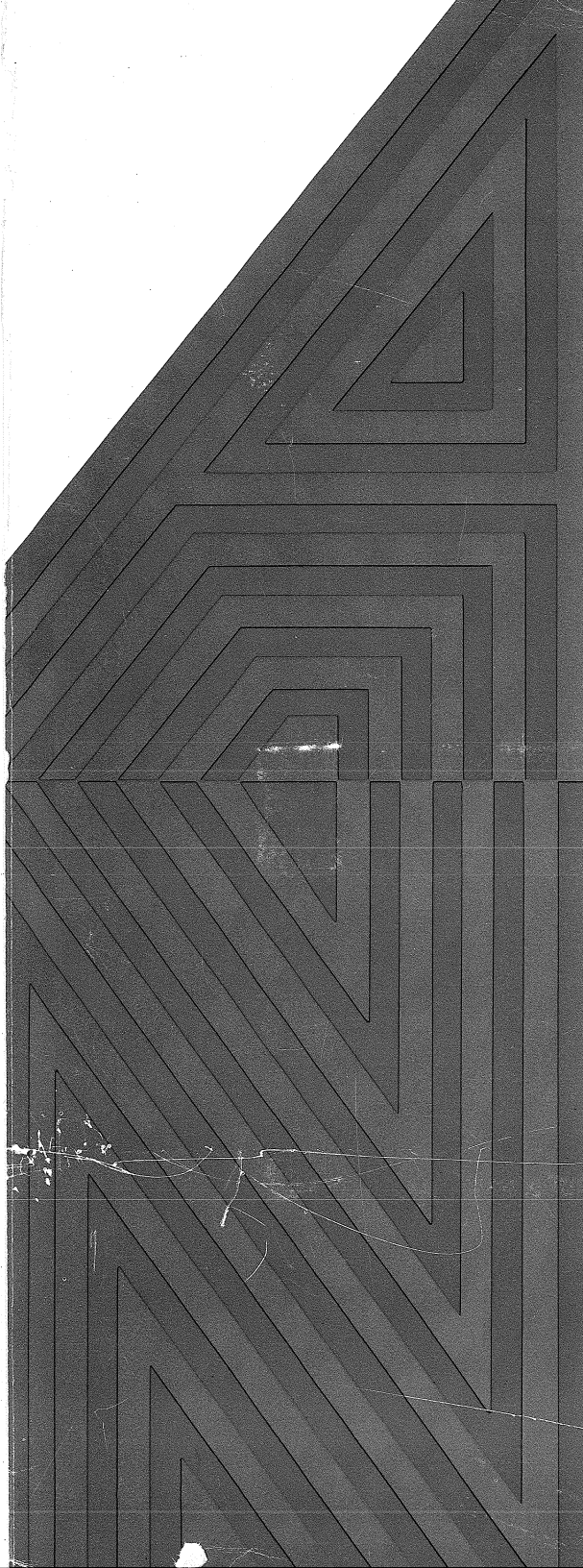




Proceedings of the

**FIRST
INTERNATIONAL
CONFERENCE
ON
AQUACULTURE
NUTRITION**

October 14-15, 1975

Sponsored by the
Sea Grant College Program
at the University of Delaware
in cooperation with the
U.S./Japan Aquaculture Panel

\$3.00

AQUACULTURE NUTRITION



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**FIRST INTERNATIONAL CONFERENCE ON
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*1st,
Lewes/Rehoboth, Del., 1975.*

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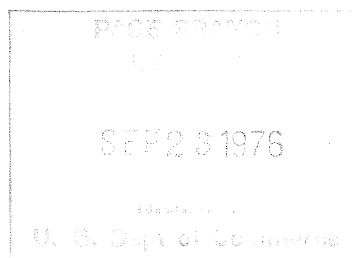
Sponsored by

the Delaware Sea Grant College Program

in cooperation with

the U.S./Japan Aquaculture Panel

Edited by: **Kent S. Price, Jr.
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PREFACE

For many thousands of years, man has pursued the goal of feeding society with convenient, economical, and nutritious foodstuffs. Curiously, the state of the art in agriculture has by far exceeded that seen in aquaculture. Now man's attention has turned to aquaculture because ever increasing population pressures are outstripping his ability to produce adequate food supplies on land.

This conference marks a milestone in the quest for knowledge concerning the production of human foods from aquatic resources. It involves representatives of other nations because in some cases their food needs are great, but in addition, their knowledge about culturing aquatic organisms for human consumption exceeds ours. There are very obvious parallels between agriculture and aquaculture, and it is the hope of the conveners and participants of this conference that for the benefit of mankind, achievements in aquaculture will eventually be equivalent to those in agriculture.

ACKNOWLEDGEMENTS

Special thanks are due the Japanese participants for providing the accumulated knowledge of the world's leading aquacultural nation, to the UJNR Panel on Aquaculture for their support and assistance in the conference, and to the National Sea Grant Program for providing the funds for conducting the conference and publishing the proceedings.

The University of Delaware Sea Grant College Program is supported in part by the Office of Sea Grant, National Oceanic and Atmospheric Administration, U. S. Department of Commerce, by the State of Delaware, and by the University of Delaware.

CONTENTS

Preface	v
Opening Remarks	
Melbourne R. Carriker.	7
Keynote Address	
<i>Nutritional Aspects of Crustacean Aquaculture</i>	
Luigi Provasoli.	13
Fish Nutrition	
<i>Nutritional Research With Salmonids</i>	
John E. Halver.	25
<i>Nutritional Studies of Red Sea Bream</i>	
Yasuo Yone.	39
<i>Studies on Nutrition and Feeds of Warmwater Fishes</i>	
Harry K. Dupree	65
<i>Diet in Yellowtail Culture</i>	
Atsushi Furukawa.	85
General Nutrition	
<i>Nutritional Consideration in the Culture of Tropical Species</i>	
John Glude	107
<i>The Role of the Feed Industry in Developing Formulated Feeds for Aquaculture</i>	
William MacGrath	119
Molluscan Nutrition	
<i>Views on Bivalve Larvae Nutrition</i>	
Ravenna Ukeles.	127
<i>Mass Rearing of Offshore-Type Bivalve Larvae in Japan</i>	
Akimitsu Koganezawa.	163
<i>Culture of Bivalve Mollusks in Recirculating Systems: Nutritional Requirements</i>	
Charles E. Epifanio	173

Recent Advances in Abalone Culture in Japan

Hisashi Kan-no195

Crustacean Nutrition

*Selected Studies of Protein and Amino Acid Requirements for
Macrobrachium rosenbergii Larvae Fed Neutral Density Formula
Diets*

Lowell V. Sick.215

Studies on the Development of Compound Diets for Prawns

John R. M. Forster229

Food and Growth of Penaeid Shrimp

Jiro Kittaka.249

Nutritional Studies of Lobsters (Homarus Americanus)

Douglas Conklin287

Summary/Participants

Summary Statement

Harold L. Goodwin299

Index of Scientific Names317

INTERNATIONAL CONFERENCE ON AQUACULTURE NUTRITION

Sponsored by
the Delaware Sea Grant Program
and the
U.S./Japan Aquaculture Panel, UJNR

October 14-15, 1975
Avenue Restaurant
Rehoboth Beach, Delaware

MONDAY, OCTOBER 13, 1975

6:00-10:00 p.m. Registration Desk Open/Pre-Conference Social

TUESDAY, OCTOBER 14, 1975

8:00-8:30 a.m.	Registration
8:30-8:45 a.m.	Welcome — Dr. Melbourne R. Carriker, Professor, College of Marine Studies, University of Delaware
8:45-9:15 a.m.	Keynote Address — Dr. Luigi Provasoli, Department of Biology, Yale University
	FISH NUTRITION
	Chairman — Dr. Jiro Kittaka, Laboratory of Aquaculture, School of Fisheries Science, Kitazato University
9:15-9:45 a.m.	Nutritional Studies of Salmonid Fishes Dr. John E. Halver, Western Fish Nutrition Lab, Bureau of Sport Fisheries and Wildlife, U.S. Department of the Interior
9:45-10:00 a.m.	Discussion
10:00-10:15 a.m.	Coffee Break
10:15-10:45 a.m.	Nutritional Studies of Red Sea Bream Dr. Yasuo Yone, Fisheries Research Laboratory, Kyushu University
10:45-11:00 a.m.	Discussion
11:00-11:30 a.m.	Nutritional Studies of Warmwater Fishes Dr. Harry K. Dupree, Director, Fish Farming Experimental Station, Stuttgart, Arkansas
11:30-11:45 a.m.	Discussion
11:45-1:00 p.m.	Lunch — Restaurant of your choice

1:00-1:30 p.m. Diet in Yellowtail Culture
Dr. Atsushi Furukawa, Director, Japan Sea Regional
Fisheries Research Laboratory

1:30-1:45 p.m. Discussion

GENERAL NUTRITION

Chairman — Dr. Atsushi Furukawa

1:45-2:15 p.m. Nutritional Considerations in Tropical Species
Mr. John Glude, National Marine Fisheries Service

2:15-2:30 p.m. Discussion

2:30-2:45 p.m. Coffee Break

2:45-3:15 p.m. Industry's Role in the Formulation of Aquacultural Feeds
Dr. William MacGrath, Ralston Purina Company

3:15-3:30 p.m. Discussion

6:00 p.m. Cash Bar and Conference Banquet/Avenue Restaurant

WEDNESDAY, OCTOBER 15, 1975

MOLLUSCAN NUTRITION

Chairman — Mr. William N. Shaw, Chairman, U.S. Aquaculture Panel, UJNR

8:30-9:00 a.m. Views on Bivalve Larvae Nutrition
Dr. Ravenna Ukeles, National Marine Fisheries Service,
Middle Atlantic Coastal Fisheries Center

9:00-9:15 a.m. Discussion

9:15-9:45 a.m. Mass Rearing of Bivalve Larvae in Japan
Mr. Akimitsu Koganezawa, Chief, Mariculture Section,
Japan Sea Regional Fisheries Research Laboratory

9:45-10:00 a.m. Discussion

10:00-10:15 a.m. Coffee Break

10:15-10:45 a.m. Bivalve Closed System Culture
Dr. Charles E. Epifanio, Assistant Professor, College of
Marine Studies, University of Delaware

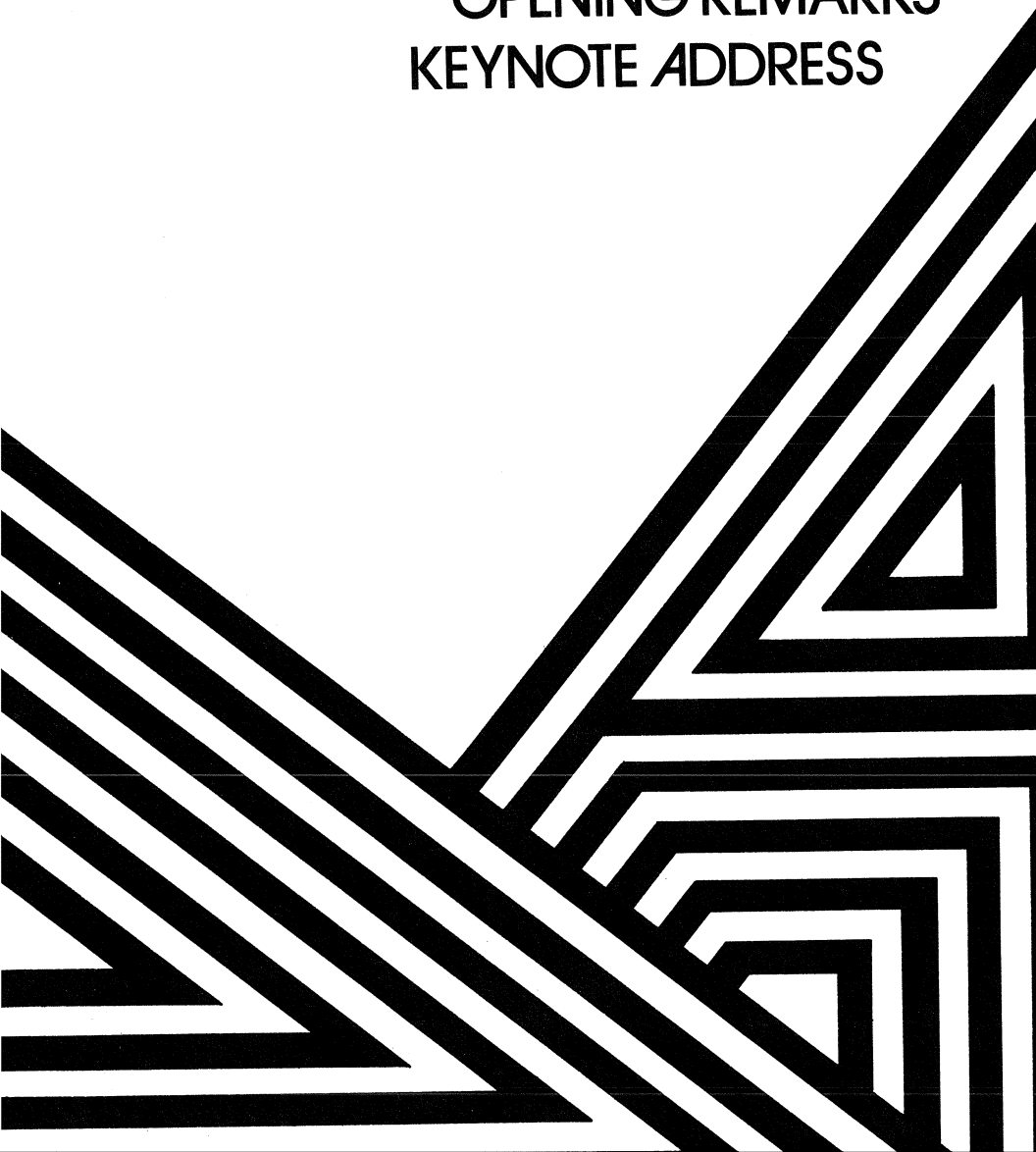
10:45-11:00 a.m. Discussion

11:00-11:30 a.m. Nutritional Considerations in Abalone Aquaculture
Dr. Hisashi Kanno, Chief, Aquaculture Laboratory,
Tohoku Regional Fisheries Research Laboratory

11:30-11:45 a.m. Discussion

- 11:45-1:00 p.m. Lunch — Restaurant of your choice
- CRUSTACEAN NUTRITION
Chairman — Dr. Hisashi Kan-no
- 1:00-1:30 p.m. Nutritional Studies of Macrobrachium
Dr. Lowell V. Sick, Assistant Professor, College of Marine
Studies, University of Delaware
- 1:30-1:45 p.m. Discussion
- 1:45-2:15 p.m. The Development of Compound Diets for Prawns
Dr. John R. M. Forster, Ministry of Aquaculture Fish and
Food, Shellfish Culture Unit, Great Britain
- 2:15-2:30 p.m. Discussion
- 2:30-3:00 p.m. Food and Growth of Penaeid Shrimp
Dr. Jiro Kittaka
- 3:00-3:15 p.m. Discussion
- 3:15-3:30 p.m. Coffee Break
- 3:30-4:00 p.m. Nutritional Studies of Lobsters
Dr. Douglas Conklin, Bodega Marine Laboratories, Univer-
sity of California
- 4:00-4:15 p.m. Discussion
- 4:15-4:30 p.m. Conference Summary
Mr. Harold L. Goodwin, Goodwin and Goodwin

OPENING REMARKS
KEYNOTE ADDRESS



Opening Comments

Melbourne R. Carriker
*Professor, College of Marine Studies
University of Delaware*

On behalf of the University of Delaware, it is my pleasure to welcome the Japanese delegation, the speakers, and the participants to this conference. The conference was developed to facilitate a better understanding of a pressing problem area, i.e., aquacultural nutrition. It is sponsored by the University of Delaware Sea Grant Program in cooperation with the United States/Japan Aquaculture Panel.

Man has practiced various forms of aquaculture for thousands of years. Most of his efforts have been directed toward manipulation of natural systems. Experience in agriculture, which enjoys the technological advantages of several hundred years of developmental effort, has shown that many crops (poultry, winter tomatoes, mushrooms, flowers, etc.) are best produced in artificial or otherwise highly controlled environments. It has become apparent to us at Delaware that such is the case with bivalve filter feeding shellfish and that the developing principles can be applied to many other marine forms.

During the late 1800's, Delaware Bay produced 23 million pounds of oysters and had substantial shad, sturgeon and other fisheries. A variety of factors, including improper management of shellfish beds (i.e. harvesting without replenishing equal quantities of shell stock), development of the watershed with attendant siltation, heavy industrialization of the Delaware River, industrial effluents and oil spills, mosquito control by ditching wetlands and spraying, and closure of harvesting areas due to sewage contamination, caused oyster production to decline steadily to 9 million pounds by 1954. By 1960, the oyster industry in the Delaware Bay region had suffered a catastrophic collapse due to a

parasitic protozoan, familiarly known as MSX (*Minchinia nelsoni* Haskin, Stauber, Mackin). In Delaware, the value of oyster landings declined from almost \$3 million to less than \$40 thousand in a few years. What has happened to the oyster in Delaware is an omen of what could happen to fisheries in other estuaries in this country and in the world.

Based on the success of artificial propagation in agriculture, scientists at the University of Delaware decided to attempt to raise marine shellfish in a closed or recycled system having a high degree of environmental control. The long range collective objective of Delaware's maricultural research is to produce successfully, on algal and/or prepared foods, desirable, fast growing, palatable, nutritious oysters, clams, and other marine forms, free of toxins and pathogens, in a controlled-environment seawater system, at reasonable cost, unhampered by legal constraints, using natural sources of energy (especially solar energy broadly considered) and recycling organic shellfish wastes. Introduction of new strains of shellfish and the successful exploitation of existing ones would provide the basis for the establishment of a new shore-based marine food industry. The College of Marine Studies/Sea Grant maricultural program on the University of Delaware Lewes campus was instituted to develop an economically and technologically viable prototype for industrial adaptation.

Successful development of this prototype will involve interdigitation of complex biological, chemical, and engineering materials and processes. It is already clear that an inclusive holistic approach on a large scale will be required. At present, there is no facility that can do more than address the problems piece-meal. Many biological and chemical aspects of maricultural research are extremely intricate, comparable in a sense to some of the research which resulted in the construction and maintenance of life-support systems for human flights in outer space. In some cases, principles and data are yet unavailable, thus designing a pilot maricultural plant is not a simple matter of scaling up an already workable technology. It includes the development of biological, microbiological, chemical, and engineering principles and data as well. The current biological and chemical research focuses on just these problems in order to provide engineers the information they require in developing the prototype for shellfish production.

The design, construction, and operation of a controlled-environment shellfish maricultural prototype is based on the following considerations:

- a strictly nontoxic physical system of economically and biologically optimal size and configuration;
- behavioral characteristics, population densities, and interactions of shellfish;
- specific nutritional requirements of shellfish at various stages in the life history;
- maximal limits of shellfish wastes consistent with a desirable marketable shellfish product;
- recurring danger of microbiota blooms resulting from dense populations of shellfish, and risks of outbreaks of shellfish diseases;
- elimination of biota potentially pathogenic or parasitic in humans; and
- the constantly changing rates of concentrations and combinations of mineral and organic chemicals in seawater.

The Delaware maricultural research and development team has demonstrated that it is possible to raise clams and oysters successfully to market size in controlled-environment recirculated seawater systems, has further refined several good algal diets that support rapid growth, and has demonstrated that certain "weed" species of algae are unsuitable shellfish foods. However, culture of the massive volumes of desired species of algae necessary to develop controlled-environment shellfish mariculture to a commercially viable system has yet to be achieved. Efforts are thus being redoubled to do this reliably. Since we do not know whether it will be possible to raise the necessary quantities of algae, we are placing additional emphasis on identification of nutritious artificial foods for possible supplemental feeding, or perhaps as algal food substitute.

Although our current research has shown that growing shellfish in closed or recycled systems is technically possible and that economic feasibility is within reach, a substantial amount of research and development remains to bring maricultural systems to the level of sophistication that has been achieved with agricultural systems.

A sample of the types of research and development activities that remain to be undertaken with shellfish include:

1. Maximum efficiency attainable in the use of sunlight by marine autotrophic organisms (i.e., food algae) as compared to the high efficiency of sorghum and very low efficiency of other land crops.

This would include research on different food species, phases of the growth curve, various nutrient media, and other environmental conditions.

2. Maximum assimilation efficiency of algal foods by filter feeders such as oysters and clams, optimum age and density of algal cells when consumed; best species for feeding; unispecies or multi-species diets; nutritional requirements to produce most nutritional algal cells (minerals, vitamins?); effect of organic catabolites from algae on duration and rates of feeding by shellfish; methods of chemically enhancing the attractiveness of algal cells to filter feeding shellfish; methods of accelerating intake of food cells and stimulating digestion; methods of improving efficiency of digestion of algae cells by shellfish.

3. Methods for avoiding contamination of closed-cycle maricultural systems by poisonous algae (like *Gonyaulax*), copepods, algal weed species, bacteria, viruses, etc.

4. Physical-chemical environment of closed-cycle maricultural systems: mineral nutrition; ionic composition of the natural seawater available; depletion of minerals by shellfish and their replacement in recycling seawater; mineral cycling through organismal chains (i.e., algae-shellfish); impact of physical and chemical perturbations on algae and shellfish as well as on the seawater itself; thermodynamic considerations (including kinetics, free energy, enthalpy, entropy); "water quality" as it applies to mariculture; possibility of discovering a ratio of ions in seawater that oysters and clams would live in, but in which parasites and disease organisms would not; search for a simple formula of a minimum number of ions (possibly sucrose, or a small molecule polysaccharide) which would satisfy osmotic requirements of shellfish and to which could be added the optimal quantities of minerals and vitamins, etc., required by the shellfish; optimal requirements in salinity, temperature, oxygen, CO₂, etc.; for ideal growth of shellfish — conditions which may not be those found in nature.

5. In genetics: breeding more nutritious, more acceptable, faster growing, algal foods; breeding thinner-shelled bivalves with larger mantle cavities and thicker mantles and other tissues that are fast growing, and more palatable to human consumers.

6. In behavior: effect of crowding of shellfish grown in closed-cycle maricultural systems on growth rate, shell size and form, taste of meats, reproduction, spawning, etc.; response of shellfish to direction and rates of flow of seawater; determining why and when shellfish open to feed, or close; shellfish adaptability to vibration, varying photo-

periods, various light intensities (photokineses); effect of elimination of such cyclic environmental phenomena as tidal cycles, lunar cycles, etc. from shellfish cultured in closed systems; effect of constant environmental conditions on the functioning of shellfish; search for alternate environmental conditions which might stimulate improved physiological activities (i.e., feeding, digestion, egestion, respiration, excretion, etc.); effect of conditions of closed-cycle mariculture on ingestion, formation of crystalline style, digestion, egestion, respiration, and excretion; possibility of reducing formation of pseudofeces.

7. Interrelation of shell deposition, i.e. shell growth, and fattening of meats; mineralogy of shell deposition in closed systems, i.e. ratios of calcite and aragonite; of mineral shell units and conchiolin etc; effect of varying concentrations of magnesium, strontium and manganese on shell composition.

8. Long term evaluation of raising algae and shellfish in artificial seawater to note effects on feeding rates, growth, quality of meats, reproduction, etc.

9. Gamete formation and spawning in closed systems, and methods of avoiding spawning in order to keep systems from being fouled by gametes; viability of gametes and development of young from shellfish grown in closed systems.

10. Chemical and taste qualities of shellfish grown in closed systems over long periods of time.

11. Chemical byproducts from shellfish: feces, excretory products (taurine, etc.); pigments from algae necessary for crustacean growth (such as carotenoid quota needed by shrimp).

12. Toxicities; oyster and algal metabolites; leechings from physical system (plastics, wood, fiber glass, etc.).

13. Potential heterotrophic habit of marine autotrophs (as per *Euglena* which is facultative): in a prototype in the absence of sunlight or artificial light, food energy can be supplemented by addition of appropriate quantities of fixed carbon (i.e., glucose).

14. Role of bacteria, viruses, yeasts, fungi, protozoa, and other microorganisms in closed systems; beneficial effects in their improving the quality of the seawater or in their use as food; potential danger from pathogens and disease.

15. Optimal sterilization procedures in maricultural operations.

16. Optimal volumes of seawater (or artificial seawater) in closed systems; rates of flow; aeration; waste removal; effect on algae of using recycled seawater.

17. Optimal engineering configurations for shellfish production ranging from family-operated units to large scale industrial units.

18. The use of solar energy in the closed-cycle maricultural production of shellfish.

19. Optimization of energy use in the production of shellfish in closed systems.

20. Processing and packaging closed system maricultural products.

In conclusion, let me emphasize that it has become readily apparent from the research conducted here and elsewhere that **significant breakthroughs** are needed in the understanding of the **nutrition** of aquacultural species, if the world is to capitalize on aquaculture as it has on agriculture. It is our hope that this conference will contribute to that understanding.

Nutritional Aspects of Crustacean Aquaculture

Luigi Provasoli

Haskins Laboratories and Department of Biology, Yale University

The long range goal of aquaculture is to emulate the success of chicken farming and commercial trout and salmon aquaculture. In both cases, knowledge of the essential nutritional requirements has allowed formulation of efficient and economical artificial diets permitting fast growth. Diets for other valuable fishes are being formulated in Japan and elsewhere. In this respect, more ingenuity will be required for finding ways to economize in labor, energy and sources of food than for methodology and new stores of basic nutritional information.

Intensive invertebrate culturing is a more severe challenge because less fundamental knowledge is available on invertebrate nutrition, physiology and behavior, and because techniques must be found to satisfy a variety of nutritional and feeding requirements. Some of these requirements, such as filter-feeding, pose particularly difficult problems. My remarks will deal with crustacean nutrition leaving fish nutrition to better qualified speakers.

My interest in crustacean nutrition started when Dr. Shiraishi, a pupil of Dr. Imai, collaborated with me. A first undefined diet was formulated for *Artemia salina* which allowed growth from new born to adults (Provasoli and Shiraishi, 1959). A more defined diet, developed in collaboration with Dr. D'Agostino, permitted identification of some of the nutritional requirements for *Artemia*: cholesterol, thiamine, pantothenic, nicotinic and folic acids, pyridoxine, riboflavin, biotin and putrescine, as well as nucleic acids were found essential for growth.

The essentiality of these requirements was confirmed in collaboration with Dr. Conklin on *Daphnia magna* (unpublished) and later on *Moina macrocopa*. Since *Moina macrocopa* is a parthenogenetic species, the problem arose of maintaining continued fertility in successive genera-

tions because the modified *Artemia* medium devised for *Moina* permitted only one or two generations. Addition of egg yolk, which alone was toxic, with incorporated vitamin E and D allowed five to six generations of *Daphnia* and over 50 of *Moina* (Provasoli, Conklin, D'Agostino, 1970). The egg yolk could be replaced by a mixture of fatty acids, lecithin, and vitamins A, D, and E. Palmitic acid and vitamin D were clearly essential for sustained fertility; addition of other fatty acids was also needed but oleic acid could replace, at least in part, linoleic and linolenic acids (Conklin, 1973). Whether vitamin E is needed as an essential molecule or acts in our diet as an antioxidant could not be established definitely. Several antioxidants do replace about half of the needed concentration of vitamin E (2 mg%) but butylated hydroxy toluene (BHT), the least toxic, became inhibitory above 1-2 mg% preventing complete replacement and leaving a doubt about the essentiality of vitamin E (Conklin, 1973).

The synthetic diet for *Moina* sustained several hundred uninterrupted generations; the only undefined component is 40-70 mg% of the Oxoid liver infusion L 25. For several reasons, work was terminated in 1973: 1) scarcity of interested graduate students; 2) the impossibility for the ONR to continue funding this type of basic research after the Mansfield amendment required military relevance; and 3) the expressed opinion that research on freshwater crustacea was not really very pertinent to research supported by a biological oceanography section. Further work indicates that hemin, biopterin, and higher concentrations of vitamins may be some of the factors present in liver infusion.

This work was done in complete asepsis, an ideal though back-breaking, experimental condition for nutritional work. Because of the lack of microorganisms and their effects, it was possible to identify with impunity the role of and need for organic substances. However, since these crustacea are filter feeders, we had our share of troubles. The imperviousness to solutes of the chitinous exoskeleton and the limited intake of environmental water during the ingestion of particulate food, demands, for effectiveness, the use of very high concentrations of soluble organics. Some of the essential organics, such as vitamins and nucleic acids, are non-toxic at the high concentration needed but all the amino acid mixtures tried were toxic for *Artemia* at a total concentration of 600 mg%. It was therefore impossible to determine the essential amino acids as well as replace energy sources provided by starch with soluble poly- or monosaccharides.

A comparison of our results and those obtained with the less chemically defined practical experimental diets for prawns and lobsters

may be interesting in singling out some common or interesting features.

In our experiments, the conditions were quite different from the practices in aquaculture. We were dealing with filter feeders grown in stagnant culture and the nutrients were offered as solutes and particulates, in the absence of microorganisms. The crustacea used in aquaculture are not filter feeders; hence, the entire diet is presented generally as "spaghetti," pellets or chunks, the water is renewed often, and bacteria are present.

Any soluble nutrient in these conditions becomes greatly diluted by the larger volume of water, is disposed of rapidly by bacteria, and therefore, cannot remain, as in our experimentation, at the high concentrations needed because of the limited imbibition of the crustacea. While a great deal of careful work has been done in obtaining appropriate texture and degree of buoyance, and in securing water stability under flowing conditions of the diets, the main preoccupation was to avoid loss of food by disintegration (a loss of 10 -15% is considered acceptable).

Decrease in weight of the bound food was observed and recorded but little consideration was given to those losses, probably because similar bonding and pelleting was used for the highly successful diets of fishes. However, it should be noted that fishes eat voraciously and rapidly consume the food given to them, while prawns, lobsters and most crustacea are nibblers and feed intermittently; therefore, most of their food remains in the water for several hours before being consumed.

My concern therefore is for the possible loss of the water soluble nutrients since in microbiology it is well known that amino acids, vitamins and minerals leach out rapidly into the supernatant from microbiological media agarized even at 3%. For these reasons, I am not surprised that supplementation of tryptophane, lysine, methionine or other amino acids added to the diet to compensate for amino acid deficiencies of the protein used, were either ineffective or effective in various degrees depending upon the other constituents of the diet (i.e., more or less leaching of solutes). The attempted replacement of proteins by a few complete amino acid mixtures (mimicking casein and other proteins), resulted in very poor growth and high mortalities of *Penaeus japonicus* (Deshimaru and Kuroki, 1974 a, b). This paralleled our failure with *Artemia* and *Moina* in replacing coagulated albumin with many amino acid mixtures even under our far more ideal conditions, i.e., no dilution in a large volume of water and no destruction because of the lack of bacteria.

Sick and Baptist (1973) noted that ingestion rates were directly proportional to amounts of freeze-dried *Artemia* in the diet and that they were inversely proportional to the length of exposure to the food: average ingestion rates were similar up to 4 h, then they declined markedly in the next 4 h, but feeding rates were still high in the high *Artemia* concentration (30%). The authors conclude that the decrease is not due to the animals being satiated but probably to the loosening of the pellets, followed by increased leaching of chemical attractants.

The possibility that high percentage loss of water-soluble essential nutrients or attractants occurs in diets could be checked easily by filtering the supernatant of the water stability test through a 0.2 μ m Millipore filter and analyzing it for C and N with conventional methods. Better still, one could include in the diet a high quantity of water soluble substances that can be either measured spectrophotometrically or seen (riboflavin, dyes) and follow their fate during compounding and after bonding by letting pieces of diet stand in a beaker with only occasional hand stirring.

Leaching could be avoided by microencapsulation of the water soluble essential nutrients. While this might be too expensive for aquaculture purposes, it would permit a reliable identification of nutritional requirements in appropriate experimental diets and would help greatly in formulating more efficient and cheap diets.

For most diets, the water soluble vitamins are supplied in a chemically-bound form as yeast cells and the fat-soluble factors as animal and vegetable oils; these conditions reduce the probability of leaching. When the vitamins are added as a mixture of pure compounds, leaching should occur; however, the diet of Kanazawa et al (1971a, b) did support growth, though only to 60 - 80% of the controls. In this case, enough vitamins for some growth should have been available to the prawns because the high proportions of refined soya protein (50%) and agar (5%) may have reduced leaching and the high concentrations of the water-soluble vitamins (mg%) used may have compensated for most of the losses by leaching. So far as I know, the essentiality of single vitamins has not been determined for the crustacea used in aquaculture and it is unfair to presume that their requirements would be the same as those for *Artemia* and *Moina*.

On the contrary, the absolute need for cholesterol of several crustacea has been established by nutritional experiments (confirming our finding for *Artemia*, *Daphnia* and *Moina*) and by demonstrating lack of synthesis from radioactive acetate and mevalonate into squalene or cholesterol (Zandee, 1964). In a series of studies, Kanazawa and collabo-

rators (1971a, b, c) have shown that *P. japonicus* utilized cholesterol better than ergo-, stigma- and β -sitosterol, that these sterols are transformed after ingestion into cholesterol, and that the spiny lobster is able to convert cholesterol into steroid hormones.

Conklin (1973) found that fatty acids are needed by *Moina* for growth and fertility and others found that the growth of prawns and lobsters benefited in appropriate diets from addition of lipids. Of the fatty acids tested by Conklin, palmitic acid was the only one that was clearly indispensable; increased fertility was directly related to concentrations of palmitic acid while the single removal from the fatty acids mix of oleic, linolenic and linoleic, lowered fertility only in a minor way. The importance of palmitic might be explained by the findings of Morris and Sargent (1973) which show that the needed fatty acids can be synthesized and that radioactive palmitic acid fed or injected in crustacea was used for the biosynthesis of wax esters and high polyunsaturated fatty acids. The beneficial effects on *Moina* of adding oleic, linoleic and linolenic acids to palmitic may confirm the hypothesis that elongation of the fatty acid chains may result from addition to pre-existing C_{16} and C_{18} polyunsaturated acids of 2-C units originating from palmitic acid.

Commercial animal and vegetable oils and fats are used in diets as sources of lipids since they supply a mixture of fatty acids, glycerides, phospholipids, sterols and fat soluble vitamins. Because these sources differ widely in their content and might be deficient in an essential component, it might be useful to investigate whether single additions of cholesterol, fatty acids, vitamin A, D, and E inhibit or elicit more growth.

The qualitative and quantitative requirements for proteins have received much attention. Some studies have correlated nutritional efficiency of the protein to their amino acids content and ratio. Deshimaru and Shigeno (1972) noted that good proteins for *P. japonicus* are rich in basic amino acids and that their content in amino acids is close to that of the short-necked clam *Venerupis philippinarum* (a good natural food) and the content of the shrimp itself.

In more defined diets, soya bean protein and egg albumin are good sources of amino acids, while casein which is widely used as the choice protein for insect diets, is inferior for crustacea. Fish, shrimp and squid protein or meals and soya bean protein seem to be considered the best sources of protein in aquaculture diets but do not support the higher growth rates elicited by fresh molluscs, squids, etc. Because feeds are often a mixture of protein sources it is difficult to express any opinion

on essentiality of amino acids. The experiments of Zandee (1966a) on *Astacus astacus* and of Cowey and Forster (1971) on *Palaemon serratus* with C₁₄-labelled precursors show that the conventional 10 amino acids are not synthesized. Knowledge of ratios of amino acids would be more important and may vary widely depending upon the type of food usually ingested in nature; vegetarians may be accustomed to and thrive on proteins deficient for omnivores and carnivores. Microencapsulated amino acid mixtures deficient in one of the ten essentials could be used to define the requirements.

Meanwhile, exploration of cheaper sources of amino acids should continue; it is interesting that petroleum yeast was found to be a good source by Deshimaru and Shigeno (1972) and the British Petroleum protein concentrate was found to be a poor source by Forster and Beard (1973). A diet based chiefly on squid meal, squid liver and meat extracts was developed by Kitabayashi et al (1971) and improved by replacing in part, the two extracts with glucosamine, CaPO₄, vitamin C and methionine.

There seems to be concordance that prawns and lobsters do far better on a high protein diet (30 - 60%). However, the ratio carbohydrates:protein has not been explored systematically — an essential factor for the formulation of cheaper diets. Since prawns are very efficient in assimilating various carbohydrate sources (Forster & Gabbott, 1971), it would be convenient to avoid as much as possible the use of proteins as energy sources.

The ratio starch:protein of 5-3:1, which was optimal for *Artemia* (Provasoli and D'Agostino, 1969), differs from the 1:1 ratio preferred by *Moina*, hence the ratio starch:protein in *Artemia* may be not typical of Crustacea. *Artemia* is an extremely euryhaline organism and changes its metabolism to osmoregulate. Hernandorena (1974, 1975a), using our media and aseptic techniques, showed the existence of a complex relationship between salinity and the ratios between energy sources, adenosine monophosphate (AMP) and protein in the diet for *Artemia*. The increase in salinity from 24 ‰ to 120 ‰ affects the metabolism of *Artemia* by simultaneously reducing the quantitative need for energy sources (either starch or lecithin), and for AMP and by increasing its quantitative need for proteins. Further experiments indicate that the increased need for protein can be relieved by addition of glutamic acid, alanine, glycine and threonine, i.e., at high salinities, non-essential amino acids take over the role of energy sources that the carbohydrates and lipids play at lower salinities. These nutritional consequences of shifts in salinity might have been overlooked in cultivating euryhalines such

as *Macrobrachium* and oysters, even though these organisms withstand minor changes in salinity.

Artemia, *Daphnia* and *Moina* are unable to synthesize the nucleic acids; the addition of a mixture of AMP (the most important), guanylic, uridylic and cytidylic acids is essential and affects total growth and growth rates. Since insects have in common with crustacea the need for cholesterol and since many insects have also an absolute requirement for nucleic acids, it is probable that most crustacea will have a similar need. Because the nucleic acids in most commercial diets are not added directly and supplied as impurities of other nutrients, it seems opportune to check periodically if shifts in constituents of diets cause a deficiency in nucleic acids. Recent work of Hernandorena (1975b) shows that the ratio protein:nucleic acid is affected also by temperature changes.

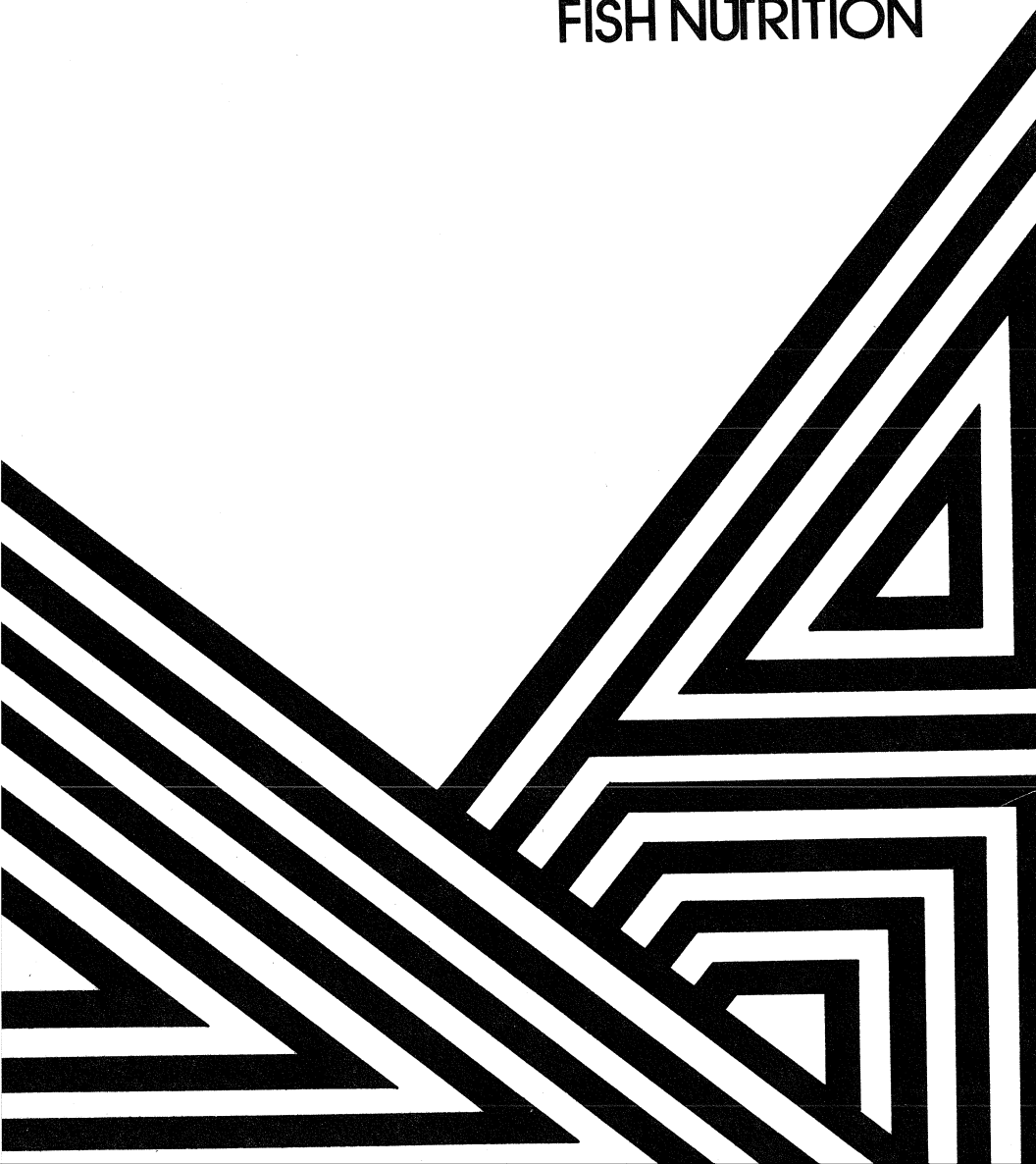
It is evident from the few examples given that formulation of complete, efficient and cheap artificial diets depends, like any other development, upon basic information in nutrition, physiology and behavior of the organism to be cultured. Many substantial, speculative investments in crustacea aquaculture have resulted in an immoral waste of capital that could have been used more profitably to foster the needed basic knowledge. The partial successes achieved are not due to formulation of efficient artificial diets but to supplementing the inadequate diets with natural food supplied by the undefined and often poorly controlled biocenosis of the artificial ponds and, economically, to the high prices that can be obtained by producing luxury food for jaded consumers. These hybrid systems have not furthered the better alternatives of either 1) controlled semi-natural food chains (polycultures) which could produce the needed protein with a minimal investment in energy, food and labor, or 2) a mass production, wholly artificial system where the high labor and energy costs are offset by a complete, efficient and cheap food as in chicken farming.

Bibliography

- Conklin, D.E. 1973. Nutritional requirements of *Moina macrocopa* in axenic culture. Ph.D. Thesis, New York University.
- Cowey, C.B. and J.R.M. Forster. 1971. The essential amino-acid requirements of the prawn *Palaemon serratus*. The growth of prawns on diets containing proteins of different amino-acid composition. *Marine Biol.* 10, 77-81.
- Deshimaru, O. and K. Shigeno. 1972. Introduction to the artificial diet for prawn *Penaeus japonicus*. *Aquaculture.* 1, 115-33.
- Deshimaru, O. and K. Kuroki. 1974-5 (a, b). Studies on a purified diet for prawn — III. A feeding experiment with amino acid test diets. *Bull. Jap. Soc. Sci. Fisheries.* 40, 1127-1131; and IV. Evaluation of protein, free amino acids and their mixture as nitrogen source. *Bull. Jap. Soc. Sci. Fisheries.* 41, 101-103.
- Forster, J.R.M. and P.A. Gabbott. 1971. The assimilation of nutrients from compounded diets by the prawns *Palaemon serratus* and *Pandalus platyceros*. *J. mar. biol. Assoc. U.K.* 51, 943-61.
- Forster, J.R.M. and T.W. Beard. 1973. Growth experiments with the prawn *Palaemon serratus* Pennant fed with fresh and compounded foods. Great Britain Ministry Agr., Fish., and Food. Fish. Investigations. Series II, 27(7), 1-16.
- Hernandorena, A. 1974. Effects of salinity on nutritional requirements of *Artemia salina*. *Biol. Bull.* 146, 238-248.
- Hernandorena, A. 1975a. Metabolic significance in nucleic acid metabolism and protein synthesis of dietary AMP requirement in *Artemia salina*. *Biol. Bull.* 148, 416-28.
- Hernandorena, A. 1975b. Effects of temperature on nutritional requirements of *Artemia salina* (L.) submitted to *Biol. Bull.*
- Kanazawa, A., N. Tanaka, S. Teshima, and K. Kashiwada. 1971. (a, b). Nutritional requirements of prawn. II. Requirement for sterols; III. Utilization of the dietary sterols. *Bull. Jap. Soc. Sci. Fisheries.* 37, 211-15 and 1015-19.
- Kanazawa, A., and S. Teshima. 1971c. *In vivo* conversion of cholesterol to steroid hormones in the spiny lobster, *Palinurus japonica*. *Bull. Jap. Soc. Sci. Fisheries.* 37, 891-97.

- Kitabayashi, K., H. Kurata, K. Shudo, K. Nakamura, and S. Ishikawa. 1971. Studies on formula feed for Kuruma prawn - I-V Bull. Tokai Reg. Rish. Res. Lab No. 65, 91-147.
- Morris, R.J. and J.R. Sargent. 1973. Studies on the lipid metabolism of some oceanic crustaceans. Mar. Biol. 22, 77-83.
- Provasoli, L. and A. D'Agostino. 1969. Development of artificial media for *Artemia salina*. Biol. Bull. 136, 434-453.
- Provasoli, L., D.E. Conklin, and A. D'Agostino. 1970. Factors inducing fertility in aseptic crustacea. Helgolander wiss. Meeresunters. 20, 443-454.
- Provasoli, L. and K. Shiraishi. 1959. Axenic cultivation of the brine shrimp *Artemia salina*. Biol. Bull. 117, 347-355.
- Sick, L.V. and G. Baptist. 1973. Effects of selected physical and nutritional factors on rates of pelleted diet ingestion by postlarval penaeid shrimp. J. Elishia Mitchell Sci. Soc. 89, 320-29.
- Zandee, D.J. 1964. Absence of sterol synthesis in some Arthropods. Nature. 202 1335-36.

FISH NUTRITION



Nutritional Research With Salmonids*

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Any balanced formula for fish diets must include an energy source plus sufficient indispensable amino acids, essential fatty acids, specific vitamins, and minerals to support life and to promote growth. Over the past twenty years, tentative requirements for the five classes of nutrients in the diet of rainbow trout (*Salmo gairdnerii*) have been accumulated (1). The requirements have been found to change with fish size, with water temperature, and with the balance of components in the ration (2). These data have been translated into practical fish diets through least cost computation using fish feed ingredient nutrient analysis. As dividends from this research and development effort, practical rainbow trout rations can now be formulated all over the world from locally available dietary ingredients and can be used with a reasonable degree of assurance to rear rainbow trout to either market size or to broodstock for replacement stock for the trout industry. The same story is not so clearly defined for other species of fish, and it is appropriate to review what we know about specific nutrient requirements for the other salmonids. Since protein is required by fish in such large amounts, and since it is often the most expensive component in satisfactory fish diets, the knowledge of protein needs that we have accumulated will be discussed first.

Every fish examined to date requires the same ten indispensable amino acids for growth (3, 4). Arginine, histidine, isoleucine, leucine,

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lysine, methionine, phenylalanine, threonine, tryptophan, and valine must be in the diet and must be present in near-balanced amounts to furnish an acceptable amino acid pattern before fish flesh can be formed. These indispensable amino acids in proper proportion are then determinants for the success or failure of any fish husbandry program.

Many practical rations used in salmonid fish production programs in the United States have been assayed for amino acid content (1, 5, 6, 7,). These values compare closely with the amino acids present in fish egg protein and with chicken egg protein (6). Therefore, most of the amino acid test diet work and subsequent determinations of quantitative amino acid requirements have used the amino acid pattern of whole egg protein as a standard reference protein for these studies. Fish that are fed diets devoid of any indispensable amino acid fail to grow, whereas other fish fed diets devoid of dispensable amino acids grow as well as control-diet fed fish receiving all amino acids in the reference protein (6). Arginine was determined to be an indispensable amino acid for salmonids (8, 9, 10). Lysine, the first probable limiting amino acid in cereal grain or oil seed protein components, also determined the rate of growth of fish. A direct response was also obtained with methionine content in fish diet, with growth rate determined directly by percent of methionine present. Likewise, tryptophan was required, and scoliosis and lordosis appeared when low levels of this amino acid were present in the ration (8, 9, 10, 11).

A new simple technique has shown that all fish tested required the same ten indispensable amino acids (12). Fish can be given intraperitoneal injections of 14-carbon uniformly labeled glucose. After radioactivity in the expired carbon dioxide has been measured for several days, the fish are sacrificed, the tissue protein is isolated and hydrolyzed, and then the constituent amino acids are resolved and purified. These amino acids are examined on paper chromatography for radioactivity. Those amino acids that contain radioactive material were obviously synthesized by the fish from other metabolites and are not essential dietary constituents. Conversely, those amino acids that do not contain radioactivity could not have been synthesized by the fish. These are classified as indispensable and necessary to be present in the dietary.

Quantitative amino acid requirements have been determined by using protein reference diets or analyzing dietary ingredients in fish feed ingredient mixtures (13). Protein test diet formulations have been used successfully in several species. Protein treatments showed that the apparent protein requirement was about fifty percent of the dry diet in-

redients for fry, with a reduction in requirement as fish increased in size (1). Trout and salmon have the same apparent protein requirement when the animals are raised at the same temperature.

