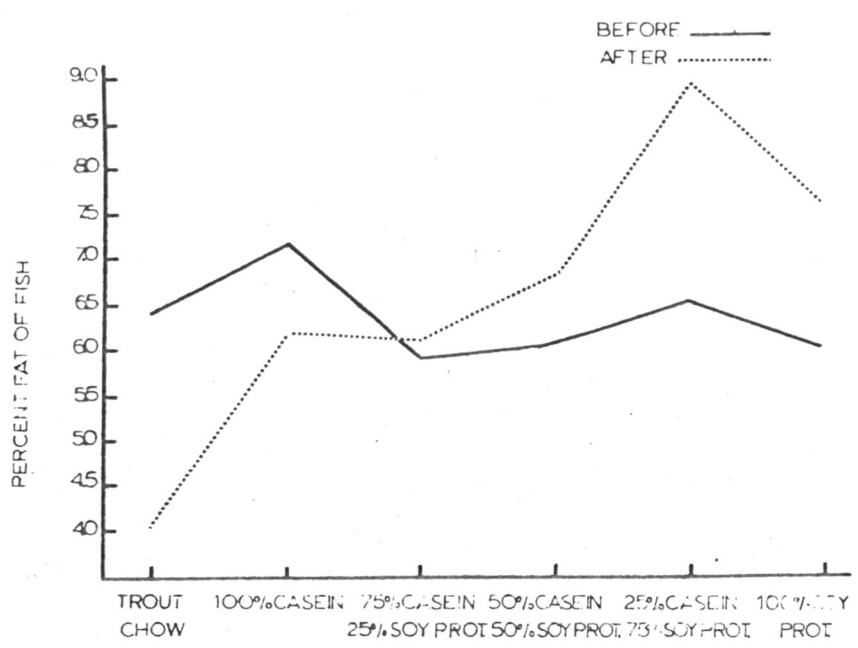


Figure 2. Average Fat Content of Fish In Each Group Before And After Study.

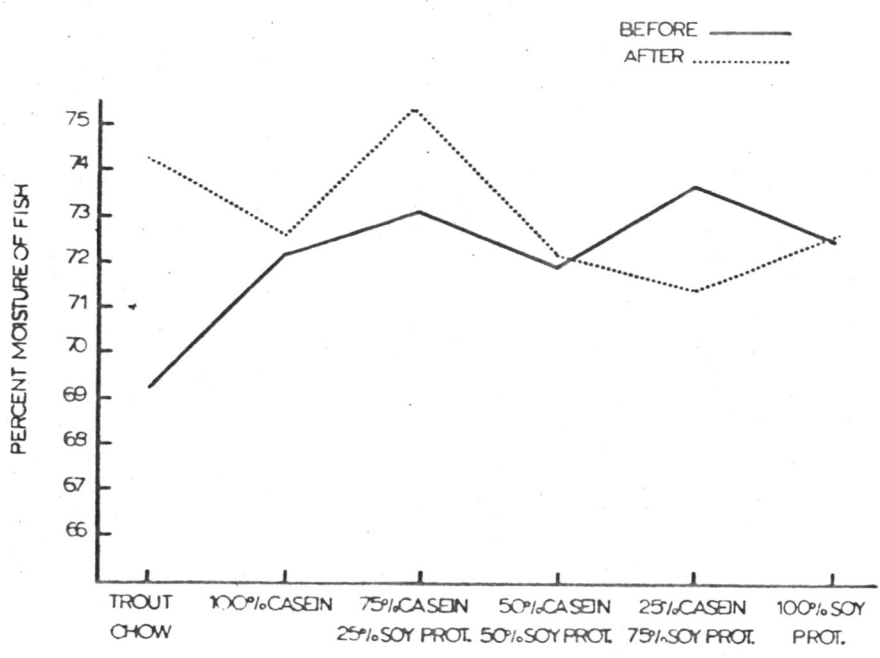


Figure 3. Average Percent Moisture Content of Fish In Each Group Before And After Study.