Some experiments have shown that the protein requirement increased in salmonids as the salinity increased and that the protein requirement of rainbow trout increased from forty percent of the diet at 10 ppt salinity to forty-five percent of the diet in waters of 20 ppt salinity (14). The same phenomenon was observed with coho salmon (*Oncorhynchus kisutch*) on feeding trials that measured brackish water saline content (15). Other salmonids also showed a high protein requirement for very young fish that decreased with age.

The protein requirement of the fish has a direct bearing upon the indispensable amino acid pattern of formulated test and practical rations. Quantitative indispensable amino acid requirement studies have been conducted on salmonids. Test diets have been prepared that enable linear increments of one amino acid at a time to be fed as different diet treatments in feeds containing some whole protein components to stimulate the digestive and absorptive processes. Almquist-type plots of the growth response to these different diet treatments produced typical inflection points for each of the ten different indispensable amino acids when the test diets contained arginine, 2.5%; histidine, 0.7%; isoleucine, 1.0%; leucine, 1.5%; lysine, 2.1%; methionine, 0.5%; phenylalanine, 2.0%; threonine, 0.8%; tryptophan, 0.2%; and valine, 1.5% (16). Subsequent experiments have showed a sparing action of cystine on the methionine requirement, and of tyrosine on the phenylalanine requirement. The combined methionine and cystine requirement appears to be about 1.5% of the dry ration, with the best growth observed when 1% of cystine and 0.5% of methionine were fed to salmonids. Approximately one-third of the phenylalanine growth requirement could be spared by L-tyrosine in some experiments (17).

Toxic effects of gross imbalance of some of the indispensable amino acids were also observed. An inhibition of growth and diet performance was observed when either isoleucine was threefold in excess of leucine or when threefold leucine was fed with the isoleucine held at the requirement. High intake of valine also inhibited growth in salmonids (18).

DL-mixtures of amino acids have been tested, and little growth was obtained in salmonids when three or more DL forms of indispensable amino acids were incorporated in the diet. D-amino acid oxidase has been isolated and measured in young salmon and trout and in actively feeding sea salmon in the ocean environment. Much variation was obtained in D-amino acid oxidase activity between fish and between

the seasons of the year in which samples were obtained and processed, indicating that it would be unreliable to expect salmonids to process excessive amounts of d-amino acids in the dietary. Toxic effects from these enantiomorphous forms of amino acids can be expected (17).

Nitrogen sources for fish have been examined, and little sparing effect on the whole egg protein requirement was obtained with urea or diammonium citrate (19). Arginine could be effectively used to spare dispensable amino acid protein, but glycine had only a minor sparing effect on protein requirement in salmonids. Thus, fish react differently from other experimental animals in their ability to use simple nitrogen sources as intermediates for dispensable amino acid synthesis.

A practical method to estimate indispensable amino acid requirements of fish is to examine the proportion of each present in the protein component of the ration. An example is the amino acid requirements of salmonids when forty percent of the diet is protein fed to young, rapidly growing fish at 10°C. A remarkable and consistent percentage of the protein for each is obtained when the gram percent quantities of the individual amino acids are compared among rainbow trout, chinook salmon (*Oncorhynchus tshawytscha*), and coho salmon (20).

Slight differences are observed only between histidine and isoleucine requirements and phenylalanine, threonine, and valine requirements. Only lysine shows a major difference between species when the gram percent quantities are extended into percent of dietary protein. The gram percent of the indispensable amino acid required may drop as the fish grow larger, but so does the protein requirement. Thus the percent of the amino acids in the dietary protein is a reasonable measurement of amino acid requirement (20).

Sufficient energy must be present in the diet to convert these ingested amino acids into tissue, and the calories per gram protein ratio will affect Protein Efficiency Ratio and Net Protein Utilization. Salmonids can use some digestible carbohydrate as an energy source, but cooked or processed starch in common fish diet ingredients must be used before many digestible energy calories can be obtained from these materials (21). Therefore, not only the type of diet ingredients but the method and degree of processing will determine the availability of the energy in this component to salmonids and several other fishes studied. Monosaccharides are best, followed in order by disaccharides, simple polysaccharides, dextrins, cooked starches, and raw starches. Some fish harbor gut bacteria which contain cellulases that can hydrolyze and

make available some monosaccharides from cellulose. The role of fat in furnishing the energy source will be discussed later.

Most of the amino acid quantitation studies have been conducted with very digestible protein components to furnish the amino acids or with amino acid mixtures which will guarantee rapid absorption of the test nutrient that is fed. This is a logical technique to catalog the indispensable amino acid requirements and to measure the sparing effect of certain dispensable amino acids on these requirements for various species of fish reared in defined experimental conditions. Much more work must be done with important commercial types of fish confined in our fish husbandry production facilities, because information on the indispensable amino acid requirements and a good pattern for fish rations will allow least cost computer formulation of practical rations and will improve the efficiency and economics of the industry. However, when testing amino acid patterns in practical rations, one must first calculate that portion of the protein component that is digested and absorbed. Only if the amino acids are absorbed and contribute to tissue synthesis can protein be deposited for fish tissue repair and for growth.

"Chemical score," the ratio of indispensable amino acids present in the finished feed component to the indispensable amino acids present in whole egg protein, is only a partial measure of what the fish actually get from the protein fed (22). The amino acids that would be limiting as far as the requirement of the animal is concerned for that particular ration, can be calculated, but a more finite prediction can be obtained when the digestibility coefficient of the protein component is measured by pepsin or papain digestion, by formal titration, or by pancreatic digestion techniques. Then a better approximation of the biological value of the protein component can be obtained. A much better approximation of the biological value of the protein component can be obtained from metabolism chamber experiments, where the actual percent of protein or specific nutrients fed is measured and compared with the metabolic wastes that are collected and analyzed for nitrogen or for other nutrient components. Large fish have been used in these metabolism chambers to measure what proportion of the feed is actually digested, absorbed, and metabolized by the fish. The assays give a tentative digestibility coefficient for that particular species of fish for the components in that diet ingredient tested, either alone or in combination with other diet components for which Gross Energy, Digestible Energy, Metabolizable Energy, and digestibility coefficients of the nitrogen and energy components have been established. More

recently, techniques have been developed in closed aquarium systems for very small fish that will adapt to the closed confinement. The results have been compared with large fish tested in metabolism chambers. Oxygen use, carbon dioxide generated, and soluble nitrogen wastes in fish feed give an estimate of the energy needs of the fish under various stages of resting or active metabolism. These values vary with the temperature, with the species, with the size, and with the stress invoked.

A combination of these techniques can be used to determine the "nutritional score" of the ingredients in the ration. Arginine will be found limiting in many of the components, since most fish tested require about six percent of the protein to be arginine for the most efficient growth. Also, arginine can be used as a nitrogen supplement to spare other whole protein requirements in the diet of salmonids or ictalurids, and therefore deserves special mention and consideration. Methionine, and methionine plus cystine, will be found limiting in single cell protein diet ingredients and in larval protein components of fish diets. Lysine is the first limiting amino acid in many cereals and in grass components. Tryptophan, because of its higher requirement, will become limiting in many rations. It deserves careful scrutiny, since it is easily destroyed under acidic conditions of diet ingredient storage such as in fish silage or in moist pellet manufacturing processes. The great reactivity of the epsilon amino group of lysine with aldehydes in sugars, forming Maillard type compounds, may mislead the investigator if diet formulations are based solely upon analysis of diet ingredients or finished feeds for indispensable amino acid content. Obviously, biological availability of these reactive amino acids is necessary, and simple amino acid analyzer values do not reflect either the optically active form of the amino acid or the biological availability of it to the fish fed. However, with these constraints in mind one can make tentative predictions for the indispensable amino acid requirement and an approximate pattern for fish to produce reasonable growth.

A first approximation for an acceptable amino acid pattern would be the indispensable amino acid requirements present in the digestible protein component absorbed by that species of fish, at the size, age, stress, and temperature measured, plus the balance of the protein component made up of dispensable amino acids in the pattern found in whole egg protein. A usable approximation is the individual percent of protein occupied by indispensable amino acids present in the digestible protein fraction of the ration, with the dispensable amino acids constrained not to exceed two times the level found in whole egg protein

or fish flesh. These values can be used for least cost computer formulation of the ration, provided the digestibility coefficient for the nitrogen component is added as a function of the calculations.

Fats are a ready source of energy for fish, and most studies indicate that fish can use 20-30% of the dry diet ingredients as fat, provided adequate amounts of choline, methionine, and tocopherol are present in the ration (24). Fats have the distinct advantage of being almost completely digestible. Fish appear to be designed to metabolize fats efficiently as an energy source, with an accompanying sparing effect on the protein requirement to produce maximum growth. The neutral lipid component of the fish ration is therefore a useful component for diet formulation and is especially desirable in fry or fingerling foods when higher energy intake is necessary to promote rapid growth. This asset is not without serious constraints, however, because some fish do not have the specific ability to elongate short chain fatty acids and then subsequently unsaturate the carbon chain to convert simple animal or vegetable fats into polyunsaturated long chain fatty acids characteristic of fish tissue lipids (25). Essential fatty acid requirements have been demonstrated for several species of fish, and while linolenic acid appears to satisfy some of the growth requirement, more polyunsaturated and longer chain fatty acids of the C₂₂ or C₂₄ omega-3 series are required for maximum growth and efficient diet use. These polyunsaturated fatty acids have accompanying requirements for adequate amounts of tocopherols to prevent fatty acid peroxidation, chain rupture, and subsequent toxic reactions in the liver and spleen. Fats do form an important part of practical fish rations for energy and, in addition, must supply the essential fatty acid requirements for that particular species of fish raised.

Vitamin deficiency diseases have been described for many species of fish (26). Test diets are available which can induce specific water soluble or fat soluble vitamin deficiency symptoms in salmonids, in ictalurids, in cyprinids, in eel, and in several marine fish. The water soluble vitamins function in fish metabolism as in homeothermal metabolism. The primary difference is one of reaction rates of the enzymes involved in poikilothermic animals reared in variable water temperatures. The water soluble vitamins are part of coenzyme systems in the same fundamental biochemical pathways as in other animals. Thus deficiencies induce specific metabolic system failures with resultant deficiency signs. These have been described and compared with other experimental animals by several groups of workers (26). Tentative requirements in terms of milligrams of vitamins per kilogram of body weight per day

have been listed as: thiamin, .13-2.0; riboflavin, 0.75-1.0; pyridoxine, .38-.42; choline, 50-70; niacin, 5-7; pantothenic acid, 1.3-2.0; inositol, 18-20; biotin, 0.03-0.04; folic acid, 0.1-0.15; vitamin B₁₂, 0.0002-0.0003; ascorbic acid, 2-3 (1). Sparing effect of one vitamin or vitamin precursor on others has not been thoroughly investigated, and specific differences in requirements of animals on different carbohydrate or fat intake and different protein intake has not been adequately measured. Since the requirement for thiamin is related to the carbohydrate intake in most other animals studied, this vitamin may become more important for certain carbohydrate-consuming species of fish such as the grass fishes, the mullets, and many others which eat phytoplankton or are herbivorous. Likewise, requirements for pyridoxine are related to the protein level in the ration; and, as a consequence, pyridoxine is one of the first vitamins exhausted in carnivorous fish fed low pyridoxine diets. The requirement for ascorbic acid may vary with the rate of protein synthesis or with the stress under which the fish is held. For example, the ascorbic acid intake to promote most rapid growth for long term salmon and trout experiments at 50°C needed to be increased three- to fivefold to promote the most rapid rate of wound repair when the fish were stressed with a 1 cm incision through the skin into the abdominal cavity or above the lateral line into the musculature (27). More work must be done in this area to determine specific requirements of these water soluble vitamins for different dietary treatments for different species, and for the different size, water temperature, and stress experienced.

The fat soluble vitamin requirements have been less discretely defined than have the water soluble vitamins. Tissue storage of the fat soluble vitamins is accumulative, and hypervitaminosis can also be expected to be encountered when high intake of diet ingredients containing large amounts of fat soluble vitamins are fed to fish for long times. Qualitative fat soluble vitamin experiments have disclosed that vitamins A, K, and E are required for normal growth and maturity of salmonids. Typical deficiency symptoms occurred when these components were absent from the diet. Vitamin D has not been shown to be required for normal growth and function of salmonids in high calcium water, but recent work has disclosed that vitamin D₃ exercises a beneficial effect to promote absorption of calcium from the gut in certain water systems and exercises a growth promoting effect in several test situations (28). Hypervitaminosis D has also been reported to occur in salmon fed diets containing large amounts of fish liver oil. It occurs as acute liver toxicity, growth inhibition, poor food conversion, and elevated alkaline phos-

phatase activity in the blood. Therefore, fish have specific quantitative requirements for the fat soluble vitamins, as do other experimental animals, and these requirements can be altered by the particular species of fish, the size, the water temperature, and the environmental and dietary conditions under which the animals are reared.

Little is known about the trace mineral requirements for these enzyme activators for fish. Macro-mineral requirements have been studied with a few species under carefully controlled experimental conditions (1). Calcium is known to be sequestered from the water through the gill tissues in trout. Likewise, calcium can be absorbed through the gut when adequate amounts of vitamin D₃ are present in the ration. Several calcium:phosphate balance ratio studies have indicated that most practical fish diets contain adequate amounts of calcium but inadequate amounts of phosphate. Also, the particular form of phosphate must be analyzed in diet ingredients because of the unavailability of phytic salts which are commonly present in agricultural products. Therefore, the type of phosphate and its availability to the fish should be measured, and gross chemical analysis should not be relied upon for computer data bank input for specific diet ingredients. A sodium:potassium balance is especially important in fish in a sea environment or in fish moving from one ionic strength environment to another such as in anadromous migrating smolts. Sodium-potassium activated gill ATPase activity offers a convenient tool for determining the ability of the young smolt to activate the sodium pump and thus convert to a sea environment. Dietary pretreatment with high sodium diets stimulates early elevated activity of this compound when the fish have been environmentally conditioned to smolt and then migrate to the sea.

One of the first recorded specific trace mineral deficiency symptoms in rainbow and brook (*Salvelinus fontinalis*) trout was described fifty years ago when fish were reared on diets deficient in iodide (29). Acute proliferation of thyroid tissue, a compensatory reaction, occurred, and fish developed typical goiter. In advanced cases, thyroid proliferation would actually invade surrounding tissue and was at first mistaken for neoplasia. However, intermediate stage thyroid proliferation could be reduced with adequate intake of iodide. Recent work with cobalt has indicated that it is not incorporated into vitamin B₁₂ in fish tissues and that the total amount of cobalt present in fish, especially in the fish embryo, is present in the form of organically bound vitamin B₁₂. The role of dietary cobalt to stimulate the production of vitamin B₁₂ by gut bacteria has not been clearly defined, and, as a consequence,

small amounts of cobalt are still used as a supplement in practical fish diets. The other classical trace mineral elements are probably also required to activate several enzyme systems in actively growing fish, and, as a result, they are included in the trace mineral supplement in diet formulations.

In summary, one can say that tentative requirements have been determined for one or two species for the water soluble vitamins, for the fat soluble vitamins, for the ten indispensable amino acids, for gross protein needs, and for some essential fatty acids. These values plus a fifty to one hundred percent safety factor have been incorporated into diet ingredients for least cost computer formulation of practical fish diets. Much more work must be done to define specific requirements for other species which are being fed these rations and to determine specific requirements of such diet cost determinants as protein and amino acid needs for a particular fish and a particular environment at a particular temperature. Tools have been fashioned, techniques have been developed, and principles have been confirmed for systematic and orderly nutritional research capability with fish that will yield (1) specific nutrient requirement values; (2) the digestibility coefficients; and (3) the sparing effect of one nutrient upon another as practical, effective, efficient fish rations are formulated from the diet ingredients available from our agricultural and aquacultural industries.

REFERENCES

1. National Academy of Sciences-National Research Council. 1973. Nutrient requirements of trout, salmon, and catfish. Nutrient requirements of domestic animal series, No. 11. National Academy of Science, Washington, D.C.
2. Halver, J. E. (ed.) 1972. Fish nutrition. Academic Press, New York, N.Y.
3. Cowey, C. B., and J. R. Sargent. 1972. Fish nutrition. Adv. Mar. Biol. 10: 383-492.
4. Mertz, E. T. 1972. The protein and amino acid needs, *In* Fish nutrition. J. E. Halver (ed.) Academic Press, New York, N.Y. p. 105-143.
5. Wood, E. M., W. T. Yasutake, A. M. Woodall, and J. E. Halver. 1957. Nutrition of salmonoid fishes, II. Studies on production diets. J. Nutr. 61: 479-488.
6. Halver, J. E. 1957. Nutrition of salmonoid fishes, IV. An amino acid test diet for chinook salmon. J. Nutr. 62: 245-254.
7. Halver, J. E. 1972. Appendix, *In* Fish nutrition. J. E. Halver (ed.) Academic Press, New York, N.Y. p. 660.
8. Halver, J. E., D. C. DeLong, and E. T. Mertz. 1957. Nutrition of salmonoid fishes, V. Classification of essential amino acids for chinook salmon. J. Nutr. 63: 95-105.
9. Halver, J. E., and W. E. Shanks. 1960. Nutrition of salmonoid fishes, VIII. Indispensable amino acids for sockeye salmon. J. Nutr. 72: 340-346.
10. Shanks, W. E., G. D. Gahimer, and J. E. Halver. 1962. The indispensable amino acids for rainbow trout. Prog. Fish Cult. 24: 68-73.
11. Dupree, H. K., and J. E. Halver. 1970. Amino acids essential for the growth of channel catfish, *Ictalurus punctatus*. Trans. Amer. Fish. Soc. 99: 90-92.
12. Cowey, C. B., J. Adron, and A. Blair. 1970. Studies on the nutrition of marine flatfish. The essential amino acid requirements of plaice and sole. J. Mar. Biol. Assoc. U. K. 50: 87-95.
13. DeLong, D. C., J. E. Halver, and E. T. Mertz. 1958. Nutrition of salmonoid fishes, VI. Protein requirements of chinook salmon at two water temperatures. J. Nutr. 65: 589-599.

14. Zeitoun, I. H., J. E. Halver, D. E. Ullrey, and P. I. Tack. 1973. Influence of salinity on protein requirements of rainbow trout (*Salmo gairdneri*) fingerlings. J. Fish. Res. Board Can. 30: 1867-1873.
15. Zeitoun, I. H., D. E. Ullrey, J. E. Halver, P. I. Tack, and W. T. Magee. 1974. Influence of salinity on protein requirements of coho salmon (*Oncorhynchus kisutch*) smolts. J. Fish. Res. Board Can. 31: 1145-1148.
16. Halver, J. E. 1970. Nutrition in marine aquaculture, *In* Marine aquaculture. W. J. McNeil (ed.) Ore. State Univ. Press, Corvallis, Ore. p. 75-102.
17. Halver, J. E. 1970. Cold-water fish nutrition requirements. U. S. Bur. Sport Fish. Wildl. Resour. Publ. 102: 141-152.
18. Chance, R. E., E. T. Mertz, and J. E. Halver. 1964. Nutrition of salmonoid fishes, XII. Isoleucine, leucine, valine and phenylalanine requirements of chinook salmon and interrelations between isoleucine and leucine for growth. J. Nutr. 83: 177-185.
19. DeLong, D. C., J. E. Halver, and E. T. Mertz. 1959. Nutrition of salmonoid fishes, VII. Nitrogen supplements for chinook salmon diets. J. Nutr. 68: 663-669.
20. Mertz, E. T. 1969. Amino acid and protein requirements of fish, *In* Fish in research. O. W. Neuhaus and J. E. Halver (eds.) Academic Press, New York, N.Y. p. 233-244.
21. Buhler, D. R., and J. E. Halver. 1961. Nutrition of salmonoid fishes, IX. Carbohydrate requirements of chinook salmon. J. Nutr. 74: 307-318.
22. Hastings, W. H. 1969. Nutritional score, *In* Fish in research. O. W. Neuhaus and J. E. Halver (eds.) Academic Press, New York, N.Y. p. 263-292.
23. Smith, R. R. 1971. A method for measuring digestibility and metabolizable energy of fish feeds. Prog. Fish Cult. 33: 132-134.
24. Lee, D. J., and R. O. Sinnhuber. 1972. Lipid requirements, *In* Fish nutrition. J. E. Halver (ed.) Academic Press, New York, N.Y. p. 145-180.
25. Yone, Y., M. Furuichi, and S. Sakamoto. 1970. Studies on nutrition of Red Sea bream—III. Nutritive value and optimum contents of lipids in diet. Rep. Fish. Res. Lab., Kyushu Univ. 1: 49-60.
26. Halver, J. E. 1972. The vitamins, *In* Fish nutrition. J. E. Halver (ed.) Academic Press, New York, N.Y. p. 29-104.

27. Halver, J. E. 1972. The role of ascorbic acid in fish disease and tissue repair. Bull. Jap. Soc. Sci. Fish. 38: 79-92.
28. Poston, H. A. 1969. Effects of massive doses of vitamin D₃ on fingerling brook trout. Cortland Hatchery Rep. 37 (1968). Fish Res. Bull. No. 32, p. 48-50. N. Y. Cons. Dep.
29. Gaylord, H. R., and M. C. Marsh. 1914. Carcinoma of the thyroid in the salmonoid fishes. U. S. Bur. Fish., Washington, D. C. Bull. 32.

Nutritional Studies of Red Sea Bream

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INTRODUCTION

Studies on nutrition of freshwater fish, e.g., salmonid, carp and channel catfish, have been done by numerous researchers. Marine fish are living in seawater possessing high osmotic pressure and various inorganic elements. Therefore, it is supposed that the appropriate dietary composition and the indispensable nutrients required by marine fish may be different to some extent from those required by freshwater fish. Studies on marine fish, however, have been few, when compared with studies on freshwater fish. Among marine fish, the nutritional requirements of red sea bream were intensively studied in our laboratory — this fish is one of the most important and expensive marine fish in Japan and its aquaculture has been under development in various districts.

In this paper, some district features in nutritional studies of red sea bream are presented and discussed.

PURIFIED TEST DIET

We found that Halver's test diet for salmonids was apparently not suitable to the taste of red sea bream (Yone, Furuichi and Shitanda, 1971). Lack of a suitable synthetic diet that is capable of sustaining normal growth of the fish for long periods, has hampered progress in studies of its nutritional requirements. To establish a purified synthetic test diet suitable for study on the nutrition of red sea bream, Yone et al made attempts to modify the test diet developed by Halver (Yone, Sakamoto and Furuichi, 1974). The modified aspects were: 1) the

addition of phenylalanine and aspartic acid capable of attracting the fish; 2) the replacement of corn oil with a pollack residual oil; 3) the modification of the mineral mixture; and 4) a further additional supplement of certain essential amino acids such as arginine, tryptophane and valine, as shown in Table 1. Red sea bream accepted the modified diet

TABLE 1

Composition of test diets for nutritional requirements of red sea bream.

diet				
ingredients	MBL	YR-1	YR-2	YR-4
Casein	52	52	52	54
Gelatin	11	11	11	12
L-Phe	0.6	0.6	0.6	0.6
L-Arg • HCL	1.3	1.3	1.3	1.5
L-Cys	0.7	0.7	0.7	
L-Try	0.2	0.2	0.2	0.2
L-His • HCl • H ₂ O	0.2	0.2	0.2	
DL-Ala	1.3	1.3	1.3	
L-Asp • Na	1.0	1.0	1.0	1.0
L-Lys • HCl	0.6	0.6	0.6	
L-Val	0.7	0.7	0.7	0.7
Gly	0.4	0.4	0.4	
P.R. Oil*	8.9	9	9	9
Dextrin	14.1	8	8	8
Minerals	4 [#]	8 ^{##}	8 ^{**}	8 ^{**}
Vitamins [#]	6.134	0.6134	0.6134	0.6134
α-Cellulose	2.3866	2.3866	4.3866	4.3866
Water	200	200	200	200

[#] The composition of mineral and vitamin mixtures were identical to those reported by HALVER.

* P.R. Oil (Pollack residual oil) is one obtained after molecular distillation of vitamin A from Alaska pollack liver oil.

^{##} P-2:NaH₂PO₄ • 2H₂O 30.81, Ca-lactate 19.74, KCl 5.18, Fe-citrate 1.49, AlCl₃ • 6H₂O 0.009, ZnSO₄ • 7H₂O 0.1785, MnSO₄ • 4~6H₂O 0.04, CuCl 0.0055, KI 0.0085, CoCl₂ • 6H₂O 0.0525, α-Cellulose 43.084.

^{**} MP-2:KCl 5.2, NaH₂PO₄ 30.8, Fe-citrate 1.5, AlCl₃ • 6H₂O 0.009, ZnSO₄ • 7H₂O 0.1785, MnSO₄ • 4-6H₂O 0.04 CuCl 0.0055, KI 0.0085, CoCl₂ • 6H₂O 0.0525, α-Cellulose 62.206.

very well, and the growth and feed efficiency were considerably improved. In our studies, a modified diet YR-1 was primarily employed as the basal diet.

PROTEIN AND AMINO ACID REQUIREMENTS

With diets containing 35-70% protein (casein and gelatin) and 40-100% carbohydrate (dextrin or glucose), the growth rate of red sea bream showed a linear elevation with the increase of dietary protein level, up to approximately 55% protein (dry weight basis), as shown in Figure 1 (Furuichi and Yone, unpublished). Since studies of an optimal dietary protein level for red sea bream are still not sufficient, it seems too early to establish the level.

An amino acid test diet was devised by making its amino acids composition closely comparable to that of beef liver, which produced the best growth in red sea bream (Table 2). In a qualitative test for

TABLE 2

Composition of test diet for amino acid requirement.

Amino acids	48.2	Minerals	
Dextrin	10.0	KCl	5.180
P. R. oil	9.0	NaH ₂ PO ₄ · 2H ₂ O	43.056
Minerals	8.0	Fe-citrate	1.485
Vitamins*	3.0	Ca-lactate	32.883
C.M.C.	10.0	Trace metals*	0.294
α -Cellulose	11.8	Dextrin	17.690
Water	40.0		

Amino acids			
L-Arg · HCl	3.73	Gly	2.93
L-Lys · HCl	4.27	L-Met	1.07
L-His · HCl · H ₂ O	1.73	L-Cys	0.60
L-Phe	2.53	L-Try	0.60
L-Tyr	1.47	L-Glu	4.80
L-Leu	3.40	L-Asp · Na	4.60
L-Ileu	2.33	DL-Ala	5.47
L-Thr	1.67	L-Pro	2.20
L-Val	3.13	L-Ser	1.67

* Halver's mix

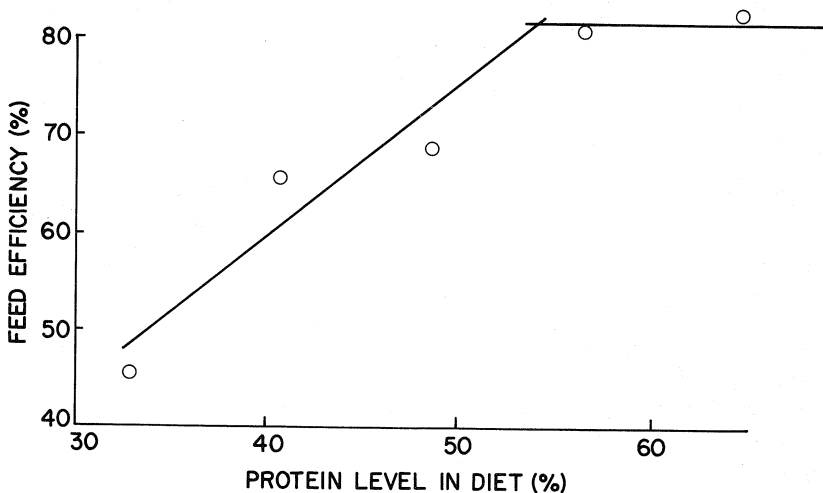


Figure 1. Relationship between feed efficiency of red sea bream and protein level in diet.

essential amino acids, it was found that the red sea bream's requirements are quite identical with those of salmonids, channel catfish and eel (Sakamoto and Yone, 1972). Figures 2 and 3 show that ten amino acids — arginine, histidine, tryptophane, lysine, phenylalanine, isoleucine, leucine, methionine, valine and threonine — are essential. A fish group fed the control diet containing all 18 amino acids, and other groups fed the diets devoid of dispensable amino acids, were feeding actively and showed equally good growth. On the contrary, the fish fed the diets devoid of essential amino acids became inactive and lost both appetite and body weight. When the essential amino acid-deficient fish were placed on the control diet, recovery of the appetite and growth was immediate. Peculiar symptoms such as scoliosis and lordosis which were observed in tryptophane-deficient chinook salmon, were not noticed.

CARBOHYDRATES

The fact that red sea bream require a high protein diet and generally feed on crustacea and others, infers that the fish may use a considerable part of its dietary protein as an energy source. If a considerable amount of carbohydrate, which is inexpensive and abundant in nature, can be

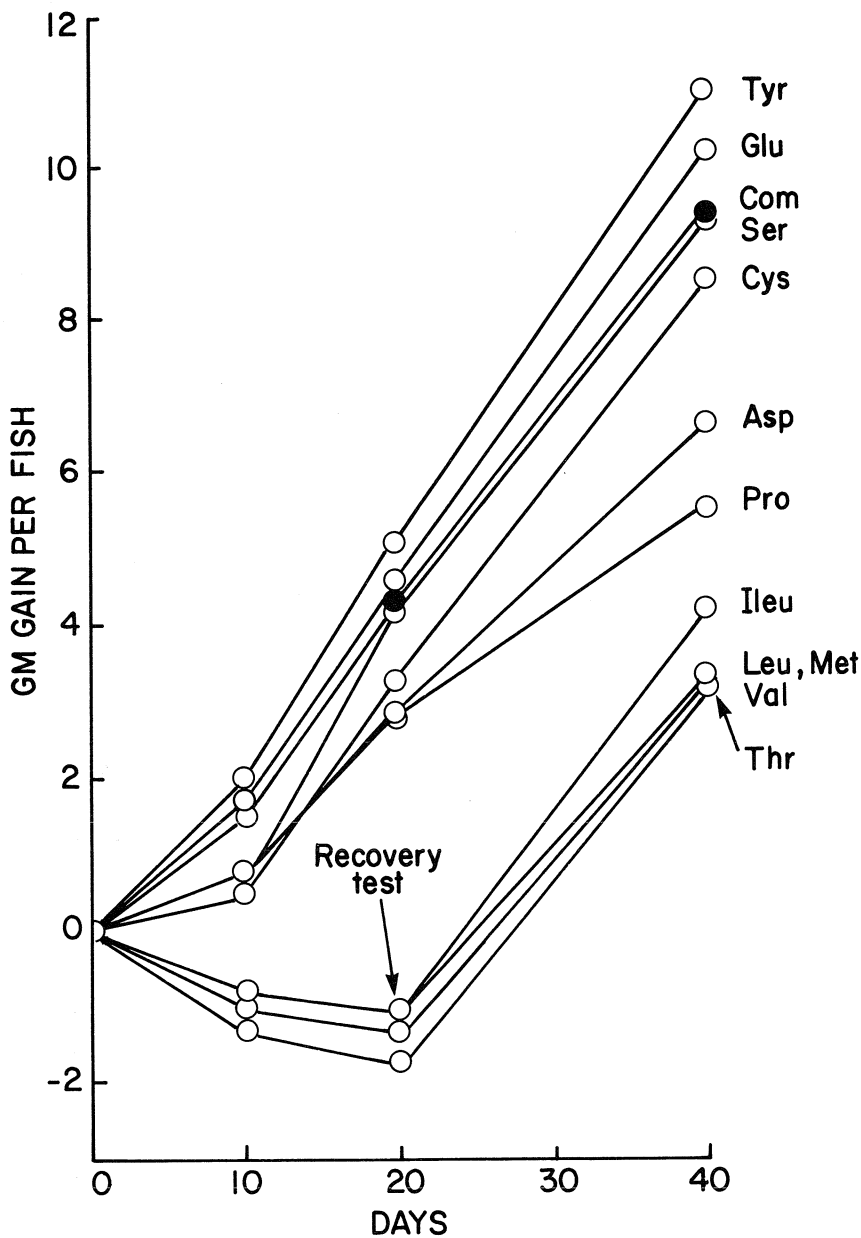


Figure 2. Weight gain of fingerlings fed an amino acid diet.

—●— amino acid complete diet, —○— amino acid deficient diet.

No. of fish and average body weight of each group at start: 17 fish and 13.6 g.

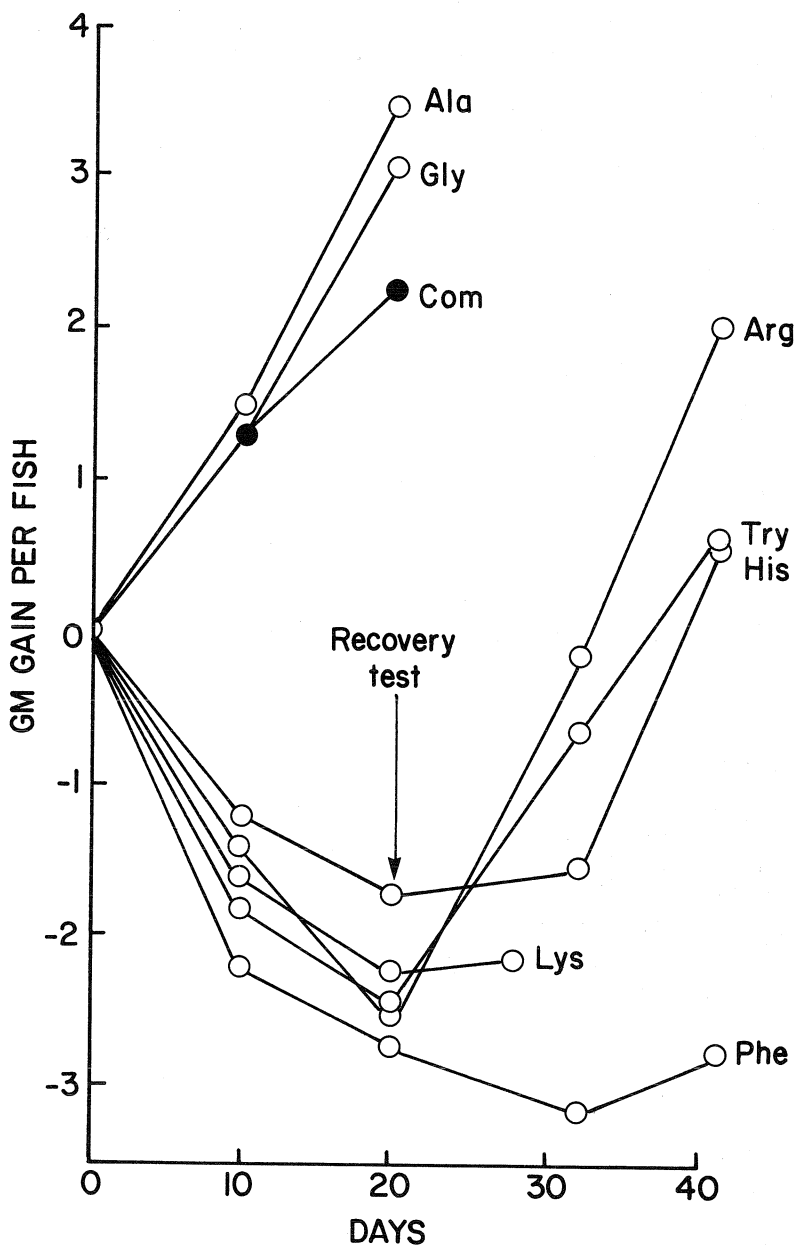


Figure 3. Weight gain of fingerlings fed an amino acid diet.

—●— amino acid complete diet, —○— amino acid deficient diet.

No. of fish and average body weight of each group at start: 20 fish and 19.6 g.

used as an energy source for the fish, it is of no doubt that fish farming can become economically feasible. Hence, we determined the nutritive value and the appropriate level of carbohydrate included in a diet containing white fish meal as a sole protein source.

In the first experiment, glucose showed the best nutritive value at a 20% level, followed by dextrin and alphaform of potato starch in that order (Furuichi and Yone, 1971). In the subsequent experiment with a 10% glucose diet group and a glucose-free diet group, almost no difference was noted in the first 30-day period in the rate of growth and the efficiency of the feed, protein and calories. In the following 20-day period, the 10% glucose diet group was inferior to the glucose-free diet group (Furuichi, Shitanda and Yone, 1971). On the other hand, fish fed diets containing 30 and 40% glucose, showed retarded growth and lower feed efficiency. A accumulation of a large amount of glycogen and lipid was found in the liver of the 40% glucose group. It was also noted that the absorption rates of protein and glucose became lower with the increase of dietary glucose.

Recently, we recognized again the low assimilability of dextrin by red sea bream using the purified test diet YR-1 (Table 3 and Figure 4).

TABLE 3

Effect of dextrin level on growth and feed efficiency of red sea bream.

Diet		D-0	D-10	D-20	D-40
Dextrin (%) in diet		0	10	20	40
No. of fish	{ Start Dead	26 0	26 1	26 0	26 0
Av. body wt. (g)	{ Start After 30 days	40.5 ±2.2* 59.2 ±6.2	40.4 ±2.0 60.2 ±6.3	40.6 ±2.2 56.4 ±6.8	40.5 ±2.1 54.0 ±7.9
"t" -- test		--	NS**	NS	S**
Gm gain (g)		18.7	19.8	15.8	13.5
Feed efficiency		74.5	75.3	57.2	43.1

* Standard deviation.

** NS = non significant, S = significant at a 5% level.

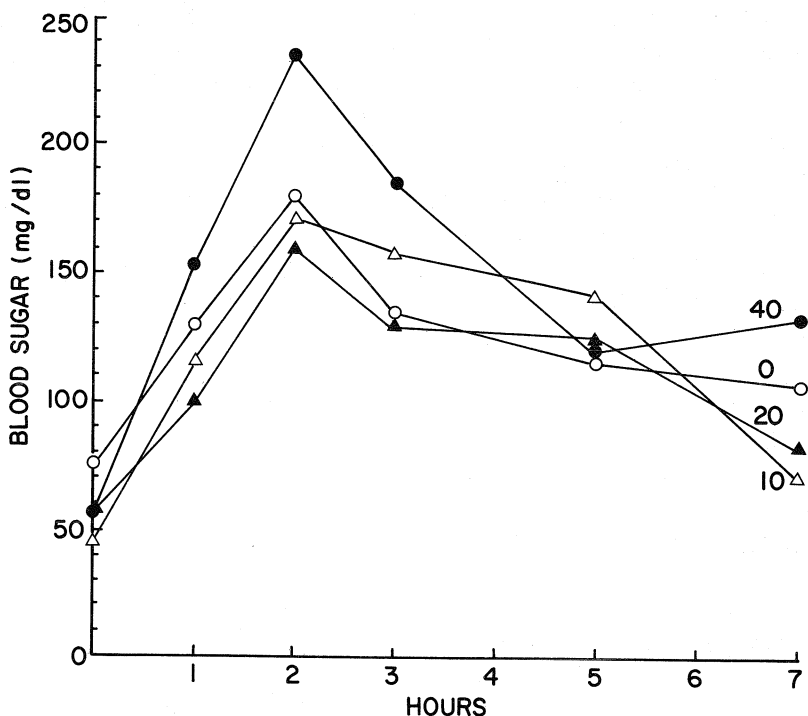


Figure 4. Change of blood sugar level of the fish in a glucose-tolerance test after a 30-day feeding with diet containing various levels of dextrin.

Orally administered glucose level: 167 mg per 100 g body weight.

- : dextrin-free diet group —△—: 10% dextrin diet group
- ▲—: 20% dextrin diet group —●—: 40% dextrin diet group

To elucidate a cause for the low assimilability of carbohydrate in red sea bream, we made a glucose-tolerance test and an insulin-glucose-tolerance test (Furuichi and Yone, 1971b). In both tests, the blood sugar increased remarkably at a 2 hour period after an oral administration of glucose and did not decrease to the original level even after a 5 hour period. The increase of blood sugar was controlled by insulin (Figure 5). From these results, we presumed that the red sea bream may be somewhat diabetic in nature and has low capability of carbohydrate assimilation which can be accounted for by a scarcity of insulin.

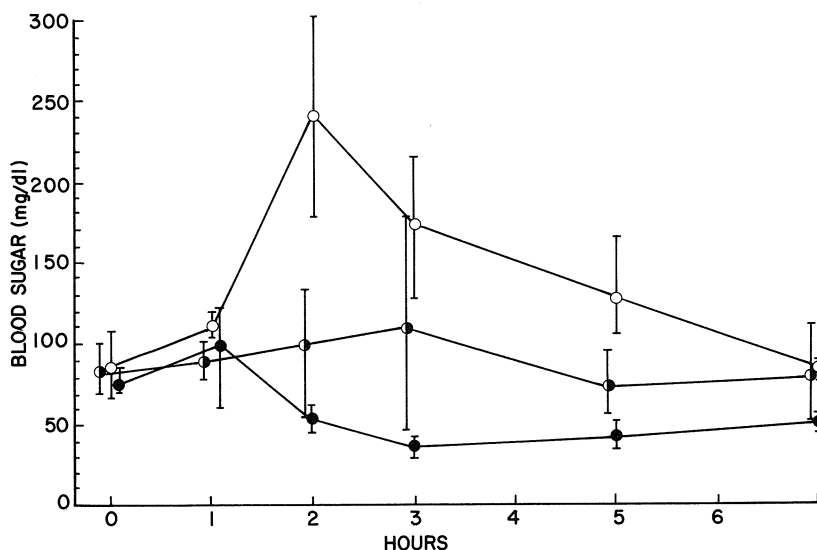


Figure 5. Change of blood sugar level of fish in glucose-tolerance and insulin-glucose-tolerance tests.

Each value is average from 3 fish and a vertical line shows a range of variation.

○ : Orally administered level, 167 mg of glucose per 100 g of body weight,
 ◐ : Orally administered level, 167 mg of glucose and injected level of insulin, 0.02 I.U. per 100 g of body weight, ● : Orally administered level, 167 mg of glucose and injected level of insulin, 2 I.U. per 100 g of body weight.

LIPID AND ESSENTIAL FATTY ACIDS

It is well known that lipid is important as an energy and essential fatty acids source. Until 1965, corn oil and soybean oil were included in purified test diets and commercial fish diets, because these vegetable oils contain the essential fatty acids, such as linoleic acid and linolenic acid. However, these oils are low in the linolenic acid content, and are very low in the content of polyunsaturated fatty acids with more than 20 carbon atoms. Marine fish are continually taking in marine organisms rich in the polyunsaturated fatty acids. Therefore, we presumed that the nutritive value of vegetable oils may be inferior to that of fish oils. In the studies on diets containing pollack meal, it was noted that the corn oil was not suitable as a dietary lipid source for carp and sea bream, and that the nutritive value of soybean oil was also inferior to that of

fish oil (Yone, 1966). In the following study, therefore, a comparison was made of the nutritive value of soybean oil and pollack residual oil by using a purified test diet (Yone, Furuichi and Sakamoto, 1971). The growth and feed efficiency of fish receiving soybean oil were noticeably inferior to those of the fish receiving pollack residual oil, as shown in Table 4.

TABLE 4

Effect of addition (8.9%) of soybean oil and pollack residual oil to synthetic diet MBL on growth rate and feed efficiency for red sea bream.

Lipid		Pollack	Soybean
No. of fish	{ at start died	20 3	20 3
Av. body weight (g)	{ at start after 28 days Gain (g)	41.2 $\pm$ 4.5* 50.9 $\pm$ 6.2 9.7	40.9 $\pm$ 4.2 46.0 $\pm$ 4.7** 5.1
Feed efficiency (%)		59.9	39.8

* Standard deviation.

** Significant at a 5% level.

Lee and coworkers also showed that corn oil in a semi-purified diet led to a poor growth and high mortality of rainbow trout (Lee, Roehm, Yu and Shinnhuber, 1967). They also demonstrated that the substitution of salmon oil or linolenic acid for a part of the dietary corn oil improved the nutritive value of corn oil, and that linolenic acid is essential to rainbow trout. However, we doubted whether the low value of vegetable oils for red sea bream is actually due to the insufficiency of linolenic acid, because pollack residual oil containing a less amount of linolenic acid than soybean oil showed a higher nutritive value than soybean oil for red sea bream.

Therefore, in order to determine the effect of linolenic acid on the growth rate and feed efficiency of red sea bream, we kept the fish on the diets containing corn oil, linolenic acid and pollack residual oil for 64 days (Table 5), (Yone and Fujii, unpublished).

The fatty acid composition of corn oil and pollack residual oil used are shown in Table 6. ω 3 polyunsaturated fatty acids scarcely exist in corn oil. Pollack residual oil contains about 13% of ω 3 polyun-

saturated fatty acids, but only 0.5% of the linolenic acid. As shown in Table 7, the growth and feed efficiency of the corn oil diet group was remarkably inferior to those of the pollack residual oil diet group. Furthermore, even a 4% linolenic acid supplement did not improve the nutritive value of corn oil.

TABLE 5

Composition of lipid source in experimental diets.

Diet No.	1	2	3	4	5	6
Corn oil	9	8.35	7.7	6.4	3.8	--
18:3 ω 3*	--	0.65 (0.5)	1.3 (1.0)	2.6 (2.0)	5.2 (4.0)	--
P. R. oil	--	--	--	--	--	9

Basal diet used is YR-1.

* Purity of methyl linolenate is 77%.

TABLE 6

Fatty acid composition of corn oil and P.R. oil.

Lipid	Corn oil	P.R. oil
14:0		5.9
15:0		0.4
16:0	3.5	12.7
16:1 ω 7	0.2	10.2
18:0	2.3	2.5
18:1 ω 9	31.5	19.4
18:2 ω 9		0.7
18:2 ω 6	57.7	0.3
18:3 ω 6	0.8	0.1
18:3 ω 3	1.7	0.5
20:1 ω 9		16.5
20:2 ω 6		0.3
20:4 ω 6		0.4
22:1		11.9
20:5 ω 3		8.0
22:4 ω 6		0.4
22:5 ω 6		0.7
22:5 ω 3		0.8
22:6 ω 3		3.8

TABLE 7

Effect of linolenic acid supplement in a corn oil diet
on growth and feed efficiency of red sea bream.

Diet No.	1	2	3	4	5	6
Lipid	Corn oil	0.5% 18:3	1% 18:3	2% 18:3	4% 18:3	P.R. oil
No. of fish	25 0	25 0	25 0	25 0	25 1	25 0
Av. {at start body wt. (g)	21.0 ±1.23	21.0 ±1.26	20.9 ±1.29	20.8 ±1.34	21.0 ±1.26	21.0 ±1.27
{after 64 days	31.0 ±3.06	30.3 ±3.72	31.5 ±3.00	31.4 ±2.92	30.8 ±2.76	48.1 ±4.91
{gain	10.0	9.3	10.6	10.6	9.8	27.1
"t" test (5%)	S	S	S	S	S	--
Feed efficiency (%)	52.0	45.0	54.8	52.3	47.8	74.8

*Standard deviation

So, in the following experiment, we examined the effect of $\omega 3$ polyunsaturated fatty acid supplement (Yone and Fujii, 1975a). The $\omega 3$ polyunsaturated fatty acid preparation contained 40% eicosapentaenoic acid and 50% docosahexaenoic acid, and was nearly free from linolenic acid and $\omega 6$ fatty acids. As shown in Table 8, no improvement in growth rate was also recognized by the supplement of linolenic acid to corn oil, but a remarkable improvement resulted from the supplement of $\omega 3$ polyunsaturated fatty acids.

Furthermore, we found that the $\omega 3$ polyunsaturated fatty acid percentage in liver phospholipid of the corn oil-linolenic acid diet group was higher than that of the corn oil diet group (Table 9), (Yone and Fujii, 1975b). This finding indicates that red sea bream possesses a metabolic pathway from linolenic acid to eicosapentaenoic acid and docosahexaenoic acid. However, the metabolic capability may not be large enough to satisfy the requirements for $\omega 3$ polyunsaturated fatty acids.

In our experiment, the best growth and feed efficiency were obtained with the diets containing approximately 10% pollack residual oil as shown in Table 10 (Yone, Furuichi and Sakamoto, 1971). We also

TABLE 8

Effect of ω 3 polyunsaturated fatty acid on the growth and feed efficiency.

Diet	C	CL	CP	CLP	YR-1
Lipid	Corn oil (9%)	Corn oil +18:3 ω 3 (0.7%)	Corn oil +PUFA (2.2%)	Corn oil +18:3 ω 3 +PUFA	Pollack residual oil (9%)
No. of fish	15 3	15 2	15 0	15 0	15 0
Av. body wt. (g)	32.8 ± 4.07 42.2 ± 4.23	32.9 ± 4.21 42.2 ± 7.31	33.1 ± 4.07 53.3 ± 6.25	33.2 ± 4.51 58.2 ± 8.84	33.6 ± 3.29 62.0 ± 7.45
"t" - test (5%)	S	S	S	NS	--
Gm gain (g)	9.4	9.3	20.2	25.0	28.4
Feed efficiency	46.4	58.7	79.7	91.9	94.1

TABLE 9

Percentage fatty acids composition of liver phospholipid

Diet	1 (C. oil)	2 (C. oil +18:3 ω 3)	3 (C. oil +PUFA)	4 (C. oil +18:3 ω 3 +PUFA)	5 (P.R. oil)
14:0	1.9	1.1	5.7	2.1	5.7
15:0	tr	2.3	1.0	2.4	1.7
16:0	4.3	4.9	16.3	23.2	15.8
16:1 ω 7	2.9	3.9	8.0	4.5	13.3
18:0	3.6	5.8	4.7	14.6	5.1
18:1 ω 9	3.8	7.6	18.1	20.2	29.9
18:2 ω 6	36.9	12.3	4.7	11.9	4.1
18:3 ω 6	tr	3.8	1.1	1.3	0.9
18:3 ω 3	1.5	3.8	0.9	1.4	0.7
20:1 ω 9	1.4	2.5	8.7	1.7	4.9
20:2 ω 6	11.8	2.2	0.8	1.1	1.2
20:3 ω 9	tr	2.1	0.7	1.0	tr
20:3 ω 6	3.5	1.8	1.1	1.6	0.6
20:4 ω 6	4.6	4.2	1.4	1.3	0.4
22:1	tr	2.0	5.9	0.9	1.8
20:5 ω 3	6.7	5.7	6.6	1.8	2.0
22:4 ω 6	tr	4.7	tr	tr	tr
22:5 ω 6	2.0	tr	tr	tr	tr
22:5 ω 3	1.0	5.5	1.2	tr	0.4
22:6 ω 3	1.5	7.6	7.7	4.2	0.8
20< ω 3	9.2	18.8	15.5	6.0	3.2
Feed effi.	46.4	58.7	79.7	91.9	94.1

recognized a tendency for the fish to become much more fatty with the increase in the quantity of the dietary oil.

From these results, it is suggested that the use of vegetable oil alone and an excessive amount of oil in the diet is undesirable for red sea bream.

VITAMINS

Using the purified test diet YR-1, we studied the qualitative requirements and deficiency symptoms of red sea bream for water-soluble

vitamins (Yone and Fujii, 1974). Red sea bream showed a poor growth response and loss of appetite when the diet is deficient in B₆, choline, pantothenic acid, B₁₂, inositol, nicotinic acid, B₂, B₁, or C. The deficiency of pantothenic acid and choline resulted in a high mortality. On the other hand, with the diet deficient in biotin, folic acid or p-aminobenzoic acid, the fish did not show any marked deficient symptoms during a 102-day feeding (Figures 6, 7, 8 and 9).

Quantitative requirements of the fish for water-soluble vitamins are still fragmentary. Fingerling requirements for inositol and vitamin B₆ have been determined by Yone et al and Takeda et al. From the relationship between the growth, inositol content of liver, cholinesterase activity in serum of the fish and the inositol content of diet, we concluded that the inositol requirement of the fish fed on the diet containing 10 to 40% glucose is 55-90 mg per 100 g of diet (Yone, Furuichi and Shitanda, 1971).

The requirement for vitamin B₆ was estimated to be about 0.02-0.085 mg per kg body weight per day (0.2-0.5 mg per 100 g of diet) from data obtained on the effect of a diet's vitamin B₆ content on body weight gain, crude fat content of muscle and liver, ratio of liver/body weight, B₆ content of liver, and blood cell count. On the basis of the variation in transaminase activity (GOT and GPT) of liver with the change in B₆ level of the diet, the B₆ requirement was also estimated about 0.1 mg per kg body weight per day (0.5-0.6 mg per 100 g of diet), (Takeda and Yone, 1971).

No study on requirements of red sea bream for fat-soluble vitamins has been carried out so far.

TABLE 10

Effect of addition levels of pollack residual oil in synthetic diet MBL on growth rate and feed efficiency for red sea bream.

Level (%)	3	9	15
No. of fish { at start died	15 0	15 0	15 0
Av. body weight (g) { at start after 28 days	33.4 + 5.4* 43.2 + 6.8**	33.8 + 5.3 45.3 + 8.5	34.0 + 5.2 40.7 + 5.2**
Gain (g)	9.8	11.5	6.7
Feed efficiency (%)	50.3	60.8	45.0

* Standard deviation.

** Non-significant at a 5% level.

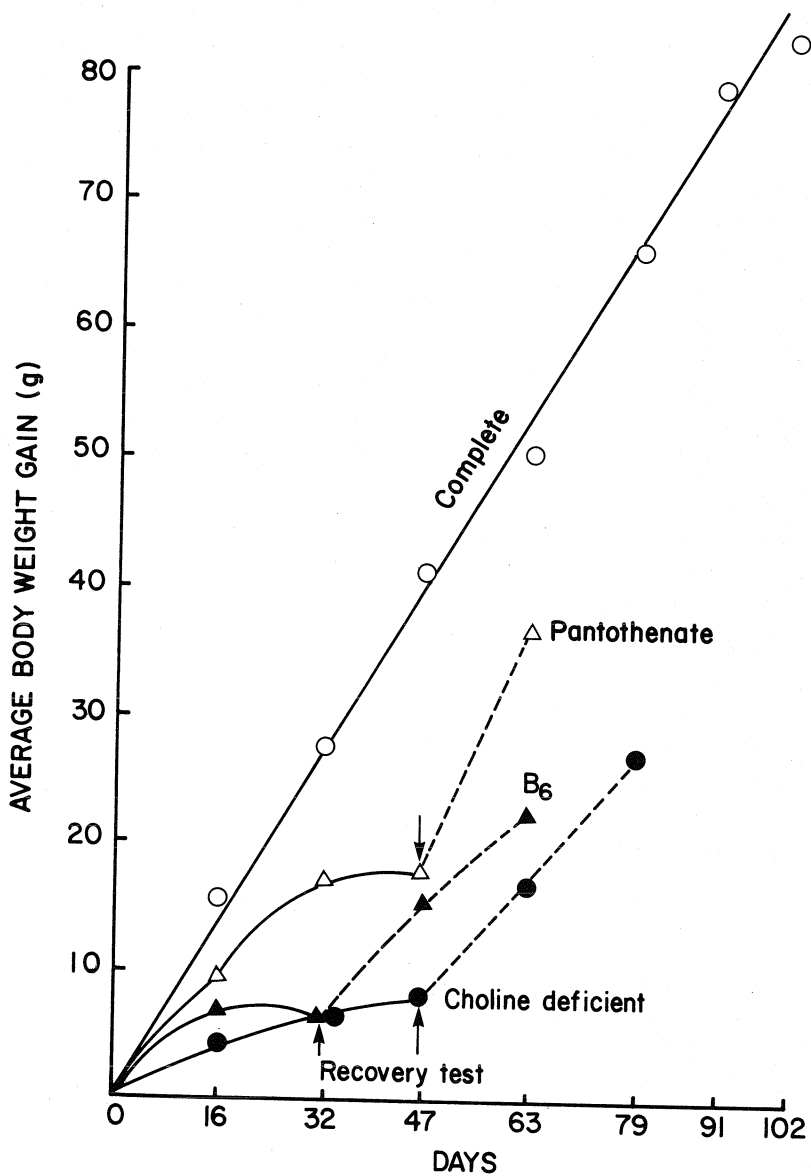


Figure 6. Growth of fish fed diets deficient in pantothenate, choline or vitamin B₆.

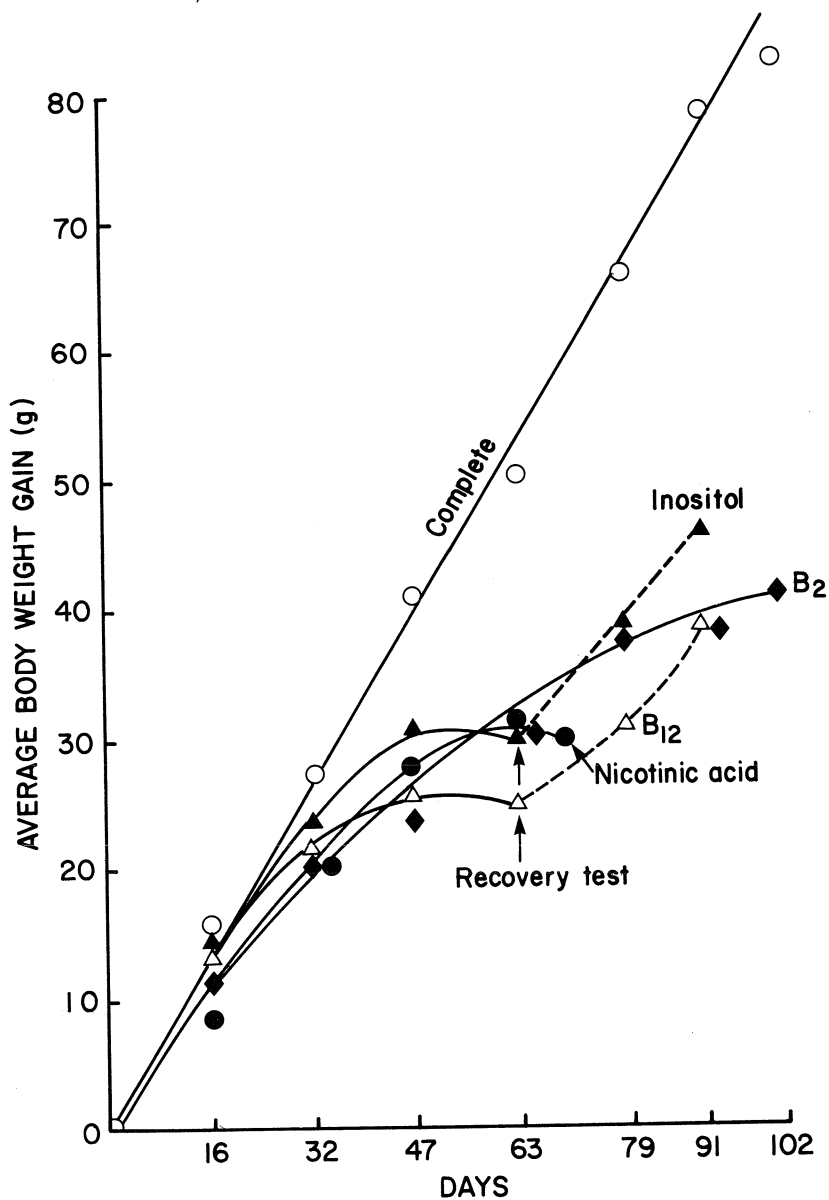


Figure 7. Growth of fish fed diets deficient in vitamin B₂, vitamin B₁₂, nicotinic acid or inositol.

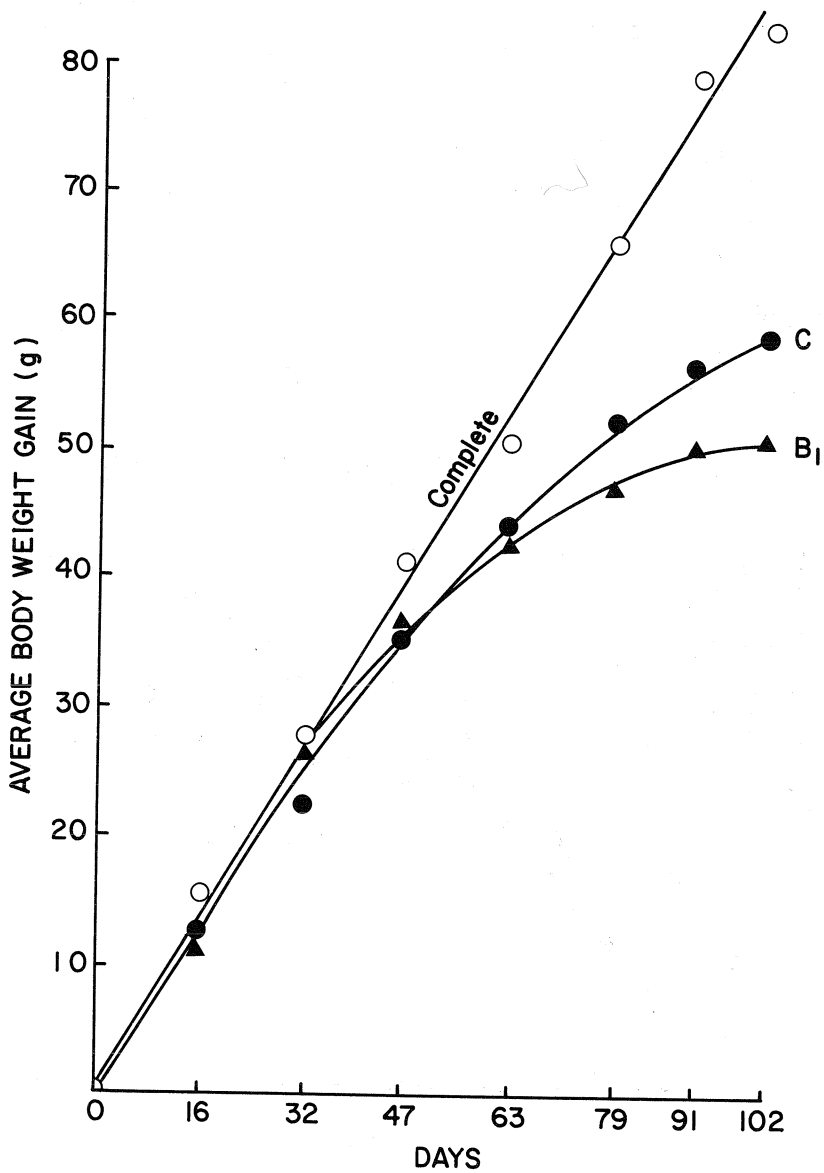


Figure 8. Growth of fish fed diets deficient in vitamin B₁ or vitamin C.

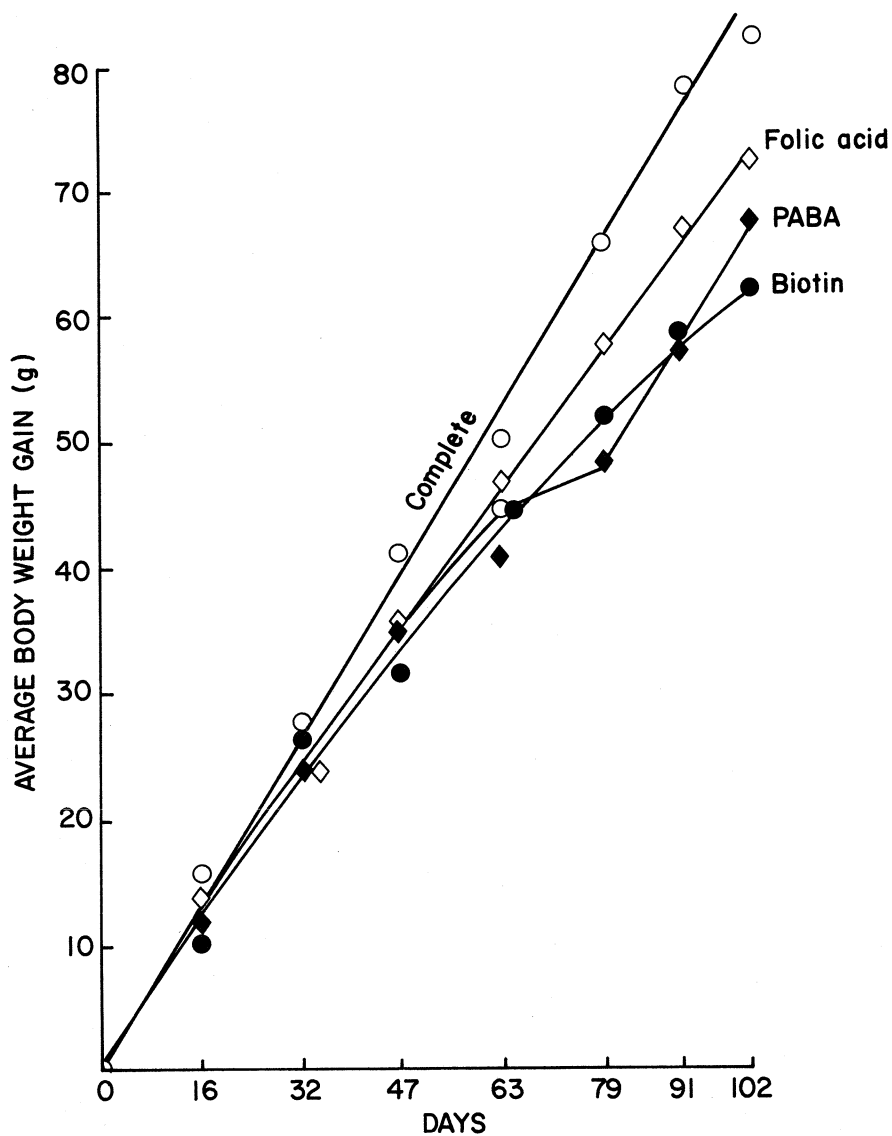


Figure 9. Growth of fish fed diets deficient in folic acid, biotin or p-amino-benzoic acid (PABA).

MINERALS

Many nutritional studies have made clear the significance of inorganic elements on the growth of domestic animals and human beings. Little attention, however, has been paid to the requirements of fish concerning the elements, probably because of the supply of minerals from water environment. Terrestrial animals absorb elements primarily from their diet, but fish can uptake elements from the water environment in addition to the diet. Hence, it is reasonable to presume that the dietary content of the elements required for the maximal growth of fish may be different from that required for terrestrial animals. Since some elements, such as calcium, magnesium, sodium, etc., abound in seawater, it can be expected that marine fish scarcely need these major elements in the diet, but fish require a comparatively large amount of some minor elements in seawater, such as phosphorus and iron. Some interesting facts have been obtained through our studies carried out on the basis of the above presumptions.

As shown in Table 11, the purified test diet without any mineral supplement produced the retarded growth, the lower feed efficiency, the lower hemoglobin content, the decrease of phosphorus in blood

TABLE 11

Effect of dietary minerals on growth and feed efficiency of red sea bream.

Minerals*	With#	Without
No. of fish		
{ at start	20	20
{ after 76 days	20	17
Average body weight (g)		
{ at start	46.0 ± 2.47**	45.8 ± 3.20
{ after 76 days	89.1 ± 13.53	68.7 ± 9.34
"t" test (5%)	--	S##
Feed efficiency (%)		
1 - 16	62.7	43.3
17 - 31	64.9	31.1
32 - 45	62.2	47.7
46 - 61	46.2	28.8
62 - 76	31.4	--

* Mineral mixture P-2, see Table 1.

YR-1 diet.

** Standard deviation.

Significant at a 5% level.

TABLE 12

Effect of dietary phosphorus levels on growth and feed efficiency of red sea bream.

P in diet* (mg%) P / Ca ratio of diet	194 0.6	340 1	680 2	1360 4
No. of fish				
{ at start	20	20	20	20
{ after 76 days	20	18	20	19
Average body weight (g)				
{ at start	46.0 ± 2.7*	46.0 ± 2.5	46.1 ± 2.4	46.0 ± 2.5
{ after 76 days	79.4 ± 10.0	76.0 ± 7.9	89.1 ± 13.5	80.7 ± 14.2
"t" test (5%)	S**	S	--	NS**
Amount of diet fed / fish (g)	77.2	72.1	79.5	65.3
Feed efficiency (%)	43.2	41.6	54.1	53.1

* YR-1 diet was used.

Standard deviation.

** S : Significant, NS : nonsignificant at a 5% level.

and bone, and the accumulation of lipid in liver and muscle (Sakamoto, Furuichi and Yone, 1971).

In the following experiment, it was found that the dietary phosphorus content noticeably affected the growth rate and feed efficiency of the fingerlings, and that the fish requirement for dietary phosphorus is more than 680 mg% in the diet (Table 12), (Sakamoto and Yone, 1973a). Lowering the dietary phosphorus level resulted in the decrease of serum phosphorus, the increase of calcium content and Ca/P ratio of serum, the decrease of ash, phosphorus, calcium and Ca/P ratio of vertebral bone, and the brittleness of bone (Table 13). The maximum and minimum values for the adequate dietary phosphorus level were recognized at 680 and 1360 mg%, respectively. These facts seem to infer that in this range of the dietary phosphorus level, red sea bream shows a higher absorption or turnover of phosphorus and a higher assimilation of calcium.

Furthermore, the insufficient amount of the dietary phosphorus caused an elevation of the hepatosomatic index and the lipid content of whole body, liver and bone (Table 14), (Sakamoto and Yone, 1973b).

These results are enough to conclude that phosphorus supplement is needed to make the diet adequate for the growth of red sea bream.

TABLE 13

Effect of dietary phosphorus level on hemoglobin, ash, calcium, phosphorus and lipid contents of blood and vertebral bone.

P in diet (mg%)	194	340	680	1360
Whole blood				
Hb (g/dl)	8.0	7.3	8.0	7.5
Total P (mg/dl)	116.6	122.2	136.1	120.0
Serum				
Ca (mg/dl)	13.42	12.38	10.52	10.47
Inorganic P (mg/dl)	12.58	14.50	20.84	19.75
Ca/P	1.07	0.85	0.50	0.53
Bone				
Ash (% . dm)	29.1	33.3	40.6	39.0
Lipid (% . dm)	33.6	27.8	25.1	24.1
Ca (mg/g . dm)	133.2	163.9	186.6	181.9
P (mg/g . dm)	80.9	92.4	100.2	101.9
Ca/P	1.65	1.77	1.86	1.79

TABLE 14

Effect of dietary phosphorus levels on hepatosomatic index and lipid content of whole body, muscle and liver.

P in diet (mg%)	194	340	680	1360
Whole body				
Moisture (%)	69.1	69.4	70.7	70.8
Lipid (%)	10.3	8.6	8.6	8.0
Crude protein (%)	18.2	19.3	18.5	18.4
Ash (%)	2.7	2.9	2.8	2.8
Total	100.3	100.2	100.6	100.0
Muscle				
Lipid (% . dm)	6.3	7.5	6.8	5.8
Liver				
Lipid (% . dm)	48.3	37.0	31.9	28.3
LW x 100 / BW (%)	1.76	1.55	1.43	1.33

In the above mentioned study on the requirement for dietary minerals, the lower hemoglobin content was recognized in the fish fed on the mineral-free diet (Sakamoto, Furuichi and Yone, 1971). Iron is a constituent of hemoglobin and is one of the substances which causes an anemia in animals if deficient. Since iron is scarce in seawater, Sakamoto and Yone studied the effect of dietary iron in the form of ferric citrate on the growth rate and blood characteristics of red sea bream (Sakamoto and Yone, 1974).

Even kept on a purified diet lacking iron, the fish showed a good growth and feed efficiency comparable to those on a diet containing a 0.118% ferric citrate for as long as 95 days. As shown in Table 15, red blood cell counts of the iron-free diet group were more than that of the iron-diet group, but other blood characteristics of the iron-free diet group were lower than those of the iron-diet group. The lower values for mean corpuscular hemoglobin (MCH) and color index (CI) of the iron-free diet group were remarkable. The mean red blood cell diameter (MCD, 7.90μ) of iron-free diet group was also smaller than that (9.39μ) of iron-diet group. These data clearly indicate that the fish showed a microcytotic-hypochromemia that is known as a characteristic of iron-deficient anemia. The iron-deficiency also caused an anisocytosis.

No dietary need for major mineral elements in seawater, such as calcium, magnesium and sulphur, was observed. Supplying the minor elements, such as aluminum, zinc, manganese, copper, iodine and cobalt

to the diet was not necessary. It seems that a sufficient amount of varying elements required by the fish can be supplied from the water environment.

TABLE 15

Effect of dietary iron on blood characteristics of red sea bream.

Iron	With	Without
Ht (%)	30.9 ± 2.5	25.8 ± 4.0
"t" test (5%)	--	S
Hb (g/dl)	7.1 ± 0.6	4.3 ± 0.5
"t" test (5%)	--	S
Rbc × 10 ⁶ / mm ³	3.181 ± 0.276	3.703 ± 0.231
"t" test (5%)	--	S
SG		
SG = (X - 1.0) × 10 ³	42 ± 2.7	37 ± 2.7
"t" test (5%)	--	S
Protein in plasma (g/dl)	3.8 ± 0.5	3.9 ± 0.7
"t" test (5%)	--	NS
MCH (γγ)	21.85 ± 0.87	11.74 ± 1.46
"t" test (5%)	--	S
MCV (μ 3)	95.91 ± 5.26	71.85 ± 8.67
"t" test (5%)	--	S
MCHC (%)	22.98 ± 1.12	16.75 ± 1.66
"t" test (5%)	--	S
CI	1	0.53
VI	1	0.74
SI	1	0.73

YR-1 diet was used.

LITERATURE CITED

- Furuichi, M., K. Shitanda, and Y. Yone. 1971. Studies on nutrition of red sea bream — V. Appropriate supply of dietary carbohydrate. Rept. Fish. Res. Lab., Kyushu Univ., No. 1: 91-100.
- Furuichi, M., and Y. Yone. 1971a. Studies on nutrition of red sea bream — IV. Nutritive value of dietary carbohydrate. Rept. Fish. Res. Lab., Kyushu Univ., No. 1: 75-81.
- Furuichi, M., and Y. Yone. 1971b. Studies on nutrition of red sea bream — VI. A study of carbohydrate utilization by glucose- and insulin-glucose-tolerance tests. Rept. Fish. Res. Lab., Kyushu Univ., No. 1: 101-106.
- Lee, D. J., J. N. Roehm, T. C. Yu, and O. Shinnhuber. 1967. J. Nutr., 92: 93-98.
- Sakamoto, S., M. Furuichi, and Y. Yone. 1971. Studies on test diet for nutrition studies of red sea bream — II. Minerals. This paper was presented to the Conference of Jap. Soci. Sci. Fish., at Hakodate in October.
- Sakamoto, S., and Y. Yone. 1972. Amino acid requirements of red sea bream. This paper was presented to the Conference of Jap. Soci. Sci. Fish. at Kochi in October.
- . 1973a. Effect of dietary calcium/phosphorus ratio upon growth, feed efficiency and blood serum Ca and P level in red sea bream. Bull. Jap. Soci. Sci. Fish., 39: 343-348.
- . 1973b. Effect of phosphorus level and P/Ca ratio of diet on approximate analysis of red sea bream. This paper was presented to the Conference of Jap. Soci. Sci. Fish. at Tokyo in April.
- . 1974. Requirement of red sea bream for dietary iron. This paper was presented to the Conference of Jap. Soci. Sci. Fish. at Tokyo in April.
- Takeda, T., and Y. Yone. 1971. Studies on nutrition of red sea bream — II. Comparison of vitamin B₆ requirement level between fish fed a synthetic diet and fish fed beef liver during pre-feeding period. Rept. Fish. Res. Lab., Kyushu Univ., No. 1: 37-47.

- Yone, Y., 1966. The aquiculture, Extra No. 6: 61-64.
- Yone, Y., M. Furuichi, and K. Shitanda. 1971. Vitamin requirements of red sea bream — I. Relationship between inositol requirements and glucose levels in the diet. Bull. Jap. Soci. Sci. Fish., 37: 149-155.
- Yone, Y., M. Furuichi, and K. Sakamoto. 1971. Studies on nutrition of red sea bream — III. Nutritive value of optimum content of lipids in diet. Rept. Fish. Res. Lab., Kyushu Univ., No. 1: 49-60.
- Yone, Y., S. Sakamoto, and M. Furuichi. 1974. Studies on nutrition of red sea bream — IX. The basal diet for nutrition studies. Rept. Fish. Res. Lab., Kyushu Univ., No. 2: 13-24.
- Yone, Y., and M. Fujii. 1974. Studies on nutrition of red sea bream — X. Qualitative requirements for water-soluble vitamins. Rept. Fish. Res. Lab., Kyushu Univ., No. 2: 25-32.
- . 1975a. Studies on nutrition of red sea bream — XI. Effect of ω 3 fatty acid supplement in a corn oil diet on growth rate and feed efficiency. Bull. Jap. Soci. Sci. Fish., 41: 73-77.
- . 1975b. Studies on nutrition of red sea bream — XII. Effect of ω 3 fatty acid supplement in a corn oil diet on fatty acid composition of fish. Bull. Jap. Soci. Sci. Fish., 41: 79-86.

Studies on Nutrition and Feeds of Warmwater Fishes

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Studies on the nutrition and feeds of warmwater fishes are relatively new when compared to those of trout, salmon, poultry and swine. Research in warmwater fish nutrition and feeds development was not required until recent years since large amounts of land for ponds were available, and acceptable fish production levels were obtainable from natural food organisms in these earthen ponds.

After World War II and especially since 1960, rising land and labor costs, and the greatly increased demand for sport and food fish brought about a need for higher production levels. Over a very short span, husbandry changed from the type where the animal obtained all its nutrients and energy from the aquatic environment, to one where these factors were supplied supplementally or totally by the fish culturist.

EXTENSIVE PRODUCTION WITH NATURAL FOODS

Natural foods, ordinarily termed "fish food organisms," include bacteria, phytoplankton, zooplankton, insects, crustacea, and, for some species even fish eggs and other fish. Production of these organisms is dependent on soil and water fertility, water temperature, and intensity of solar radiation. Fish populations will harvest natural food organisms and some plants as rapidly as produced. Artificially fertilized ponds and some ponds constructed in fertile soil areas, often produce over 200 kg/ha of sustained fish harvest annually. Production of fish in unmanaged ponds or reservoirs is usually less than production of cereal grains or livestock on the same acreage. Stocking of compatible species of fish (polyculture) usually does not result in a significant increase in total production in these unmanaged ponds.

The most limiting factor in fish production after environmental restrictions is food. Supplemental feeding supports annual harvests of 500 to 1000 kg/ha of bait fish, 1000-2000 kg/ha of food fish, and 2000 to 4000 kg/ha of exotic fish. Feed efficiency values of 1:1.5 are not unusual.

INTENSIVE PRODUCTION WITH ARTIFICIAL FEEDS

Fish stocked in numbers greater than found in natural conditions soon will reach the production limit imposed by the ability of the environment to produce fish food organisms. At this point growth will cease, and additional growth occurs only when some individual fish are removed (by death, fishing, etc.) or when an external food source is added. Many fish species can convert these feeds to flesh relatively efficiently. The first limiting nutrient for growth in most warmwater cultures is protein, and the fish culturist expects the natural fish food organisms to supply the vitamins, mineral, and other nutrient factors. However, when the natural food organisms become diluted because of over-production of fish, or reduced because of pollution or disease, it becomes necessary to fortify these warmwater fish pond feeds with the essential growth factors. So in this respect, content of warmwater fish feeds is approaching that of coldwater fishes.

Purified diets modified from those used so successfully in determining the nutritional requirements for trout and salmon have provided the basis for the relatively rapid expansion of warmwater fish nutrition knowledge (Dupree and Sneed, 1966). Using the results of these and other studies with purified diets, the fish nutritionist and feed formulator has been able to develop feeds suitable for earthen ponds, raceways, and cages that will result in economical production of healthy fish.

NUTRITIONAL REQUIREMENTS

Amino Acids

Protein supplies the amino acids essential for growth of warmwater fishes and all other animals. One of the main factors affecting feed efficiency is the amino acid composition of the dietary protein relative to the indispensable amino acid requirement of the species. It would appear at first thought that knowledge of the specific amino acid requirements of the fish species coupled with chemical score values of the protein would allow the feed formulator to prepare mixtures of proteins supplemented with the deficient amino acids and thus achieve maximum growth and protein efficiency. Although it sounds reasonable at first,

more understanding of the problems involved shows the difficulty in implementing this theory.

Amino acid test diets developed by Halver (1975b) in studies with chinook salmon were modified and used to show that channel catfish (Dupree and Halver, 1970), common carp (Nose et al., 1974) and two species of eel (Arai et al., 1971) require the same 10 amino acids as do all other animals tested (Figure 1). These 10 amino acids are: arginine, histidine, leucine, isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. The nine others found to be dispensable are: tyrosine, glycine, alanine, aspartic acid, glutamic acid, cystine, proline, serine, and hydroxyproline.

Halver (1975b) reported that salmon and Arai et al., (1972) that eel fed the amino acid control diet, and those fed the diets devoid of the dispensable amino acids grew at a rate that approached that of the whole protein rations. In contrast, Dupree and Halver (1970) with the catfish, and Nose et al., (1974) with the carp, reported no growth with the Halver (1975b) diet, and only after extensive testing of various modifications including different salt mixtures, binders, and neutralizations did these investigators obtain measurable growth. Thus there appears to be some difference in the mechanisms of digestion, absorption, or possibly use of amino acids by these fish species.

Complete quantitative amino acid requirements appear to be available only for the chinook salmon, but some work has been conducted on trout, catfish and eel. Requirements for specific amino acids appear to be high when compared to poultry, swine, and rats but Mertz (1969) noted that when these amino acid requirements are expressed as a percentage of dietary protein, considerable similarity is observed. In the nutrition studies with catfish and eel, similar values to those of salmon were obtained.

Use of supplemental amino acids to improve the nutritional value of proteins has been used extensively in mammalian and poultry feeds, but has had mixed and rather disappointing results in fish feeds. A positive effect was measured by Hastings and Dupree (1969) by replacing the 12% fish meal in a catfish ration with soybean meal supplemented with lysine at the same level as found in the fish meal. They found that growth approached that of the fish fed the feed that contained fish meal, and that the growth was statistically equal. However when methionine, or lysine and methionine in combination were added to the soybean protein-substituted ration, growth was reduced.

The reduction in growth caused by the methionine, and methionine-lysine supplementation was similar to the results obtained by Arai

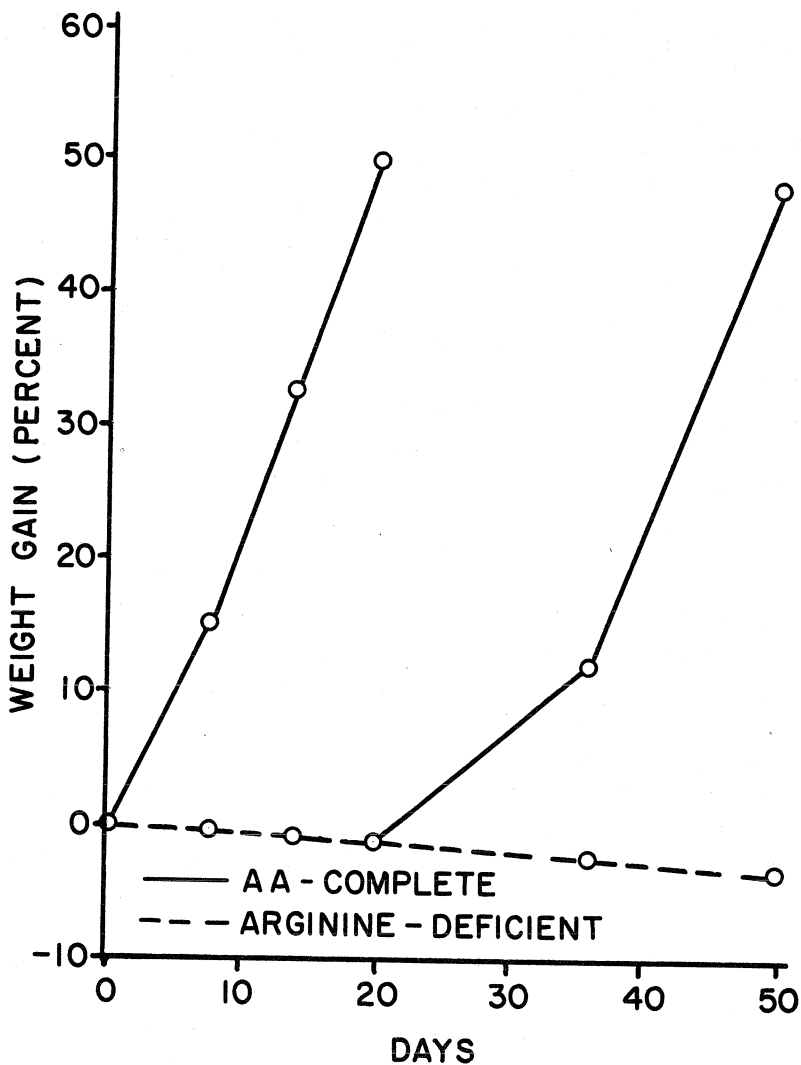


Figure 1. Growth response of fingerling channel catfish fed a purified diet that contained all the amino acids (AA complete) and an identical diet devoid of arginine. At day 20, the group of fish fed the arginine-deficient diet were divided: one-half continued on the deficient ration and the other half started on the AA complete diet. Resumption of growth of fish on the arginine-deficient diet was prompt when the missing indispensable amino acid was replaced in the diet (Dupree and Halver, 1970).

et al., (1972). They found that the growth stimulation resulting from supplementation of purified diets for eels with L-arginine, L-alanine, and L-aspartic acid could be counteracted by the addition of 0.5 percent L-threonine.

In a recent study with coldwater fish species, Rumsey and Ketola (1975) reported that the methionine-leucine-lysine-valine-threonine-histidine-tryptophan-tyrosine combination as well as the methionine-leucine-lysine-valine-threonine combination significantly increased the growth rate of rainbow trout fed diets that contained primarily soybean meal as the protein component. In contrast, supplements of methionine, histidine, lysine, and leucine, alone and in combination, had no significant effect on growth.

Other workers have reported poor use of amino acids in isolated amino acid diets — Aoe et al., (1970 and 1974) and Nose et al., (1974) for the carp; Arai et al., (1972) for the eel; and Dupree and Halver (1970) for the channel catfish. Doyoe et al., (1968), Lovell (1973), and Page (1975) reported that amino acid-supplemented intact proteins were not used by the channel catfish.

Protein

The numerical value representing the quantitative protein requirement of a particular fish species is the question most frequently asked the fish nutritionist. There are no limits to the number of values in the scientific literature, but each of these values is influenced by water temperature, fish size, fish age, protein source and quantity, biological value of the protein, levels and types of other nutrients present, feeding rate, and a multitude of other factors.

Most studies on quantitative protein requirements have been conducted with fry to fingerling-sized fish and the results based on isocaloric diets containing a number of protein increments. DeLong et al., (1958) using casein-gelatin diets determined the optimum level of protein for fingerling chinook salmon to be 40% at 8°C., and 55% at 14°C. Ogino and Saito (1970) reported the optimum protein level to be 38% at 23°C for carp, and Nose and Arai (1972) reported 45% at 25°C for eel. Dupree and Sneed (1966) tested the effect of seven levels of three proteins at two water temperatures and found that gain was linear with protein from 12 to 40% of the diet, but 52% protein retarded growth (Figure 2). At the higher water temperature (24°C), the three proteins were used almost equally. At the lower temperature (20°C), casein was used almost as well as at the warmer temperature, but soybean use was only about one half as efficient at 20°C as at 24°C. Gluten use was

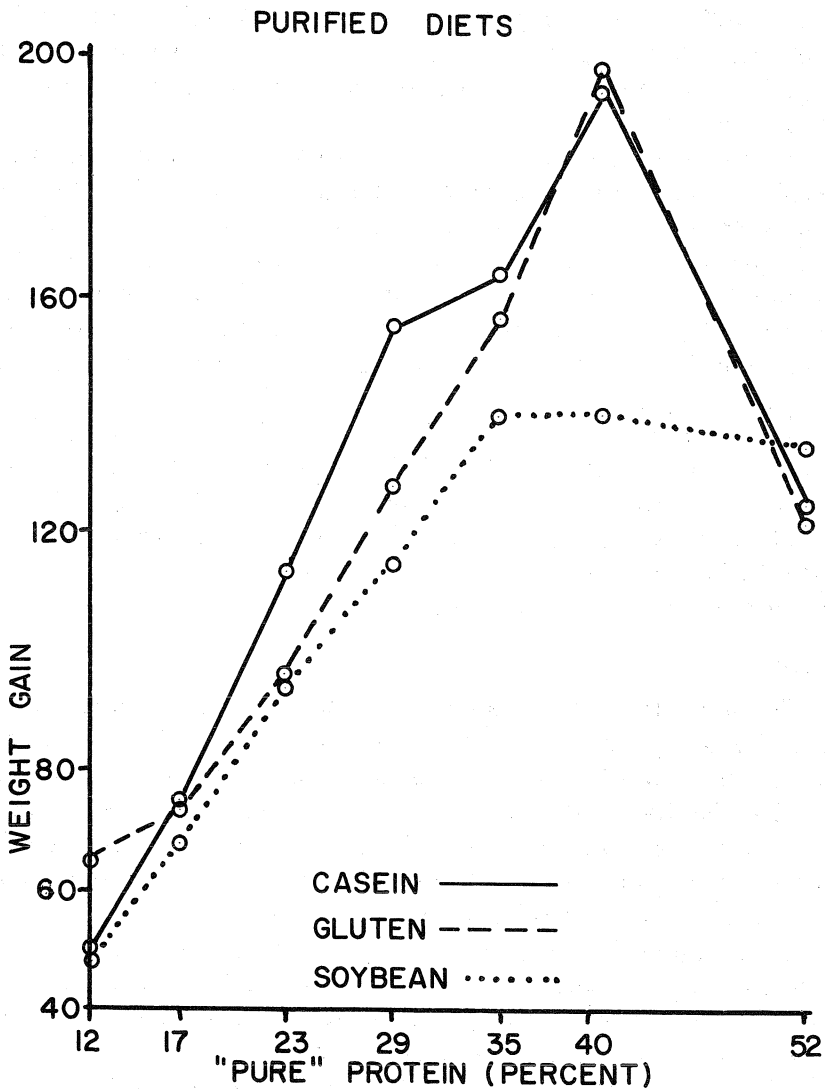


Figure 2. Weight gain of fingerling channel catfish fed seven levels of three purified proteins at 24°C. Growth of fish fed the casein and gluten diets was essentially identical and linear with protein content of the diets from 12 to 40%. Growth was reduced when protein content of the diets reached 52% (Dupree and Sneed, 1966a).

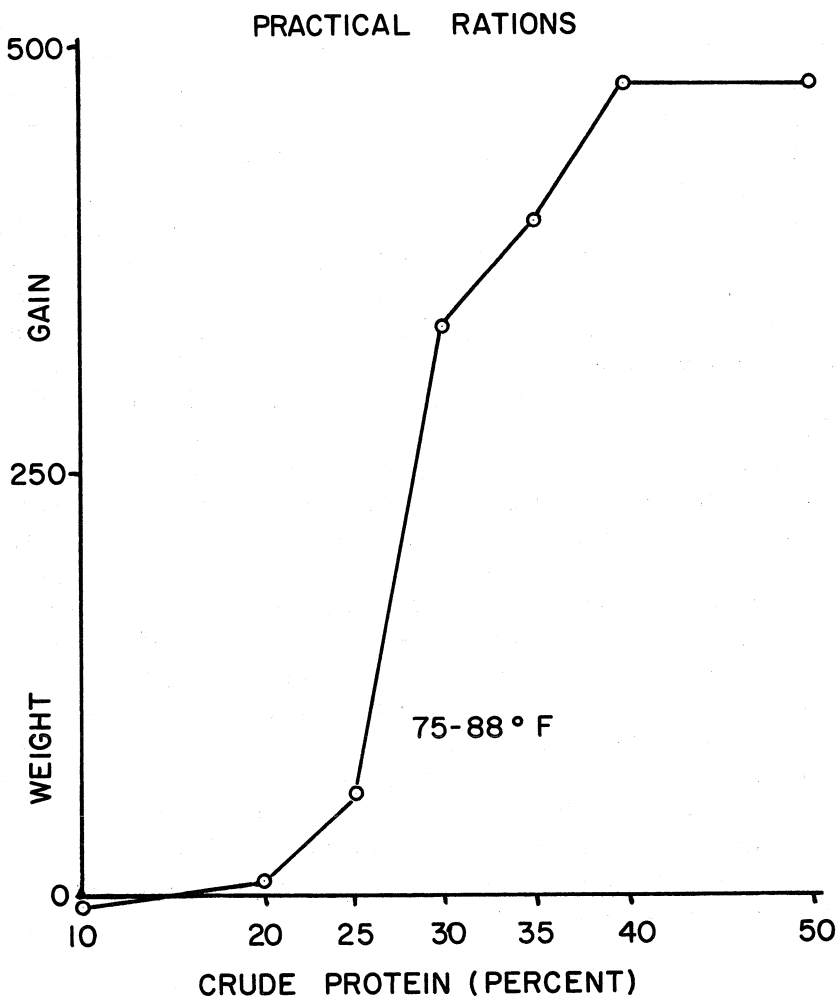


Figure 3. Growth of channel catfish fed a series of practical-type feeds to channel catfish fingerlings in aquaria. Weight gain was essentially linear with protein content of the diet from 20 to 40%, but 50% protein did not improve growth (Hastings and Dupree, 1969).

almost unmeasurable at the lower temperature. Similar observations are noted with commercially-available rations (Figure 3).

Size of fish does have an appreciable effect on protein requirements although data are meager. Inspection of published data and unpublished

reports supports the conclusion that protein requirements for warm-water fish species of similar sizes and exposed to similar environmental influences are approximately the same, i.e., 50% for fry, 40% for small fingerlings decreasing to 35% for large fingerlings, and 30% for large fish.

Of interest are the measurements on nutritional quality of the different proteins as affected by the relatively high requirement by fish for the indispensable amino acids. Ogino and Chen (1973) reported differences in biological values of egg yolk, fish meal, and gluten when fed at 10 to 40% of the diet. However, most other animals, including the rat, that show different biological values at low feed intakes will, in contrast, show considerable similarity in the biological values of the same proteins when offered at a higher feed intake (Miller and Payne, 1961).

Dupree (1967) compared weight gain of fingerling channel catfish fed 12 nutritionally complete purified diets that contained seven animal proteins, three plant proteins, a mixture of the animal proteins and a mixture of the plant proteins (Table 1).

In this experiment, better efficiency was obtained for all single tested proteins when offered at 24-31°C than when offered at 18°C. The mixture of animal proteins resulted in better weight gain and feed conversion than the mixture of plant proteins or any protein tested alone. Probably the most interesting observation was that the mixture of animal protein was used almost equally at both water temperatures. Also of note was the observation that protein particle size (12 mesh to 100 mesh) in these purified diets had no measurable effect on protein use at either temperature. Use was affected only by the protein type and source, and water temperature.

Vitamins

Dupree (1966) in the late 1950's modified slightly the purified vitamin test diet of Halver (1957a) and determined the qualitative vitamin requirements of channel catfish. The effect of the pyridoxine-deficient diet on growth and mortality is shown in Figure 4. This study marked the first success of the use of a purified diet for warmwater fish in which the various dietary components were controllable.

Qualitative requirements and deficiency symptoms for the salmonoids, carp, and channel catfish are similar and have been reported in detail by Hashimoto (1972). Arai et al., (1972) reported that the deficiency symptoms for ascorbic acid, niacin, choline, and inositol were different from those reported for other fishes suggesting species

TABLE 1

Results of the effect of protein and water temperature¹ on the weight gain and food conversion of fingerling channel catfish (Dupree, 1967).

Protein	Experiment I ³			Experiment II ³		
	Grams	Gain	Percent	Feed Conversion	Gain	Feed Conversion
Fish meal	248		165	1.6	129	3.1
Zein ²	13		7	31.6	-5	
Casein	264		176	1.6	136	2.9
Whole egg	303		202	1.4	187	2.1
Wheat gluten	85		57	5.0	27	14.6
Soybean	138		92	3.0	92	4.3
Gelatin ²	84		56	4.9	45	8.8
Blood plasma					95	4.2
Whole blood					8	49.2
Blood albumin					15	26.3
All animal protein mixture	352		235	1.2	203	1.9
All plant protein mixture	136		91	3.0	58	6.8

¹In the first experiment, water temperature initially averaged approximately 31°C and decreased to approximately 24°C at the conclusion. In the second experiment, water temperature was a constant 18°C.

²Proteins supplemented with tryptophan in second experiment.

³Total feed offered (dry weight) equaled 411 and 394 grams in experiments I and II, respectively.

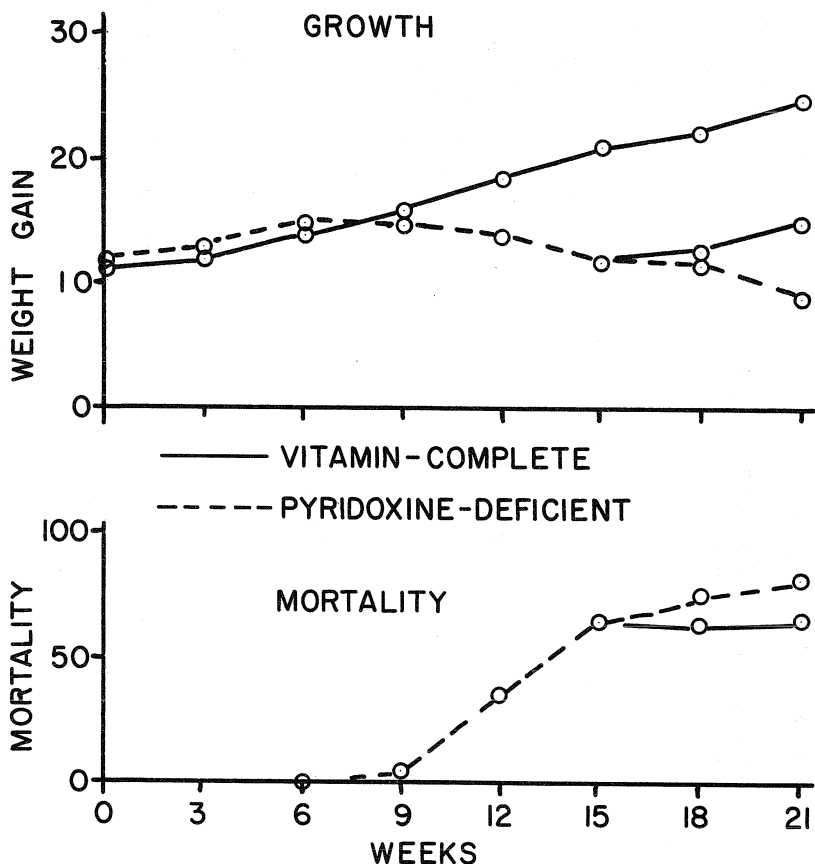


Figure 4. Growth response and mortality of fingerling channel catfish fed a purified diet that contained all the vitamins (vitamin-complete) and an identical diet devoid of pyridoxine. At week 15, the group of fish fed the pyridoxine-deficient diet was divided: one-half continued on the deficient diet and the other half started on the vitamin-complete diet. Growth of fish on the pyridoxine-deficient diet was resumed, and mortality stopped when the missing vitamin was replaced in the diet (Dupree, 1966).

specificity. No demonstration of need for Para-aminobenzoic acid was observed for any fish species tested. Yone and Fujii (1974) as Dupree (1966) could not show a need for biotin in test diets for red sea bream and channel catfish, respectively. Aoe et al., (1957) and Yone and

Fujii (1974) could not demonstrate a need for folic acid in the diet of carp and red sea bream, respectively. Thus, there are unexplained differences in qualitative requirements of vitamins by fishes.