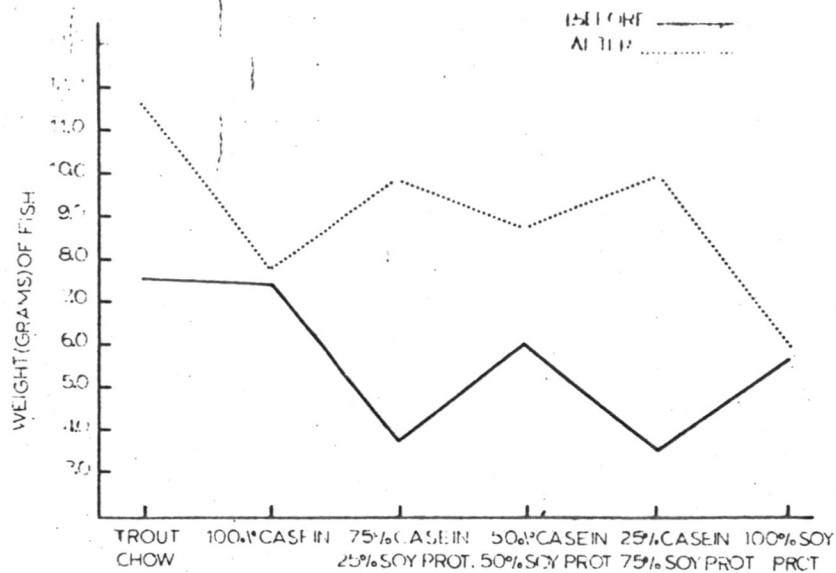


Figure 4. Average Weight Of Fish In Each Group Before And After Study.

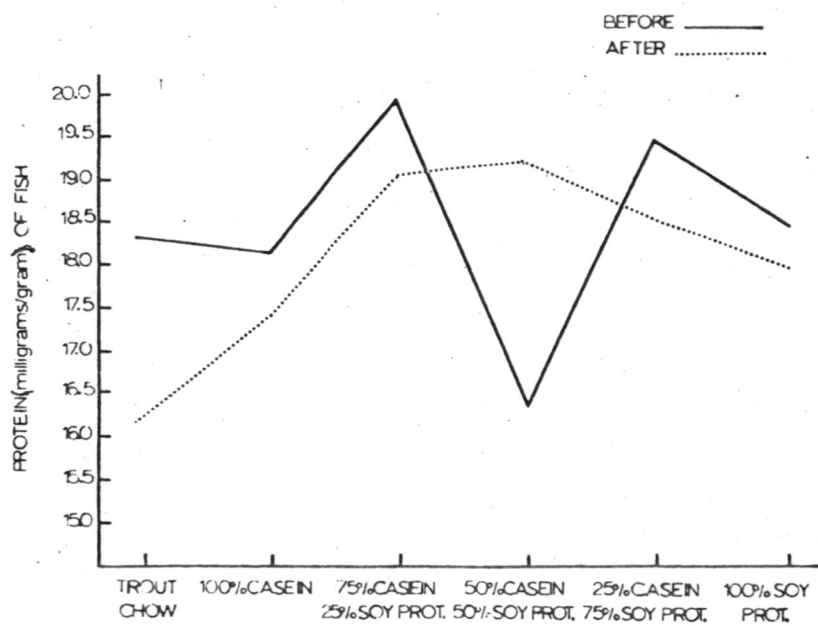


Figure 5. Average Protein Concentration Of Fish In Each Group Before And After Study.

Multiple Regression Analysis of Diets

Before the feeding study and then after the feeding trials, fish in each diet group were compared to (1) fish fed the same diet, and (2) other diet groups using the variables protein, moisture and fat content to explain weight trends of fish in each diet group. The equation which expresses this relationship is

$$\text{Wt Fish} = f (\text{Protein} + \text{Moisture} + \text{Fat}).$$

Regression analysis was conducted using SPSS computer programs to determine if significant trends could be obtained between weight of fish and the variables, protein, moisture and fat. Earlier linear regression analysis showed no significant correlation among fish in the same diet group or between fish in other diet groups, while log transformation of data provided a better model than did raw data.

Multiple Regression Analysis of All Diet Groups Combined

The regression results indicate that 29% of the variation in weight trend of the combined fish in all diet groups before the feeding study began can be explained by moisture content of the fish. Fat and protein contribute a total of 2.7% of the weight. Therefore, of the three variables, moisture would be the best predictor of weight in the above combined fish groups. Regression analysis on combined diet groups after the feeding study showed that protein, fat and moisture content were not significant in explaining weight trends of the fish.

Multiple Regression Analysis of Separate Diet Groups

(Fish Fed the Same Diet)

Fingerlings fed diets of common trout chow, 100% casein, 75% casein,

50% casein, and 100% soy protein showed no weight trends before the feeding study that could be explained by the variables used in this study. This may be possibly due to the limited sampling of fish before the feeding study and the unknown protein, fat and moisture composition of the fish prior to the initial sampling.

Data gathered from fish fed 25% casein before the feeding study indicated that all variables were significant in explaining weight trends in this diet group. After the feeding study, fish fed diet 5 showed no explainable weight trend.