Quantitative water-soluble vitamin requirements for warmwater fishes have been established only for carp, although some data exist for catfish and red sea bream. Values that have been obtained are almost identical to those for trout and salmon. Aoe et al., (1971) reported on the utility of reported minimum vitamin requirements of carp by testing a mixture containing these minimum vitamin levels. This vitamin mixture failed to support growth of young carp, but if enriched with thiamine, riboflavin, and niacin, growth was almost normal. This study suggested that the minimum requirements of these vitamins are influenced by the quantity of other vitamins present.

Studies on fat-soluble vitamin A requirements of warmwater fishes are limited almost entirely to catfish (Dupree, 1970) and carp (Aoe et al., 1968). Dupree (1966) reported vitamin A deficiency symptoms for catfish that included popeye, hemorrhagic kidneys, and edema of the body cavity. Later, Dupree (1970) added to these observations protruding and opaque eyes, and a small white spot in the epidermis between the eyes and over the pineal body. Of particular note was that no deficiency symptoms or differences in weight gain were measured for the first 13 months of the test, and not until intense light was placed over the tanks, thus possibly increasing the turnover of vitamin A-containing-rhodopsin to retinene. Beta carotene was almost valueless as a provitamin for vitamin A (Figure 5). Catfish require vitamin K (Dupree, 1966). Catfish (Dupree, 1969a) and carp (Watanabe et al., 1970a; 1970b) require vitamin E (alpha-tocopherol).

Carbohydrates

In studies to determine the protein sparing effect of white dextrin Dupree and Sneed (1966) found that this carbohydrate and also corn oil spared protein in low energy diets but not in high energy diets.

Furuichi and Yone (1971a; 1971b) and Furuichi et al., (1971) reported that red sea bream used glucose best, followed by dextrin and then starch. Glucose at 30% and 40% of the diet resulted in reduced growth and feed efficiency, and an accumulation of glycogen and lipid in the liver. Glucose tolerance tests and other observations suggest that the red sea bream is normally diabetic and thus lacks the ability to use large amounts of carbohydrate.

The channel catfish is not diabetic, having been found to synthesize a very active insulin molecule in islet tissues in the liver and in dis-

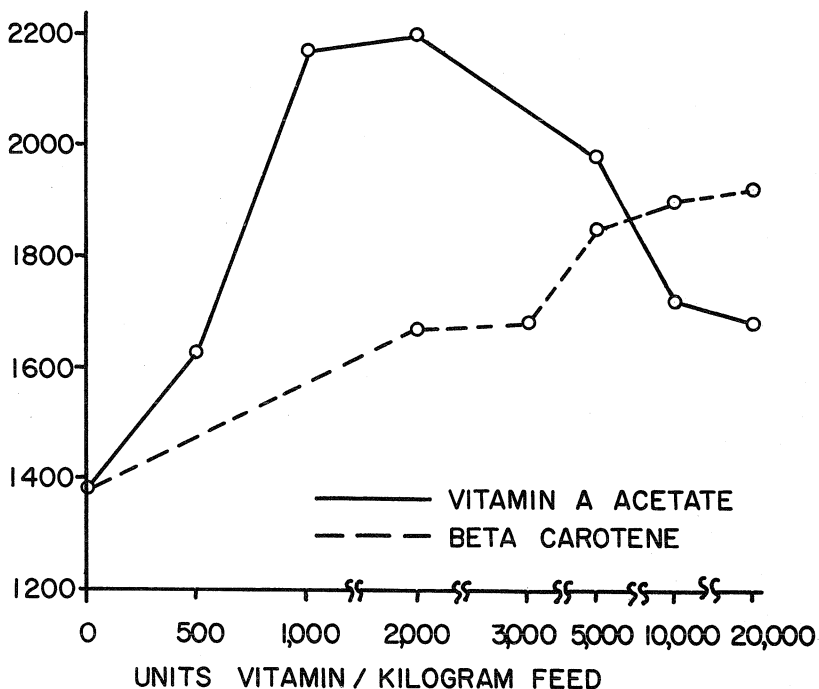


Figure 5. Effect of vitamin A acetate and beta carotene on the growth of channel catfish. Growth was linear with vitamin A acetate content of the purified diets from 0 to 1000 units/kg feed. Higher levels of vitamin suppressed growth. Levels of beta carotene up to 20,000 units/kg feed did not result in growth equal to that obtained with 1000-2000 units vitamin A acetate/kg feed (Dupree, 1970a).

tinct nodules on the intestinal mesenteries (Young, 1970). Also in contrast to the observations with the red sea bream, Dupree (1966b) reported that better growth was obtained with the polysaccharides, starch and dextrin, than with the monosaccharides and disaccharides, glucose, and maltose. With 20% and 35% protein in the diet, weight increased with level of starch and dextrin (7.5, 15, and 30%), but gains remained essentially the same regardless of the amounts of dextrose or maltose in the diets (Figure 6). Blood glucose measurements were made on samples of fish before feeding, and at intervals of 1, 3, 7, 14 and 26 hours after feeding. Maximum blood glucose levels for all diets occurred after three hours, and then decreased to the base level (15 to 20 mg/

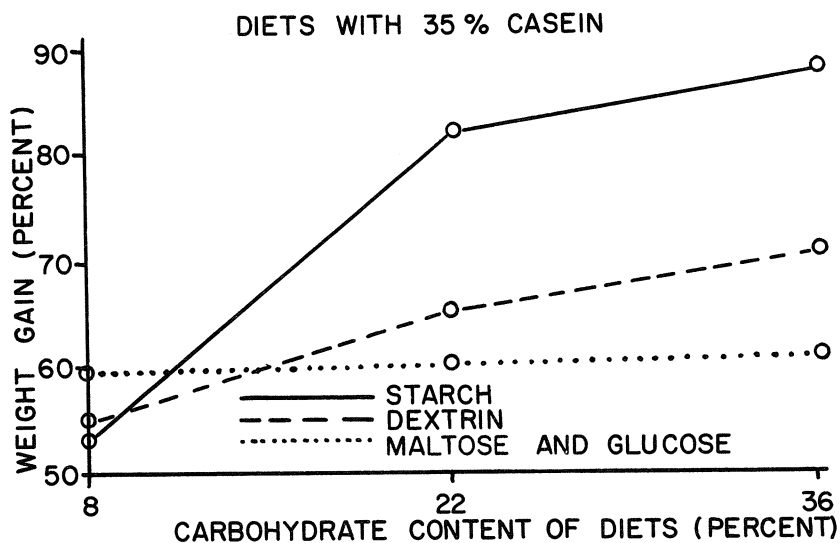


Figure 6. Effect of starch, dextrin, maltose and glucose in purified diets on weight gain of channel catfish. Weight gains increased with amount of starch and dextrin in the diet, but gains remained essentially the same regardless of the amounts of dextrose and maltose (Dupree, 1966).

100 ml) within 7 to 26 hours. Highest glucose levels (120 mg/100 ml) were measured in those fish fed the glucose diets followed by maltose (60 to 86 mg/100 ml), dextrin (55 to 60 mg/100 ml), and starch (25 to 35 mg/100 ml).

Fats

The use of fats as an energy source to spare protein, and the requirement for specific fatty acids in metabolism has been only incompletely investigated in warmwater fish nutrition. Most practical rations contain only about 5% crude fat and this is usually as a residue from extractions of grain and fish proteins.

In studies conducted in 1960, before the importance of essential fatty acids was recognized, Dupree and Sneed (1966) measured the best gain with purified rations that contained 5% corn oil. Dupree (1969b) later found that corn oil and beef tallow diets which contained 16% dextrin resulted in increased gains with fat up to 16%. With 8% dextrin

diets, gains increased with fat content up to 8%, but then decreased when fat was increased to 16%. In the best diets, 1 gram each of liquid corn oil, solid corn oil and beef tallow, spared 0.38, 0.73, and 0.27 grams of protein dry weight, respectively.

In studies in which fish oil levels were varied, Yone et al., (1971) and Tsukahara et al., (1967) reported the best growth and feed conversion with diets that contained 10% and 5% fish oil for the red sea bream and yellowtail, respectively. Fat up to 12% of the diet had little effect on eel (Arai et al., 1971).

Dupree (1970a; 1970b) fed a series of purified diets that contained 0 to 20% corn oil, 0 to 20% "bleached" menhaden oil, 12% bleached fish oil with and without an antioxidant, and 12% "crude" fish oil. Weight gain and protein deposition increased as the level of bleached fish oil was elevated from 0 to 15% of the dry diet, but gain decreased at 20% (Figure 7). At practical dietary levels, the addition of 1.5 grams of fish oil to the ration resulted in 1.0 grams of additional weight gain. Gains of fish fed corn oil diets were inferior to those fed fish oil diets,

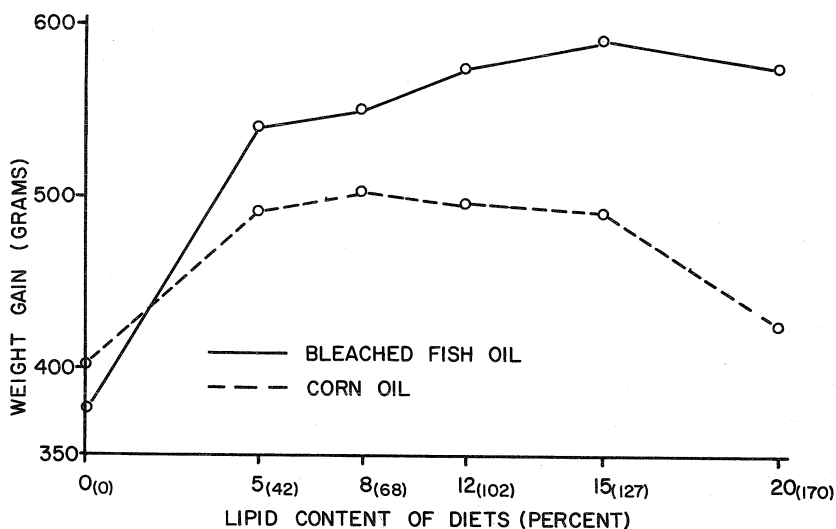


Figure 7. Effect of different amounts of fish oil and corn oil on the growth of channel catfish. Weight gain increased with dietary level of fish oil up to 15% of the diet; 20% dietary fish oil was not beneficial. Corn oil levels greater than 5% of the diet retarded growth (Dupree, 1969b; 1970b).

demonstrating little benefit from corn oil. Lipid, protein, ash, and moisture content of fillets fed 0 to 15% fish oil were little affected by the dietary oil level. At 20% fish oil, tissue stored oil was greater and tissue protein less when compared with fish fed the lower fed levels. There were no significant differences in the liver glycogen content or liver histology of the fish fed corn oil or fish oil at any level.

Addition of corn oil as the sole lipid source has been reported in numerous papers to retard the growth of eel, carp, yellowtail, red sea bream and catfish. (Tsukahara et al., 1967; Yone et al., 1971; Arai et al., 1971; Dupree, 1969b; and Watanabe et al., 1975a; 1975b). The essentiality of the w 6 and w 3 fatty acids for carp has been shown by Watanabe et al., (1975a), and will probably be found to be required by the other warmwater fish species.

LITERATURE CITED

- Aoe, H., K. Ikeda, and T. Saito. 1974. Nutrition of protein in young carp. II. Nutritive value of protein hydrolyzates. Bull. Jap. Soc. Sci. Fish. 40(4): 375-379.
- Aoe, H., I. Masuda, T. Mimura, T. Saito, and A. Komo. 1968. Requirement of young carp for vitamin A. Bull. Jap. Soc. Sci. Fish. 34(10): 959-964.
- Aoe, H., I. Masuda, I. Abe, T. Saito, T. Toyoda, and S. Kitamura. 1970. Nutrition of protein in young carp. I. Nutritive value of free amino acids. Bull. Jap. Soc. Sci. Fish. 36(4): 407-413.
- Aoe, H., I. Masuda, I. Abe, T. Saito, and Y. Tajima. 1971. Water-soluble vitamin requirements of carp. III. Some examinations of the utility of the reported minimum requirements. Bull. Jap. Soc. Sci. Fish. 37(2): 124-129.
- Aoe, H., I. Masuda, T. Saito, and T. Takada. 1957. Water-soluble vitamin requirements of carp. V. Requirement for folic acid. Bull. Jap. Soc. Sci. Fish. 33(11): 1068-1071.
- Arai, S., T. Nose, and Y. Hashimoto. 1971. A purified test diet for the eel, *Anguilla japonica*. Bull. Freshwater Fish. Res. Lab. 21(2): 161-178.
- . 1972. Amino acids essential for the growth of eels, *Anguilla anguilla* and *A. japonica*. Bull. Jap. Soc. Sci. Fish. 38(7): 753-759.
- . 1972. Qualitative requirements of young eels *Anguilla japonica* for water-soluble vitamins and their deficiency symptoms. Bull. Freshwater Fish. Res. Lab. (Japan). 22(1): 69-83.
- DeLong, D. C., J. E. Halver, and E. T. Mertz. 1958. Nutrition of salmonid fishes VI. Protein requirements of chinook salmon at two water temperatures. J. Nutrition 65: 589-599.
- Deyoe, C. W., O. W. Tiemeier, and C. Suppes. 1968. Effects of protein, amino acid levels, and feeding methods on growth of fingerling channel catfish. Prog. Fish-Cult. 30(4): 187-195.
- Dupree, H. K. 1966a. Vitamins essential for the growth of channel catfish. U. S. Dept. of Interior, Bureau of Sport Fisheries and Wildlife, Technical Paper 7, 12 p.

- _____. 1966b. Carbohydrate molecular size. *In* U. S. Dept of Interior, Bureau of Sport Fisheries and Wildlife, Progress in Sport Fisheries Research, 1965, Resource Publication 17: 45-46.
- _____. 1967. Effect of type of protein, protein particle size, and water temperature on fingerling channel catfish growth. *In* U. S. Dept. Interior, Bureau of Sport Fisheries and Wildlife, Progress in Sport Fisheries Research, 1966, Resource Publication 39, pp. 129-130.
- _____. 1969a. Vitamin E requirement of channel catfish. *In* U. S. Dept. of Interior, Bureau of Sport Fisheries and Wildlife, Progress in Sport Fisheries Research, 1968, Resource Publication 77, pp. 220-221.
- _____. 1969b. Influence of corn oil and beef tallow on growth of channel catfish. U. S. Dept. of Interior, Bureau of Sport Fisheries and Wildlife, Technical Paper 27, 13 p.
- _____. 1970a. Dietary requirement of vitamin A acetate and beta carotene. *In* U. S. Dept. of Interior, Bureau of Sport Fisheries and Wildlife, Progress in Sport Fisheries Research, 1969, Resource Publication 88, pp. 148-150.
- _____. 1970b. Effect of fish oil and corn oil on growth and flesh quality of channel catfish. *In* U. S. Dept. of Interior, Bureau of Sport Fisheries and Wildlife, Progress in Sport Fisheries Research, 1969, Resource Publication 88, pp. 150-151.
- _____. 1970c. Further studies on the effect of fish oil and corn oil in the diet of channel catfish, *In* U. S. Dept. of Interior, Bureau of Sport Fisheries and Wildlife. Progress in Sport Fisheries Research, 1970, Resource Publication 106. pp. 191-192.
- Dupree, H. K., and J. E. Halver, 1970. Amino acids essential for the growth of channel catfish, *Ictalurus punctatus*. Trans Amer. Fish. Soc. 99(1): 90-92.
- Dupree, H. K., and K. E. Sneed. 1966. Response of channel catfish fingerlings to different levels of major nutrients in purified diets. U. S. Dept. Interior, Bureau of Sport Fisheries and Wildlife, Technical Paper 9, 21 p.

- Furuichi, M., and Y. Yone. 1971a. Studies on the red sea bream. IV. Nutritive value of dietary carbohydrates. Rep. Fish. Res. Lab., Kyushu Univ. (Japan). No. 1: 75-81.
- . 1971b. Studies on the red sea bream VI. A study of carbohydrate utilization by glucose-and insulin glucose-tolerance tests. Rep. Fish. Res. Lab., Kyushu Univ. (Japan). No. 1: 101-106.
- Furuichi, M., K. Shitanda, and Y. Yone. 1971. Studies on nutrition of red sea bream. V. An appropriate supply of dietary carbohydrate. Rep. Fish. Res. Lab., Kyushu Univ. (Japan). No. 18: 91-100.
- Halver, J. E. 1957a. Nutrition of salmonid fishes. III. Water-soluble vitamin requirements of chinook salmon. J. Nutrition 62(2): 225-243.
- . 1975b. Nutrition of salmonid fishes. IV. An amino acid test diet for chinook salmon. J. Nutrition. 62(2): 245-254.
- Hashimoto, Y. 1975. Nutritional requirements of warmwater fish. Proc. 9th Int. Congr. Nutrition, Mexico 1972, Vol. 3, pp. 158-175. (Pub. 5. Karger, Basel, Switzerland.)
- Hastings, W. H., and H. K. Dupree. 1969a. Practical diets for channel catfish. In U. S. Dept. Interior, Bureau of Sport Fisheries and Wildlife, Progress in Sport Fisheries Research, 1968, Resource Publication 77, pp. 224-226.
- . 1969b. Formula feeds for channel catfish. Prog. Fish.-Cult. 31(4): 187-196.
- Lovell, R. T. 1973. Protein requirements of cage-cultured channel catfish. Proc. Twenty-sixth annual Southeast Assoc. of Game and Fish Commrs., Knoxville, TN., pp. 357-361.
- Mertz, E. T. 1969. Amino acid and protein requirements of fish. In O. W. Neuhaus and J. E. Halver (ed.), Fish in Research, Academic Press, New York: 233-244.
- Miller, D. S., and P. R. Payne. 1961. Problems in the prediction of protein values of diets. The influence of protein concentration. British J. Nutrition 15: 11-19.
- Nose, T., and S. Arai. 1972. Optimum level of protein in purified diet for eel, *Anguilla japonica*. Bull. Freshwater Fisheries Res. Lab. 22(2): 145-155.

- Nose, T., S. Arai, D. Lee, D. L. Lee, and Y. Hashimoto. 1974. A note on amino acids essential for the growth of young carp. Bull. Jap. Soc. Sci. Fish. 40(9): 903-908.
- Ogino, C., and M. Chen. 1973. Protein nutrition in fish. IV. Biological value of dietary proteins in carp. Bull. Jap. Soc. Sci. Fish. 39(7): 797-800.
- Ogino, C., and K. Saito, 1970. Protein nutrition in fish. I. The utilization of dietary protein by young carp. Bull. Jap. Soc. Sci. Fish. 36(3): 250-254.
- Page, J. W. 1975. Utilization of free amino acids by channel catfish. Proceedings of the Third Fish Feed and Nutrition Workshop, Sept. 24-25, 1974, at the Tunison Laboratory of Fish Nutrition, Cortland, N. Y. pp. 9-12.
- Rumsey, G. L., and H. G. Ketola. 1975. Amino acid supplementation of casein in diets of Atlantic salmon (*Salmo salar*) and of soybean meal for rainbow trout. (*Salmo gairdneri*) fingerlings. J. Fish. Res. Bd. Can. 32(3): 422-426.
- Tsukahara, H., A. Furukawa, and K. Funae. 1967. Studies on feed for fish. VII. The effects of dietary fat on the growth of yellowtail (*Seriola quinqueradiata* TEMMINCK ET SCHLEGEL). Bull. Naikai Reg. Fish. Lab. (Japan). No. 24: 29-50.
- Watanabe, T., F. Takashima, C. Ogino, and T. Hibiya. 1970a. Effects of alpha-tocopherol deficiency in carp. Bull. Jap. Soc. Sci. Fish. 36(6): 623-630.
- _____. 1970b. Requirement of young carp for alpha-tocopherol. Bull. Jap. Soc. Sci. Fish. 36(9): 972-976.
- Watanabe, T., O. Utsue, I. Kobayashi, and C. Ogino. 1975. Effect of dietary methyl linoleate and linolenate on growth of carp. I. Bull. Jap. Soc. Sci. Fish. 41(2): 257-262.
- Watanabe, T., T. Takeuchi, and C. Ogino. 1975. Effect of dietary methyl linoleate and linolenate on growth of carp II. Bull. Jap. Soc. Sci. Fish. 41(2): 263-269.

- Yone, Y., M. Furuichi, and S. Sakamoto. 1971. Studies on the nutrition of red sea bream. III. Nutritive value and optimum content of lipids in diet. Rep. Fish. Res. Lab., Kyushu Univ. (Japan). No. 1: 49-60.
- Yone, Y., and M. Fujii. 1974. Studies on nutrition of red sea bream. X. Qualitative requirements for water-soluble vitamins. Rep. Fish. Res. Lab., Kyushu Univ. (Japan). No. 2: 25-32.
- Young, J. E. 1970. Catfish: A new source of insulin for diabetics. The Catfish Farmer 2(4): 11.

Diet in Yellowtail Culture

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INTRODUCTION

Yellowtail (*Seriola quinqueradiata*) culture is one of the most popular mariculture processes in Japan. As is well known, the Japanese derive a greater proportion of their food from the sea than any other major nation. There are also various food customs pertaining to seafood. For example in the western part of Japan, people consider the yellowtail an indispensable food for the New Year. About 30 years ago before yellowtail culture was begun, yellowtail that could be kept alive brought higher prices and were primarily sold at fish markets in winter. In order to make a better profit, fishermen had to develop methods of keeping the fish alive during transport.

Considering these circumstances, it is natural that yellowtail culture was started in the Seto Inland Sea, which is surrounded by such major cities as Osaka, Kyoto, etc. As in the culture of other freshwater or marine finfish available, inexpensive fresh fish were used as feed in the beginning. As the yellowtail culture developed, availability of feed decreased and production costs gradually increased. In addition, various problems occurred in the operation of the business, primarily a rapid increase in labor costs.

In this paper, the present status of yellowtail diet and an outline of the studies on diet are reviewed and discussed.

Production and Amount of Diet

The annual production of yellowtail during the past decade is shown in Table 1. From the table the following observations can be

made: 1) Quantities obtained by both fishing and culture increased during this period; 2) The rate of increase per year for the culture production was not only higher than that for fishing production but also, since 1971, cultural yield per year has surpassed the total fishing yield per year; 3) In 1973, the quantity of cultured yellowtail was about 1.4 times that of fished yellowtail.

The value of the aquacultural product increased remarkably during this period and reached about \$150 million in 1973 — the rate of increase in value for the aquacultural product was higher than that for the fishing product. This phenomenon was caused by the improvement of cultural techniques and by a rise in the unit price of the aquacultural product. Consequently, the cultured yellowtail has been established as a unique product at the fishmarket.

Annual statistics of diets used in yellowtail culture are shown in Table 2. As is obvious from the table, the majority of the diets consist of fresh fish supplemented by frozen fish — anchovy (*Engraulis japonica*), sand lance (*Ammodytes personatus*), horse mackerel (*Trachurus*

TABLE I. Annual production of cultured and naturally caught yellowtail

	Fisheries		Culture	
	Yield (ton)	Value (Million\$)	Yield (ton)	Value (Million\$)
1964	42,800	29.9	9,493	10.0
1965	43,819	36.1	18,083	21.3
1966	38,950	37.6	19,629	22.8
1967	48,623	43.7	26,712	28.2
1968	48,363	44.4	30,774	43.3
1969	54,192	53.2	36,630	50.6
1970	54,875	65.1	50,855	70.9
1971	55,463	67.2	69,414	114.3
1972	59,260	63.6	73,946	151.3
1973	65,523	78.6	88,483	150.8

TABLE 2. Annual amount of diets in yellowtail culture

	Wet weight of diets (ton)			F.C.E.*	F.C.F.**
	Total	Artificial	Fresh-fish		
1965	138,543	?	138,543	13.1%	7.7
1966	161,392	?	161,392	12.2	8.2
1967	215,389	?	215,389	12.4	8.1
1968	268,237	?	268,237	11.5	8.7
1969	348,338	341	347,997	10.5	9.5
1970	437,572	131	437,441	11.6	8.6
1971	502,079	1,675	500,404	13.8	7.2
1972	576,304	6,810	569,494	12.8	7.8
1973	679,657	10,058	669,599	13.0	7.7
Mean				12.3	8.2

* F.C.E. (Feed Conversion Efficiency) = $\frac{\text{Wet weight of fish}}{\text{Wet weight of diet}} \times 100$

** F.C.F. (Feed Conversion Factor) = $\frac{\text{Wet weight of diet}}{\text{Wet weight of fish}}$

japonicus) and others. Use of artificial diets mixed with fresh fish began several years ago and gradually increased, but in 1973 the artificial diet accounted for only 1.4% of the total diet. Usually, the artificial diet has been mixed with frozen fresh fish.

Feed conversion efficiency (F.C.E.) and feed conversion factor (F.C.F.) were calculated from statistics in the table. Average values for nine years were 12.3% and 8.2, respectively. In 1973, assuming a 70% moisture content in fresh fish, F.C.E. and F.C.F. (dry basis) were calculated as 44% and 2.3, respectively.

Dietary Problems in Yellowtail Culture

Dietary problems in yellowtail culture are divided into two types — technical and economic or management related. Both types are not independent of each other — rather, a strong dependence is observed between both.

One of the technical problems is the use of fresh fish. Today fresh fish diets are being replaced with frozen fish, but the frozen fish has several problems with transportation, stock and feeding. Dry diets are preferred over frozen fish because studies of freshwater fish culture show the merits of dry diets (D.O. Locke et al. 1969): 1) lower conversions and a corresponding decrease in the cost per unit fish weight are obtained; 2) less waste occurs with dry diets than with meat diets; 3) the time-consuming, messy job of preparing wet feeds is eliminated; 4) expensive freezer operations are unnecessary; 5) less contamination of water and rearing pools occurs with dry diets than with wet diets or fresh fish; 6) automated feeding is more practical.

In the management of yellowtail culture, the cost of the diet equals about 60-70% of the total culture expenses; therefore, a key point of successful operation is to maintain an inexpensive and good quality diet.

Fresh Fish Diet

As described above, frozen fish occupies a large portion of the fresh fish diet. Three major fish species — anchovy, horse mackerel and sand lance — are sometimes caught in great quantities within a short period, resulting in a sudden decrease of their prices. Taking advantage of the low price, a large amount of this diet fish is deep-frozen and stocked for future use.

Chemical analyses of these frozen fish used in diets are given in Table 3. (A. Furukawa et al. 1965).

TABLE 3. Quality of fresh fish

Fresh fish species	Proximate chemical composition				Volatile basic N (mg %)	Acid value	Size (cm)
	Moisture	Protein (wet basis%)	Fat	Ash			
Sand lance★	72.7±0.6	16.0±1.2	6.3±1.7	2.7±0.2	25-80	6-23	4-5
	71.3±1.3	17.2±0.7	9.0±0.9	2.8±0.9	0-0.1?	9-15	15
	65.8±1.8	17.1±1.6	11.6±0.9	2.8±0.4	22-41	14-38	15
	71.9±2.2	17.5	7.8	2.8±0.2	14	5, 15	5, 15
	72.5±1.9	15.1±0.1	8.3±0.7	2.4±0.1		12-15	12-15
	62.7	19.1	11.0				
Jack mackerel★	76.0±1.4	17.0±0.1	5.9±0.2	2.9±0.1	16-30	5-8	15
	72.9	19.5	4.8	2.8	8-19		12-15
	73.9±0.5	15.0±1.0	2.8±0.6	3.5±0.1			15
	76.2	18.8	1.0	3.9			
	77.5	16.7	2.9				
	76.5	18.4	1.4	3.6			7-8
Anchovy★★	72.3	19.2	5.7	3.2			
	72.1	17.9	8.3	3.5			
	68.7	18.2	10.4	3.2			
	71.4	16.8	6.9	2.8			
	70.6	20.6	6.2	2.7			
	71.3	19.4	5.1				

★—A. Furukawa et al (1965)

★★—H. Sakai et al (1968) and S. Yano et al. (1967)

As a matter of course, there were differences between species as to the proximate chemical composition. In the same species, however, the freshness of the diet fish, indicated by the volatile basic N and the acid value, has more influence on the cultured yellowtail than does the chemical composition of the diet fish. In a practical sense, a mass mortality of cultured yellowtail caused by feeding them putrefied frozen fish has often been experienced.

On the other hand, amino acid content in horse mackerel used in a diet and yellowtail meat fed the above fresh fish diet are shown in Table 4 (S. Yano et al. 1967). Although there is little knowledge of the amino acid content in the fresh fish diet, this table can be considered an example, showing the outline of features of a fresh fish diet.

As mentioned above, the frozen fish diets are perishable and cause dietary diseases, but these diets are nevertheless practically used in the present yellowtail culture industry.

Availability of Diet

There are various points of view when determining the quality of diet used in yellowtail culture. The nutrients must be completely contained in the diet, it must be preferred by the fish, and it must be easy

TABLE 4. Composition of amino acids in dietary fresh fish and yellowtail meat

	Horse mackerel		Yellowtail meat		
	Values as Tryptophan 1.0	g./Protein 100g.	Values as Tryptophan 1.0	Start	35 days 65 days
Tryptophan	1.0	1.3	1.0	1.0	1.0
Lysine	9.8	12.7	7.3	8.5	11.2
Histidine	2.8	3.6	1.8	2.2	2.0
Arginine	5.3	6.9	4.7	5.5	5.0
Threonine	3.6	4.7	3.0	3.7	4.3
Valine	6.5	8.4	4.3	4.5	3.7
Methionine	2.3	3.0	2.0	2.6	7.4
Isoleucine	4.8	6.3	3.8	5.1	5.8
Leucine	7.2	9.3	6.2	7.5	9.7
Phenylalanine	4.1	5.3	2.6	3.5	2.5
Aspartic acid	7.3	9.5	7.2	6.8	8.8
Serine	2.9	3.8	2.4	3.1	4.0
Glutamic acid	9.2	12.0	9.1	6.5	11.0
Proline	3.1	4.0	2.3	3.2	2.7
Glycine	4.8	6.2	2.9	3.5	4.2
Alanine	6.0	7.8	4.4	5.5	7.3
Cystine	—	—	—	—	—
Tyrosine	2.8	3.7	2.0	2.8	3.8

in treatment. Diets of various types are necessary when feeding is to be done economically.

In this next section, the feed conversion factor, and digestibility of diet components will be reviewed and discussed.

Feed conversion factor. Evaluation of diet in the practical yellowtail culture industry is usually done by using the feed conversion factor. One of the reasons for this is economic — feed expenses make up a very large portion of the total expenses, and obtaining the necessary amount of diet for the expected production is important to get good results in the intensive culture of yellowtail. In order to estimate the amount of diet required, application of the feed conversion factor is practical.

The feed conversion factor is calculated as a relative value of the body weight gain to the quantity of diet fed to the fish. Therefore, the utility value of the diet is not always shown by the feed conversion factor, which is affected by such factors as the water temperature, fish size, and feeding method. Several feed conversion factors obtained under various conditions are shown in Table 5. The conversion values in Table 5 are, therefore, independent of each other, and the comparison is not always meaningful. However, Table 5 may provide some ideas about the feed conversion factor.

The results of a rearing test, done under similar conditions are given in Figure 1. One can observe that the frozen fish shows maximum utility as a yellowtail diet, the mixed diet medium utility and the artificial diet, minimum.

Digestibility of dietary components. Protein digestibility. Protein digestibilities in various yellowtail diets are shown in Table 6. Protein of raw, frozen, and boiled fish, and gluten gave higher values of digestibility than did protein of the mixed diet and fishmeal. In comparison with the results reported for rainbow trout, carp, channel catfish and red sea bream (Y. Hashimoto 1972), it seems that the digestibility of fish meal protein in yellowtail is inferior.

The effect of carbohydrate on protein digestibility. Relationship between the quantity of starch added to the diet and protein digestibility is summarized in Figure 2. The data were gathered from various papers and plotted regardless of the various experimental conditions of the respective authors. Conditions include not only differences in composition or quality of the tested diets but also differences in rearing conditions. Therefore, it is difficult to discern a clear relation between both factors, but it may be roughly determined that the decreased protein digestibility is caused by the increased starch content in the diet. Also, the effect of carbohydrate in decreasing protein digestibility in yellow-

TABLE 6. Protein digestibility of various diets

Composition of diet	Digestibility (%)	Remarks
Raw Sand lance	84	A. Furukawa et al. (1965)
Raw Sand lance	av. 86.5	H. Tsukahara et al. (1967)
Boiled Sand lance	av. 89.2	" " "
Horse mackerel	91.5	M. Kitamikado et al. (1965)
Mixed diet		
Sand lance 40% + W.F.M. 40% + S 10% + others	89.88	A. Furukawa et al. (1965)
Horse mackerel 40% + W.F.M. 42% + S 10.2% + others	av. 75.5	S. Yano et al. (1966)
Horse mackerel 40% + W.F.M. 45.4% + G 6% + others	av. 73.4	S. Yano et al. (1966)
Frozen Horse mackerel 10% + Horse mackerel meal 90%	74	M. Kitamikado et al. (1965)
Frozen Horse mackerel 85% + S 15%	85	" " "
Artificial diet		
W.F.M. 44% + S 25.4% + Corn oil 10% + others	av. 56	A. Furukawa et al. (1965)
W.F.M. 73.6% + S 16.7% + others	av. 31.6	H. Tsukahara et al. (1967)
G 85.5% + S 4.75% + Oil 5% + others	av. 91.2	" " "
W.F.M. 70% + Corn oil 15% + S 15%	51.3	M. Sato et al. (1965)
W.F.M. 69.8% + S 17% + others	av. 51.3	S. Yano et al. (1966)
W.F.M. 75.7% + G 10% + others	av. 64.1	" " "
W.F.M. 73.8% + S 9.5% + others	av. 87.7	A. Furukawa (1967)

W.F.M. — White fish meal

S — Starch

G — Gluten

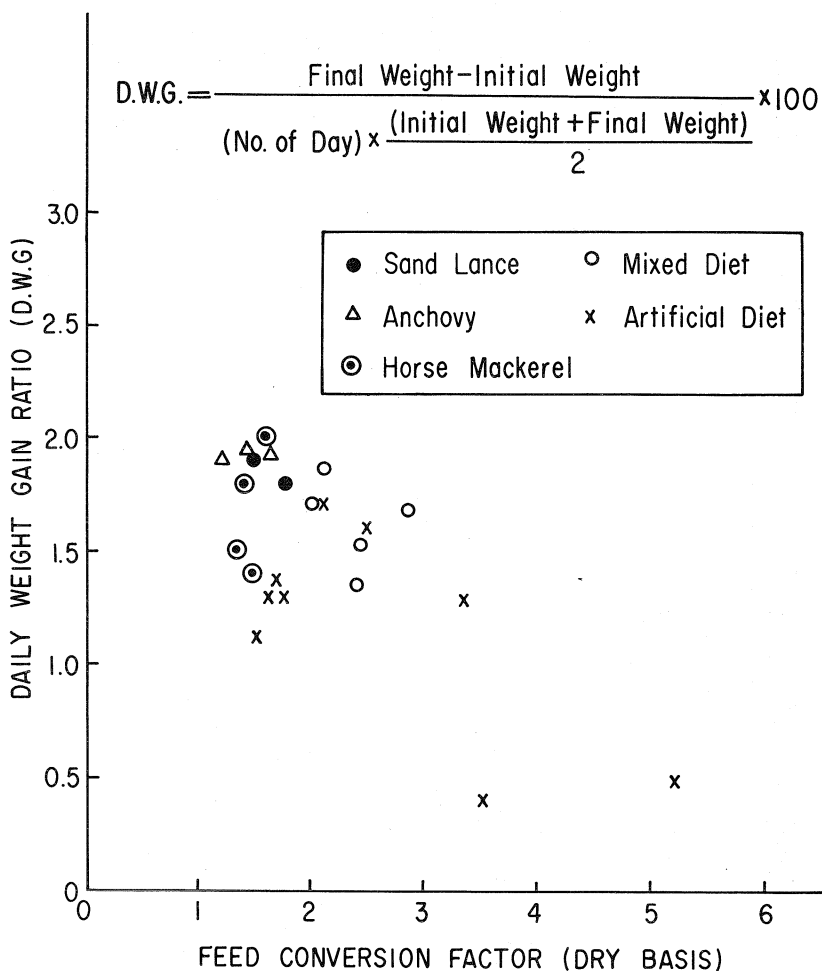


FIGURE I. Utility of diet shown by the feed conversion factor and daily weight gain ratio (D.W.G.)

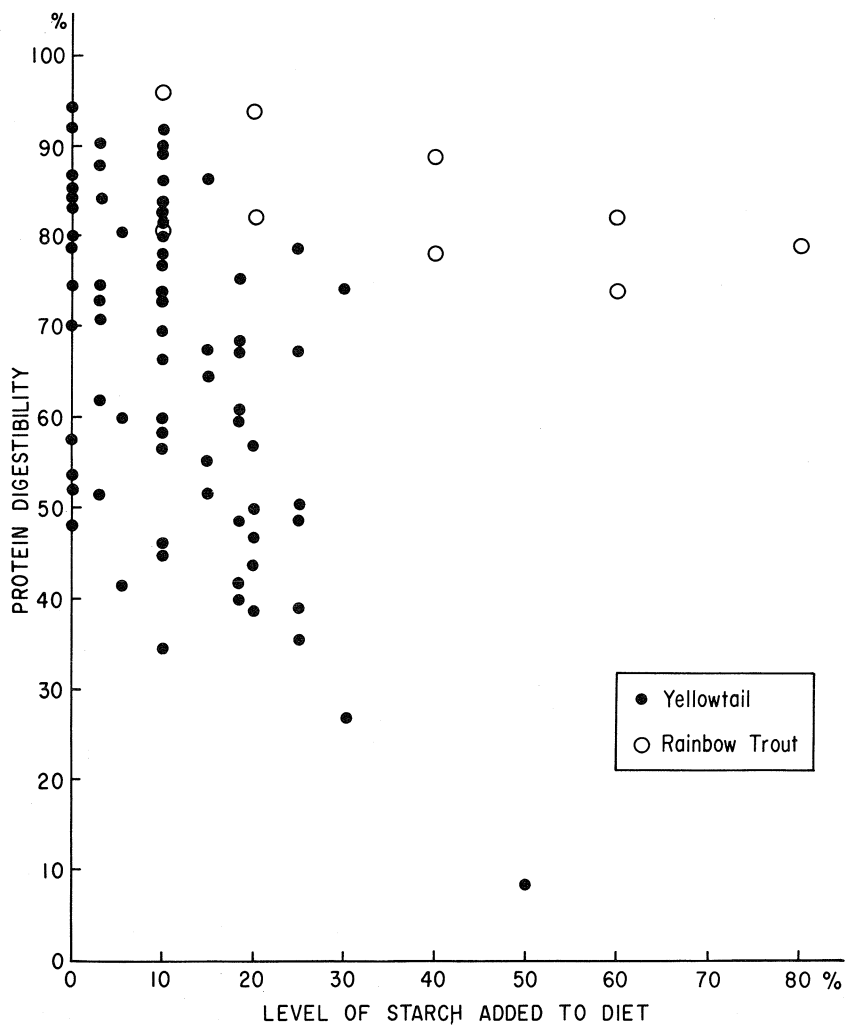


FIGURE 2. The relationship between starch level in diet and protein digestibility

tail is larger than that in rainbow trout. In any case, the concentration of starch added to the yellowtail diet is to be kept less than 10% of total weight of the diet.

Change of protein digestibility. Figure 3 represents the protein digestibility value of an artificial diet (74.8% white fish meal + 17% corn starch + 5% fat + other nutrients) and the amount of feces produced every hour in a feeding experiment. Within the first seven hours after feeding, the protein digestibility showed not only a low value (under 80%) but also violent variation. Thereafter, the digestibility increased until 11 hours, showing a rather stable value (around 85%) between 12 hours and 24 hours after feeding. On the other hand, for the first eight hours, more than 40% of the total feces was excreted, therefore, it is possible that a large quantity of indigested diet was excreted in the early period.

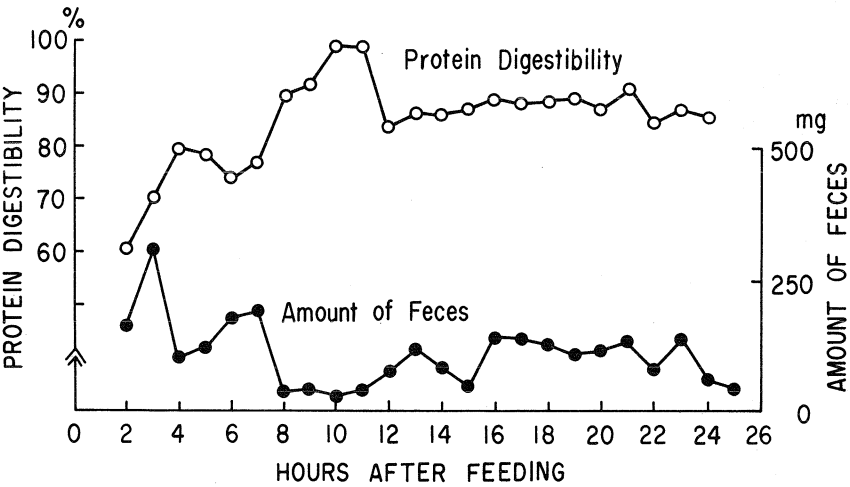


FIGURE 3. Digestibility of protein every hour after feeding

Figure 4 shows the change in protein digestibility of minced sand lance meat in another experiment. The value of protein digestibility was determined at hour intervals. In this case, the maximum value appeared three hours after feeding and then decreased gradually. The minimum was obtained eight hours after feeding. On the other hand, M. Sato et al. (1966) reported that the maximum protein digestibility value was obtained two hours after feeding. In any case, the protein digestibility was changed by the collection time of feces after feeding — so it is

necessary to collect the whole feces excreted after feeding. In order to achieve this, the sack method for feces collection was applied and good results were obtained, in spite of the fact that the contents of the sack were contaminated by urinary N (A. Furukawa 1967).

Digestibility of protein materials prepared by different treatment.

In order to detect the differences among protein digestibilities of processed and non-processed fish meat, a series of feeding experiments was carried out, using raw, boiled, sun-dried, and heat (120°C) dried sand lance meat (H. Tsukahara et al. 1967).

Raw and boiled sand lance meat resulted in higher digestibilities than sun-dried and heat-dried meats.

There was an observed insignificant difference between the digestibilities of raw and boiled meat, while heat-dried meat showed inferior digestibility to that of sun-dried meat as a whole. However, the digestibilities of both dried meat materials showed different sequences when observed at various time intervals.

Carbohydrates. Carbohydrates are used as a substitute for protein and as binders in diets. Since the protein component of diets is probably the most expensive major ingredient, attempts were made to replace protein with carbohydrate. It is a very difficult problem to decide the optimum level for this nutrient, since the digestive efficiency is greatly affected not only by its quality but also by its quantity relative to all other broad classes of nutrients present in the diet (Y. Hashimoto 1974).

Information on carbohydrate metabolism in yellowtail is still scanty.

According to M. Sato et al. (1967) glucose and glycogen are best as a carbohydrate source of growth, followed by dextrin and *alpha*-starch in that order. The same result was obtained by testing the red sea bream (M. Furuichi et al. 1971a, 1971b; M. Furuichi et al. 1971). When the experiment was carried out with diets containing 0, 3, 6% glucose for 103 days, fish fed diets containing 3% glucose showed the best growth, feed efficiency and healthy condition. On the contrary, the diet containing 6% glucose showed retarded growth, but the feed conversion factor of this diet was not affected greatly (M. Sato et al 1968).

Starch digestibility. Several results of starch digestibility tests are given in Table 7. In general, it has been considered that the starch digestibility of yellowtail is relatively low, compared to that of dietary protein and fat. Almost all data obtained by various authors show the low level of starch digestibility, but some data (A. Furukawa 1967) show a considerably high level. The origin of such difference is not clear and may be a future problem. It can be suggested that the different carbohydrate digestibilities shown by various experiments are the result of

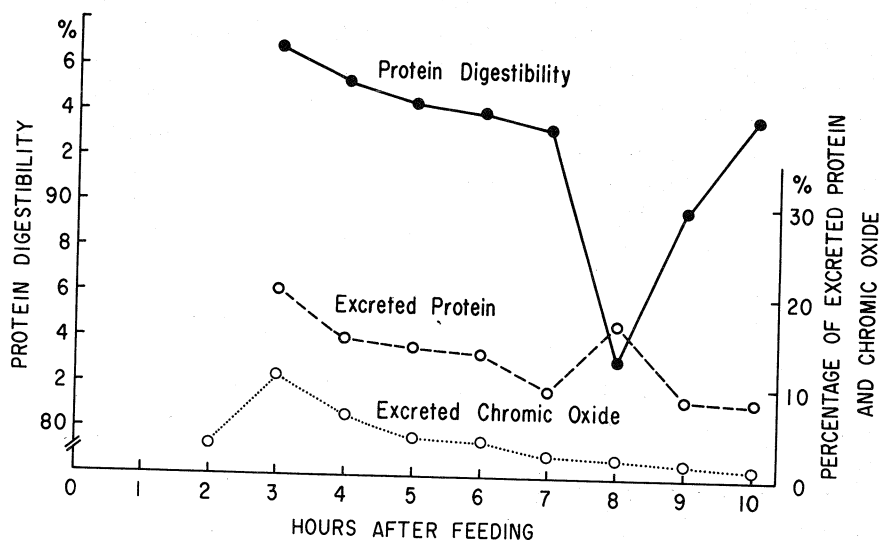


FIGURE 4. Availability of protein every hour after feeding

differences in the methods of collecting rejected feces. The lower values were obtained in experiments applying incision or press methods in collecting feces, while higher values were obtained in the investigation using the sack method. Of course, each method has advantages and disadvantages in collecting the sample feces, but as was shown in protein digestibility changes, the value of starch digestibility varies at every moment (H. Tsukahara et al. 1967). So, if the complete sack feces collection method is used, the results may be more reliable than that of other methods.

Fat. In general, nutritional studies on fat for yellowtail are still scarce too. Authors (H. Tsukahara et al. 1967) have reported on the effect of dietary fat on the growth of yellowtail. Differences in weight and length were apparent between the fish fed corn oil diets and cod liver oil diets. Thus, it is recognized that cod liver oil effectively induces rapid growth in the yellowtail, as it does in trout. The results of blood examinations on red cell counting, hematocrit value and hemoglobin content showed that cod liver oil was more suitable for healthy physiological conditions. Yellowtail preferred the cod liver oil diet more than the corn oil diet, and it may be supposed that the differences in growth rate and blood property already observed resulted from the difference in total protein intake.

TABLE 7. Starch digestibility of various diets

Composition of diet (%)				Digestibility (%)	Remarks
Horse mackerel	Frozen fish		Potato starch	21 39 28	M. Kitamikado et al. (1965) (Operation method)
	85		15		
	- 85 - 70		15 30		
Protein	Carbohydrate		Others	av. 27.9 av. 26.7 av. 15.5 av. 25.5	H. Tsukahara et al. (1967) (Press method)
	22.7		+		
	18.3		+		
	16.9		+		
	16.5		+		
Protein	Carbohydrate		Fat	26.7	M. Sato et al. (1965) (Press method)
	16		15.5		
			Others +		
White fish meal	Potato starch		Others	av. 67.0 av. 75.7 av. 74.3 83 av. 67.0 av. 72.7	A. Furukawa (1967) (Sack method)
	9.5		+		
	9.2		+		
	8.0		+		
	7.9		+		
	7.4		+		
	7.3		+		
★ D.M.	Potato starch		Corn oil	67.1 66.9 72.8 75.6 74.1 82.5	H. Tsukahara et al. (1967) (Sack method)
	4.3		5		
	3.9		15		
	3.2		30		
	4.3		-		
	3.9		5		
	3.2		15		
	3.9		30		
	3.2		-		
			Cod liver oil		
D.M.	Gluten		Others	+ + + + + +	
	8.6		+		

★ D.M. White fish meal: Casein = 1:1

Food conversion and protein efficiency showed that the diet supplied with 5% cod liver oil was the most efficient among all artificial diets used in this experiment. The hepatosomatic value was altered by the influence of the ratio of dietary ingredients.

Fat deposition in liver and flesh increased with the increase of added fat in the diet. Mean fat digestibility was 92.7% and there was no difference in the value of fat digestibility between the cod liver oil diet and the corn oil diet, but the fat digestibility was lower in the case of excess fat content (30%) in diet.

The fatty acid compositions of the flesh and liver of the yellowtail were dependent on the amount of supplied fat and combination of dietary ingredients. Remarkably, the oleic acid was deposited more when the artificial diet was fed than when the raw sand lance diet was fed. The pattern of fatty acid composition of liver lipid changed more rapidly and remarkably than that of flesh lipid.

Vitamins. In spite of various endeavors to prepare the complete artificial diets or the purified test diets for yellowtail, as yet satisfactory results have not been obtained. Under these situations, the study of vitamin requirements for yellowtail remains only at the initial and unsatisfactory stage as shown by Hashimoto (1975).

The first experiment on vitamin testing for yellowtail was carried out with vitamin E (A. Furukawa et al. 1965). High mortality occurs sometimes under the practical operation and it may be caused directly or indirectly by dietary disease. When the putrefied raw fish diet was fed to yellowtail for a long time (these feeding conditions were often observed in summer) a symptom similar to the blackened fish (M. Kimura 1963) was frequently observed, and ultimately mass mortality followed. In our experiment, we observed an obvious effect of dietary vitamin E on the young yellowtail fed a putrefied raw fish diet. The fish tested was fed with sufficient putrefied raw minced sand lance. One lot was given this food as a vitamin E-deficient diet, while the other was fed the food enriched with vitamin E (ca. 34 I.U./100 g. of diet) as a vitamin E-supplied diet. The difference between the two groups was shown in feeding activity, growth and the number of red corpuscles.

H. Sakaguchi and A. Hamaguchi (1969) investigated the effects of oxidized oil and vitamin E administration on yellowtail fed with artificial diets. In their experiment, fish were fed for 93 days with diets containing 8.5% of fresh (P.O.V. 11-13) or oxidized (P.O.V. 480-500) cod liver oil to which DL-alpha-tocopheryl acetate was either added or not (0.6 - 150 I.U. of DL-alpha-tocopheryl acetate was added until 71st day, thereafter increased to 1.2 g.). The fish fed oxidized oil, without

vitamin E, showed a slightly poor appetite after about 40 days of feeding, and at the termination of the experiment a leaning of the dorsal muscle was observed in about 10% of the tested fish. The authors concluded that these symptoms are probably due to muscle dystrophy, as reported with the carp (Y. Hashimoto et al. 1966). However such symptoms were not found in the vitamin E-supplemented group. The muscle of fish fed with oxidized oil but without the addition of vitamin E showed a slightly higher TBA value than that of the vitamin E-supplemented fish, but there was no extreme difference in the TBA values of the hepatopancreas of these two groups. At the termination of the experiment, the glycogen contents in the hepatopancreas of the three groups (sand lance, fresh cod, and vitamin E) were elevated to about two times the content at the 70th day. However, such elevation of the glycogen level was not observed in the group fed with oxidized oil. On the other hand, the activities of transaminases (GOT, GPT) were higher in the fish fed with oxidized oil than the fish fed with fresh oil, and these enzyme activities could not be reduced even by the administration of vitamin E. The alkaline phosphatase activity showed little, if any, difference between these groups.

On the vitamin B₆ and vitamin C deficiency symptoms for yellowtail, H. Sakaguchi et al. (1969) reported as follows: both vitamin B₆ and vitamin C deficiency symptoms appeared after about 40 days. Characteristics of B₆ avitaminosis of the yellowtail were anorexia, retarded growth, nervous disorder, high mortality, injury at mouth and rostrum regions, exophthalma and scoliosis. The fish fed with a diet lacking vitamin C showed loss of activity and appetite, retarded growth, change of body color, poor growth of operculum, scoliosis, amputation of snout, and hemorrhage in the laterodorsal region. Fat content in the liver decreased in both B₆ and C deficient fish. Serum protein of the yellowtail was separated into four fractions by electrophoresis on cellulose acetate membrane. In the serum of the fish fed with vitamin B₆ or C deficient diet, fraction I decreased but fractions III and IV increased.

Compared to other diets fed to salmon, trout and other fish, the artificial diet tests, growth test, feed conversion test, etc. for yellowtail, were generally conducted with much more vitamin fortification to avoid the occurrence of vitamin deficiency. A test of three premixed vitamin supplementation levels in the artificial diet was carried out by M. Sato et al. (1968). Three test groups were set up by the vitamin supplementation levels as 0.4 group, 2.2 group and 3.8 group. These figures are percentages of vitamin mixture in the diets. High mortality was observed in the 0.4 group and several dead fish were observed in the 2.2 group, but

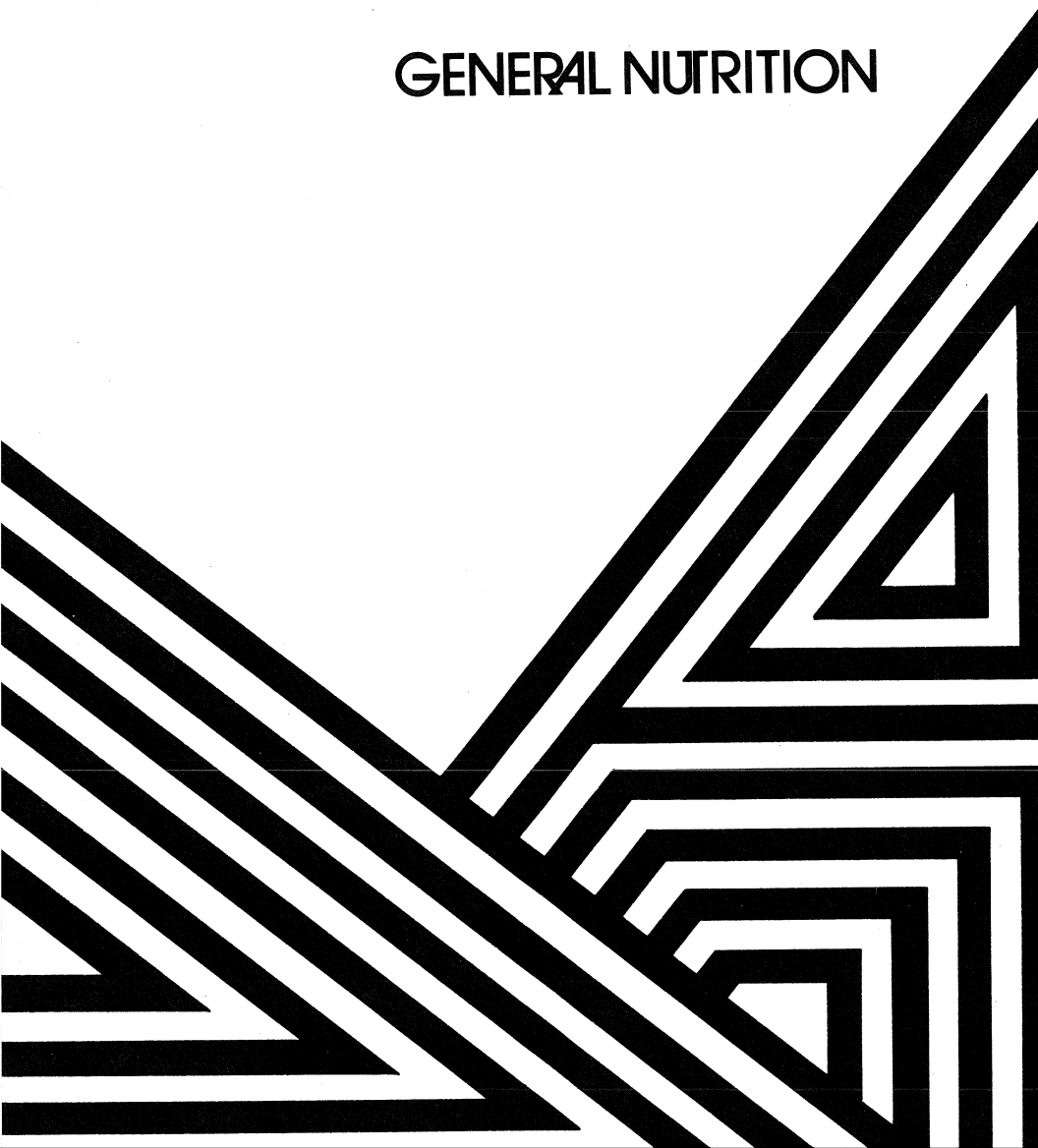
this phenomenon was not observed in the 3.8 group. Differences in growth response were apparent at the termination of the experiment. The rate of growth increased with the increasing premix in the diet. Anemia was recognized obviously in the 0.4 group but could not be observed in other groups.

LITERATURE CITED

- Furuichi, M. and Y. Yone. 1971a. Rep. Fish Res. Lab., Kyushu Univ. No. 1.
- . 1971b. Rep. Fish. Res. Lab., Kyushu Univ. No. 1.
- Furuichi, M., K. Shitanda and Y. Yone. 1971. Rep. Fish. Res. Lab., Kyushu Univ. No. 1.
- Furukawa, A. 1967. The Aquaculture Vol. 14, No. 4.
- Furukawa, A. and T. Umezu. 1965. Publication C of Naikai Regional Fisheries Research Laboratory No. 4.
- Furukawa, A., T. Umezu, H. Tsukahara and I. Iwata. 1965. Publication C Naikai Regional Fisheries Research Laboratory No. 4.
- Furukawa, A., T. Umezu, H. Tsukahara, K. Funae and I. Iwata. 1966. Bulletin of Naikai Regional Fisheries Research Laboratory No. 23.
- Hashimoto, Y. 1972. Yogyo-Shiryo-Gaku; Koseisha-Koseikaku. Tokyo.
- . 1974. Proc. 9th Int. Congr. Nutrition Mexico 1972, Vol. 3.
- Hashimoto, Y., T. Okaichi, T. Watanabe, A. Furukawa and T. Umezu. 1966. Bull. Japan. Soc. Sci. Fish. Vol. 32, No. 1.
- Kimura, M. 1963. Bull. Japan Soc. Sci. Fish. Vol. 35, No. 10.
- Kitamikado, M., T. Takahashi, H. Noda, T. Morishita and S. Tachino. 1965. Bull. Japan. Soc. Sci. Fish. Vol. 31. No. 2.
- Locke, D. O., and Stanley P. Linscott. 1969. The Progressive Fish-Culturist Vol. 31, No. 1.
- Sakaguchi, H. and A. Hamaguchi. 1969. Bull. Japan. Soc. Sci. Fish. Vol. 35, No. 12.
- Sakaguchi, H., F. Takeda, and K. Tange. 1969. Bull. Japan. Soc. Sci. Fish. Vol. 35, No. 12.
- Sakai, H. 1968. Bull. of Ehime Prefectural Fisheries Research Station.
- . 1969. Bull. Ehime Prefectural Fisheries Research Station.

- Sakai, H., and Y. Oiwa. 1971. Bull. of Ehime Prefectural Fisheries Research Station.
- Sato, M., M. Kawanishi, T. Kitazima and Y. Arakawa. 1965. Bull. of Hiroshima Prefectural Fisheries Research Station Vol. 25, No. 1.
- Sato, M., M. Kawanishi and T. Kitazima. 1967. Bull. of Hiroshima Prefectural Fisheries Research Station Vol. 28.
- . 1968. New Bull. of Hiroshima Prefectural Fisheries Research Station No. 1.
- Tsukahara, H., A. Furukawa and M. Hagihara. 1967. Bull. of Naikai Regional Fisheries Research Laboratory No. 24.
- Yano, S., Y. Oiwa, H. Sakai and Y. Nakamura. 1966. Bull. of Ehime Prefectural Fisheries Research Station.
- Yano, S., H. Sakai, Y. Oiwa, A. Minamizawa and Y. Nakamura. 1967. Bull. of Ehime Prefectural Fisheries Research Station.

GENERAL NUTRITION



Nutritional Consideration In The Culture Of Tropical Species

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Tropical aquaculture, especially in the Pacific islands, has been the subject of several investigations and conferences. The major portion of studies under the South Pacific Islands Fishery Development Agency (SPIFDA) during 1970 to 1973, involved identifying species with best potential for aquaculture and geographical locations best suited to such ventures. (Glude, 1972 and Villaluz, 1972)

The Pacific Island Mariculture Conference held in Hawaii February 6-8, 1973, called together representatives from various organizations and locations to identify fish and shellfish with the best potential for mariculture in the Pacific. (University of Hawaii, 1973) Among the species of fish considered by this conference to have potential for culture in the tropics were: rabbitfish, *Siganus* sp.; mullet, *Mugil* sp.; milkfish, *Chanos* sp.; baitfish, (Topminnows or mollies, *Poecilia* sp. cardinal fish, *Apogon* sp., etc.). Invertebrates considered to be highest priority for aquaculture included the freshwater prawn *Macrobrachium* sp. and the marine shrimp of the family *Penaeidae*. Two crabs, the mangrove crab *Scylla serrata* and the coconut crab *Birgus latro*, fell within the second priority for aquaculture.

More recently Dr. Robert May and his associates at Hawaii Institute of Marine Biology have grown the threadfin *Polydactylus sexfilis*, known in Hawaii as moi, to market size in floating cages in less than a year. With high local demand, rapid growth of commercial fish foods and good conversion ratios, *Polydactylus sexfilis* is a likely species for tropical aquaculture.

The dolphin fish *Coryphaena hippurus*, known in Hawaii as mahi mahi has considerable potential as an aquaculture species. William Hassler (1975) at North Carolina State University reported extremely rapid growth with good conversion rates for dolphin held in pens.

Aquaculture in tropical island environments of all the species listed above would require an economical source of suitable food. This requirement might be difficult to satisfy in view of transportation costs in the area, difficult logistics, and the lack of local sources of suitable components for fish feeds.

This short paper will discuss some local food sources which might be used in tropical aquaculture.

KOA HAOLE, *LEUCAENA LEUCOCEPHALA*

Duane Rodman (1973) working with the Marine Resources Division at Pago Pago, American Samoa, reported success in feeding a local mollie *Poecilia mexicana* Steindachner (*Poeciliium mexicana*) on the dried leaves of a local plant, *Leucaena leucocephala* (formerly *L. glauca*) (known locally as the lopa Samoa, in Hawaii as the koa haole, in Guam as tangen tangen and in French Polynesia as acacia). In these experiments, the leaves of this plant were air-dried, after which they were so brittle they could easily be crushed into small pieces. The fact that these fish were able to use koa haole as their only source of food and continue to survive and grow indicated a fairly significant protein content. Subsequent analysis of the dried leaves by the Pacific Utilization Research Center of the National Marine Fisheries Service in Seattle indicated a total crude protein level of 33%.

A similar sample of *L. leucocephala* collected by Dennis Tufts, Micronesian Mariculture Demonstration Center at Koror, Palau, in April, 1973, had a crude protein level of 28%. This sample included some fine stems (petioles) along with the leaves, which may explain its lower protein content.

Amino acid content of the protein fraction of koa haole from American Samoa was determined by Dr. John Halver, Bureau of Sport Fisheries and Wildlife, Western Fish Nutrition Laboratory, Cook, Washington. The sample from Palau Islands was examined for amino acid content by a commercial laboratory in Seattle. Results are indicated in Table I with comparative amino acid content of dried shrimp waste and meal prepared from Atlantic red hake. The analyses indicate that most of the amino acids are present in koa haole at levels similar to those in shrimp wastes and hake meal.

TABLE 1

Amino acid content of the protein fraction of koa haole (*Leucaena leucocephala*) from American Samoa and Palau Islands, and comparative values for shrimp wastes and red hake meal.⁵

Amino Acid	Haole koa from ¹ American Samoa (% of protein)	Haole koa from ² Palau Islands (% of protein)	Shrimp Wastes (Dried) (% of protein)	Atlantic Red Hake Meal (Urophycis chuss) (% of protein)
Alanine	6.10	6.4	5.85	6.5
Arginine	5.70	6.4	7.35	6.8
Aspartic	13.2	10.5	11.5	10.7
Cystine/ ³	N.A. ⁴	1.6	1.43	0.9
Glycine	5.65	5.9	4.77	7.6
Glutamic	11.05	12.2	13.5	14.4
Histidine	2.03	1.74	2.76	1.9
Isoleucine	5.58	5.0	5.4	4.2
Leucine	5.70	10.3	8.23	7.5
Lysine	5.63	6.9	7.77	8.3
Methionine	1.32	1.65	1.92	3.2
Phenylalanine	6.03	6.35	5.37	4.0
Proline	7.34	5.05	3.51	5.1
Serine	5.28	5.22	3.73	4.2
Threonine	5.68	5.10	4.73	4.3
Tryptophan	N.A.	N.A.	N.A.	N.A.
Tyrosine	4.02	4.36	4.5	3.1
Valine	6.37	6.72	6.25	5.1
Total Crude Protein (Nitrogen x 6.25)	33%	28%	75%	Approx. 70%

¹ Dried leaves without stems received from Duane Rodman March 7, 1973; analyzed by Dr. John Halver, BSWF, Western Fish Nutrition Laboratory, Cook, Washington.

² Dried leaves with stems received from Dennis Tufts April 17, 1973; analyzed by Triple A Labs, Seattle Washington.

³ Performic oxidation. All other values from 24 hour acid hydrolyseate.

⁴ Not available.

⁵ Prepared by NMFS Pacific Utilization Research Center, Seattle, Washington, June 1973.

It appears from these analyses that the leaves of the koa haole plant provide a reasonably good source of protein which might be used by herbivorous or omnivorous fish, and perhaps crustaceans. However, use depends on biological availability. It is possible that part of the protein, and therefore the amino acids of that fraction, may be indigestible. As described by Pirie (1971), the first limiting amino acid of plant proteins seems to be methionine, but synthetic methionine is fairly inexpensive. It is added to poultry rations in which soy is the primary protein source.

Dr. D. K. Villaluz (1972) included a comment in his report on aquaculture possibilities in some islands in the South Pacific, that the leaves of *Leucaena glauca* (Now *L. leucocephala*) were a possible source of local food for fishes in the Fiji Islands, Gilbert Islands, Cook Islands, Tonga, Western and American Samoa, Palau Islands and New Caledonia. Although Dr. Villaluz did not elaborate, it appears that the leaves of this plant have been considered useful for culture of some aquatic species in Southeast Asia.

Feeding Tests With Land Animals

Brewbaker, Plucknett and Gonzales (1972) describe studies in Hawaii concerning the yield of several varieties of *Leucaena*, which indicate maximum production of 10.8 tons dry weight, equivalent to 4,320 pounds of crude protein, per acre per year. This report and a personal letter from Dr. Brewbaker dated June 21, 1973, indicate that a toxic amino acid [B (N-(3-hydroxypyridone-4))d-aminopropionic acid], a lysine derivative named mimosine, occurs in all *Leucaena* species. Although this alkaloid causes no difficulty with ruminant animals, provided the intake is graduated, as would be true with any other legume, mimosine delivered at high doses causes hair loss in horses and humans. Several other symptoms of its intake have been reported in poultry and rats. Poultry rations successfully employ about 15% of *Leucaena* by dry weight but higher doses reduce egg production (Oakes, 1968). *Leucaena* is also satisfactory for growing and fattening swine, but large amounts may have adverse effects on reproduction (Kinch and Ripperton, 1962).

Several methods have been developed for reducing the mimosine content and toxicity of koa haole. Matsumoto, Smith and Sherman (1951) reported that storage of fresh koa haole leaves with mimosine content of 6.4 to 8.7% at 70°C for four days or at 80°C or higher for two days reduced the mimosine by 50%. Leaves in which moisture was first removed by drying in a forced-draft oven and then stored at 90°C for as long as five days showed no loss in mimosine content. All material stored at elevated temperatures turned dark and developed

a strong meat-like odor which apparently was not objectionable to the albino rats used in the experiment, since they did not refuse the heated leaf meal when incorporated in their rations.

Matsumoto et al., also studied the effect of temperature on mimosine in koa haole seeds in an experiment similar to that used for the leaves. Storage of mature seeds at elevated temperatures had no effect on mimosine content but in the immature seeds mimosine content decreased from 5.3% when the seeds were collected to 2.8% after two days storage at 70°C. These authors also found that mimosine in koa haole leaves could be reduced from 6.4% to 3.5% — a 54.7% reduction by subjecting the leaves to steam for two hours.

Matsumoto et al., tested the effectiveness of iron as a detoxicating agent based upon earlier observations by Yoshida (1944). Diets with and without ferrous sulphate ($\text{Fe SO}_4 \cdot 7\text{H}_2\text{O}$) were used in feeding experiments with albino rats. Test animals fed the ration containing koa haole without added ferrous sulphate showed signs of mimosine toxicity (mainly severe hair loss) whereas those fed the ration with ferrous sulphate showed only minor signs of hair loss and their fur condition was excellent at the end of the experiment.

Ross and Springhall (1963) in experiments with chickens found that the addition of ferrous sulphate in solution to koa haole prior to mixing with a balance of the ration was effective in reducing the toxicity. Additional improvement was obtained when the iron-treated koa haole was allowed to stand at least one week prior to mixing with other ingredients in the ration.

In later experiments, Springhall and Ross (1965a) added ferrous sulphate to *L. leucocephala* in rations fed to chicks because it had been shown to reduce the growth depressing effect. In these experiments, pullets fed grower rations containing up to 85% of a combination of sago, copra meal and koa haole did not differ significantly in egg production, egg weights or feed conversion although groups receiving the experimental grower rations took significantly longer to reach sexual maturity and were significantly lighter after eight months production.

In a second series of experiments, Springhall and Ross (1965b), fed laying rations containing 80-85% copra meal, sago, coconut oil and koa haole to pullets for an eight month period. There were no significant differences noted in egg production, feed conversion, fertility, hatchability or mortality, although eggs laid by hens fed the experimental rations were significantly lighter in weight than the controls, and the hens laying these eggs gained significantly less weight than control hens.

Feeding Tests With Fish And Shellfish

The use of koa haole as a food ingredient for fishes was tested in American Samoa in 1972-73 using a local mollie, *Poecilia mexicana* Steindachner, which was being reared for use as bait for skipjack tuna (Rodman, 1973). Growth of newborn fry in freshwater fed only on koa haole was satisfactory and a suitable baitfish size was reached in 60-90 days. Average length on a diet of koa haole after 47 days was 31.66 mm — somewhat better than growth on a fish meal diet, (20.33mm after 43 days).

In a similar project, Dr. Wayne J. Baldwin at Hawaii Institute of Marine Biology developed methods for culturing large numbers of the topminnow *Poecilia vittata* Cuvier and Valenciennes for possible use as tuna baitfish (Baldwin, 1974; and Herrick and Baldwin, 1975). Dr. Baldwin found that this species also will consume koa haole and grow at satisfactory rates.

Dr. Robert May (1975) at the Hawaii Institute of Marine Biology conducted an experiment in which a test lot of *P. vittata* were fed 15% gelatin and 85% untreated koa haole leaf meal over a 12-week period. A control lot was fed 15% gelatin and 85% commercial catfish food. Growth of the control lot exceeded that of the test fish but survival was slightly better in the experimental group. No pathological symptoms were noted in either group. At the end of the experiment six fish in the control group and four in the experimental group were gravid. Apparently there is no adverse effect of the koa haole on reproduction of this species as has been reported for some mammals and fowls.

Dr. Robert May in experiments with the threadfin *Polydactylus sexfilis* Cuvier and Valenciennes conducted experiments in 1975 using koa haole as a food ingredient (May, 1975). In these experiments, koa haole was fed to two test groups at 25% of the diet. In one group the koa haole was heated to reduce the mimosine content. In the other group the koa haole was used untreated to evaluate the effect of mimosine on growth and survival. A control group received a similar diet except that 13% soybean meal and 12% cellulose was used instead of koa haole leaves. The test fish consumed more of the diet containing koa haole with reduced mimosine than of the diet with untreated koa haole but both diets with koa haole were consumed much less than the control diet. Growth was much better in the control group but no pathological symptoms were noted in the test lots. Dr. May commented that tests with lower levels of koa haole were needed to determine the usefulness of this legume in the feeding of threadfin.

Also the koa haole could be treated at higher temperatures to further reduce mimosine content. In Dr. May's experiments the leaves were heated to 50°C for 72 hours which reduced mimosine by 19%. According to Matsumoto, Smith and Sherman (1951) heating to 70°C for four days or 80°C for two days will reduce mimosine to about half of the original level.

Test feedings of *Leucaena leucocephala* to various species of rabbit-fish genus *Siganus* have been conducted at Guam and Palau. Preliminary trials on a wild stock of *Siganus canaliculatus* in Palau, revealed that this species will eat this legume in a fresh state. Experiments at Guam with *Siganus spinus* and *S. argenteus* indicate adverse effects of a diet based on a mixture of the green alga *Enteromorpha* and *L. leucocephala*. In this experiment, test lots of each species of fish were fed *Enteromorpha* plus trout chow and *Enteromorpha* plus *Leucaena*.

Preliminary results after eight weeks indicated an adverse effect on fishes fed the legume. *S. argenteus* and *S. spinus* fed *Enteromorpha* and *Leucaena* grew one-third and one-fourth slower, respectively, than *S. argenteus* and *S. spinus* fed *Enteromorpha* and trout chow. All of the fish fed a diet of *Enteromorpha* and *Leucaena* were dwarfed compared to fishes fed *Enteromorpha* and trout chow with large heads and abnormally thin bodies characteristic of fish in a starved condition. Red areas appeared on the base of the pectorals, caudal peduncle and lips. (Tsuda et al., 1974). Experiments at the University of Guam Marine Laboratory are continuing.

Koa haole has been tested as a food ingredient in the diet of freshwater prawn, *Macrobrachium rosenbergii* at the Centre Nationale pour L'Exploitation Des Oceans (CNEXO) Tahiti Laboratory. Gerard Cuzon (1975a, 1975b) reported successful results in growth experiments using the leaves of *L. leucocephala*, known locally as acacia, at a level of 40%. According to Cuzon, no harmful effects from mimosine were observed in the different nutritional tests, but he suggests that this substance may be inhibited during the processing of the food. Cuzon also reported increased pigmentation of test animals especially in the diet composed of 40% acacia meal, probably because of the large quantity of beta carotene present in this legume.

It is not known whether koa haole has been tested as a food ingredient for marine shrimp of the family *Penaeidae*. However, Dr. Harold Webber (1975) has offered to test *Leucaena* along with other native plant materials as a food for *Penaeid* shrimp in Costa Rica where it is indigenous.

THE MIMOSA, *PROSOPIS INSULARUM*

This legume, formally designated *Leucaena insularum*, and recently designated *Prosopis insularum* (Guill) Bret. is relatively widespread throughout the Pacific and contains no mimosine. (Brewbaker and Hylin, 1965). Brewbaker and Hylin also tested ten other mimosaceous genera but found none containing mimosine and therefore concluded that this toxic alkaloid is apparently unique to the genus *Leucaena*. Therefore, if mimosine should prove to be a limiting factor in the use of *Leucaena* for fish food, there is a possibility of using *Prosopis* or other tropical mimosaceous species.

COCONUT

Copra is widely available in the tropical islands and contains substantial quantities of protein. Copra meal, a byproduct of the extraction of coconut oil from copra, has also been suggested as a food for various species since it contains 10 to 20% protein. Springhall and Ross, (1965a and 1965b) report experimental feeding of poultry with copra in combination with sago in attempt to maximize the use of feedstuffs indigenous to the territory of Papua and New Guinea. Although copra meal is produced only at a few widespread areas where copra mills are located, substantial quantities are available. A new coconut oil extraction process using fresh coconuts instead of copra, reportedly requires a much less costly plant and could be installed on nearly any island group. If this extraction method is applied, a local source of coconut presscake would become available.

Cuzon (1975) used copra meal at levels between 40% and 60% in growth rate experiments with the freshwater prawn *Macrobrachium rosenbergii* at Tahiti and found this to be a satisfactory food ingredient. Similar experiments are needed with other aquaculture species.

FISH SCRAPS OR FISH MEAL

Fish scraps or fish meal are standard ingredients in the diet of most carnivorous or omnivorous species reared by aquaculture methods. In the tropical Pacific, tuna meal or tuna scraps are available at several locations including Palau Islands, Fiji Islands, Hawaiian Islands and Samoa. Development of tropical aquaculture would provide a local market for fish scraps or fish meal which might be more attractive economically than shipment of meal to distant markets. Combinations of fish scraps or fish meal with koa haole, coconut presscake or other tropical plants might provide satisfactory diets for aquaculture species.

CONCLUSION

At the present time, there is a good indication that *Leucaena leucocephala* can be used as a food ingredient by mollies and topminnows of the genus *Poecilia*, by the freshwater prawn *Macrobrachium rosenbergii* and possibly by the rabbitfish *Siganus canaliculatus*. Experiments with the threadfin *Polydactylus sexfilis* are in progress.

Definitive feeding experiments with the species listed above and other species considered for tropical aquaculture are needed to determine the percentage of the diet which can be composed of koa haole. Experiments to evaluate other local food sources for aquaculture of tropical fish and shellfish, similar to those of Springhall and Ross for poultry, are indicated.



Palau Haole Koa Leucaena leucocephala.

LITERATURE CITED

- Baldwin, W. J., 1974. Raising mollies for skipjack bait may eliminate use of frail nehu. *National Fisherman* (54):7-C.
- Brewbaker, J. L., and J. W. Hylin, 1965. Variations in mimosine content among *Leucaena* species and related *Mimosaceae*. *Crop Science*, 5: 348-349.
- Brewbaker, J. L., D. L. Plucknett and V. Gonzalez, 1972. Varietal variation and yield trials of *Leucaena leucocephala* (Koa Haole) in Hawaii. Hawaii Agricultural Experiment Station, University of Hawaii, Research Bulletin 166, 29 pp.
- Cuzon, G., 1975a. Incorporation of vegetal proteins in a compounded diet for the freshwater prawn, *Macrobrachium rosenbergii*. *Aquaculture*: (in press).
- _____, 1975b. Personal communication May, 1975.
- Glude, J. B., 1972. Report on the potential for shellfish aquaculture in Palau Islands, Yap Islands, Guam, Truk, Ponape, Ellice Islands, American Samoa, Cook Islands, Fiji Islands, New Caledonia and French Polynesia. Food and Agriculture Organization of the United Nations (unpublished report), WS/C6976:99pp.
- Gonzalez, V., J. L. Brewbaker and D. Elizabeth Hamill, 1967. *Leucaena* cytogenetics in relation to the breeding of low mimosine lines. *Crop Science*, 7:140-143.
- Hassler, W. W., 1975. Growth and culture of the dolphin, *Coryphaena hippurus*, in North Carolina (in press).
- Herrick, S. F. and W. J. Baldwin, 1975. The commercial production of topminnow. A preliminary economic analysis. University of Hawaii Sea Grant Report, HIMB contribution no. 464 (unpublished):18 pp.
- Kinch, D. and J. C. Ripperton, 1962. Koa haole production and processing. University of Hawaii, Hawaii Agricultural Experiment Station, Bulletin 129, 58 pp.
- Matsumoto, H., A. G. Smith and G. D. Sherman, 1951. The effect of elevated temperatures on the mimosine and toxicity of koa haole (*Leucaena glauca*). *Arch. Biochem. Biophys.*, 33:201-211.

- Oakes, A. J., 1968. *Leucaena leucocephala* — description, culture, utilization. *Advancing Frontiers Plant Science*, 20:1-114.
- Pirie, N. W., 1971. Leaf protein: its agronomy, preparation, quality and use. IBP Handbook No. 20, Blackwell Scientific Publications, Oxford and Edinburg:192 pp.
- Rodman, D. T., 1973. Culture of baitfish for a skipjack tuna pole-and-line fishery in American Samoa, Government of American Samoa, Pago, Pago, First Annual Report July 1, 1972 — June 30, 1973 (unpublished):17 pp.
- Ross, E. and J. A. Springhall, 1963. Evaluation of ferrous sulphate as a detoxifying agent for mimosine in *Leucaena glauca* rations for chickens. *Australian Veterinary Journal*, 39:394-397.
- Springhall, J. A. and E. Ross, 1965a. Preliminary studies with poultry rations for the Territory of Papua and New Guinea. I-Grower rations with copra, sago and *Leucaena leucocephala*. *Papua and New Guinea Agricultural Journal*, 17:117-121.
- , 1965b. Preliminary studies with poultry rations for the Territory of Papua and New Guinea. II-Layer rations with copra, sago and *Leucaena leucocephala*. *Papua and New Guinea Agricultural Journal*, 17:122-126.
- Tsuda, R. T. Personal communication, May 6, 1975.
- Tsuda, R. T., P. G. Bryan, W. J. Fitzgerald and W. J. Tobias, 1974. Juvenile adult rearing of *Siganus* (Pisces: Siganidae) in Guam. South Pacific Commission 7th Technical Meeting on Fisheries, Nuku'alofa, Tonga, July 15-19, 1974 (unpublished).
- University of Hawaii, 1973. Pacific Island Mariculture Conference Report, February 6-8, 1973. University of Hawaii Institute of Marine Biology, 1973, (unpublished report), 109 pp.
- Villaluz, D. K., 1972. Aquaculture possibilities in some islands of the South Pacific. Food & Agriculture Organization of the United Nations (unpublished report), 453/72:51 pp.
- Webber, H. H. Personal communication, May 7, 1975.
- Yoshida, R. K., 1944. A chemical and physiological study on the nature and properties of the toxic principle in *Leucaena glauca* (koa haole). Doctoral dissertation, University of Minnesota.

The Role Of The Feed Industry In Developing Formulated Feeds For Aquaculture

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The feed industry basically has one key role in the aquaculture field — the development and distribution of a convenient feed that will provide profitable growth and performance for the producer of aquaculture products.

The feed industry has a tremendous resource in:

- ingredient purchasing power
- understanding nutrient availability of ingredients
- general nutritional technology
- ability to least cost a formulated feed
- manufacture of finished product in an economic form

In 1974 the feed industry manufactured an estimated 72.3 million tons of primary or complete feeds and another 40 million tons of secondary or incomplete feeds in 9,641 feed mills. (Feedstuffs, Vol. 47, No. 38, 1975). According to the U.S. Department of Agriculture, the farmers or purchasers of feed products spent nearly \$15 billion for feeds in 1974. These figures indicate not only the purchasing power but the volume of over 112 million tons of ingredients purchased by feed manufacturers in 1974.

Aquaculture feeds produced in the U.S. that are complete or primary feeds represented 125-130 thousand tons in 1974 (SRI, Fish Farming, 1974) which is less than 0.2% of the total primary feed manufactured in the U.S. However, the buying power of the feed industry as a result of other animal feeds is reflected in aquaculture feeds, even

though aquaculture feeds represent a very small percent of the total. If aquaculture feeds are to maintain this buying power advantage, it is imperative that the same ingredients commonly used today are also used in existing and new aquaculture feeds.

The nutrient availability of ingredients is a key factor in determining the nutritional dollar value of a product. Various tables are available on the nutrient content of ingredients and every feed company undoubtedly has a separate nutrient listing for ingredients commonly used in their production facilities. At any rate, the feed manufacturer realizes that 100% of the nutrients reported in ingredients are not necessarily available to the animals being fed. Therefore, the feed manufacturer must and will carefully select ingredients and/or combinations of ingredients that will supply the available nutrients required by the animal.

In the process of feeding aquatic animals, a general understanding of the type of digestive system found in the animal is essential. Answers must be found to questions such as does the animal have the ability to chew or break feed particles into smaller units thus increasing the surface area of feed particles for greater ease of digestion? does the animal have a simple stomach or a multiple compartmental stomach? does the animal have a grinding apparatus in the stomach to facilitate breakdown of feed particles? does the animal have a short or long digestive tract? are there histologically absorptive mechanisms similar to other animals?

With these basic questions answered, the nutritionist can begin to study diet forms, nutritional requirements, general digestibility or a assimilation of diets and nutritional environmental interactions.

If the nutritional requirements are known and an assessment of the ingredient availability of nutrients has been made, a least cost, formulated diet can be developed. In the process of developing the least cost formula, the nutritionist will be able to assess the cost effect of nutrients and/or ingredients. For example, the vitamin biotin costs approximately \$6 per pound for a .1% concentrate. If the formulated diet contains 0.1% of this biotin concentrate, the cost in the diet is \$12 per ton of feed manufactured. It is therefore of economic interest to determine the biotin requirement since its cost in the diet is high. Another example is fish meal, which at present, costs \$275 per ton. If a diet contains 20% fish meal, this means the ingredient cost is \$55 for every ton of diet formulated. For a 1% drop in fish meal, the nutritionist could save as much as \$2.50 per ton of feed depending on the cost of the replacement ingredient. It is therefore economically essential for the nutritionist to reevaluate the protein and/or quantitative amino acid requirements to facilitate use of lower cost protein ingredients.

Most aquaculture feeds cannot have a fixed feed formulation because changes in ingredient supply, seasonal and climatic changes, and anticipation of export can all interact to influence ingredient prices from week to week. Feed manufacturers must constantly reformulate feeds so as to minimize price fluctuations of the finished feed.

The manufacture of aquaculture feeds can fall into one of four basic processing categories: **meal**, **flaking**, **pelletizing** and **extrusion cooking**. The type of manufacture selected is largely dependent upon the aquaculture production system and the eating habits of the animal under cultivation.

Meal manufacture is essentially nothing more than grinding and mixing various ingredients together. The meal-type feed can be sized in various ranges which would be compatible to the animal or system being fed. Examples of uses of meal-type feeds would be tropical fish farming, starter feeds for trout and catfish, and starter feeds for small shrimp.

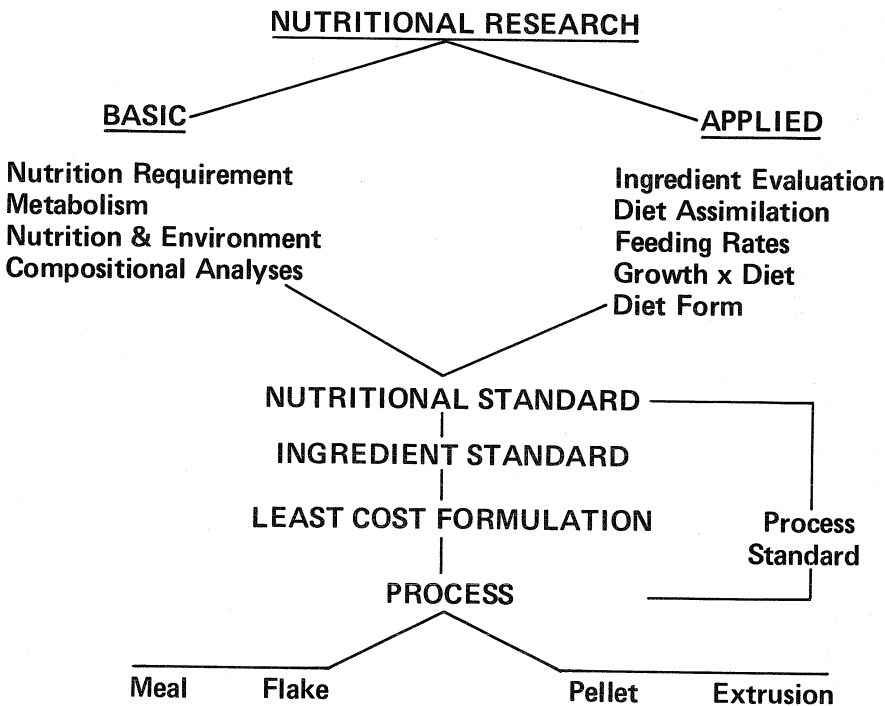
The **flaking** process involves treating a fine meal feed with water and passing the wet mix between large heated rolls. Flaking, by virtue of the heat treatment under the pressure of the rolls, results in thin partially gelatinized flakes that when dried maintain their integrity in water. The flaking process is used primarily in the production of tropical fish feeds and in some cases has been used for larval foods for shrimp.

The **pelletizing** process is the most popular method of manufacture of aquaculture feeds. This process essentially involves the compacting under steam and pressure of a meal-type feed into various sizes and shapes. The pelleted diets are used extensively in the fish feeding industry and to some extent in the experimental shrimp farming industry. The pellet does have one distinct disadvantage in that it will not maintain its form or integrity in water for long periods of time. Pellet binders have been used, but in general, they are expensive to incorporate in a finished feed.

The most exciting form of feed manufacture today is **extrusion cooking**. Essentially, extrusion is a cooking process whereby a wet or moist meal type diet is heated with or without steam under various degrees of pressure and formed into a multitude of shapes or configurations. When the formed diet is dried, it can be observed that the ingredients are "bound together" so as to make it virtually impossible to separate the ingredients. In addition, the process of binding the ingredients results in a finished product which has excellent stability in water, particularly suitable for bottom-feeding animals such as shrimp. Extruded

feeds are used principally in the catfish, trout and shrimp farming industries.

To summarize, the following flow chart will put the discussed points into perspective.



Any new or existing aquaculture feed must evolve from a research program. In the feed industry, nutritional research falls in two major categories, **basic** and **applied**, and these two areas are managed simultaneously in the development of a new feed or continuous improvement of an existing feed.

The **basic nutritional research**, as the word implies, is a basic understanding of the animal. The animal's method of eating should be studied carefully as should the digestive process. The general metabolism of the animal should be understood, if only the optimum temperature and metabolic rate is correlated. The essential nutrients should be qualitatively defined via depletion/repletion studies or radioactive isotope

labeling, as can be done with amino acids. The interaction of nutrition and environment should be explored, such as stress due to salinity, water quality, density, or other observed environmental factors. Compositional analysis, particularly changes during a life cycle, can be an interesting way for a nutritionist to evaluate the effects of diets or nutrients being studied.

The **applied nutritional research** is in actuality, the practical application of the basic nutritional knowledge. Diet assimilation or ingredient availability of nutrients must be assessed. Diet form as it relates to the animal's feeding habits should be evaluated. Feeding rates or growth-diet interaction must be carefully studied so a diet can be evaluated as to economic performance.

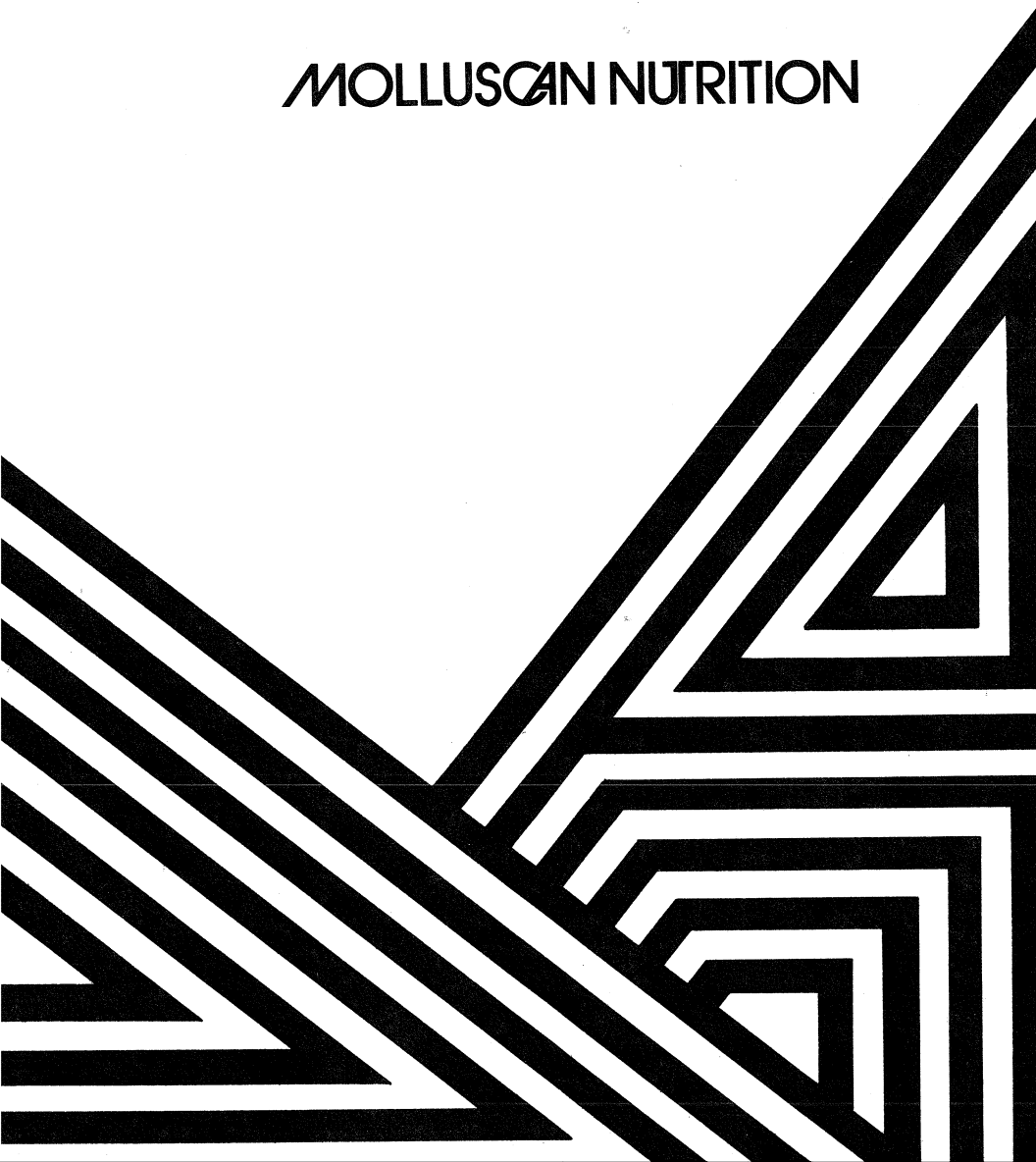
A nutritional standard must then be developed and constantly reassessed, as well as an ingredient standard established for as many ingredients as possible. The computer, with the assistance of the ingredient buyer, does the rest and a least cost formulation is developed.

The manufacturing process is next and, as indicated before, is dependent upon the type of animal and/or system of aquaculture production. Every process has a standard method of manufacture and in essence, that defines the quality and quantity of ingredients needed to produce the desired finished product.

The finished product is then ready for distribution but before the product is shipped, it must be registered, tested to meet quality standards and packaged in a convenient form for the aquaculture producer.

This has been a very simple overview of the role of the feed industry in producing aquaculture products. The emphasis of this presentation should be for the nutritionist, both basic and applied, to consider that a product of his/her research must someday be manufactured and the finished product must provide profitable growth and performance for the producer of aquaculture products.

MOLLUSCAN NUTRITION



Views On Bivalve Larvae Nutrition

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At the present time, a very great concern is expressed in both professional and lay literature over our ability to meet demands for food as both population and worldwide pressures for higher standards of living increase. Shellfish aquaculture is considered one method of increasing food supplies that may be of some small benefit in meeting our future needs. In fact, as far back as the early 1900's, literature was published on methods for laboratory cultivation of the scallop, American oyster, quahog and soft shell clam (Belding, 1910, 1912, 1930; Wells, 1920, 1927) but many problems still remain in rearing of these species under controlled conditions. There are now about 35 pilot plants and commercial shellfish hatcheries in the United States and a considerable number abroad. No doubt this number would be substantially increased if more information were available offering solutions to practical and biological problems, which would ensure a consistent financial gain. In questioning individuals who are building and maintaining commercial aquaculture plants, as well as investigators pursuing research that requires the laboratory rearing of bivalves, similar problems are identified time and time again as being the most significant. Leading the list is the one of providing an optimal nutritional support that is efficient and economical for culturing animals under controlled laboratory conditions (Goodrich et al., 1968; Landers, 1971; Dassow and Steinberg, 1973; Mardela Corp., 1973). The need for this information is more critical for some species of bivalves than others, e.g., oyster larvae seem to have more specific dietary requirements than clam larvae. Juvenile stages of bivalves are, in general, less demanding in their dietary requirements

than veligers, but since juveniles require vast amounts of food for rapid growth, the problem here lies in providing an abundant food supply.

The American oyster, *Crassostrea virginica*, and the northern hard shell clam, *Mercenaria mercenaria*, are the primary bivalves that are being reared for market by aquaculture methods in the United States. With small adaptations of the techniques used in oyster aquaculture (Loosanoff and Davis, 1963), other pelecypods can be cultured from larval stage through metamorphosis in essentially the same manner. The following species have all been reared from fertilized egg to past metamorphosis by aquaculture methods: American oyster, *C. virginica* (Loosanoff and Davis, 1963); European oyster, *Ostrea edulis* (Walne, 1974); Japanese oyster, *C. gigas* (Imai and Hatanaka, 1949); Olympia oyster, *O. lurida* (Imai et al., 1954); pearl oyster, *Pinctada maximus* (Minaur, 1969); quahog, *M. mercenaria* (Menzel, 1966); butter clam, *Saxidomus giganteus* (Breese and Phibbs, 1970); soft shell clam, *Mya arenaria* (Stickney, 1964); horse clam, *Tresus capax* (Bourne and Smith, 1972); mussel, *Mytilus edulis* (Bayne, 1965); hooked mussel, *Brachidontes recurvus* (Chanley, 1970); bay scallop, *Aequipecten (Argopecten) irradians* (Castagna, 1975; Castagna and Duggan, 1971) and *Pecten maximus* (Gruffydd and Beaumont, 1972); and giant scallop, *Placopecten magellanicus* (Gmelin) (Culliney, 1974). Descriptions for rearing other bivalves are found in Loosanoff and Davis (1963), Chanley (1965a, b; 1966; 1969) and Chanley and Castagna (1966).