Data gathered on separate diets after the feeding study indicate that fish fed 75% casein showed weight trends which could be explained by fat and moisture. Fish fed diet 3 showed weight trends mainly due to fat (88%) and secondly due to moisture (7%). Protein did not contribute significantly to this weight trend. The Log Regression Equation which explains this relationship is: $\text{Log Wt} = 11.84 + 2.059 (\text{Log Fat}) + 6.383 (\text{Log Moisture})$. A more desirable diet to the aquaculturist would be one in which weight trends are due mainly to protein gain (fish muscle) instead of fat or moisture.

Regression analysis on both combined and separate diet groups showed that protein was not a significant determinate of weight trends. Therefore, a practical approach is to examine the graphic changes observed in body composition of fingerlings in each diet group.

DISCUSSION

The purified diets in this study differed only in the source of protein available to the fingerling striped bass. The ability of the fish to utilize the plant, animal, or combination of both proteins was reflected in the metabolism of the fish in each diet group. This was demonstrated earlier by Slaughter (unpublished data) in showing that fingerling striped bass utilized a combination of 50% casein-50% soy protein diet to a higher degree of energy efficiency than compared to diets of 100% animal protein (casein) and common trout chow. The diet of 50% casein-50% soy protein used in this study also showed higher protein disposition than fingerlings fed diets of common trout chow, 100% animal protein, 100% plant protein, or other combinations.

Plant and animal sources of protein used in the diets is reflected in the changes in protein, fat, and moisture content in the fingerlings. The protein utilization by the fingerlings can be attributed to several factors--the quality of protein, digestibility of the protein, toxicants present in proteins, and possibly excessive amounts of protein in the diets.

Animal proteins generally are of high quality, vegetable proteins can vary considerably in quality. Soy protein is approximately of the same quality as most animal proteins. The amino-acid content of casein (mg/gm nitrogen) is 228, that of soy protein is 197. Halver et al (1957) was able to demonstrate that fish require the ten essential amino-acids needed by growing rats. Casein is given a higher essential amino-acid score (81.7) compared to soy protein (71.0) when based on chinook

salmon requirements. The possibility of essential amino-acid limitations in 100% soy protein diet may explain the increased mortality rates and lack of growth response of fingerlings fed this diet.

The feeding behavior of the fish in all diet groups were essentially the same at the beginning of the study. The fish fed 100% soy protein showed a decreased response to feeding as compared to the other diet groups at the end of the feeding study. This may be due to the build-up of toxic factors over the duration of the study.

The fingerling striped bass fed the 100% soy protein diet showed the least weight gain (Figure 4), and a mortality rate twice that (25%) of any other group of fish in the study.

Toxic factors have been associated with protein rich seeds, such as soybean (Halver, 1972). These toxic substances can interfere with the utilization of amino-acids; and, therefore, decrease availability of essential amino-acids. The digestibility of proteins in the diet has been shown to have an effect on other levels of nutrients in the diet being utilized by the fish. Kitamikado (1964) found a drop in protein digestion when fish meal was replaced by potato starch in a diet for rainbow trout. The fish fed a diet of 50% soy protein showed increased fat and protein disposition when compared to diets of 100% soy protein or 100% animal protein. The digestive coefficient for casein fed to rainbow trout is 79%, that for soy protein fed to channel catfish is only 56%

The presence of soy protein in the diets affected fat metabolism of the fingerlings. The fat metabolism showed increase with increases in soy protein content in the diet. The percent fat increased from 4.8%

in the 25% soy protein diet to 36.8% in the 75% soy protein diet. However, the 100% soy protein diet showed decreased fat gains when compared to the 75% soy protein diet, possibly due to increase of fibrous material over the optimum level (Halver, 1972). Excessive protein in the diets is not very likely since fish have the ability to eliminate ammonia excesses through the gills with slight or moderate energy requirements, in addition to fecal elimination.

Animal protein is an important protein source as shown in fingerlings fed the 100% soy protein diet. This is indicated in the nutrition scores, soy protein scoring 71.0 and animal protein (casein) having a score of 81.7. Weight gains of fingerlings in this group were less than any diet in the study (14%). Fat gains were less than the 75% soy protein diet and the mortality rate was twice that of any other diet group. Fish fed common trout chow, the control diet currently used in fish hatcheries, showed the highest percentage of protein and fat loss of any diet studied. The weight gain (89%) of the fingerlings fed common trout chow was shown to be due to increases in moisture retention by the fish, a 7.3% increase, which was the highest of any diet. The diets of soy protein showed increased fat metabolism which could be beneficial to fingerlings raised in fish hatcheries if the fingerlings were released in the winter months.