It is a general opinion that veliger larvae of bivalves are so similar that they cannot be distinguished and that differentiation of the species takes place only in the post-larval stage (McBride, 1914; Jorgensen, 1946). Many larvae of the pelagic type possess similar anatomical features, particularly the internal ones (Loosanoff et al., 1966) and bivalve identification depends only on shell shape, size and hinge structure, including teeth (Rees, 1950; Chanley and Andrews, 1971). Bivalve larvae are characterized by well-developed ciliary organs and a swimming appendage, the velum. Cilia of the velum direct food particles toward ciliary tracts leading to the mouth. Lamellibranchs exhibit an active speed at $1\text{--}60\text{ cm}\cdot\text{min}^{-1}$ (Mileikovsky, 1973); for *Mercenaria*, $7\text{--}8\text{ cm}\cdot\text{min}^{-1}$ (Turner and George, 1955; Carriker, 1961) and for *Crassostrea*, $60\text{ cm}\cdot\text{min}^{-1}$ (Wood and Hargis, 1971). The assumption is that larvae do not drift but are able to control their position in vertical water columns and are continuously feeding (Kirby-Smith and Barber, 1974). The concentration of food digested and assimilated is only a finite quantity of that consumed in a given time (Ukeles and Sweeney, 1969;

Winter, 1973); hence, once the assimilation rate is saturated, growth rate is independent of the food supply.

According to Yonge (1937) the digestive tract is divided into five functional regions: 1) receptors, the mouth and mouth cavity (supposedly free of glands in particulate feeders); 2) conduction and storage (glands may be present); 3) digestion and internal trituration (contains the conspicuous digestive diverticula, which may function by increasing the surface for intracellular digestion, and also digestive glands; 4) absorption (this overlaps the previous region and is indistinguishable from it); and 5) conduction and feces formation (comprises the whole digestive tract where ciliary action is the mechanism of particle movement through the tract). Molluscs show a variety of intracellular and extracellular digestive mechanisms (Yonge, 1926). Lamellibranchs digest protein and fat inside cells of the digestive diverticula and in wandering amoebocytes. Amoebocytes are abundant throughout the gut, and migrate into the wall of the gut lumen, where the particles are digested and nutrients make their way to the tissue spaces. Starch is digested extracellularly by the crystalline style, which is secreted by the crystalline style sac. The structure differentiates fairly early in the veliger stage from the posterior part of the stomach as a blind sac (Kume and Dan, 1968).

Since digestive systems of veliger larvae are similar, this suggests that the food requirements of different species may be satisfied by similar diets. While this is generally true, (e.g., the chrysophyte flagellates, *Monochrysis lutheri* and *Isochrysis galbana* have been used successfully as larval food for virtually all species of bivalves in culture and diets of some larvae in culture appear to be restricted to these flagellates) other larvae are less exacting. Larvae in laboratory culture are fed for the first time at the early veliger stage, either 24 or 48 hours. At the Milford laboratory, 48 hours is used routinely, but in an earlier work feeding was delayed to 60 hours (Amemiya, 1926) and, in fact, the ability of bivalve larvae to develop normally to metamorphosis even after 8 or more days of starvation has been reported (Bayne, 1965; Millar and Scott, 1967b; Gruffydd and Beaumont, 1972). In some commercial hatcheries, phytoplankton foods are included in seawater containing suspensions of fertilized eggs. This is considered a practical method because it combines two manipulatory operations into one step. It is also advantageous to larvae since the food is available as soon as they are capable of feeding. In fact, large size differences are apparent at 48 hours between larvae that received food during early development of the fertilized egg and those whose feeding commenced at 24 or 48 hours. The nutritional

value of diets for larvae is determined by the proportion of larvae that successfully reach metamorphosis, by the extent to which different food sources support different rates of shell growth, or by whole live weight.

Investigations into the importance of good nutrition for larvae should originate with the parent spawners. It has been generally observed that well-fed adult bivalves produce healthier progeny than adults receiving a minimal amount of food. Recent experimental work with *O. edulis* demonstrated that enriching the seawater supply to oyster breeding stocks with the laboratory cultured flagellate, *Tetraselmis*, produced earlier broods, more rapid growth of larvae, and greater yields of spat than from larvae of adult stocks receiving only natural phytoplankton in the seawater supply (Helm et al., 1973).

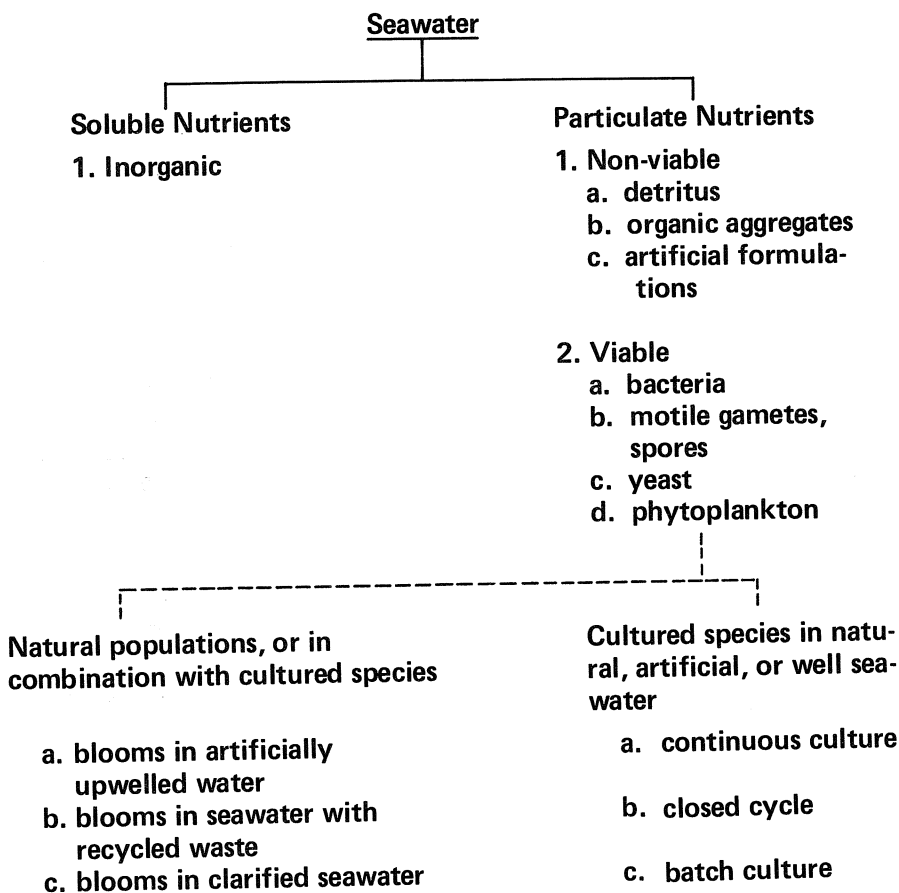
No matter what feeding methods are proposed, three fundamental requirements must be fulfilled: 1) the food source must be inherently non-toxic and not be rendered toxic by some manipulatory process; 2) it must be easily accessible by the animal; and 3) it must provide the nutrients needed for normal growth and development. For commercial aquaculture the cost of food production is a critical consideration.

We are far from the goal of knowing the qualitative and quantitative requirements of bivalve larvae in general or of specific species. However, one can be guided by the nutritional principles that have already been clarified for other animals in marine aquaculture (Halver, 1968). There are five major classes of nutrients that must be made available: 1) specific vitamins or growth factors; 2) nitrogen sources, amino acids and proteins; 3) reduced carbon sources; 4) lipids; and 5) inorganic anions and cations. The question then is: what is the best method of providing these nutrients to bivalve larvae? The potential sources of nutrients for bivalves may be considered in an overview according to the scheme outlined in Figure 1. The balance of the discussion will be based on this scheme.

SEAWATER

Seawater composition is fundamental to all nutritional considerations since, aside from its osmotic and respiratory functions, it is also a source of nutrients. Seawater is the carrier of dietary components, both particulate and soluble, in addition to a variety of industrial and domestic wastes. Many observations in research laboratories, pilot plants and commercial hatcheries emphasize the importance of seawater quality to the successful culture of bivalves. Seawater quality can deter-

Figure 1. Potential Sources of Diets for Bivalve Larvae



mine the type of supplementary diet that is needed by bivalves, while poor quality seawater can inhibit larval growth even if nutritional requirements are being fulfilled.

A major problem in the design of laboratory or hatchery facilities is to avoid bodies of water that are likely to be polluted by heavy metals, detergents or pesticides. Direct toxicity of these materials to young larvae has been amply documented (Davis and Hidu, 1969b; Calabrese, 1972). Indirect toxicity can take place through the chelation

of essential trace elements, inactivation of cilia movement, or inactivation of digestive enzymes. Another source of concern in seawater quality is the presence of toxic strains of bacteria, such as from the genus *Vibrio* (Tubiash et al., 1965; Brown, 1973) or fungi that are potential larval parasites (Davis et al., 1954; Vishniac, 1955). There is a general consensus of opinion that even non-pathogenic bacteria in high numbers will have a deleterious effect on larval growth. This effect can be caused by bacteria depleting the oxygen supply or removing essential ions from the water. Mixed populations of non-toxic marine bacteria in high numbers can also inhibit the uptake of useful phytoplankton foods by oyster larvae (Ukeles and Sweeney, 1969). Protists in seawater have been observed to adversely affect the availability of a phytoplankton food supply. A colorless flagellate of the genus *Monas* has been observed to be a predator in cultures of the phytoplankton food *Phaeodactylum tricornutum*, rapidly destroying the food species (Raymont and Adams, 1958; Ansell et al., 1963b). Blooms of the gelatinous alga, *Phaeocystis*, have also been a serious problem in seawater used by the Conway laboratory (Walne, 1970).

Various devices are used in marine laboratories and hatcheries to treat seawater so that the quality is suitable for larval culture; in particular, to remove particulate matter and to decrease the bacterial and fungal loads in the water. Ultra-violet irradiation of seawater has been employed for many years at the Milford laboratory with a laboratory constructed ultra-violet apparatus (Loosanoff and Davis, 1963). The efficiency in reducing bacteria and fungi in seawater is only partial, but could probably be improved with some modification in design. In a more elaborate piece of equipment, commercially available (9 U.V. lamps in a chamber 12" x 32" long), one investigator found that bacteria were eliminated only at a flow rate of less than 10 ml/min (Culliney et al., 1975). A study of Kelly (1961) showed that ultra-violet irradiation, of seawater is an effective method for the destruction of coliform organisms. Although there are no apparent toxic by-products from ultra-violet irradiation, such treatment may remove organic material and destroy thiamine (Armstrong et al., 1966; Button, 1968). The possibility that these soluble nutrients are useful to bivalves has not been ruled out. The use of chlorine for sterilization of seawater is contra-indicated since chlorine has an adverse effect on feeding activity that is not completely corrected by dichlorination reagents (Kelley et al., 1960).

As early as 1910, Allen and Nelson employed ozonization to eliminate bacteria and other organisms in seawater used for culturing marine phytoplankton. Recently, there is again considerable interest in ozoniza-

tion of seawater as a method of reducing the bacterial count and improving water quality (Blogoslawski et al., 1975). Considerable work must be done before this can become a practical technique for aquaculture. Preliminary experiments at Milford have shown that caution must be exercised in the use of ozone. Detrimental effects of ozonated water on larvae growth have been observed (Landers, personal communication) and MacLean et al., (1973) reported chromosome damage of oyster eggs in ozonated seawater. Toner and Brooks (1975) observed that low residual concentrations of ozone had an adverse effect on a variety of organisms, including phytoplankton. The formation of positive free radicals, organic peroxides, and the chelation of trace metals predict an adverse effect from residual ozone in seawater on viable phytoplankton foods, as well as larvae.

The leaching of toxic materials into seawater must be avoided in larval rearing vessels and in preparation of foods for larvae. The presence of toxicity, of course, will occlude nutrient benefits of food. Guidelines for avoiding toxic materials in laboratory culture of microscopic algae have been fairly well developed (Dyer and Richardson, 1962; Bernhard and Zattera, 1970; Blankley, 1973). It is possible for the growth of an alga in culture to be unaffected by some material with which it is in contact, yet it becomes useless as a food because a substance toxic to larvae is incorporated into the algal cell. At the Milford laboratory we observed that food cultures in contact with certain types of stainless steel, e.g., in filter support screens or blender blades, become a toxic food for bivalve larvae. Various types of filters are useful for removing debris and particles from large volumes of seawater, e.g., cartridges of orlon wound on polyvinyl cores, snap-on filter bags of polypropylene-nylon, and sand bed filters. At Conway a plastic filter apparatus is employed, which is composed of plates of filters and uses Kieselguhr (a powder prepared from the siliceous shells of fossil diatoms) as the filter medium. By varying the number of plates the capacity of the filter can be altered, depending on the volume. Seawater containing 10,000-20,000 particles greater than $3.2 \mu\text{m}$ in diameter was reduced to seawater containing about 2,000 by this procedure (Walne, 1974). Wherever it has been possible to dig a seawater well, water of low particle content and of excellent quality is produced.

The use of antibiotics and other antibacterial agents appears to be an obvious method of controlling bacterial numbers of pathogenic and non-pathogenic forms in phytoplankton food cultures and larval rearing tanks. The use of these agents, however, is not recommended as a method of improving food quality on a routine basis (it may still be

useful for some emergency measures) for the following reasons: 1) on a large scale the cost of purified reagents is apt to be prohibitive for a commercial venture; 2) routine use of reagent grade antibiotics will no doubt induce the development of resistant strains; 3) the use of technical grade antibiotics may contain fillers that could have unexpected side effects on the larvae; 4) inhibition of bacterial populations may stimulate growth of molds (Ukeles, unpublished observation); and 5) certain antibiotics may result in the formation of larval anomalies (Ukeles and Rose, 1975). As an alternative to the use of additives for reduction of contaminants in culture, strict attention should be paid to good housekeeping, frequent washing of larvae and avoidance of static water conditions. A careful balance must be met in the treatment of seawater that will remove its harmful components, while still retaining the nutritive osmotic and respiratory functions of the seawater.

SOLUBLE NUTRIENTS

Inorganic

The importance of soluble inorganic nutrients in seawater is suggested by some recent experiments in our laboratory in which oyster larvae were reared from the fertilized egg in an artificial seawater formulation developed by Zaroogian et al. (1969). The formulation includes major elements of natural seawater (NaCl , $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, KCl , KBr , H_3BO_3 , $\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$, NaF , NaHCO_3 , EDTA). Development from fertilized egg to straight-hinge larva in this artificial seawater was normal; veliger larvae were motile and appeared healthy. New growth, however, was not added even though larval phytoplankton foods were added during the incubation period of 7 days, at 26.5 C.

Organic

Putter's (1909) theory on nutrition of aquatic species by dissolved organic matter received considerable attention until it was thoroughly criticized by Krogh (1931) because of insufficient evidence that any free-living animals, except protozoa and sponges, are capable of living on dissolved substances. Even Krogh, however, agreed that at least in the case of oysters and a few other species "nutrient substances can be absorbed from solution and when the solutions are fairly concentrated such absorption may be a significant source of nourishment for the animal in question." In recent years the appearance of numerous reports showing that seawater contains a considerable amount of dissolved low molecular weight organic substances (Walsh, 1965; Wagner, 1969;

Andrews and Williams, 1971; North, 1975) has renewed interest in the possibility that the uptake of dissolved organic matter by marine animals may have nutritional benefit.

Sorokin and Wyshkwarzev (1973) reported on the uptake of dissolved organic matter and its incorporation into the metabolic pool in the form of C^{14} labeled algal hydrolyzates in many marine animals, including bivalve molluscs. All exhibited significant capacity for uptake (10-40%) of the total metabolic requirement when exposed to 0.50 mg C/l of dissolved organic matter. The mechanism is assumed to be similar to the assimilation process taking place during intestinal digestion with the participation of ciliated epithelia. Epithelium cells of bivalves have a wide spectrum of enzymes (permeases) participating in the active transport of organic molecules through the cell walls (Purchon, 1971). Although this latter report is based on studies with adult animals, it suggests that this type of feeding deserves a careful study of its efficiency, mechanisms and importance in larval nutrition. Several other reports with adult oysters also indicate use of dissolved organic material. Collier et al., (1953) reported that an unidentified carbohydrate at high concentrations in seawater was retained by oysters. Gillespie et al. (1964) observed that oysters fed glucose (5-15 mg/l) appeared to concentrate the sugar and had a longer life span than those not receiving the glucose. Starch was also assimilated and appeared to increase tissue weight in oysters (Haven, 1965). Some authors concluded that the capacity to remove amino acids from solution in seawater is broadly distributed among marine animals (Stephens and Schinske, 1961). Sorokin and Wyshkwarzev (1973) stated that the "the direct consumption of dissolved organic matter by animals is one of the quite real and important ways of transformation of organic substances and energy in natural and artificial aquatic ecosystems."

It is still a question whether the uptake of soluble organic material makes a substantial contribution to the foods of bivalve larvae. Davis and Chanley (1956) investigated the effect of some water soluble vitamins on the growth of oyster and clam larvae. Riboflavin alone or in combination with calcium pantothenate, thiamine hydrochloride, and pyridoxine hydrochloride significantly increased the rate of growth of *C. virginica* and *O. lurida* larvae but had no effect on *M. mercenaria*. Chanley and Normandin (1967) reported that solutions of lactose broth, amino acids, and carbohydrates did not support growth of *M. mercenaria* larvae. The larvae failed to grow at all or grew more slowly than controls fed live phytoplankton. In a review of the subject of dissolved organic material as a nutritional source for marine and estuarine invertebrates,

Stephens (1967) stated that "in defending the potential significance of dissolved organic material, there is no intent to deny the significance of other means by which marine invertebrates obtain organic material."

PARTICULATE NUTRIENTS

Non-viable

In proposing a definition of an animal from a nutritional point of view, Hutner and Provasoli (1951) stated that "an animal ingests particulate food; it is phagotrophic; it eats." With this capacity are developed various adaptations such as secretion of digestive enzymes, flexibility of cells to engulf foods, food gathering and locomotor devices. The overwhelming evidence is in favor of a nutritional support of bivalve larvae on particulate food.

The role of detritus in feeding zooplankton is still questioned, but probably should be considered individually for each species. The chemical composition of detritus suggests that it could act as a food source (Parsons and Strickland, 1962). Results of a study on the use of detritus by *Calanus helgolandicus* show that there can be a different response to different detrital particles (Paffenhofer and Strickland, 1970). Blegvad (1914) considered organic detritus, principally from eelgrass (*Zostera*), to be an important part of the diet of many marine organisms. Stickney (1964) fed clam larvae ground and strained *Zostera* leaves, but the larvae did not grow on this food mixture with or without the addition of antibiotics to control the bacterial population.

The process of conversion of filterable matter to organic particulates may be important in considering the role of soluble vs. particle nutrition. It has been suggested that micelles of organic material may be absorbed on inorganic particles of colloidal dimensions and so become available to filter-feeding animals as particulate nutrients (Fox et al., 1953).

Baylor et al. (1962) and Sutcliffe et al. (1963) showed that organic particles could be produced in seawater through the adsorption of organic matter on air-water interfaces in rising bubbles, and also within the water column by aggregation of smaller particles. The particulate organic matter so produced could serve as food to support the growth of filter-feeding crustaceans (Baylor and Sutcliffe, 1963). Results of various investigators have differed in attempts to produce a significant number of particles by bubbling (Barber, 1966; Menzel, 1966), but the conversion of filterable material to organic particulates is believed to

be of undoubted importance by some investigators (Riley, 1963, 1970; Batoosingh et al., 1969).

Although the question of use of organically rich detrital particles and other organic particulates as a food remains open, there is evidence that some particles have a detrimental effect on larval growth. For example, laboratory experiments showed that a suspension of dinoflagellate trichocysts will inhibit uptake of food in oyster larvae (Ukeles and Sweeney, 1969). Davis (1960) and Davis and Hidu (1969a) reported on the effect of silt, clay and Fuller's earth on survival and growth of larvae of the hard clam, *M. mercenaria*, and the American and European oysters. These authors also concluded that particles inhibit the use of algal foods. American and European oysters both displayed a severe reduction in growth at 0.75 g/l, and 2 g/l or silt did not grow at all; clam larvae, however, were not seriously affected until concentrations exceeded 2 g/l. Conversely, both species of oysters tolerated kaolin and Fuller's earth better than clam larvae did.

Despite the success of pelleted foods in fish farming, comparable progress in marine aquaculture has not been made. Pelleted diets ensure continuous availability and uniformity of food, ease of storage, feeding of controlled rations, and diminishing risks of introducing disease. "The advantages conferred by a pelleted ration are so manifold as to make its use almost mandatory for successful marine fish farming under western conditions" (Cowey and Sargent, 1972). It is the expectation of molluscan aquaculture industries that modern nutrition scientists will apply their technology to the development of non-living prepared diets. If this becomes economically feasible, it opens the exciting possibility that molluscan aquaculture industries will become free of the burden of cultivating living food sources, and will be in a position to provide a highly nutritious diet that will result in good predictability of bivalve growth.

Artificial food particles are known to be acceptable to a wide range of filter feeders, but food particles supplied in the form of minced tissue or organic slurry adsorbed on particles bring about problems associated with high populations of bacteria and other contaminants. An alternative approach to particulate feeding is to use encapsulated diets. Encapsulation is the process by which liquid or particulate materials are enclosed within a specially designed artificial membrane wall or shell made of natural polymers, as gelatin, gums, waxes, or the synthetic polymers of ethyl cellulose, polyvinyl alcohols or polyethylene. The type of capsule required depends on the mode of feeding, e.g., molluscs ingest the food whole and must be provided with a capsule

whose walls are stable to seawater but readily attacked in the digestive tract of the animal. Capsules of this type needed by molluscan larvae have not yet been described, but are available for larvae or adults that masticate their food. Encapsulation is a process that is being actively pursued among shrimp aquaculturists as an artificial food supply (Meyers et al., 1971; Meyers and Zein-Elden, 1972; Jones et al., 1974). Nevertheless, although considerable progress in microencapsulation has occurred in shrimp aquaculture, there is still much to be accomplished before a complete food is developed. In one series of experiments only 25% of the *Artemia* fed with capsules (containing 5% soluble starch and 10% hemoglobin) remained alive after 10 days, whereas 80% of those fed *Isochrysis galbana* were alive (Jones et al., 1974). There has been almost no progress in designing an artificial diet for molluscan larvae. Many investigators have made sporadic attempts to induce larval growth on various concoctions of organic materials, such as yeast, cereals, baby foods, etc. These attempts have remained largely unsuccessful and unreported.

Recent work reported by Castell and Trider (1974) on feeding artificial diets to adult oysters shows that there was a tenfold faster growth rate of oysters fed a natural diet, compared to those fed artificial diets. The results demonstrated that the quality and quantity of nutrients in the artificial diet (mixtures of water soluble nutrients and suspended particles consisting of corn starch, corn oil, cod liver oil, casein, cellulose, vitamin mix, mineral mix, choline, and vitamin E) are far from optimum.

A mixture of soluble nutrients and suspended particles seems to have some capacity to support growth of clam larvae (*M. mercenaria*). Clam larvae fed dried particles of *Ulva*, *Fucus*, or *Laminaria* grew to metamorphosis in 13-17 days. Larvae receiving frozen *Ulva* did not grow as rapidly as those receiving live foods, but reached metamorphosis in 8-13 days and eventually increased in size to 350-450 μ (Chanley and Normandin, 1967). Lyophilized cultures of the flagellates, *Dunaliella euchlora* and *Isochrysis galbana*, as well as heat-dried samples of *Scenedesmus obliquus*, fed to clam larvae produced survival and growth comparable to that obtained when clams were fed living algae. American oyster larvae, *C. virginica*, however, failed to grow on lyophilized preparations of *I. galbana* (Hidu and Ukeles, 1962). Spray-dried *Chlorella* cultures from Japan, a culture of *Monochrysis lutheri* vacuum-dried in manitol, and lyophilized *I. galbana* gave similar results with larvae of European oysters, *O. edulis* (Walne, 1974).

Viable

Although high bacterial populations are generally to be avoided in bivalve culture (Walne, 1956; Davis, personal communication), there have been occasions in which bacteria were implicated as a source of food. Carriker (1956) reared clam larvae on an extract of cereal and concluded that good growth was a result of the bacterial population appearing as a result of the rich cereal extract. However, pure cultures of nine different species of marine bacteria did not support growth of oyster larvae in an earlier report (Davis, 1953).

A further suggestion that bacteria may be used as food sources comes from the work of Imai and Hatanaka (1949) in whose laboratory bacterized cultures of the colorless flagellate, *Monas*, were used as food for rearing of the Japanese oyster. Filtered seawater enriched with nitrate, phosphate, and glucose was used to induce an abundant bacterial population upon which the colorless holozoic flagellate could feed. The success of the method in Imai's laboratory and lack of success in other institutions suggest that beneficial species of bacteria were induced under a particular set of conditions in Imai's laboratory and used as a food together with flagellate. Hidu and Tubiash (1963) reported that the formulation known as "Combistrep" (dihydrostreptomycin sulfate, streptomycin sulfate, phenol, sodium citrate, sodium bisulfate, water), which consistently resulted in a 25-100% increase in the rate of larval growth, produced an increase in numbers of bacteria in direct correlation to the concentration of "Combistrep." These authors concluded that the improved growth rate of the larvae was related to an increase in a selected group of bacteria. Bacteria may or may not have a beneficial effect on larval growth, but Millar and Scott (1967a) considered the growth of bacteria-free cultures of *O. edulis* larvae fed the alga, *M. lutheri*, as evidence that bacteria did not provide an essential nutritional component for growth.

In early studies on food use by larvae the possibility of using *Fucus* antherozoids was explored, and the successful culture of European oyster larvae through metamorphosis was obtained in 1930 (Walne, 1974). This technique, however, was not pursued. Yeast cells were also tried as a food source in the early studies but without marked success.

It has been known since the early 1900's that bivalve larvae can be reared in laboratory culture on a diet of small naked flagellates. Demonstrations by Cole (1937, 1938) and Bruce et al. (1939) on the large-scale culture of *O. edulis* were followed by a number of studies on use of phytoplankton by larvae of the American oyster. Davis (1950, 1953)

and Davis and Guillard (1958) studied food use by *C. virginica* and *M. mercenaria* larvae. Walne (1963, 1964, 1965, 1966) conducted experiments on the nutritional value of various algal species for growth of *O. edulis* larvae. Bayne (1965) reported increases in growth of *Mytilus edulis* on diets of the flagellate, *I. galbana*.

All investigators observed that phytoplankters were not equal in nutritive value. In addition, it was frequently observed that a mixture of algal species provided a better food source than any individual species. These observations indicate that no one algal species is a complete food source. Similar conclusions were reported from results of experiments with *Tigropus* (Provasoli et al., 1959; Shiraishi and Provasoli, 1959) and with the marine amoeba, *Oxyrrhis* (Droop, 1966). The basis for the dietary preferences of bivalve larvae is not well-defined. Such factors as size of cells or production of toxic metabolites by particular species are obvious limitations to the use of particular species as food. Information is generally lacking on important factors such as cell wall structures and susceptibility of cells to mechanical damage or enzymatic digestion. Differences in chemical composition of algal cells would provide the most obvious explanation for the nutritional benefits of some algae and the lack of them in others. It is interesting to find that, in general, striking differences in chemical composition between algae have not been found. Riley and Roth (1971) analyzed 15 species of phytoplankton, representing several classes, for 18 trace metals. Most of the elements sought were accumulated, but there were no differences in their distribution pattern that could be correlated with the classification of the organisms. Results of chemical analysis of *I. galbana*, *M. lutheri*, *P. tricornutum*, and *D. tertiolecta* showed that differences in chemical composition between the species were no greater than those within a species brought about by changes in the media or time of subculturing (Walne, 1970). Similarly, the analysis of 11 species of marine planktonic algae representing 5 classes for chemical components (protein, carbohydrate, fat, total pigment, ash) demonstrated that marine phytoplankters have very similar organic composition when grown under similar physical and chemical conditions, irrespective of the size or class to which the organism belongs (Parsons et al., 1961). The available evidence does suggest vaguely that the lipid content of food organisms may be significant. Millar and Scott (1967b) showed that lipid was the major energy reserve used during starvation of newly released *O. edulis* larvae and the viability of *O. edulis* larvae was correlated with the lipid content upon liberation (Helm et al., 1973). Developing

larvae accumulated neutral lipids which increased from 8.8% of total organic matter in newly released larvae to 23.8% at metamorphosis. It was concluded by Holland and Spencer (1973) that neutral lipid is the major energy reserve of larvae and young spat.

Diatoms are usually not fed to young oyster larvae as most species are too large and difficult to digest. *Cyclotella nana*, however, is used by some hatcheries as a single food species or in combination with others to feed larvae approaching metamorphosis. This species is considered a very good food for young quahog larvae and later larval stages of oysters (Matthiessen and Toner, 1966), but some hatchery operators find it difficult to maintain in culture. *Phaeodactylum tricornutum* is also sometimes used as part of a food mixture for later larval stages of oysters. Larvae of the quahog have been reared on a variety of foods, including diatoms from straight-hinge to metamorphosis. The soft shell clam was reared on a mixture of *P. tricornutum* and *C. nana*, but the diatom, *Thalassiosira*, was toxic (Stickney, 1964). Larvae of the bay scallop were successfully reared on a mixture of *C. nana*, *Skeletonema costatum*, and the chrysophyte, *I. galbana* (Matthiessen and Toner, 1966). In Japan, the diatoms, *Chaetoceros calcitrans*, *C. simplex*, and *C. gracilis*, have been used to rear a variety of molluscs (Koganezawa, 1975). The diatom, *C. calcitrans* (Paulsen) Takano, used by Kan-no (1965) as a food for clams, was found to be of equal value for *O. edulis* larvae as the flagellates, *I. galbana* and *Tetraselmis*. Liberated larvae cultured for eight days on a mixture of two of these species resulted in larvae that were significantly larger, a greater proportion that were eyed, and a larger number of spat developed than from any of the foods fed singly. A mixture of all three foods did not produce particularly better results than two species (Walne, 1970). A mixture of *C. calcitrans* and *I. galbana* was also used for the successful rearing of early stages of the scallop, *Pecten maximus*. At later stages (14 days) the chlorophyte, *Pyramimonas obnata*, was added and still later (25 days), *Tetraselmis suecica* (Gruffydd and Beaumont, 1972).

Results of feeding species of cryptophytes to oyster larvae are variable and a clear evaluation of its food potential has not been possible (Walne, 1964).

Some of the yellow-brown flagellates of the Chrysophyceae comprise the best foods for rearing larvae of oysters and other bivalves. The following flagellates supported good growth of oyster (*C. virginica*) larvae and larvae of the hard clam, *M. mercenaria*: *Chromulina pleiades*, *Dicrateria inornata*, *Hemiselmis rufescens*, *Monochrysis lutheri*, and *Iso-*

chrysis galbana, but *Prymnesium parvum* was toxic (Davis, 1950, 1953; Davis and Guillard, 1958). *D. inornata*, *M. lutheri* and *I. galbana* were also used to rear the soft shell clam (Stickney, 1964). Of eight species fed to the larvae of the pearl oyster, *P. maximus*, *M. lutheri* and *I. galbana* produced growth far superior to the other species, *D. primolecta*, *Chlorococcum* sp, *Pyramimonas grossi*, *Dicrateria* sp, *Nitzschia closterium*, and *Nannochloris* sp.

The motile chlorophytes, such as species of the genus *Dunaliella* and *Pyramimonas*, produced excellent results in growth of *O. edulis* larvae (Bruce et al., 1939; Walne, 1964) but moderate growth of *C. virginica* larvae. Forms with a cell wall, such as *Chlamydomonas* sp, did not produce good growth of oyster larvae, but could be used by clam larvae (Walne, 1964; Davis and Guillard, 1958). Non-motile species of the genus *Chlorella* were described as an "indifferent food" by Walne (1964) although an earlier report indicated that a mixed culture of *Chlorella* species could serve as an adequate food for several different species of bivalve larvae (Davis, 1953). In one study the non-motile chlorophyte, *Chlorococcum*, appeared to be as good an oyster larval food as *M. lutheri* or *I. galbana*, but the non-motile species, *Stichococcus*, was found to be highly toxic (Davis and Guillard, 1958).

The relationship between the quantity of algae fed to larvae and the density of the larvae in culture is of great importance to good survival and growth of the larvae. As the larvae grow, the density should be reduced to prevent crowding, and to discourage predators and bacterial proliferation — factors which have a marked influence on survival. Individual investigators have developed an optimum larval and food concentration for their own culture systems. These values range approximately between 15-20 larvae/ml although 1/ml or less has also been used. The numbers of algal cells vary widely, depending on the algal species, the age of the larvae, and the frequency of feeding. Davis and Guillard (1958) fed straight-hinge larvae (15/ml) daily with *M. lutheri* or *I. galbana* at 200,000-250,000 cells/ml, while Walne (1964) cultured larvae of *O. edulis* (1/ml) with 60,000 cells/ml of *I. galbana*.

There seems to be an optimum concentration for feeding at different stages in the life cycle of bivalves. This value, called "the critical cell concentration" in a study on mussels by Schulte (1975), is one in which volumes are swept clear, all food particles are ingested, and no pseudofeces produced. As food concentrations higher than the critical concentration are consumed, there is a steady decrease in sweeping rates, together with an increase in pseudofeces formation. Ansell et al. (1963a) suggested that bivalves have a maximum growth rate which

is temperature-controlled and relatively independent of food concentrations, as long as the concentration is maintained above a certain minimum level. The critical level varies at different larval sizes. Rhodes and Landers (1973) showed that the optimum feeding concentration of *C. virginica* larvae between 75μ and 250μ will vary. A 46% economy in the use of the larval food, *I. galbana*, was effected by feeding animals according to a graduated schedule, depending on the size of the larva, rather than at a steady feeding rate throughout this growth period. Although a similar study on a diet of mixed food species has not been conducted, it is probably correct to assume a similar economy would be effected.

With the minimum information available on specific dietary requirements of bivalve larvae and the rather specialized laboratory prototypes for culturing useful phytoplankton foods, enterprising shellfish companies have applied this information to aquaculture of molluscs and operate hatcheries as pilot plants or, hopefully, commercially feasible operations. The two most important characteristics necessary for the production of food in commercial aquaculture are 1) a minimum cost of materials and labor consistent with a maximum productivity; and 2) predictability and consistency of results. With careful management phytoplankton foods can meet these requirements. Various procedures have been adopted for obtaining phytoplankton to be used in the large-scale rearing of bivalve larvae; many procedures are still experimental. Some of these techniques depend on harvesting of naturally occurring blooms, and others, on culture of microscopic algae that are useful foods.

NATURAL POPULATIONS OR NATURAL POPULATIONS IN COMBINATION WITH CULTURED SPECIES

Blooms in artificially upwelled seawater.

The significance of clean water to the success of aquaculture is clearly demonstrated by the artificial upwelling projects that are taking place under the direction of Dr. Oswald Roels in St. Croix. In this operation relatively sterile nutrient-rich deep water is pumped (from a depth of 3,000 ft) to the surface and stored in 12,000-gallon concrete pools. The phosphates and minerals in this clean water are derived from the oxidation of organic matter sinking through the water column. The pools are inoculated with diatoms from a culture system. The algae grow rapidly to a concentration of 10^{4-5} /ml and are then pumped at a metered rate to epoxy-coated plywood tanks containing shellfish. In

reports by Roels et al. (1971) and Baab et al. (1973) hatchery reared shellfish were used in all growth experiments and excellent adults of eight species of shellfish were produced in rapid time. This source of nutrient-rich cold water has recently been tested as a growth medium for larval food organisms at the St. Croix laboratory, and there is no reason why it should not yield good food cultures. The prohibitive cost of an artificial upwelling installation and the limited geographical areas to which it is applicable would preclude the widespread use of this culture system in commercial hatchery operations. Nevertheless, in isolated situations excellent results can be anticipated.

Blooms in seawater with recycled water

The possibility of using waste with the intent to recycle it, while at the same time culturing phytoplankton food, has been explored from time to time. By-products of a power plant were used in the culture of *Phaeodactylum tricornutum* in concrete tanks, containing 100 liters of seawater, built on the grounds of the Poole Electric generating station in Dorset, England. Nitrogen and phosphorus fertilizer was added to the tanks and waste flue gas from the station boilers, containing 8-15% carbon dioxide (scrubbed to remove oxides of sulfur and other impurities), was used as a source of CO₂ (Ansell et al., 1963b). Oswald et al. (1959) and Oswald and Golueke (1968) proposed that the large quantities of inorganic nitrogen and phosphorous remaining in the effluent of a secondary sewage treatment plant could be recycled into the biomass of freshwater algae. Ryther et al. (1972) and Goldman et al. (1975) applied this concept to marine systems in which algae grow in mixtures of wastewater and seawater. Filtered seawater is blended with unchlorinated secondary treated sewage effluent in 2000-1 ponds and mixed through rotating arms and recirculating pumps. Ponds are not inoculated, but a virtual unialgal culture of a marine diatom is established from the diatoms present in the seawater; the predominant species (*P. tricornutum* dominates during May and June, followed by *Amphirora* until late August and then *Amphora*) varies at different times of the year and, as would be expected, the yield is temperature-dependent (Dunstan and Menzel, 1971; Dunstan and Tenore, 1972). The culture has no adverse effect on adult oysters, *C. virginica*, bay scallops, *Aequipecten irradians*, or blue mussels, *Mytilus edulis* (Tenore and Dunstan, 1973). This phytoplankton composition would make these cultures unsuitable for food for young larvae. If the use of secondary treated sewage were adapted to the culture of species suitable for rearing

larvae, other problems would still remain. Aside from nitrates and phosphates, secondary treated sewage effluent may contain a number of other substances such as trace metals, organic pesticides, and viral particles (Berger, 1970; Homma et al., 1973; Kollins, 1966). Average trace metal concentrations may be in order of magnitude that could be harmful to larvae: zinc, 0.18 ppm; chromium, 0.04 ppm; copper, 0.06 ppm; and cadmium, 0.01 ppm (Dunstan and Menzel, 1971). The results of a study by Vaughn and Ryther (1974) suggest that viruses in sewage effluent used as culture media for phytoplankton foods could expose the molluscs to viruses through the food chain.

Blooms in clarified seawater

Several pelecypod larvae have been reared successfully on phytoplankton foods that have been cultured on what is known as the "Glancy-Wells Method." Whenever it has been possible to use this method, it has been found less expensive and yields a better increase in larval growth than conventional methods of algal culture. Although the method is used with great success in certain commercial hatcheries, i.e., in Virginia for bay scallops (Castagna, 1975; Castagna and Duggan, 1971) and in one hatchery on Long Island Sound for the American oyster, other installations, e.g., VIMS at Gloucester Point (Hidu, 1971), have found it unsatisfactory because of poor phytoplankton yields or, at best, unreliable yields on a year-round basis. The method was developed by Wells and Glancy in the early 1900's but a clear design was worked out recently for a patent submission (Glancy, 1965). Seawater used for this purpose is first clarified by filtering in polypropylene nylon filter bags or by continuous centrifugation in a large Sharples centrifuge (3,200 g) to remove detritus, large particles and suspended plants and animals. The effluent is piped into fiberglass tanks located under a translucent roof where adequate light is available to sustain photosynthesis. The greenhouse effect raises the temperature and a rich bloom of diatoms, flagellates, and nanoplankters results in 24-48 hrs. The faintly colored water is referred to as cultured water and is used as the medium for growing larvae and juvenile bivalves. It is interesting to note that recently-bloomed water results in faster growth and better survival of larvae than water allowed to bloom for more than 48 hrs. It has been noted frequently that the mixed wild cultures from this system were better foods (as measured by excellent survival and fast larval growth) than any phytoplankters tested as individual species or in combination. One difficulty is that a natural food popula-

tion may also contain a zooplankton population and care must be exercised to exclude it by appropriate screenings. Growth rates with natural algal foods show an initial lag followed by very rapid growth in later stages, in contrast with cultured foods which produce a slow steady increase in larval size (Hidu, 1971).

CULTURED SPECIES

Continuous culture

In theory, continuous culture has several inherent benefits over batch cultures for algal production, such as 1) maintaining growth rates and productivities near maximum; 2) producing uniformity of cell populations; and 3) partially automating the culture system. Simple continuous culture algal production lines have been described for short-term experiments using natural seawater in a sterile system (Ukeles, 1973) and a similar type of apparatus in a long-term experiment using a partially sterile system and artificial seawater (Palmer et al. 1976). The latter authors believe that this system of culture has a potential for hatchery use, but this writer is of the opinion that in its current scale and state of development, continuous culture is not practical for hatchery operations.

Closed cycle culture

Similarly, information on the technology of closed cycle systems has not been applied to the rearing and feeding of bivalve larvae on a practical basis. Closed cycle systems are being developed as a rearing technique for post-larval oysters to overcome the uncertainties of water quality and to exercise a complete control over the factors of oyster cultivation (Epifanio et al., 1975). It is still questionable whether such a system could be applied to larval rearing because of the great sensitivity of animals at this stage to waste products, disease, and other factors. In any event, if these problems could be overcome by refinements in techniques, the methodology would still represent a considerable increase in capital investments over that now in use by commercial aquaculture companies. The additional expenditure would have to be justified by a commensurate greater productivity.

Batch culture

The large-scale culture of phytoplankton for commercial hatcheries and pilot plants is conducted by various procedures, but the most

general approach is to use a batch culture system in increasing volumes, and dilutions according to the prototype developed at the Milford laboratory. Hatcheries fortunate enough to possess a seawater well have water of very high quality available. These hatcheries achieve excellent results in phytoplankton culture, as well as larval growth, again emphasizing the significance of clean water to success in aquaculture practices. Bivalve larvae are reared at the Milford laboratory for research investigations, as well as for pilot plant hatchery programs; the food culture system was developed to fulfill varied requirements. Numerous strains of food organisms are maintained as axenic cultures in Erlenmeyer flasks (125 and 500 ml). These cultures are allowed to grow to a high density and then transferred to Fernbach flasks (2800 ml). After a suitable growth period cultures are inoculated into 5-gal Pyrex carboys, which is the final growth vessel for the production of larval foods. The growth medium consists of an enriched seawater that is autoclaved before use. Carboys are maintained on a semi-continuous basis (harvested once or twice weekly) for as long as cultures produce good yields of algae with minimum numbers of bacteria. These procedures, as well as the types of growth media used, have already been described in considerable detail (Ukeles, 1971, 1973). Foods for larvae approaching metamorphosis are cultured in open fiberglass tanks, both indoors and outdoors, using a growth medium based on artificial sea salts (Ukeles, 1965, 1971). Commercial hatcheries that employ this batch culture system keep stock cultures in Erlenmeyer flasks and use cultures in 5-gal Pyrex carboys to inoculate the final growth vessels. In hatcheries these are open fiberglass or polyethylene tanks on the order of 3000-gal capacity. These tanks are kept well-aerated and illuminated by suspended fluorescent lamps. Translucent plastic material is used liberally in the hatchery construction as part of the roof and walls so that an abundance of natural light, in addition to artificial illumination, reaches the cultures in these tanks. Seawater pumped from the source is filtered to remove large particles and enriched according to published formulations (Guillard and Ryther, 1962; Ukeles, 1971). After a brief growth period cultures are pumped directly into larval culture tanks to the appropriate density. Post-larval foods are generally dispensed at a continuous or intermittent rate to holding trays of juveniles.

The possibility of harvesting and storing phytoplankton food as a concentrated suspension has always remained an attractive idea and a step in the direction of improving the efficiency of algal food preparation. The long-term efficiency of the aquaculture process could probably

be improved by a separation in time of the process of algal food culture from that of larval culture. With such a system it would then be possible to "pre-test" food "lots" as a means of uncovering toxicity in the food supply on developing egg or 48-hour larval assay systems before they are fed to large populations of larval or juvenile bivalves (Ukeles, unpublished). At the Milford laboratory concentrated suspensions of algae are often harvested and stored in the dark at 10°C for several days before being fed to larvae. This method reduces the number of times each culture carboy is harvested, but the method is dependent upon the availability of axenic food cultures or those with very low numbers of bacteria. When highly concentrated samples are required we have used a small Sharples centrifuge to collect algal cells on a paper lining inserted into the sedimentation chamber. Cells are then washed off with seawater and stored or dried. The common practice in hatcheries is to pump algal cells directly from dilute suspensions in large tanks to larval rearing tanks. Cells from dilute suspensions can be concentrated by filtration, as well as centrifugation. Filtration on membrane filters with the aid of a vacuum pump (Clark and Sigler, 1963) is not suitable for large volumes because of rapid clogging of the filter and possible damage to delicate cells. A reverse-flow filter designed by Dodson and Thomas (1964) and modified by Holm-Hansen et al. (1970) and Morris and Yentsch (1972) provides a more gentle process and avoids packing of cells. Harvesting algae by froth flotation is another possibility. The method developed by Levin et al. (1962) is particularly useful for harvesting food cells as it does not depend on the addition of the flotants.

CONCLUSIONS

Future developments in studies of bivalve larvae nutrition are dependent upon finding better and more definitive methods of evaluating the effect of various diets upon larval growth and development. There is need for additional investigations in each of the areas of potential diet sources outlined in this discussion. It is important, however, that more critical standards in experimental designs and evaluations be employed than in previous work to avoid unclear results and needless duplication of effort.

LITERATURE CITED

- Allen, E. J. and E. W. Nelson, 1910. On the artificial culture of marine plankton organisms. J. Mar. Biol. Assn. U.K. 8, 421-474.
- Amemiya, I., 1926. Notes on experiments on the early developmental stages of the Portuguese, American and English native oysters, with special reference to the effect of varying salinity. J. Mar. Biol. Assn. U.K. 14, 161-175.
- Andrews, P. and P. J. Le B. Williams, 1971. Heterotrophic utilization of dissolved organic compounds in the sea. III. Measurement of the oxidation rates and concentrations of glucose and amino acids in seawater. J. Mar. Biol. Assn. U.K. 51, 111-125.
- Ansell, A. D., J. E. G. Rayment, and K. F. Lander, 1963a. Studies on the mass culture of *Phaeodactylum*. III. Small-scale experiments. Limnol. Oceanogr. 8, 207-213.
- Ansell, A. D., J. E. G. Rayment, K. F. Lander, E. Crowley, and P. Shackley, 1963b. Studies on the mass culture of *Phaeodactylum*. II. The growth of *Phaeodactylum* and other species in outdoor tanks. Limnol. Oceanogr. 8, 184-206.
- Armstrong, F. A. J., P. M. Williams, and J. D. H. Strickland, 1966. Photo-oxidation of organic matter in seawater by ultra-violet radiation, analytical and other applications. Nature 211, 481-483.
- Baab, J. S., G. L. Hamm, K. C. Haines, A. Chu, and O. A. Roels, 1973. Shellfish mariculture in an artificial upwelling system. Proc. Nat. Shellfish. Assn. 63, 63-67.
- Barber, R. T., 1966. Interaction of bubbles and bacteria in the formation of organic aggregates in seawater. Nature 211, 257-258.
- Batoosingh, E., G. A. Riley, and B. Keshwar, 1969. An analysis of experimental methods for producing particulate organic matter in seawater by bubbling. Deep-Sea Res. 213-219.
- Baylor, E. R. and W. H. Sutcliffe, Jr. 1963. Dissolved organic matter in seawater as a source of particulate food. Limnol. Oceanogr. 8, 369-381.
- Baylor, E. R. and W. H. Sutcliffe, Jr., and D. S. Hirschfeld, 1962. Adsorption of phosphate onto bubbles. Deep-Sea Res. 9, 120-124.

- Bayne, B. L., 1965. Growth and delay of metamorphosis of the larvae of *Mytilus edulis* (L.). *Ophelia* 2, 1-47.
- Belding, D. L., 1910. The scallop fishery of Massachusetts. Marine Fisheries Series - No. 2. Mass. Div. Marine Fisheries, Dept. Natural Resources, Boston, Mass. 51 p.
- , 1912. The quahaug fishery of Massachusetts. Marine Fisheries Series - No. 2 Mass. Div. Marine Fisheries, Dept. Natural Resources, Boston, Mass. 41 p.
- , 1930. The soft-shelled clam fishery of Massachusetts. Marine Fisheries Series - No. 1. Mass. Div. Marine Fisheries, Dept. Natural Resources, Boston, Mass. 65 p.
- Berger, B. B., 1970. Current perspectives in disinfection. Proc. Nat. Spec. Cont. Disinfect., Univ. Mass., Amherst, 1-10. Amer. Soc. Civ. Eng., New York, N. Y.
- Bernhard, M. and A. Zattera, 1970. The importance of avoiding chemical contamination for a successful cultivation of marine organisms. *Helgolander wiss. Meeresunters.* 20, 655-675.
- Blankley, W. F., 1973. Toxic and inhibitory materials associated with culturing. In J. Stein (ed), *Handbook of phycological methods*. Cambridge Univ. Press, 207-229.
- Blegvad, H., 1914. Food and conditions of nourishment among the communities of invertebrate animals found on or in the sea bottom in Danish waters. *Rep. Danish Biol. Sta.* 22, 41-78.
- Blogoslawski, W. J., C. Brown, E. W. Rhodes, and M. Broadhurst, 1975. Ozone disinfection of a seawater supply system. First international Symposium on Ozone for Water and Wastewater Treatment, International Ozone Institute Proc. 674-687.
- Bourne, N. and D. W. Smith, 1972. Breeding and growth of the horse clam, *Tresus capax* (Gould), in southern British Columbia. *Proc. Nat. Shellfish. Assn.* 62, 38-46.
- Breese, W. P. and F. D. Phibbs, 1970. Some observations on the spawning and early development of the butter clam *Saxidomus giganteus* (Deshayes). *Proc. Nat. Shellfish. Assn.* 60, 95-98.

- Brown, C., 1973. The effects of some selected bacteria on embryos and larvae of the American oyster, *Crassostrea virginica*. J. Invert. Pathol. 21, 215-223.
- Bruce, J. R., M. Knight, and M. W. Parke, 1939. The rearing of oyster larvae on an algal diet. J. Mar. Biol. Assn. U. K. 24, 337-374.
- Button, D. K., 1968. Selective thiamine removal from culture media by ultra-violet irradiation. Appl. Microbiol. 16, 530-531.
- Calabrese, A., 1972. How some pollutants affect embryos and larvae of American oyster and hard-shell clam. Mar. Fish Rev. 34, 66-77.
- Carriker, M. R., 1956. Biology and propagation of young hard clams, *Mercenaria mercenaria*. J. Elisha Mitchell Sci. Soc. 72, 57-60.
- , 1961. Interrelation of functional morphology, behavior, and autecology in early stages of the bivalve *Mercenaria mercenaria* J. Elisha Mitchell Sci. Soc. 77, 168-241.
- Castagna, M., 1975. Culture of the bay scallop, *Argopecten irradians*, in Virginia. Mar. Fish. Rev. 37, 19-24.
- Castagna, M. and W. Duggan, 1971. Rearing the bay scallop, *Aequipecten irradians*. Proc. Nat. Shellfish. Assn. 61, 80-85.
- Castell, J. D. and D. J. Trider, 1974. Preliminary feeding trials using artificial diets to study the nutritional requirements of oysters (*Crassostrea virginica*). J. Fish. Res. Bd. Canada 31, 95-99.
- Chanley, P., 1965a. Larval development of a boring clam, *Barnea truncata*. Chesapeake Sci. 6, 162-166.
- , 1965b. Larval development of the brackish water clam, *Rangia cuneata*. Chesapeake Sci. 6, 209-213.
- , 1966. Larval development of the large blood clam, *Noetia ponderosa* (Say). Proc. Nat. Shellfish. Assn. 56, 53-57.
- , 1969. Larval development of the coquina clam, *Donax variabilis* Say, with a discussion of the structure of the early hinge in the Tellinacea. Bull. Mar. Sci. 19, 214-224.
- , 1970. Larval development of the hooked mussel, *Brachidontes recurvus* Rafinesque (Bivalvia: Mytilidae) including a literature review of larval characteristics of the Mytilidae. Proc. Nat. Shellfish. Assn. 60, 86-94.

- Chanley, P. and J. D. Andrews, 1971. Aids for identification of bivalve larvae of Virginia. *Malacologia* 11, 45-119.
- Chanley, P. and M. Castagna, 1966. Larval development of the pelecypod *Lyonsia hyalina*. *Nautilus* 79, 123-128.
- Chanley, P. and R. F. Normandin, 1967. Use of artificial foods for larvae of the hard clam, *Mercenaria mercenaria*. *Proc. Nat. Shellfish. Assn.* 57, 31-37.
- Clark, W. J. and W. F. Sigler, 1963. Method of concentrating phytoplankton samples using membrane filters. *Limnol. Oceanogr.* 8, 127-129.
- Cole, H. A., 1937. Experiments in the breeding of oysters (*Ostrea edulis*) in tanks, with special reference to the food of the larva and spat. *Fish. Invest., Min. Agric. Fish., Lond., Ser. II*, 15, 24 p.
- , 1938. A system of oyster culture. *J. Cons. Perm. Int. Explor. Mer.* 13, 221-235.
- Collier, A., S. M. Ray, A. W. Magnitzky, and J. O. Bell, 1953. Effect of dissolved organic substances on oysters. *U. S. Fish Wildl. Serv. Fish. Bull.* 84, 54, 167-185.
- Cowey, C. B. and J. R. Sargent, 1972. Fish nutrition. In F. S. Russell and M. Yonge (eds), *Advances in marine biology*. Academic Press, Inc., London and New York, 10, 383-492.
- Culliney, J. L., 1974. Larval development of the giant scallop *Placopecten magellanicus* (Gmelin). *Biol. Bull.* 147, 321-332.
- Culliney, J. L., P. J. Boyle, and R. D. Turner, 1975. New approaches and techniques for studying bivalve larvae. In W. L. Smith and M. H. Chanley (eds), *Culture of marine invertebrate animals*. Plenum Publ. Corp., New York, 257-271.
- Dassow, J. A. and M. A. Steinberg, 1973. The technological basis for development of aquaculture to produce low-cost food fish. *Mar. Fish. Rev.* 35, 6-13.
- Davis, H. C., 1950. On food requirements of larvae of *Ostrea virginica*. *Anat. Rec.* 108, 132-133.
- , 1953. On food and feeding of larvae of the American oyster, *C. virginica*. *Biol. Bull.* 104, 334-350.

- _____, 1960. Effects of turbidity-producing materials in sea water on eggs and larvae of the clam (*Venus (Mercenaria) mercenaria*). Biol. Bull. 118, 48-54.
- Davis, H. C. and P. E. Chanley, 1956. Effects of some dissolved substances on bivalve larvae. Proc. Nat. Shellfish. Assn. 46, 59-74.
- Davis, H. C. and R. R. Guillard, 1958. Relative value of ten genera of micro-organisms as foods for oyster and clam larvae. U. S. Fish Wildl. Serv. Fish. Bull. 136, 58, 293-304.
- Davis, H. C. and H. Hidu, 1969a. Effects of turbidity-producing substances in sea water on eggs and larvae of three genera of bivalve mollusks. The Veliger 11, 316-323.
- _____, 1969b. Effects of pesticides on embryonic development of clams and oysters and on survival and growth of the larvae. U. S. Fish. Wildl. Serv. Fish. Bull. 67, 393-404.
- Davis, H. C., V. L. Loosanoff, W. H. Weston, and C. Martin, 1954. A fungus disease in clam and oyster larvae. Science 120, 36-38.
- Dodson, A. N. and W. H. Thomas, 1964. Concentrating plankton in a gentle fashion Limnol. Oceanogr. 9, 455-456.
- Droop, M. R., 1966. The role of algae in the nutrition of *Heteramoeba clara* Droop, with notes on *Oxyrrhis marina* Dujardin and *Philodina roseola* Ehrenberg. In H. Barnes (eds), Some contemporary studies in marine science. George Allen and Unwin Ltd., Lond., 269-282.
- Dunstan, W. M. and D. W. Menzel, 1971. Continuous cultures of natural populations of phytoplankton in dilute, treated sewage effluent. Limnol. Oceanogr. 16, 623-632.
- Dunstan, W. M. and K. R. Tenore, 1972. Intensive outdoor culture of marine phytoplankton enriched with treated sewage effluent. Aquaculture 1, 181-192.
- Dyer, D. L. and D. E. Richardson, 1962. Materials of construction in algal culture. Appl. Microbiol. 10, 129-131.
- Epifanio, C. E., R. Srna, and G. Pruder, 1975. Mariculture of shellfish in controlled environments: A prognosis. Aquaculture 5, 227-241.

- Fox, D. L., C. H. Oppenheimer, and J. S. Kittredge, 1953. Microfiltration in oceanographic research. II Retention of colloidal micelles by adsorption filters and by filter-feeding invertebrates: proportions of dispersed organic to dispersed inorganic matter and to organic solutes. *J. Mar. Res.* 12, 233-243.
- Gillespie, L., R. M. Ingle, and W. K. Havens, 1964. Glucose nutrition and longevity in oysters. *Quart. J. Florida Acad. Sci.* 27, 279-288.
- Glancy, J. B., 1965. Method of raising shellfish seed in a simulated habitat. U. S. Patent Office, Patent No. 3,196,833.
- Goldman, J. C., J. H. Ryther, and V. D. Williams, 1975. Mass production of marine algae in outdoor cultures. *Nature* 254, 594-595.
- Goodrich, D. B., L. J. Calbo, A. Perlmutter, and R. B. Wainwright, 1968. New engineering approaches for the production of Connecticut oysters. I, II. American Cyanamid Co. Central Res. Div., Stamford, Connecticut.
- Gruffydd, L. D. and A. R. Beaumont, 1972. A method for rearing *Pecten maximus* larvae in the laboratory. *Mar. Biol.* 15, 350-355.
- Guillard, R. R. and J. H. Ryther, 1962. Studies on marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea* (Cleve) Gran. *Can. J. Microbiol.* 8, 229-239.
- Halver, J. E., 1968. Nutrition in marine aquaculture. In W. J. McNeil (ed), *Marine aquaculture, selected papers from conference in marine aquaculture*. Oregon State Univ. Marine Science Center, Newport, Oregon. Oregon State Univ. Press, Corvallis.
- Haven, D. S., 1965. Supplemental feeding of oysters with starch. *Chesapeake Sci.* 6, 43-51.
- Helm, M. M., D. L. Holland, and R. R. Stephenson, 1973. The effect of supplementary algal feeding of a hatchery breeding stock of *Ostrea edulis* L. on larval vigour. *J. Mar. Biol. Assn. U. K.* 53, 673-684.
- Hidu, H., 1971. The feasibility of oyster hatcheries in the Delaware-Chesapeake Bay region. In K. S. Price Jr. and D. L. Maurer (eds), *Proc. conf. on artificial propagation of commercially valuable shellfish — oysters*. Univ. Delaware, Newark, Delaware, 111-132.

- Hidu, H. and H. S. Tubiash, 1963. A bacterial basis for the growth of antibiotic-treated bivalve larvae. Proc. Nat. Shellfish. Assn. 54, 25-39.
- Hidu, H. And R. Ukeles, 1962. Dried unicellular algae as food for larvae of the hard shell clam, *Mercenaria mercenaria*. Proc. Nat. Shellfish. Assn. 53, 85-101.
- Holland, D. L. and B. E. Spencer, 1973. Biochemical changes in fed and starved oysters, *Ostrea edulis* L., during larval development, metamorphosis and early spat growth. J. Mar. Biol. Assn. U. K. 53, 287-298.
- Holm-Hansen, O., T. T. Packard, and L. R. Pomeroy, 1970. Efficiency of the reverse flow filter technique for concentration of particulate matter. Limnol. Oceanogr. 15, 350-355.
- Homma, A., M. Sobsey, C. Wallis, and J. L. Melnick, 1973. Virus concentration from sewage. Water Res. 7, 945-950.
- Hutner, S. H. and L. Provasoli, 1951. The phytoflagellates. In A. Lwoff (ed), Biochemistry and physiology of protozoa. Academic Press, Inc., New York, 1, 27-128.
- Imai, T. and M. Hatanaka, 1949. On the artificial propagation of the Japanese common oyster, *Ostrea gigas* Thun., by non-colored naked flagellates. Bull. Inst. Agric. Res., Tohoku Univ. 1, 33-46.
- Imai, T., S. Sakai, H. Okada, and T. Yoshida, 1954. Breeding of the Olympia oyster in tanks and culture experiments in Japanese waters. Tohoku J. Agric. Res. 5, 13-25.
- Jones, D. A., J. G. Munford, and P. A. Gabott, 1974. Microcapsules as artificial food particles for aquatic filter feeders. Nature 247, 233-235.
- Jørgensen, C. B., 1946. Lamellibranchia. In C. Thorson (ed), Reproduction and larval development of Danish marine bottom invertebrates. Meddel. fra Komm. for Danmarks Fiskeri - og Havundersog. KBH., Ser. (d): Plankton 4, 277-311.
- Kan-no, H., 1965. Experiments concerning the tank breeding of the surf clam *Spisula sachalinensis* (Schrieber). Bull. Tohoku Reg. Fish. Res. Lab. 25, 131-141.

- Kelly, C. B., 1961. Disinfection of seawater by ultraviolet radiation. Amer. J. Publ. Health 51, 1670-1680.
- Kelly, C. B., W. Arcisz, M. W. Presnell, and E. K. Harris, 1960. Bacterial accumulation by the Oyster *Crassostrea virginica* on the Gulf coast Cincinnati, Ohio: Robert A. Taft Sanitary Engineering Center, Tech. Rept. F60-4.
- Kirby-Smith, W. W. and R. T. Barber, 1974. Suspension-feeding aquaculture systems: Effects of phytoplankton concentration and temperature on growth of the bay scallop. Aquaculture 3, 135-145.
- Koganezawa, A., 1975. Mass rearing of off-shore bivalve larvae in Japan. Paper presented at International conference on aquaculture nutrition, Rehoboth Beach, Delaware.
- Kollins, S. A., 1966. The presence of human enteric virus in sewage and their removal by conventional sewage treatment methods. In W. B. Umbreit (ed), Advances in applied microbiology. Academic Press, Inc., 8, 145-193.
- Krogh, A., 1931. Dissolved substances as food of aquatic organisms. Biol. Rev. 6, 412-442.
- Kume, M. and K. Dan (eds), 1968. Invertebrate embryology. Transl. by J. C. Dan. NOLIT Publ., Belgrade, Yugoslavia, 485-521.
- Landers, W., 1971. Some problems in the culture of oyster larvae. In J. W. Avault (ed), Proc. 2nd Annual Workshop World Mariculture Soc., Louisiana State Univ., Baton Rouge, Louisiana, 37-50.
- Levin, G. V., J. R. Clendenning, A. Gibor, and F. D. Bogar, 1962. Harvesting of algae by froth flotation. Appl. Microbiol. 10, 169-175.
- Loosanoff, V. L. and H. C. Davis, 1963. Rearing of bivalve mollusks. In F. S. Russell (ed), Advances in marine biology. Academic Press, Inc., London and New York, 1, 1-136.
- Loosanoff, V. L., H. C. Davis, and P. E. Chanley, 1966. Dimensions and shapes of larvae of some marine bivalve mollusks. Malacologia 4, 351-435.
- McBride, E. W., 1914. Text-book of embryology. Invertebrata. Macmillan and Co., New York, 1, 1-692.

- MacLean, S. A., A. C. Longwell, and W. J. Blogoslawski, 1973. Effects of ozone-treated seawater on the spawned, fertilized, meiotic, and cleaving eggs of the commercial American oyster. *Mutation Res.* 21, 283-285.
- Mardela Corp., 1973. Summary report to participants. NOAA regional aquaculture workshops. U. S. Dept. Commerce.-Mardela Corp., Burlingame, California.
- Matthiessen, G. C. and R. C. Toner, 1966. Possible methods of improving the shellfish industry of Martha's Vineyard, Duke's County, Massachusetts. Marine Research Foundation, Edgartown, Mass. 138 p.
- Menzel, D. W., 1966. Bubbling of seawater and the production of organic particles: a re-evaluation. *Deep-Sea Res.* 13, 963-966.
- Menzel, R. W., 1971. Quahog clams and their possible mariculture. In J. W. Avault, Jr. (ed), *Proc. 2nd Annual Workshop World Mariculture Soc.*, Galveston, Texas, Louisiana State Univ., Baton Rouge, Louisiana.
- Meyers, S. P. and Z. P. Zein-Eldin, 1972. Binders and pellet stability in development of crustacean diets. *Proc. 3rd Annual Workshop World Mariculture Soc.*, Louisiana State Univ., Baton Rouge, Louisiana, 351-364.
- Meyers, S. P., D. P. Butler, and G. F. Sirine, 1971. Encapsulation, a new approach to larval feeding. *American Fish Farmer* 2, 15-16, 18, 20.
- Mileikovsky, S. A., 1973. Speed of active movement of pelagic larvae of marine bottom invertebrates and their ability to regulate their vertical position. *Mar. Biol.* 23, 11-17.
- Millar, R. H. and J. M. Scott, 1967a. Bacteria-free culture of oyster larvae. *Nature* 216, 1139-1140.
- , 1967b. The larvae of the oyster *Ostrea edulis* during starvation. *J. Mar. Biol. Assn. U. K.* 47, 475-484.
- Minaur, J., 1969. Experiments on the artificial rearing of the larvae of *Pinctada maxima* (Jameson) (Lamellibranchia). *Aust. J. Mar. Freshwater Res.* 20, 175-187.

- Morris, I. and C. S. Yentsch, 1972. A new method for concentrating phytoplankton by filtration with continuous stirring. *Limnol. Oceanogr.* 17, 490-493.
- North, B. B., 1975. Primary amines in California coastal waters: Utilization by phytoplankton. *Limnol. Oceanogr.* 20, 20-27.
- Oswald, W. J. and C. G. Golueke, 1968. Harvesting and processing of waste-grown microalgae. In D. F. Jackson (ed), *Algae, man, and the environment*. Syracuse Univ. Press, New York, 371-389.
- Oswald, W. J., C. G. Golueke, and H. K. Gee, 1959. Waste water reclamation through production of algae. *Univ. Calif. Sanit. Eng. Res. Lab. and Water Resources Center, Contrib.* 22.
- Paffenhof, G. A. and J. D. H. Strickland, 1970. A note on the feeding of *Calanus helgolandicus* on detritus. *Mar. Biol.* 5, 97-99.
- Palmer, F. E., K. A. Ballard, and F. B. Taub, 1976. A continuous culture apparatus for the mass production of algae. *Aquaculture* (in press).
- Parsons, T. R. and J. D. H. Strickland, 1962. Oceanic detritus. *Science* 136, 313-314.
- Parsons, T. R., K. Stephens, and J. D. H. Strickland, 1961. On the chemical composition of eleven species of marine phytoplankters. *J. Fish. Res. Bd. Canada* 18, 1001-1016.
- Provasoli, L., K. Shiraishi, and J. R. Lance, 1959. Nutritional idiosyncrasies of *Artemia* and *Tigriopus* in monoxenic culture. *Ann. N. Y. Acad. Sci.* 77, 250-261.
- Purchon, R. D., 1971. Digestion in filter feeding bivalves — a new concept. *Proc. Malacol. Soc. Lond.* 39, 253-262.
- Putter, A., 1909. *Die Ernährung der Wassertiere und der Stoffhaushalt der Gewässer*. Fischer, Jena 1-168.
- Raymont, J. E. G. and M. N. E. Adams, 1958. Studies on the mass culture of *Phaeodactylum*. *Limnol. Oceanogr.* 3, 119-136.
- Rees, C. B., 1950. The identification and classification of lamellibranch larvae. *Hull Bull. Mar. Ecol.* 3, 73-104.

- Rhodes, E. W. and W. S. Landers, 1973. Growth of oyster larvae, *Crassostrea virginica*, of various sizes in different concentrations of the chrysophyte, *Isochrysis galbana*. Proc. Nat. Shellfish. Assn. 63, 53-59.
- Riley, G. A., 1963. Organic aggregates in seawater and the dynamics of their formation and utilization. Limnol. Oceanogr. 8, 372-381.
- , 1970. Particulate organic matter in sea water. In F. S. Russell and M. Yonge (eds), Advances in marine biology. Academic Press, Inc., London and New York, 8, 1-118.
- Riley, J. P. and I. Roth, 1971. The distribution of trace elements in some species of phytoplankton grown in culture. J. Mar. Biol. Assn. U. K. 51, 63-72.
- Roels, O. A., L. Van Hemelrijk, R. D. Gerard, and J. L. Worzel, 1971. Cold, nutrient rich water: the most abundant resource of the deep sea. CNEXO, Colloque international sur l'exploitation des oceans. Bordeaux, March 9-12, 1971, Th. IV, Sect-G.2-02, 1-21.
- Ryther, J. H., W. M. Dunstan, K. R. Tenore, and J. E. Huguenin, 1972. Controlled eutrophication-increasing food production from the sea by recycling human wastes. BioScience 22, 144-152.
- Schulte, E. H., 1975. Influence of algal concentration and temperature on the filtration rate of *Mytilus edulis*. Mar. Biol. 30, 331-341.
- Shiraishi, K. and L. Provasoli, 1959. Growth factors as supplements to inadequate algal foods for *Tigriopus japonicus*. Tohoku J. Agric. Res. 10, 89-96.
- Sorokin, Y. I. and D. I. Wyshkwarzev, 1973. Feeding on dissolved organic matter by some marine animals. Aquaculture 2, 141-148.
- Stephens, G. C., 1967. Dissolved organic material as a nutritional source for marine and estuarine invertebrates. In G. H. Lauff (ed), Estuaries. Amer. Assn. Advance. Sci., Publ. No. 83, Washington, D. C.
- Stephens, G. C. and R. A. Schinske, 1961. Uptake of amino acids by marine invertebrates. Limnol. Oceanogr. 6, 175-181.
- Stickney, A. P., 1964. Salinity, temperature, and food requirements of soft-shell clam larvae in laboratory culture. Ecology 45, 283-291.

- Sutcliffe, W. H., Jr., E. R. Baylor, and D. W. Menzel, 1963. Sea surface chemistry and Langmuir circulation. *Deep-Sea Res.* 10, 233-243.
- Tenore, K. R. and W. M. Dunstan, 1973. Growth comparisons of oysters, mussels and scallops cultivated on algae grown with artificial medium and treated sewage effluent. *Chesapeake Sci.* 14, 64-66.
- Toner, R. C. and B. Brooks, 1975. The effects of ozone on four species of phytoplankton, crab zoea and megalops and the Atlantic silver-side, *Menidia menidia*. In W. J. Blogoslawski and R. G. Rice (eds), *Aquatic applications of ozone*. International Ozone Institute, Hampson Press, 154-160.
- Tubiash, H. S., P. E. Chanley, and E. Leifson, 1965. Bacillary necrosis, a disease of larval and juvenile bivalve mollusks. I. Etiology and epizootiology. *J. Bact.* 90, 1036-1044.
- Turner, H. J. and C. J. George, 1955. Some aspects of the behavior of the quahog, *Venus mercenaria*, during the early stages. *Dept. Nat. Res., Commonwealth Mass., Div. Mar. Fish., 8th Rept. Invest. Shellfish. Mass.* 5-14.
- Ukeles, R., 1965. A simple method for the mass culture of marine algae. *Limnol. Oceanogr.* 10, 492-495.
- , 1971. Nutritional requirements in shellfish culture. In K. S. Price Jr. and D. L. Maurer (eds), *Proc. conf. on artificial propagation of commercially valuable shellfish — oysters*. Univ. Delaware, Newark, Delaware, 43-64.
- , 1973. Continuous culture - a method for the production of unicellular algal foods. In J. R. Stein (ed), *Handbook of phycological methods - Culture methods and growth measurements*. Cambridge Univ. Press, 233-256.
- Ukeles, R. and W. E. Rose, 1975. Induced adhesion in *Crassostrea virginica* larvae. *Science* 189, 51-53.
- Ukeles, R. and B. M. Sweeney, 1969. Influence of dinoflagellate trichocysts and other factors on the feeding of *Crassostrea virginica* larvae on *Monochrysis lutheri*. *Limnol. Oceanogr.* 14, 403-410.
- Vaughn, J. M. and J. H. Ryther, 1974. Bacteriophage survival patterns in a tertiary sewage treatment-aquaculture model system. *Aquaculture* 4, 399-406.

- Vishniac, H. S., 1955. The morphology and nutrition of a new species of *Sirolopidium*, Mycologia 47, 633-645.
- Wagner, F. S., Jr., 1969. Composition of the dissolved organic compounds in seawater: A review. Contrib. Mar. Sci. Univ. Texas 14, 115-153.
- Walne, P. R., 1956. Experimental rearing of the larvae of *Ostrea edulis* L. in the laboratory. Fish Invest., Min. Agric. Fish., Lond., Ser. II. 20, 23 p.
- , 1963. Observations on the food value of seven species of algae to the larvae of *Ostrea edulis*. 1. Feeding experiments. J. Mar. Biol. Assn. U. K. 43, 767-784.
- , 1964. The culture of marine bivalve larvae. In K. M. Wilbur and C. M. Yonge (eds), Physiology of mollusca. Academic Press, Inc., New York and London, 197-210.
- , 1965. Observations on the influence of food supply and temperature on the feeding and growth of the larvae of *Ostrea edulis* L. Fish. Invest., Min. Agric. Fish., Lond., Ser. 2. 24, 45 p.
- , 1966. Experiments in the large-scale culture of the larvae of *Ostrea edulis* L. Fish. Invest., Min. Agric. Fish., Lond., Ser. 2. 25, 53 p.
- , 1970. Present problems in the culture of the larvae of *Ostrea edulis*. Helgolander wiss. Meeresunters. 20, 514-552.
- , 1974. Culture of bivalve molluscs. Fishing News (Books) Ltd., Surrey, England. 173 p.
- Walsh, G. E., 1965. Studies on dissolved carbohydrates in Cape Cod waters. II. Diurnal fluctuations in Oyster Pond. Limnol. Oceanogr. 10, 577-582.
- Wells, W. F., 1920. Growing oysters artificially. Conservationist 3, 151, New York Conservation Com.
- , 1927. Report of the experimental shellfish station. Rept. New York State Conserv. Dept. No. 16, 1-22.
- Winter, J. E., 1973. The filtration rate of *Mytilus edulis* and its dependence on algal concentration measured by a continuous automatic recording apparatus. Mar. Biol. 22, 317-328.

- Wood, L. and W. J. Hargis, Jr., 1971. Transport of bivalve larvae in a tidal estuary. In D. J. Crisp (ed), Fourth European marine biology symposium, Bangor Wales, 1969. Cambridge, Univ. Press, 20-44.
- Yonge, C. M., 1926. Structure and physiology of the organs of feeding and digestion in *Ostrea edulis*. J. Mar. Biol. Assn. U. K., 14, 295-386.
- , 1937. Review of digestion in metazoa. Biol. Rev. 12, 87-115.
- Zaroogian, G. E., G. Pesch, and G. Morrison, 1969. Formulation of an artificial sea water media suitable for oyster larvae development. Amer. Zool. 9, 1144.

Mass Rearing Of Offshore-Type Bivalve Larvae In Japan

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INTRODUCTION

In recent years the technique of rearing the larvae of marine molluscs has developed rapidly in various countries. This is mainly due to progress in the culture of algal food organisms suitable for rearing the larvae. In Japan, researchers at seedling hatcheries have tried to develop methods of producing on a commercial scale, juveniles of various molluscs and then growing them to market size under captive conditions. The results obtained so far in experimental work with offshore molluscs will be presented in this paper.

Laboratories in Japan have worked with the following offshore types of molluscs: pecten, ark shell, fan shell, and hard shell. These species and the researchers who worked with them are shown in Table 1.

The characteristic feature of the methods used is that the larvae, juveniles and adults are cultured continuously and completely in tanks on land or suspended under racks in the sea. In the case of tank culture, the seawater is sand-filtered and when supplied to the tank, it is cartridge filtered to remove particles as small as $4\ \mu$ in size. Compressed air is also supplied to each tank for aeration.

CONTROL AND INDUCTION OF SPAWNING

Annual variations of seawater temperature and the periods for larval culture are different for different species. Scallops spawn naturally at 8°C in early spring. Because a temperature above 12°C is desirable for tank breeding, the matured individuals are kept in chilled seawater of 5°C until May when the ocean temperature reaches more than 10°C . Spawning can be induced by subjecting the matured scallop to tem-

TABLE 1

Size of eggs and full grown larvae of off-shore type molluscs.

Kind of molluscs	Size of eggs (μ)	Name of authors	Size of full grown larvae (mm)	Name of authors
<u>Anadara broughtonii</u>			0.23 - 0.28 0.28 - 0.30	Imai ('69) Kanno ('68)
<u>Anadara subcrenata</u>			0.28 x 0.20 - 0.30 x 0.22	Yoshida ('64)
<u>Artina pectinata</u>			0.558 x 0.54 - 0.63 x 0.593	Yoshida ('64)
<u>Patinopecten yessoensis</u>	90 - 100	Yamamoto ('43)	0.30 - 0.35	Imai ('69)
<u>Pecten albicans</u>			0.24 - 0.26	Imai ('69)
<u>Chlamys nobilis</u>			0.23 - 0.24	Hotta ('72)
<u>Fulvia mutica</u>	75 - 100	Inoue ('55)	0.19 -	Hotta ('72)
			0.23 -	Hotta ('72)
<u>Meretrix lusoria</u>	72 - 75	Tanaka ('69)	0.25 x 0.23 - 0.27 x 0.25	Yoshida ('64)
	60 - 80		0.18 x 0.16 - 0.21 x 0.19	Yoshida ('64)
<u>Meretrix lamarckii</u>	82 - 84	Tanaka ('69)		
<u>Mactra sulcataria</u>	55	Miyazaki ('36)		
<u>Spisula sachalinensis</u>	70 - 80	Imai ('53)		
	50	Kinoshita et al. ('34)		
<u>Schizothaerus keenae</u>	62 - 68	Mizogami et al. ('71)		
			0.17 - 0.20	Tanaka ('69)
			0.26 - 0.27	Kan-no ('65)
			0.25 - 0.27	Yokoyama et al. ('70)
			0.24 -	Hotta ('72)

perature fluctuations between 10 and 15°C. The ark shell spawns naturally in late July or early August when the temperature is around 21°C. Spawning and larval swarming can be delayed until mid-August when the sea temperature exceeds 25°C, an optimum temperature for tank rearing, by keeping the ark shell first in warm, then in chilled seawater.

Control and induction of spawning by temperature in scallops and ark shells are summarized in Figure 1. Temperature fluctuations required for inducing spawning in various molluscs can be obtained simply by running water through a heat exchanger.

FOOD ORGANISMS

The principal food organisms used to rear offshore-type bivalve larvae, are a mixture of a pure culture of *Monochrysis lutherii*, a colored flagellate, and *Chaetoceros calcitrans*, a diatom (Table 2). Food organisms are grown in a room kept at a constant temperature of 20 - 25°C. The culture solution is Miquel's seawater with vitamin B12 and P6-Methal added. The culture solution is sterilized by autoclaving. For large-scale culture, the solution is prepared by a high speed centrifuge

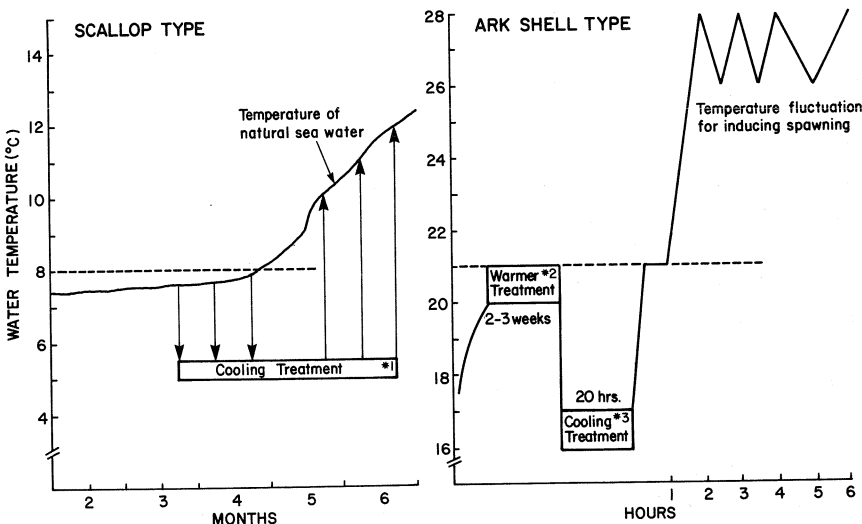


Figure 1. Control and induction of spawning by temperature fluctuation.

----- Temperature of natural spawning
 * Temperature for controlling spawning (*1;5°C, *2;20-21°C, *3;16.5°C)

(10,000 - 14,000 revolutions per minute) and by exposure to ultraviolet light in order to kill as many microbes as possible.

TANK REARING

The culture tank is filled with filtered seawater. Larvae are divided into tanks at the rate of 2,000 - 3,000 larvae per liter. The larvae are fed with appropriate food organisms (Table 2). The density of food organisms in each tank ranges from 3,000 to 40,000 organisms/ml according to the growth of larvae. Food efficiency for larvae and the relationships of each food value are summarized in Tables 3 and 4.

SEEDLING PRODUCTION

When the larvae reach setting stage, collectors are put in the tanks to catch the juveniles. Collectors for scallop and ark shell types are made of palm fibers fixed on wire frames; hard shell types settle on a sandy bottom.

Juvenile scallops and ark shells are kept in a tank with running water until they grow big enough to be transferred to nets in the sea. Hard shell types are kept in double bottom sandy tanks with running water until they grow big enough to be transferred to natural seawater. In the case of scallop culture, juveniles over 1 cm in shell length are put in wire framed nets and hung in the sea. Growers can grow them to a market size of 12 cm in shell length in one to two years. When ark shells on collectors reach 5 mm in shell length, they are put in net bags and hung in the sea for further culture. Ark shell and hard shell type bivalves grow to 3 cm in shell length in about 15 months after spawning. They are then ready to be released in the natural habitat as seed. If culture in net bags or double bottom sandy tanks is continued, most grow to a market size of over 10 cm in shell length two to three years after spawning.

CONCLUSION

The results of cultivation of larvae and juveniles show an overall survival rate of 10 - 30%. Though the percentage is rather low as compared to that of oyster larvae rearing, the survival rate of offshore-type bivalves could be increased considerably by future technical improvements. Though the study of offshore-type bivalves in Japan is still in the exploratory stage, we are hoping to make significant contributions to the fisheries by improving techniques for hatchery operations and also those for through-culture from larvae to market size.

Kind of food organism for rearing larvae

167

TABLE 3

Condition of rearing larvae

Kind of molluscs	Swimming period (day)	Larval density /ml	Density of food organisms* larvae/day	Food efficiency for larvae
<u>Anadara broughtonii</u>	23 - 39	0.1 - 3.0	6,000 ~ 10,000	MO > CH > PH
<u>Artina pectinata</u>		0.1 - 0.3	6,000 ~ 10,000	MO
<u>Patinopecten yessoensis</u>	40	1.2 - 3.0	3,000 ~ 20,000	MO, CH > PH, MO > MY > GR
<u>Pecten albicans</u>	60	1.0 - 4.0	5,000 ~ 40,000	MO, CH
<u>Chlamys nobilis</u>	11 - 25	1.0	5,000 ~ 40,000	MO, CH
<u>Fulvia mutica</u>	45	1.0 - 2.0	5,000 ~ 30,000	CH > MO
<u>Spisula sachalinensis</u>	22 - 30	3.0 - 10.0	3,000 ~ 30,000	CH > MO > PH = GR > MY
<u>Meretrix lamarckii</u>	9	5.0		CH > MO > GR > MY
<u>Schizothaerus keenae</u>	24 - 38	0.7 - 1.0	5,000 ~ 20,000	CH > MO

MO: Monochrysis lutheri, CH: Chaetoceros calcitrans, PH: Phaeodactylum tricornutum, MY: Marine Yeast, GR: Green (Chlorella sp.).

*Chaetoceros calcitrans, see Table 4 for conversion to other species.

TABLE 4

A conversion table of each food for larvae

Kind of food for larvae	Dry weight (mg)	Number of cell	Packed cell volume* (ml)
<u>Chaetoceros calcitrans</u>	0.000054	4500	0.000000678
<u>Monochrysis lutheri</u>	0.000054	2000	0.000000121
<u>Phaeodactylum tricornutum</u>	0.000054	4950	0.000000256
<u>Green (Chlorella sp.)</u>	0.000054	15423	0.000000200
Marine Yeast	0.000054	7100	

*This value is prepared by centrifuge of high speed (3,000 revolutions per min.).

LITERATURE CITED

Anadara broughtonii, Anadara subcrenata

- Kan-no, H. 1963. Bull. Tohoku Reg. Res. Lab. No. 23, pp. 108-116.
- Ito, S., H. Kanno, A. Chiba. 1964. Ann. Res. Aquaculture Center, Aomori Pref. No. 9, pp. 54-63.
- Hasegawa, I. 1968. Saibai Gyogyo 6 (1).
- Kanno, H. 1968. Fish Culture Vol. 5, pp. 88-100.
- Ito, S., K. Takeda, S. Akaboshi. 1968. Ann. Rep. Aquaculture Center, Aomori Pref. No. 10, pp. 75-79.
- Itami, K., K. Yamauchi, A. Hamaguchi. 1969. Res. Rep. Hyogo Pref. Fish. Exp. St. pp. 1-24.
- Imai, T., N. Nishikawa. 1969. The Aquaculture 16 (6), pp. 309-316.
- Itami, K. 1971. Bull. Hyogo Pref. Fish. Exp. St., No. 10, pp. 27-30.
- Itami, K. 1970. Res. Rep. Hyogo Pref. Fish. Exp. St. pp. 1-17.
- Itami, K., K. Tange, K. Yamauchi, F. Takeda, A. Hamaguchi. 1970. The Aquaculture 18 (1), pp. 25-34.
- Sato, A., K. Yokoyama, S. Naoe. 1971. Res. Rep. Aquaculture Center, Aomori Pref. S. 46, No. 4.
- Hotta, M., A. Mizogami. 1972. Res. Rep. Hiroshima Pref. Fish Exp. St. pp. 12-14.
- Itami, K., T. Matsuda, K. Sakaguchi. 1972. Res. Rep. Hyogo Pref. Fish. Exp. St. pp. 1-16.
- Itami, K., T. Matsuda. 1973. Bull. Hyogo Pref. Fish. Exp. St. 13, pp. 51-55.
- Yoshida, M., T. Ichiki. 1973. Bull. Nagasaki Pref. Fish. Exp. St. pp. 33-38.
- Itami, K., T. Matsuda. 1974. Bull. Hyogo Pref. Fish. Exp. St. 14, pp. 37-43.
- Nakamura, M., K. Tateishi. 1974. Technical Rep., SAIBAI GYOGYO, 3 (2), pp. 7-9.

Tani, Y., K. Igita, T. Nishida. 1974. Bull. Saga Pref. Fish. Exp. St. pp. 2-28.

Tani, Y., T. Nishida. 1964. Ibid, pp. 19-22.

Artina pectinata

Akiyama, H. 1963. Bull. Yamaguchi Pref. Fish. Exp. St. 13 (1), pp. 81-82.

Itami, K. 1970. Res. Rep. Hyogo Pref. Fish. Exp. St. pp. 1-17.

Patinopecten yessoensis

Sato, A., K. Yokoyama, K. Ogawa, H. Aoyama, S. Ito. 1967. Res. Rep. Aquaculture Center Aomori Pref. S. 42 No. 2, pp. 1-12.

Imai, T., N. Nishikawa. 1969. The Aquaculture, 16 (6) pp. 309-316.

Tanaka, S., A. Sato, K. Kawamura. 1975. Bull. Aquaculture Center, Aomori Pref. 4, pp. 47-50.

Pecten albicans

Hotta, M. 1972. Bull. Hiroshima Fish. Exp. St. pp. 7-9.

Chlamys nobilis

Kan-no, H. 1962. Bull. Tohoku Reg. Fish. Res. Lab., 20, pp. 114-120.

Naniwa, T., T. Minami, T. Nishiyama. 1971. Bull. Wakayama Pref. Aquaculture Exp. St. 4, pp. 48-65.

Hotta, M. 1972. Bull. Hiroshima Fish. Exp. St. pp. 10-12.

Naniwa, T., T. Nishiyama. 1972. Bull. Wakayama Pref. Aquaculture Exp. St. pp. 68-95.

Naniwa, T. 1973. Bull. Wakayama Pref. Aquaculture Exp. St. 6, pp. 112-125, 126-133.

Meretrix lamarckii

Nakagawa, M. 1968. Bull. Shizuoka Fish. Exp. St. 1, pp. 53-58.

Tanaka, Y. 1969. Bull. Tokai Reg. Fish Res. Lab., 58, pp. 163-168.

Spisula sachalinensis

- Imai, T., M. Hatanaka, R. Sato, S. Sakai. 1953. Sci. Rep. Res. Inst. Tohoku Univ., D-4, pp. 121-131.
- Kan-no, H. 1965. Bull. Tohoku Reg. Fish. Res. Lab., 25, pp. 131-141.
- Ito, S. 1970. Res. Rep. Aquaculture Center, Aomori Pref. S-44, No. 11, pp. 1-30.
- Ito, S. 1970. Cir. Rep. The Bivalve, No. 1, pp. 1-10.
- Yokoyama, K., K. Kawamura. 1971. Res. Rep. Aquaculture Center, Aomori Pref. S-45, No. 16, pp. 1-8.
- Ito, S., K. Yokoyama, K. Kawamura, K. Ogawa, Y. Hayakawa, T. Ueki, K. Kawamura. 1972. Res. Rep. Aquaculture Center, Aomori Pref. S-46, No. 25, pp. 1-18.

Schzothaerus keenae

- Akiyama, H. 1965. Res. Rep. Yamaguchi Pref. Fish. Exp. St. pp. 1-14.
- Akiyama, H. 1966. Aquaculture 14 (3), pp. 151-156.
- Mizogami, A., M. Hotta. 1971. Aquaculture 18 (5/6), pp. 237-246.

Food & others

- Hirano, R., Y. Oshima. 1963. Bull. Jap. Soc. Sci. Fish. 23 (3), pp. 282-297.
- Yoshida, Y. Hokuryukan, Tokyo.
- Imai, T. 1967. Venus 25 (3/4), pp. 159-167.
- Sato, S., H. Kan-no. 1967. Bull. Tohoku Reg. Fish Res. Lab. 27, pp. 101-108.
- Sato, C., S. Serikawa. 1968. Bull. Plankton Soc. Jap., 15 (1), pp. 13-16.
- Ito, S., H. Naoe. 1971. Res. Rep. Aquaculture Center, Aomori Pref. S. 44, No. 14, pp. 1-13.
- Iwasaki, H., T. Tanaka, T. Fujiyama. 1971. Bull. Jap. Soc. Sci. Fish., 37 (11), 1044-1048.

Culture Of Bivalve Mollusks In Recirculating Systems: Nutritional Requirements

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INTRODUCTION

Knowledge of nutritional requirements of bivalve mollusks lags considerably behind that of nutritional requirements of fish and decapod crustaceans. This is not surprising since until very recently all attempts at culturing bivalves have used natural estuarine waters where food was supplied by natural processes. There was, of course, considerable speculation concerning the food sources of bivalves in nature and, while there is evidence that the organisms satisfy some of their nutritional needs through uptake of organic compounds dissolved in seawater or through ingestion of detritus, most present students of the problem agree that phytoplankton serve as the main food source of filter-feeding bivalves (See Ukeles, 1971, for review). With the availability of techniques for culturing marine phytoplankton under controlled conditions, it became possible to test the value of various species of these algae as foods for bivalves. Because of the small amounts of food which the larval stages of bivalves require and because of commercial interest in bivalve hatcheries, most of this work has concerned larvae. During the past twenty-five years many papers have been published in this area (See Loosanoff and Davis, 1963; Walne, 1965 for reviews) and several species of algae have been identified as excellent foods and employed as standard diets in bivalve hatcheries.

Similar studies with juvenile and adult bivalves are much more difficult because of their prodigious appetites and the limited capacities of most algal culture systems, but the development of recirculating seawater systems has allowed much more efficient use of algae in feeding

studies and several reports of growth of post-larval bivalves on defined algal diets have appeared in recent years. More sophisticated studies along these lines have defined various efficiencies (assimilation, growth, etc.) of bivalves fed defined algal diets. The absence of any formulated diet that will support growth of bivalves has virtually precluded any studies that would give a detailed picture of nutritional needs of bivalves at the biochemical level — and it is at this level that knowledge of the needs of bivalves falls so far behind that of other mariculturally important groups of animals.

The purpose of the present paper is to review the various areas of study summarized above and to present a projection of future work in the field.

ALGAL DIETS

Early workers attempted to deduce the natural foods of bivalves from an analysis of stomach contents. Most of these studies (Dean, 1887; Moore, 1908; Kellogg, 1915; Grave, 1916) led to the conclusion that diatoms constituted the major food source of bivalves. Inferences from analysis of stomach contents, however, are prejudiced by different rates of digestion of different foods. The indigestible frustules of diatoms, for example, would certainly be more easily identified in stomach contents than the remains of unarmored algae and hence lead to an overestimation of the importance of diatoms. Martin (1927, 1928) circumvented this problem in a series of experiments where he fed defined diets, some of which were composed of cultured algae, to oysters. In one study lasting fifteen days he fed four species of diatoms, three species of green algae, and an unidentified flagellate to juvenile oysters kept in jars of standing, filtered seawater. His experimental design did not allow a comparison of the relative value of the different algae as foods but he did demonstrate beyond doubt that oysters are capable of growing on a diet consisting solely of cultured algae (and their associated bacteria).

During the next thirty years there was virtually no study of the value of various algal species to juvenile or adult bivalves. Extensive progress was made, although, in the culture of larval bivalves. The identification of various naked flagellates as excellent foods for larvae made feasible the idea of mollusk hatcheries that could produce large amounts of high quality seed oysters. Dean's Ph.D. dissertation (1957) was the next important study of the value of algal foods to juveniles and adults. He compared the food value of the diatom, *Skeletonema costatum*, which field observations had indicated to be a good food (Nelson,

1947), to that of a mixture of algae dominated by the Chryptophyte, *Cryptomonas* sp. (= *Chroomonas salina* Butcher) and the Chlorophyte, *Chlamydomonas* sp. Oysters were held in a recirculating seawater system which had no capability of detoxifying the animal's waste products; however, the water was changed every two weeks. Dean did not achieve very rapid growth of the oysters over periods as long as 45 days but he did find differences in the food value of the two diets. Animals fed the mixture deposited considerably more shell than animals fed *Skeletonema*. Dean concluded that the mixture was a more valuable diet than *Skeletonema* alone, but that the daily rations in the 45-day experiment were not large enough to support normal growth. He subsequently conducted a 16-day experiment in which he found that the growth rate of yearling oysters increased with larger rations. He concluded from this experiment and another in which he tested the effect of food density upon the production of pseudofeces that 2.4×10^8 cells/oyster/day represented a maximum ration for yearling oysters at 20°C.

Guillard (1957) was able to achieve more rapid growth of oyster spat fed a diet consisting solely of the same Chryptophyte found in Dean's mixture. He fed oyster spat a ration of 0.03 ml packed cell volume of *Chroomonas salina* per liter per day and was able to grow oyster spat from 0.7 mm mean shell height to 4.5 mm in 30 days. Guillard also reported (but presented no data) that certain small diatoms were also excellent food for juvenile oysters and clams. Guillard further pointed out that some forms such as the Chryptophyte, *Prymnesium parvum*, were distinctly toxic to juvenile oysters and clams and even species which are generally good foods may become toxic under some conditions of culture.

After another period of relatively little study of the food value of algae to post-larval bivalves, Walne (1970) published an excellent report of an extensive series of studies of the value of 25 species of algae to several bivalves (Table 1). The procedure used to assess the food value of an algal species was to grow the animals for a three-week period on several concentrations of the food. These tests were generally conducted in highly filtered seawater which was changed every few days, but in some experiments, food was added to crudely filtered seawater to see if the small amounts of natural food available in the seawater would enhance the food value of the particular species of cultured algae. Walne's results (Table 2) showed very clearly that bivalves fed similar quantities of different species of algae exhibited different growth rates and that these growth rates were further influenced by the concentration of algae in the water. He found, with some variation, that algae which

TABLE 1

Species of algae fed to four genera of bivalves.
(Modified from Walne, 1970.)

Species

HAPTHOPHYCEAE

Dicrateria inornata ParkeIsochrysis galbana ParkeCricosphaera carterae (Braarud and Fagerland) Braarud

CHRYSTOPHYCEAE

Monochrysis lutheri DroopPavlova gyraus Butcher

PRASINOPHYCEAE

Micromonas pusilla (Butch.) Manton et Parke == Chromulina pusilla (Butch.)Micromonas squamata Manton et ParkeTetraselmis suecica (Kylin) Butch.T. tetrahele (G. S. West) Butch.T. inconspicua Butch.T. chui E. G. Prings, ex Butch.Pyramimonas grossii Parke

CHLOROPHYCEAE

Dunaliella tertiolecta Butch.D. euehloria LercheChlamydomonas coccoides Butch.Chlorella stigmatophora Butch.Chlorella autotrophica, Shihira and KraussNannochloris atomus Butch.Brachiomonas sub-marina Bohl

XANTHOPHYCEAE

Olisthodiscus sp.

CRYPTOPHYCEAE

Cryptomonas sp.

BACILLARIOPHYCEAE

Phaeodactylum tricornutum BohlinThalassiosira fluviatilis HustedtSkeletonema costatum (Greville) CleveChaetoceros calcitrans (Paulsen) Takano

TABLE 2

The growth of bivalve spat when fed on various foods compared with the growth of controls on Isochrysis. The index has been calculated by dividing, for each experimental series, the largest mean size in the series at the measurement made on the nearest to 21 days from the commencement of the test, by the mean size of the control on that day. (Modified from Walne, 1970.)

A. <u>Ostrea edulis</u>	Index of food value	Average
Species	Individual experiments	
<u>Monochrysis lutherii</u>	1.70, 1.03	1.36
<u>Chaetoceros calcitrans</u>	1.28	1.28
<u>Tetraselmis suecica</u>	1.42, 1.06, 1.12	1.20
<u>Skeletonema costatum</u>	0.93, 1.09	1.01
<u>Isochrysis galbana</u>	--	1.00
<u>Dicrateria inornata</u>	0.85, 1.04	0.94
<u>Cryptomonas</u> sp.	0.54, 0.74	0.64
<u>Cricosphaera carterae</u>	0.41, 0.83, 0.61	0.62
<u>Chlorella stigmatophora</u>	0.65, 0.56	0.60
<u>Phaeodactylum tricornutum</u>	0.62, 0.43, 0.73	0.59
<u>Olisthodiscus</u> sp.	0.47, 0.71	0.56
<u>Nannochloris atomus</u>	0.60, 0.60, 0.41	0.54
<u>Chlorella autotrophica</u>	0.37, 0.66	0.52
<u>Pavlova gyrams</u>	0.69, 0.32	0.50
<u>Micromonas pusilla</u>	0.50, 0.40, 0.42	0.44
<u>Dunaliella euchlora</u>	0.40	0.40
<u>Dunaliella tertiolecta</u>	0.36, 0.42	0.39
<u>Chlamydomonas coccoides</u>	0.26, 0.33	0.30
B. <u>Mercenaria mercenaria</u>		
Species		
<u>Skeletonema costatum</u>	3.30	3.30
<u>Pyramimonas grossii</u>	1.19	1.19
<u>Tetraselmis suecica</u>	1.17, 1.07, 1.09	1.11
<u>Isochrysis galbana</u>	--	1.00
<u>Nannochloris atomus</u>	0.95, 0.23, 1.57	0.92
<u>Olisthodiscus</u> sp.	0.72, 0.55, 0.97	0.75
<u>Micromonas pusilla</u>	0.73, 0.76	0.74
<u>Cricosphaera carterae</u>	1.10, 0.50, 0.50	0.70
<u>Dicrateria inornata</u>	0.28, 1.04, 0.70	0.67
<u>Monochrysis lutherii</u>	0.62, 0.63, 0.53	0.59
<u>Phaeodactylum tricornutum</u>	0.44	0.44
<u>Chlorella stigmatophora</u>	0.45, 0.17	0.31
<u>Chlamydomonas coccoides</u>	0.19	0.19
<u>Dunaliella tertiolecta</u>	0.14	0.14

were good or bad foods for one species of bivalve were of similar value to the other species of bivalves. Analyses of amino acid composition of representative good foods and bad foods showed no substantial differences in quantity or quality. Walne concluded that the cause of differences in food value of most algal species probably lies in an area other than that of their protein chemistry.