Various combinations of the plant and animal protein in the purified diets were utilized by the fish to a greater extent than fish fed diets of only plant protein or animal protein. In the comparison of the changes in the body composition of fat, moisture, and protein in the fingerlings, only the purified diet of 50% casein-50% soy protein showed

increased fat, moisture, and protein content in the fingerlings--13%, 0.4%, and 18% respectively. The ratio of animal to plant protein (essentially amino-acids) in the 50% casein-50% soy protein diet allowed for more efficient utilization of all the nutrients in the diet than of any other experimental diet in this study. This is reflected in the 56% weight gain of the fingerlings in this diet group.

The diet of 50% casein allowed the fingerlings to build up both fat and protein (muscle tissue), a diet which the aquaculturist would find beneficial for the rearing of fingerling striped bass. To raise fingerling striped bass in unnatural conditions, such as fish hatcheries, specialized diets such as the ones in this study or natural diets must be made available to provide for proper growth and maintenance of the fingerling striped bass.

CONCLUSIONS

1. Specialized diets are needed to assure that fish are receiving the proper amounts and rations of proteins for proper growth when raised under artificial conditions.
2. Proteins used in purified diets should provide the fish with all the necessary amounts of essential amino-acids, be of high digestibility to the fish and contain no toxic substances which may interfere with protein utilization.
3. Plant protein can be used as a partial substitute for animal protein (casein) in purified diets for fingerling striped bass.
4. A lack of acceptance was indicated by fingerlings fed a diet of 100% soy protein, as reflected by the least weight gain of any fish group and a mortality rate twice that of any other group.
5. The 75% casein showed that 95% of the weight trends of the fish in this group can be explained by the fat and moisture changes in the fish. The equation which expressed this relationship is:
$$\text{Log weight} = 11.84 + 2.059 (\text{Log Fat}) + 6.383 (\text{Log Moisture}).$$
6. Fingerling striped bass fed purified diets consisting of more than 50% soy protein showed an increase in fat metabolism.
7. The only diet in the study which produced an increase in body protein, and fat moisture of the fingerlings was the purified diet of 50% casein.

8. An acceptable diet for one species of fish (common trout chow) is not necessarily adequate for other species, such as striped bass.
9. Repeated studies are needed to determine that similar results can be obtained using the same diets and fed to the same age group of fingerling striped bass.

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APPENDIX I

Closed System Design

A closed water system consisting of a fiberglass reserve tank with a one-horsepower submersible pump and a series of six 80 liter feeding tanks was designed for feeding studies with fingerling striped bass. Water from the reserve tank passed into polyvinylchloride pipe across the top of the feeding tanks. Each tank has a separate water outlet leading from the PVC pipe. Water leaving from the bottom of each feeding tank emptied into a common drainage unit of PVC pipe sloped in the direction of the reserve tank. Situated on top of the reserve tank was a gravel filter unit into which water flowed from the drainage unit (Figure 1).

The gravel filter unit consisted of a rectangular box made of plywood sides coated with epoxy and with a plexyglass bottom perforated with holes to allow water to be filtered as it flowed down into the reserve tank for recycling. A depth of 30 centimeters of gravel ranging in size from coarse sand to pebbles was used as the filtering substrata. Oyster shells were placed into the upper portion of the filter to act as a buffering system in maintaining a pH above seven. A one-half horsepower Blissfield cooling unit located in the reservoir tank maintained the water temperature between 20 - 21^oC.

APPENDIX II

Diet Preparation

The following procedure was used in the preparation of the purified diets. The de-ionized water was brought to a boil, the salt mixture was added and the pH adjusted to 8 before adding the agar. After cooling the mixture below 80°C, the vitamins and oils were added and blended. The other ingredients were then added and blended using a stainless steel rod with a propeller head, powered by a 3/8 inch electric drill. The diets were then cooled at 7°C for 3 hours and made into pellets by passing the contents through a grinder. The grinder was equipped with a head having small holes to allow contents to extrude through into long rod shapes which were then divided into smaller lengths. Diets were stored in the refrigerator between feedings of the fish.

APPENDIX III

Average Calories/Gram of Each Purified Diet

<u>Diet</u>	<u>Average Caloric Value Per Gram</u>
1. Commercial Trout Chow	4643
2. 100% Casein	5590
3. 75% Casein	5600
4. 50% Casein	5537
5. 25% Casein	5413
6. 100% Soy Protein	5497