In a subsequent study, however, Walne (1973) concluded that the difference in the value of *Isochrysis galbana*, *Tetraselmis chuii*, and *Phaeodactylum tricornutum* as foods for the clam *Saxidomus giganteus* lay in the protein fraction of the algae since large amounts of glycogen were accumulated by the clams even on the poorest diet (*Phaeodactylum*). He speculated that this difference lay not in amino acid quality or quantity but in the availability of various amino acids in the protein molecules of the different algae, i.e. there was a large difference in the clams' ability to assimilate N from the different algal proteins.

Subsequent studies of the food value of different algae used rather unique culturing systems. Baab et al. (1973) reported growth rates of several species of shellfish in an artificial upwelling system where seawater, relatively rich in nutrients, was pumped from 870 m depth into 45,000 liter pools in which three species of diatoms (*Bellerophon sp.*, *Chaetoceros simplex*, *Thalassiosira pseudonana*) were grown as foods. While these workers were able to achieve rapid growth of hybrid clams (*Mercenaria mercenaria* x *M. campechiensis*) and European oysters, *Ostrea edulis*, in the system, they were not able to differentiate the relative food values of the three diatoms.

The studies of Hartman et al. (1973) and Epifanio and Mootz (1976) were conducted in recirculating seawater systems where a relatively large number of parameters of water quality were monitored and controlled. These and subsequent studies in that system were reviewed in a recent paper by Epifanio et al. (1976).

In one experiment, hard clams, *M. mercenaria*, and oysters, *Crassostrea virginica*, were fed eight different diets which consisted of four species of algae, either singly or in some combination. The diet consisting of the diatom, *P. tricornutum*, alone, proved to be very poor. Neither clams nor oysters grew appreciably when fed this diet and all had died within twenty-five weeks from setting.

All other diets sustained growth over a 46-week experimental period with a diet consisting of equal cell numbers of *P. tricornutum*, *Carteria chuii* (Chlorophyceae), *I. galbana* (Haptophyceae), and *C. salina* (Cryptophyceae) producing fastest growth among both clams and oysters. These fastest growing groups of clams and oysters were main-

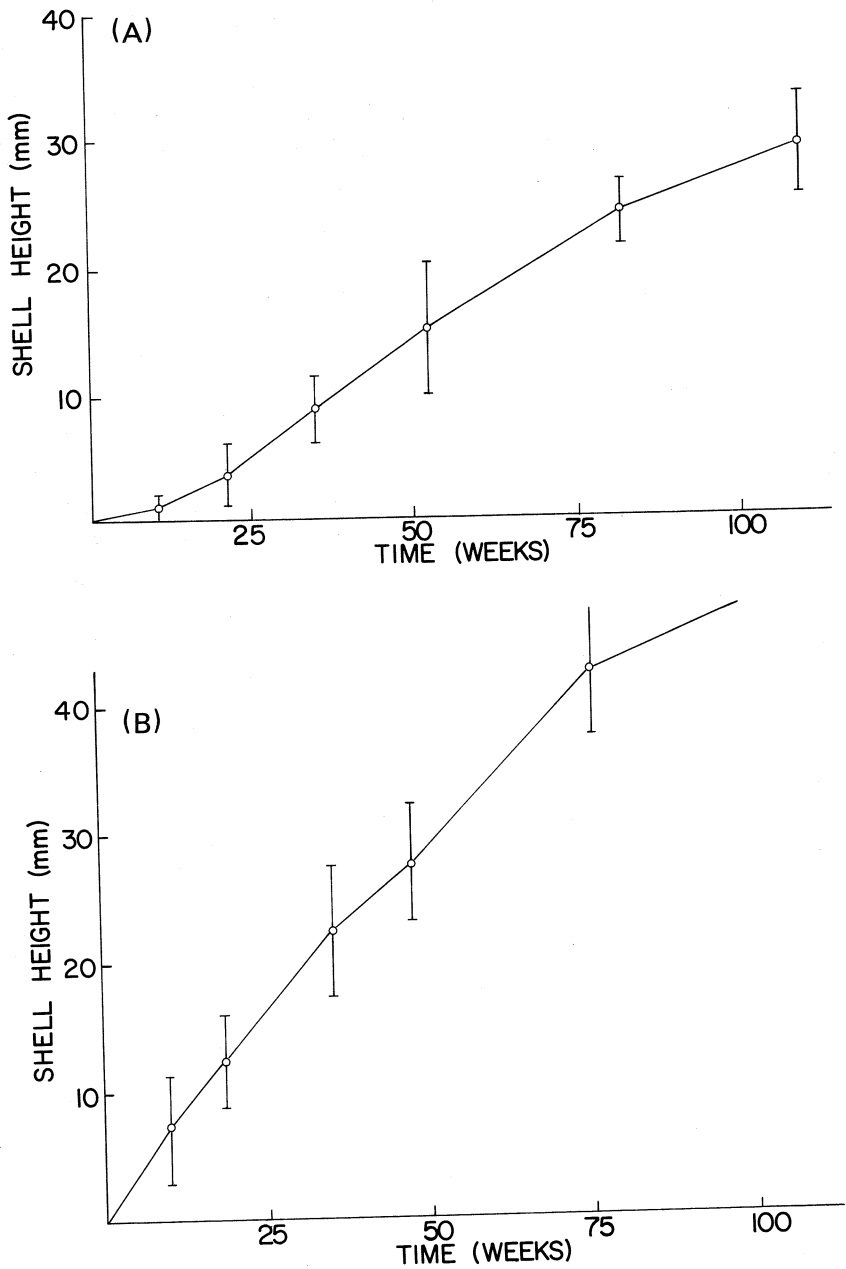


Figure 1. Growth of *Crassostrea virginica* (A) and *Mercenaria mercenaria* (B) fed defined algal diets in recirculating seawater system. (From Epifanio et al., 1976)

tained under the same conditions until they reached an age of 110 weeks post-setting (Figure 1). The yearly growth rate of the oysters was nearly that of wild oysters in the Mid-Atlantic region of the United States and that of the cultured clams was somewhat greater than wild clams. Since growth in this recirculating system continued on a year-round basis, however, the daily growth of wild clams and oysters during their relatively short growing season was somewhat greater than daily growth of the cultured shellfish.

In a subsequent experiment, another diatom *Thalassiosira pseudonana*, was substituted for *P. tricornutum* and combined with *C. chuii*, *I. galbana* and *C. salina* and fed to juvenile hard clams. The rate of growth of clams fed this diet was more than twice that of those fed the initial diet (Figure 2). In still another experiment *Thalassiosira* alone was shown to support growth of oysters comparable to the four-part diet of *T. pseudonana*, *C. chuii*, *I. galbana* and *C. salina*. In a final experiment *Thalassiosira*, the best four-part diet, and *Phaeodactylum* were compared as foods for *C. gigas*, *Tapes semidecussata*, *O. edulis*, and

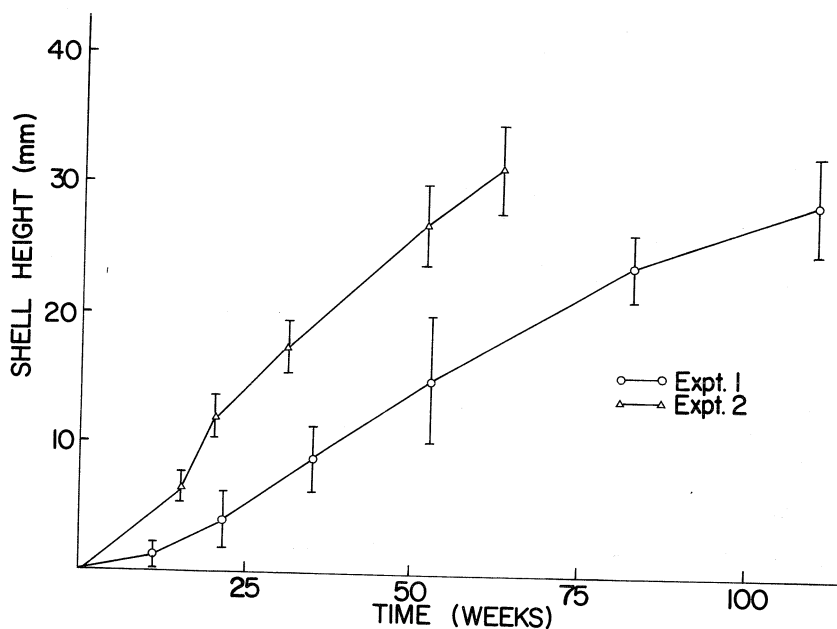


Figure 2. Comparison of growth of *Mercenaria mercenaria* fed different algal diets in a recirculating seawater system. See text for explanation. (From Epifanio et al., 1976).

Mytilus edulis (Figure 3). The growth responses of these species to the three diets were remarkably similar to the responses of *C. virginica* and *M. mercenaria*. Both *Thalassiosira* and the four-part diet supported good growth of all species of bivalves and only *Mytilus* showed any growth when fed *Phaeodactylum*.

It appears then, that bivalves are quite capable of normal and even accelerated growth when fed diets consisting solely of cultured algae. Diets consisting of mixtures of several species of algae generally yield

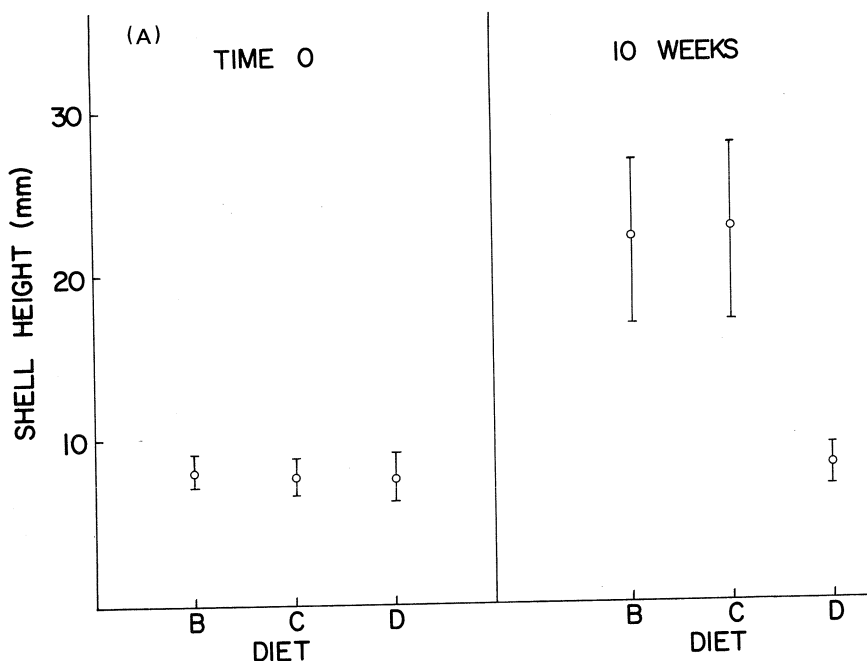
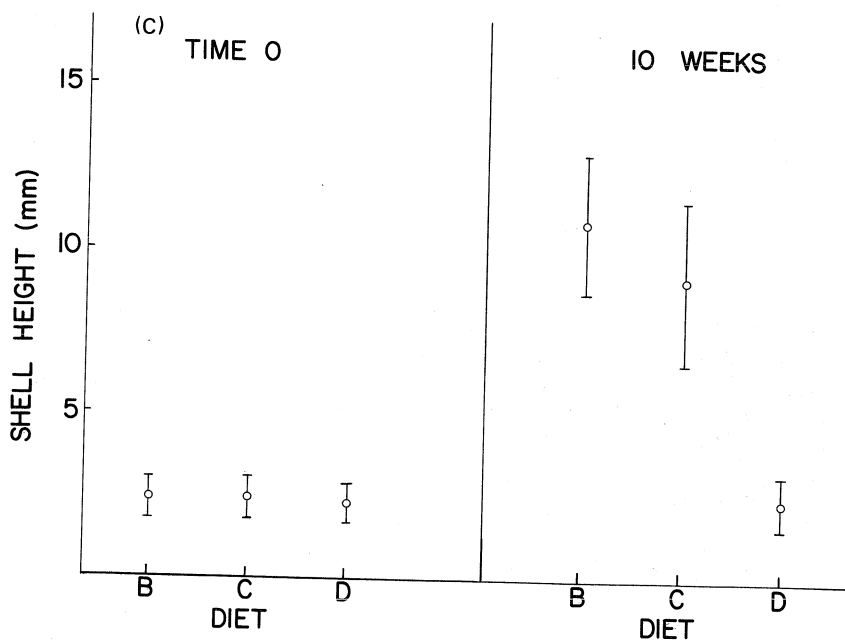
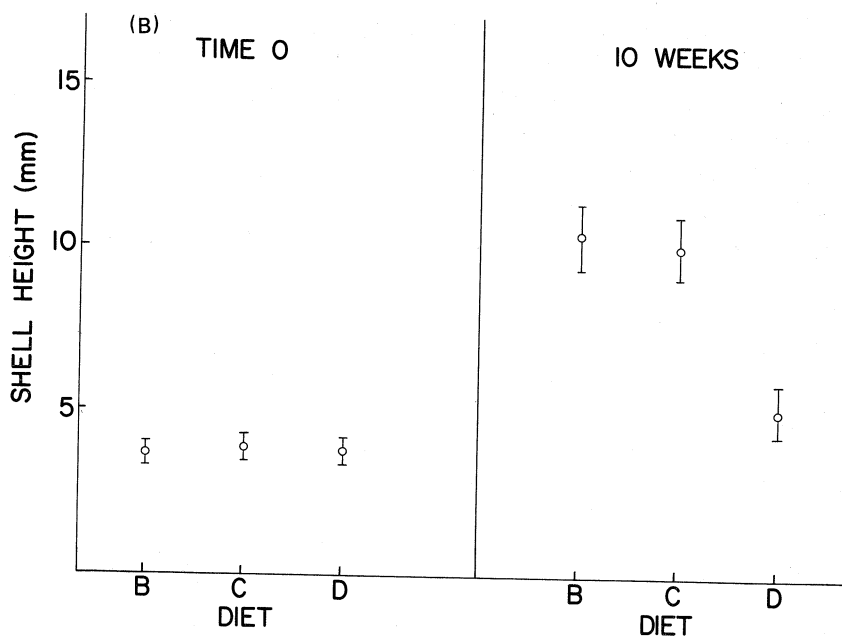
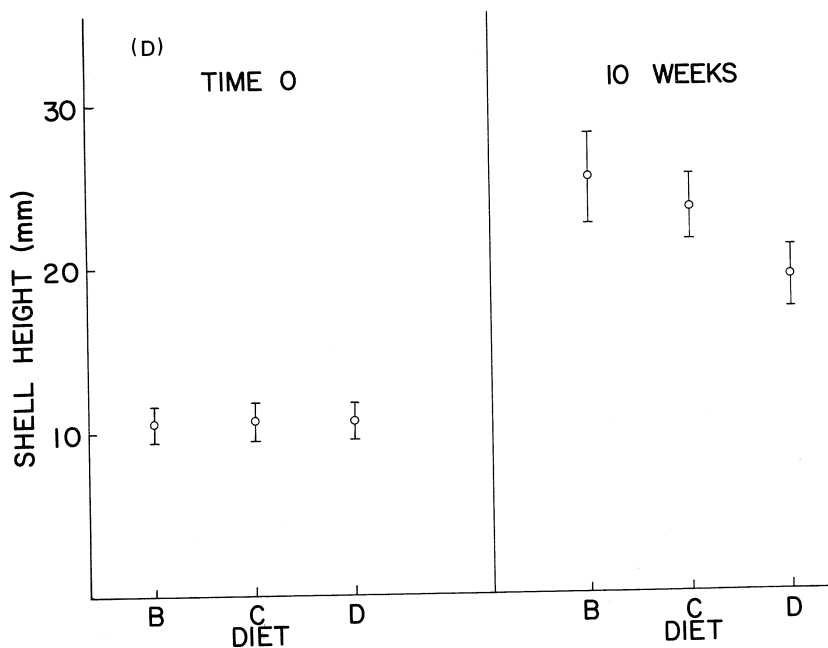


Figure 3. Comparison of growth of four species of bivalves fed Diets B (*Isochrysis galbana* + *Chroomonas salina* + *Carteria chuii* + *Thalassiosira pseudonana*), C (*Thalassiosira pseudonana*), and D (*Phaeodactylum tricornutum*) for 10 weeks. Bars around points signify $\pm$ one standard deviation. TIME 0 refers to initiation of the experiment.

- a. *Crassostrea gigas*
- b. *Tapes semidecussata*
- c. *Ostrea edulis*
- d. *Mytilus edulis*

(From Epifanio et al., 1976)





excellent growth but certain species, e.g. *T. pseudonana*, appear to be nutritionally adequate by themselves. The nutritional importance of bacteria normally associated with algal cultures is poorly known but it might be inferred to be minimal since Epifanio et al. (1976) showed the bacterial flora not to vary much in quantity or quality among cultures of species of algae which were found to have grossly different food values. The chemical basis for the different food values of different algae remains a mystery. Present available data (Parsons et al., 1961; Walne, 1970) indicate little gross difference in the composition of good and bad foods.

ENERGETIC EFFICIENCIES

One of the ways of assessing the value of a diet is to determine the efficiency with which it is used by an organism. This can be done by quantifying the amounts of various parameters of the diet, (e.g. its energy content) that are used by the organism in various processes (Figure 4). By comparing the amount of energy used in one process to that in another, one can draw strong inferences concerning the relative value of a diet. For example, if the assimilation efficiency

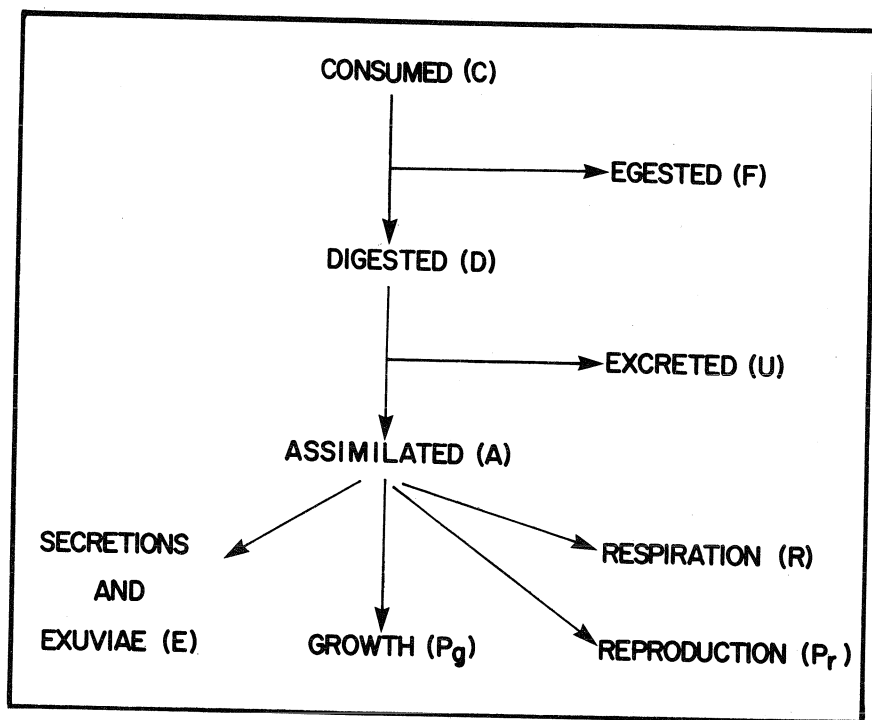


Figure 4. Possible flows of food-energy. (Modified from Petrusewicz and Macfadyen, 1970).

$$\left(= \frac{\text{CONSUMPTION-EGESTION}}{\text{CONSUMPTION}} \right)$$

is low, it can be predicted that growth will be poor since the food is largely indigestible. Even if this efficiency is high, however, the growth efficiency

$$\left(= \frac{\text{GROWTH}}{\text{CONSUMPTION}} \right)$$

may be low. This would indicate that large amounts of energy are being used in excretion, respiration, reproduction or secretion. This could be the result of improper culture conditions or the lack of subtle "growth promoters," e.g. vitamins, in the diet. The availability of various energetic efficiencies constitutes a useful tool for the nutritionist and there does exist considerable information concerning various aspects of energy flow in bivalves.

Consumption

Since the rate of consumption of food by bivalves is dependent on the rate at which particles of food are filtered from the surrounding water, data concerning filtering are very important in the construction of energy budgets. The volume of water from which all particles are removed in a given time period is termed the filtering rate (Fox et al., 1937). Filtering rate is equivalent to pumping rate if all suspended particles are removed from water passing through the filtering mechanism. Since retention of particles by bivalves is rarely 100% efficient (Jorgensen, 1955), pumping rate is usually greater than filtering rate. The rate of consumption is dependent upon, but not necessarily linearly related to, filtering rate since some of the particles removed from suspension may not be eaten but may be rejected as pseudofeces. Rates of filtration are affected by a host of environmental factors (Epifanio et al., 1975) and are related to the size of the bivalve by the equation:

$$Y = aW^b$$

where Y is rate of filtration, a is the filtration rate of a bivalve of unit weight, W is the weight of the bivalve and b is a constant, usually near 0.75 (Winter, 1973). Techniques for measuring rates of filtration are well developed and usually involve radio-nuclide tagged algae or the use of automated particle counting devices.

Egestion

Since the term, **egestion**, refers only to feces, and since bivalves may also produce pseudofeces (particles which are filtered from suspension but not consumed by the bivalve), the total biodeposition rate (feces + pseudofeces) of the organism is of more importance in terms of energetic efficiencies than egestion. The rate of biodeposition is affected by various environmental factors including the concentration of food in suspension (Tenore and Dunstan, 1973) and is relatively easy to measure.

Respiration

There have been a large number of studies of oxygen consumption in commercially important bivalves (See Epifanio et al., 1975, for review), but varying methodologies and results make comparison difficult. Techniques for determination of respiration rates in undisturbed bivalves are rather difficult but good data do exist for *C. virginica* and *M. mercenaria* (Dame, 1975; Loveland and Chu, 1969).

Reproduction

There has been extensive study of reproduction and gonadal development in bivalves (Galtsoff, 1964) with the major point emerging that bivalves normally shunt very large amounts of energy into the production of gonads. Previous workers have identified this as a potential problem in the mariculture of bivalves in controlled environments where the culturist must supply the food (Goodrich et al., 1968). No one, however, has included the quantity of energy going into reproduction in energy budgets for bivalves.

Excretion

There is relatively little information concerning excretion in bivalves. Hammen et al. (1966) studied rates of nitrogenous excretion by *C. virginica* and more recently Srna and Baggaley (1976) reported rates of excretion of both *C. virginica* and *M. mercenaria*. Both studies indicated that ammonia is the major excretory product of bivalves and that energy loss through excretion is relatively small.

Energy Budgets

Various energetic efficiencies have recently been determined for commercially important bivalves but none take all possible energy flows into account. As might be expected from the above review of the various components of an energy budget, assimilation and growth have received the most attention. Only Dame (1972) and Widdows and Bayne (1971) have quantified the amount of energy channeled into respiration. Dame studied the effect of temperature upon the respiration rate of intertidal *C. virginica* while Widdows and Bayne (1971) determined the effect of both temperature and food concentration on respiration of *M. edulis*. Dame found Q_{10} for respiration to be higher at low temperatures than at intermediate or high temperatures. He found that maximum growth occurred at temperatures somewhat less than maximum environmental temperature and concluded that assimilation efficiencies were also maximal at this temperature. Dame gave no data concerning seasonal variations in the amount of food available to the oysters, however, so it is difficult to judge the validity of his conclusions concerning temperature.

Widdows and Bayne also found increasing rates of respiration with increasing temperature but they found the effect of temperature upon their "index of energy balance" (= Assimilation-Respiration) dependent upon the amount of food available to the organism. Widdows and Bayne

(1971) also showed the assimilation efficiency of *M. edulis* to be higher when fed low concentrations of *Tetraselmis suecica* than when fed higher concentrations at every temperature tested.

Other studies have tested the effect of food concentration upon various energetic efficiencies in bivalves. Walne and Spencer (1974) conducted a very complicated series of factorial experiments where the effects of several variables, including size of ration, upon growth efficiency of *O. edulis* spat were determined. The results were somewhat variable but it can be concluded that spat fed smaller rations of *T. suecica* converted the food to dry tissue weight more efficiently than those fed higher rations. Growth efficiencies ranged from 10% – 20% over the range of rations tested (0.4 – 2.4 mg dry weight algae per spat over a three-week period) and the total increase in live weight (shell included) was significantly higher for spat fed the higher rations. This indicates that larger rations may favor shell growth over growth of soft tissues.

The results of Langefoss (1973) contradict those of Walne and Spencer as Langefoss found little effect of different rations of the diatom *P. tricornutum* upon assimilation efficiency of *C. virginica*. In a related study Tenore and Dunstan (1973) compared the assimilation efficiencies of *C. virginica*, *M. mercenaria*, and *M. edulis* fed different rations of algae in a flowing system. Flow of water through the system was 300 ml/min and concentrations of algae were approximately 1×10^5 , 2×10^5 , 4×10^5 , and 6×10^5 cells/ml. Algae were from a mixed culture dominated by the diatom *S. costatum*. They found that assimilation efficiencies varied as much as 10% with oysters with a maximum occurring at 4×10^5 cells/ml while assimilation efficiency varied very little in the other species. The actual amount of food assimilated continued to increase over the range of algal concentrations tested, but the authors determined that ingestion increased arithmetically with algal concentration while deposition increased logarithmically. They concluded that at food concentrations beyond those used in their experiment, assimilation efficiency would quickly decrease.

General conclusions from published energetic efficiency studies are that assimilation efficiencies of bivalves are quite high (70% – 90%) and that even algae which do not support good growth, such as *P. tricornutum*, may be assimilated at a high level (Langefoss, 1973). Growth efficiency may be related to the size of the ration available to the shellfish and may be as high as 20% for soft tissue growth in very young animals. High temperature may favor shell growth relative to growth of

soft tissue in some species (Walne and Spencer, 1974) and bivalves appear to assimilate food very efficiently at food concentrations considerably higher than found in nature (Tenore and Dunstan, 1973).

NON-ALGAL DIETS

Early literature concerning the value of non-algal foods such as bacteria, detritus, and dissolved organic compounds has been reviewed by Ukeles (1971). The results of these early studies were generally inconclusive and there is little quantitative information to support the tenet that detritus or dissolved organic compounds are major food sources for filter-feeding bivalve molluscs. A study by Zobell and Feltham (1938) seemed to show that mussels are capable of long-term growth and survival when fed a diet consisting solely of bacteria at 10^8 to 10^9 cells/ml but subsequent studies (Davis, 1953; Galtsoff and Arcisz, 1954; Walne, 1963) have failed to show the adequacy of bacteria alone as a diet for bivalves. Other studies (Carriker, 1956; Imai et al., 1950; Hidu and Tubiash, 1963) however, suggest that bacteria may be of some nutritional value to bivalves in combination with algae.

More recent investigations of non-algal foods have concentrated on the development of techniques for building up glycogen reserves ("fattening") of bivalves through the addition of supplemental feeds to natural diets and upon the problem of developing a non-algal diet which would support survival and growth of bivalves. Haven (1965) reported significant increase in weights of soft tissues but no increase in shell size of *C. virginica* held in flowing seawater to which 5 ppm cornstarch or wheatflour were added. In a similar study Dunathan et al. (1969) fed the meal of various grains to oysters in both flowing and static systems and again were able to increase weight and glycogen content of soft tissues but were not able to achieve significant shell growth. It is not at all clear, however, whether increase in weight of soft tissues through massive glycogen storage should properly be considered growth.

Another interesting study of the value of a non-algal, natural product as food for bivalves is that of Claus and Adler (1970) who reported rapid growth of oysters fed a diet consisting solely of tanned, human red blood corpuscles. Since blood stored in blood banks beyond a certain period of time is not suitable for use in transfusions, and since large amounts of livestock blood are produced in slaughter houses, it might seem that there would exist a cheap, readily available supply of food for bivalves. A subsequent study of the value of red blood corpuscles as food for *C. gigas* (Kern, 1973) failed to corroborate the re-

port of Claus and Alder, however, and I know of no further work in the area.

There is only one published study of the value of a formulated feed as a diet for bivalves. Castell and Trider (1974) fed four formulated diets to *C. virginica* held in filtered, flowing seawater, and each of the diets, which differed mainly in carbohydrate quantity and lipid quality, supported more rapid growth in a 12-week test than unfed controls. Growth of other controls held in trays in the natural environment was ten times that of any of the experimental groups, however. While differences among the experimental groups were not nearly as striking, the authors did conclude that higher levels of carbohydrates in the diet produced higher glycogen contents in the tissues of the oysters and that the substitution of cod for corn oil significantly increased the value of the diet. Regardless, all the formulated diets supported very poor growth.

CONCLUSIONS

Interest in the nutritional requirements of juvenile and adult bivalves has increased considerably in the past ten years but the level of knowledge is still low when compared to that for decapod crustaceans or fish. This stems largely from the fact that no nutritionally adequate, formulated diet for bivalves has yet been developed. As a consequence of this, research has largely been limited to determining which commonly cultured algal species are good foods and to elucidating various energetic efficiencies of bivalves fed algal diets. Further work with algal diets might profitably explore the possibility of manipulating the chemical composition of algae through changes in algal culture techniques. Kern (1973), for example, was able to control the protein content of *M. lutheri* by adjusting the flow of nutrients into a continuous culture (Taub and Dollar, 1965) and found little difference in the value of algae of higher protein content (50% dry weight) and lower protein content (30% dry weight) as foods for juvenile *C. gigas*. Flaak (1976) was able to achieve a two-fold difference in protein content per cell of *T. pseudonana* by harvesting the cells at different points on the growth curve, but also found little difference in the value of the algae of different protein concentrations as foods for oysters. Admittedly, the amount of control which one can exert over the chemical composition of algae is small compared to that possible with formulated diets, but in the absence of the latter, it seems reasonable to continue experimentation with modified algae.

The reasons for the failure of formulated diets to support normal growth of bivalves may be as much related to the physical as to the

biochemical properties of the feeds. Development of different techniques for producing particles of the appropriate size, texture, taste, etc. (Jones et al., 1974) should probably continue along with further study of the appropriate chemical composition of the particles. More detailed chemical analyses of algal species which have proven to be good foods are in order.

Of course, from the practical maricultural point of view, suitable algal diets already exist, with the major bottleneck to commercialization of intensive bivalve culture systems lying in the inability to economically culture massive quantities of these suitable algal species. Intensive study of problems associated with such mass culture of algae is certainly warranted and should be conducted coincidentally with studies aimed at determining the nutritional requirements of bivalves at the biochemical level.

LITERATURE CITED

- Baab, J. S., G. L. Hamm, K. C. Haines, A. Chu, and O. A. Roels. 1973. Shellfish mariculture in an artificial upwelling system. Proc. Nat. Shellf. Assoc., 63: 63-67.
- Carriker, M. R. 1956. Biology and propagation of young hard clams, *Mercenaria mercenaria*. J. Elish Mitchell Sci. Soc. 72: 57-60.
- Castell, J. D., and D. J. Trider. 1974. Preliminary feeding trials using artificial diets to study the nutritional requirements of oysters (*Crassostrea virginica*). J. Fish. Res. Board Can. 31: 95-99.
- Claus, G. and C. Adler. 1970. Optimal conditions for oysters grown in closed environments. Proc. Mar. Tech. Soc. 1: 671-679.
- Dame, R. F. 1972. The ecological energies of growth, respiration, and assimilation in the intertidal American oyster *Crassostrea virginica*. Mar. Biol. 17: 243-250.
- Davis, H. C. 1953. On food and feeding of larvae of American oyster, *C. virginica*. Biol. Bull. 104: 334-350.
- Dean, B., 1887. The food of the oyster: its conditions and variations. *Second Rep. Oyster Invest.*, State of New York, Albany.
- Dean, D. 1957. Experimental feeding of oysters. Ph.D. Dissertation, Rutgers University. New Brunswick, N.J.
- Dunathan, J. P., R. M. Ingle, and W. K. Havens, Jr. 1969. Effects of artificial foods upon oyster fattening with potential commercial applications. Technical Series No. 58. Marine Research Laboratory. Florida Dept. Nat. Res., St. Petersburg, Fla.
- Epifanio, C. E., R. Srna, and G. Pruder. 1975. Mariculture of shellfish in controlled environments: a prognosis. Aquaculture 5: 227-241.
- Epifanio, C. E., C. M. Logan, and C. Turk. 1976. Culture of six species of bivalves in a recirculating seawater system. Proc. 10th Eur. Symp. Mar. Biol. In Press.
- Epifanio, C. E. and C. A. Mootz. 1976. Growth of oysters in a recirculating maricultural system. Proc. Nat. Shellf. Assoc. In Press.

- Flaak, R. A. 1976. The effect of light of different wavelength on the growth and protein content of the marine diatom *Thalassiosira pseudonana* and its nutritional value to the American oyster, *Crassostrea virginica*. M. S. Thesis. University of Delaware. Newark, Del.
- Fox, D. L., H. N. Sverdrup, and J. P. Cunningham. 1937. The rate of water propulsion by the California mussel. Biol. Bull. 72: 417-438.
- Galtsoff, P. S. 1964. The American oyster. Fish. Bull. U. S., 64: 1-480.
- Galtsoff, P. S. and W. Arcisz. 1954. Observations on the rate of propulsion of water and retention of coliform bacteria by the oyster. Proc. Nat. Shellfish. Assn. 44, 1-8.
- Goodrich, D. R., R. B. Wainwright, L. J. Balbo, and A. Perlmutter. 1968. New Approaches for the Production of Connecticut Oysters. I. A Problem Analysis. American Cyanamid Co., Central Res. Div., Stanford, Conn., 140 pp.
- Grave, C. 1916. The process of feeding in the oyster. *Science* 44, 178-181.
- Guillard, R. R. 1957. Some factors in the use of nannoplankton cultures as food for larval and juvenile bivalves. Proc. Nat. Shellf. Assoc. 48: 134-142.
- Hammen, C. S., H. F. Miller, and W. H. Geer. 1966. Nitrogen excretion of *Crassostrea virginica*. Comp. Biochem. Physiol 12: 185-190.
- Hartman, M., C. E. Epifanio, G. Pruder, and R. Srna. 1973. Farming the artificial sea: growth of clams in a recirculating seawater system. Proc. 26th Annu. Session Gulf and Carib. Fish. Inst.: 59-74.
- Haven, D. S., 1965. Supplemental feeding of oysters with starch. *Chesapeake Sci.* 6, 43-51.
- Hidu, H. and H. S. Tubiash. 1963. A bacterial basis for the growth of antibiotic-treated bivalve larvae. Proc. Nat. Shellfish. Assn. 54, 25-39.
- Imai, T., M. Hatanaka, R. Sato, S. Sakai, and R. Yuki. 1950. Artificial breeding of oysters in tanks. Tohoku J. of Agric. Res. 1, 69-86.
- Jones, D. A., J. G. Munford, P. A. Gabbot. 1974. Microcapsules as artificial food particles for aquatic filter feeders. *Nature* 247: 233-35.

- Jørgensen, C. B. 1955. Quantitative aspects of filter feeding in invertebrates. *Biol. Rev.*, 30: 391-454.
- Kellogg, J. L., 1915. Ciliary mechanisms of lamellibranchs with descriptions of anatomy. *J. Morph.* 26, 625-701.
- Kern, R. B. 1973. A survey of some potential artificial foods for juvenile oysters. M. S. Thesis. University of Washington. Seattle, Washington.
- Langefoss, C. M. 1973. Energy budget of the American oyster *Crassostrea virginica* (Gmelin). M. S. Thesis. University of Delaware. Newark, Del.
- Loosanoff, V. L. and H. C. Davis. 1963. Rearing of bivalve mollusks. In *Advances in Marine Biology* 1, 1-136.
- Loveland, R. E. and S. K. Chu. 1969. Oxygen consumption and water movements in *Mercenaria mercenaria*. *Comp. Biochem. Physiol.*, 29: 173-184.
- Martin, G. W. 1927. Utilization of food by young oysters. *N. J. Agric. Experimental Sta. Bull.* 442.
- . 1928. Experimental feeding of oysters. *Ecology* 9, 49-55.
- Moore, H. F. 1908. Volumetric studies of the food and feeding of oysters. *Bull. U. S. Bur. Fish.* 28, 1297-1308.
- Nelson, T. C. 1947. Some contributions from the land in determining conditions of life in the sea. *Ecol. Monogr.* 17: 337-346.
- Parsons, T. R., K. Stephens, and J. D. H. Strickland. 1961. On the chemical compositions of eleven species of marine phytoplankters. *J. Fish. Res. Bd. Can.* 18: 1000-1016.
- Petrusewicz, K. and A. Macfadyen. 1970. *Productivity of terrestrial animals: principles and methods*. Blackwell Scientific Publications, Oxford and Edinburgh.
- Srna, R. and A. Baggaley. 1976. Excretion of ammonia by the hard clam *Mercenaria mercenaria* and oyster *Crassostrea virginica*. *Mar. Biol.* In press.
- Taub, F. B. and A. M. Dollar. 1968. The nutritional inadequacy of *Chlorella* and *Chlamydomonas* as food for *Daphnia pulex*. *Limnol. Oceanog.* 13: 607-617.

- Tenore, K. R., and Dunstan, W. M., 1973. Comparison of feeding and biodeposition of three bivalves at different food levels. *Mar. Biol.*, 21: 190-195.
- Ukeles, R. 1971. Nutritional requirements for shellfish culture. In: *Artificial Propagation of Commercially Valuable Shellfish*. (K. Price and D. Maurer, eds.) University of Delaware. 212 pp.
- Walne, P. R. 1963. Observations on the food value of seven species of algae to the larvae of *Ostrea edulis*. I. Feeding experiments. *J. Mar. Biol. Assn. U. K.* 43, 767-784.
- . 1965. Observations on the influence of food supply and temperature on the feeding and growth of the larvae of *Ostrea edulis*. *L. Fishery Invest., Lond. Ser. 2*, 24(1): 45 pp.
- . 1970. Studies on the food value of nineteen genera of algae to juvenile bivalves of the genera *Ostrea*, *Crassostrea*, *Mercenaria*, and *Mytilus*. *Fishery Invest., Lond. Ser. 2*, 25 (5): 62 pp.
- . 1973. Growth rates and nitrogen and carbohydrate contents of juvenile clams, *Saxidomus giganteus* fed three species of algae. *J. Fish. Res. Bd. Can.* 30: 1825-1830.
- Walne, P. R. and B. E. Spencer. 1974. Experiments on growth and food conversion efficiency of the spat of *Ostrea edulis* in a recirculating system. *Cons. Int. Explor. Mer.* 35: 303-318.
- Widdows, J. and B. L. Bayne. 1971. Temperature acclimation of *Mytilus edulis* with reference to its energy budget. *J. Mar. Biol. Ass. U. K.* 51: 827-43.
- Winter, J. E. 1973. The filtration rate of *Mytilus edulis* and its dependence on algal concentration, measured by a continuous automatic recording apparatus. *Mar. Biol.* 22: 317-328.
- ZoBell, C. E. and C. G. Feltham. 1938. Bacteria as food for certain marine invertebrates. *J. Mar. Res.* 312-327.

Recent Advances In Abalone Culture In Japan

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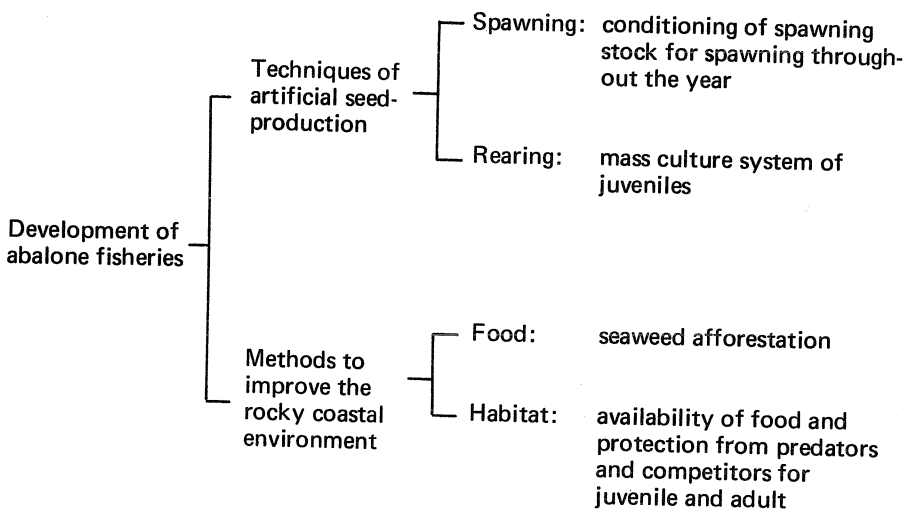
INTRODUCTION

It is well known that abalone is one of the major marine products in Japan. The natural population in rocky areas along Japan's coast has been subjected to management practices (e.g., fishing season limitations and individual and harvest size restrictions) that have resulted in stabilization of the annual yield at approximately 5,000 tons (with shell). In 1973 the harvest totaled 5,939 tons, and was valued at 9.8 billion yen, approximately 32 million U. S. dollars.

In occasional reports during the past several years, American scientists have summarized the results of pure and applied abalone research in Japan. Loosanoff (1969), Leighton (1972), Imai (1970), and Bardach et al., (1972) all described progress made in abalone research. Rapid advances in the past five years, however, require that this report be written for the benefit of those wishing to understand the techniques as they are practiced today.

GENERAL ASPECTS OF AQUACULTURE SYSTEM FOR ABALONE

In preparation for a nationwide project, comprehensive research aimed at developing natural fishing areas and technology for fish and shellfish culture in coastal areas was undertaken by our group from 1970 through 1974. A system of development for abalone fisheries was sketched out as follows.



We considered two principal approaches to the development of abalone aquaculture: mass production of juveniles in hatcheries; and the artificial production of food in the field by seaweed afforestation. Current harvesting techniques of the natural abalone population have led to such proficiency in the harvest that any further expansion of Japan's abalone industry will occur only through the successful application of innovative culture methods. We had already discovered that increased returns could be achieved by releasing juveniles with shell length of 2-3 cm and subsequent harvesting three to four years later.

RECENT TECHNICAL DEVELOPMENTS IN ABALONE SEED PRODUCTION

Baseline studies on the early development and growth of *Haliotis gigantea* by Murayama (1935) and of *H. discus* by Ino (1952) formed the research framework with which abalone hatchery work began in the 1960's. The techniques painstakingly developed by the hatcheries, however, resulted in unprofitably low yields. Ino (1960) had predicted four problem areas. The first was ignorance of the conditioning necessary for producing spawning stock — naturally matured adults were unsuitable for use as spawning stock. The second was lack of a fertilization technique that ensured an optimal number of viable zygotes. The third problem was the inability to supply proper food to the settling larvae and juveniles. The last was the lack of control of the behavior of the

settling larvae. There seemed to be no way of controlling the settlement on, and subsequent loss from, the substrate provided them.

Since 1972 each of these fundamental problems has been examined in detail at the Tohoku Laboratory and Oyster Research Institute, located on Mohne Bay. Solutions were discovered through both patient experimental deduction and unexpected breakthroughs. In these ways suitable methods evolved, such as the conditioning of adults in indoor tanks and inducement of spawning with U.V. irradiated water (Kikuchi and Uki, 1974a and b). Each improvement facilitated the development of the next; consequently progress was rapid. Annual abalone juvenile production at the Oyster Research Institute pilot hatcheries at the Sendai power station and at Mohne Bay now exceeds one million.

CONDITIONING OF THE ADULT

Optimal water temperature for conditioning mature adults for spawning was determined for the northern species, *H. discus hannai*, by Kikuchi and Uki (1974a). The experiment was carried out in our laboratory at six different temperatures ranging from 8-22°C during the period January to July 1972, which is outside the natural spawning season that occurs during September and October. Fifty adult abalone were housed in each experimental tank made of waterproof canvas, measuring 150 x 90 x 80 cm, and containing about one ton (1000 liter) of seawater. A photoperiod of 12 hours each of alternating light and dark was maintained (Figure 1). Light conditions of approximately 150 Lux at the bottom of the rearing tank simulated average daylight conditions at 7-8 m, the preferred depth of the adult in nature. Two bent shelters made of vinyl plate were placed at the bottom of each tank. Filtered seawater flowed continuously into the bottom of the tank, and water was siphoned from the bottom. Food such as the fresh seaweeds, *Undaria* and *Laminaria*, were supplied every day (about 20% of their weight). Under these rearing conditions the adults maintained in 17°C water temperature experienced the greatest increase of weight and shell length, as well as the greatest extent of maturation as determined by gonad color and gravid condition (Figure 2).

Initially during this experiment, the increase in gonad maturity of *H. discus hannai* was expressed by four categories (0 to 3rd grade) representing immature to fully gravid, respectively. However, by laboratory experimentation a more quantitative expression of the rate of gonad development related to temperature exposure was developed (Figure 3). According to the equation for the slope in the graph (Figure 3), the theoretically calculated water temperature minimum at which

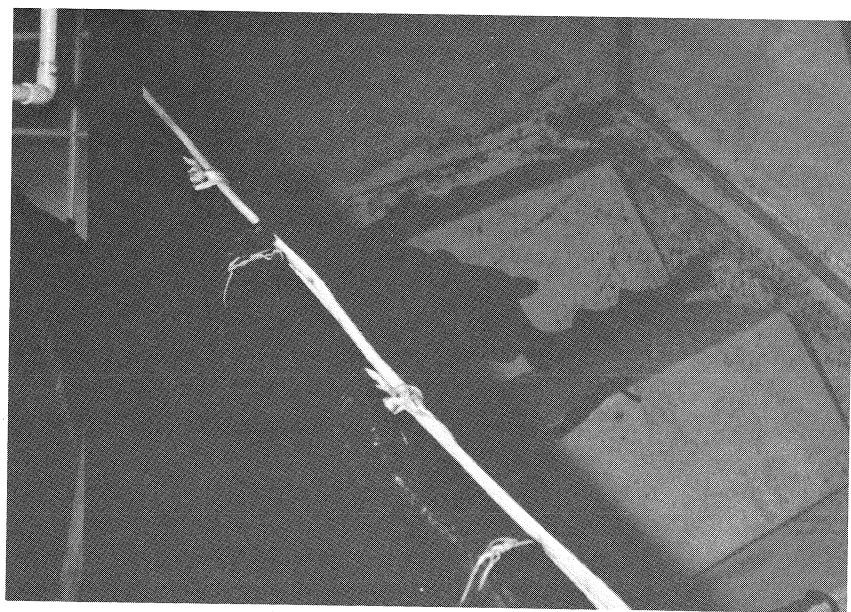
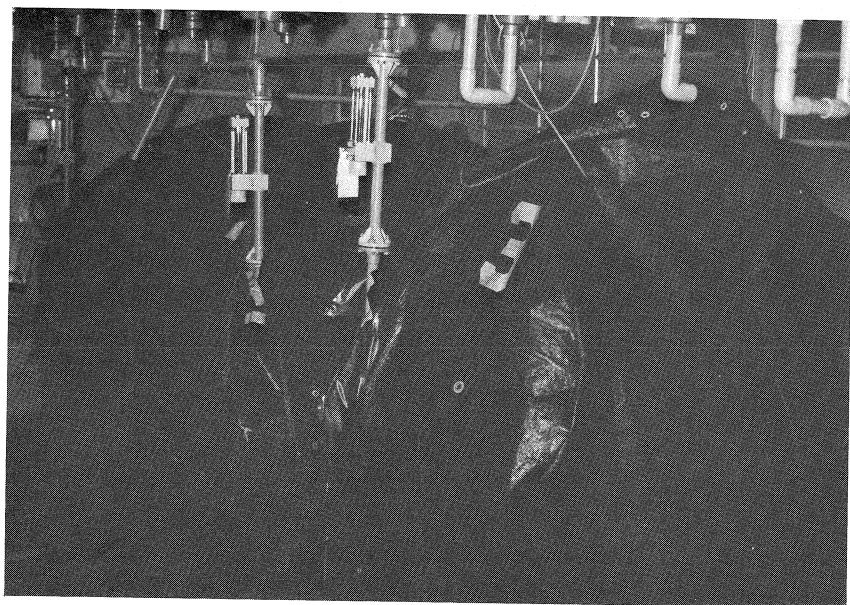


Figure 1. Conditioning of Haliotis discus hannai in rearing tank; outside view (above), inside view (below), Oyster Research Institute.

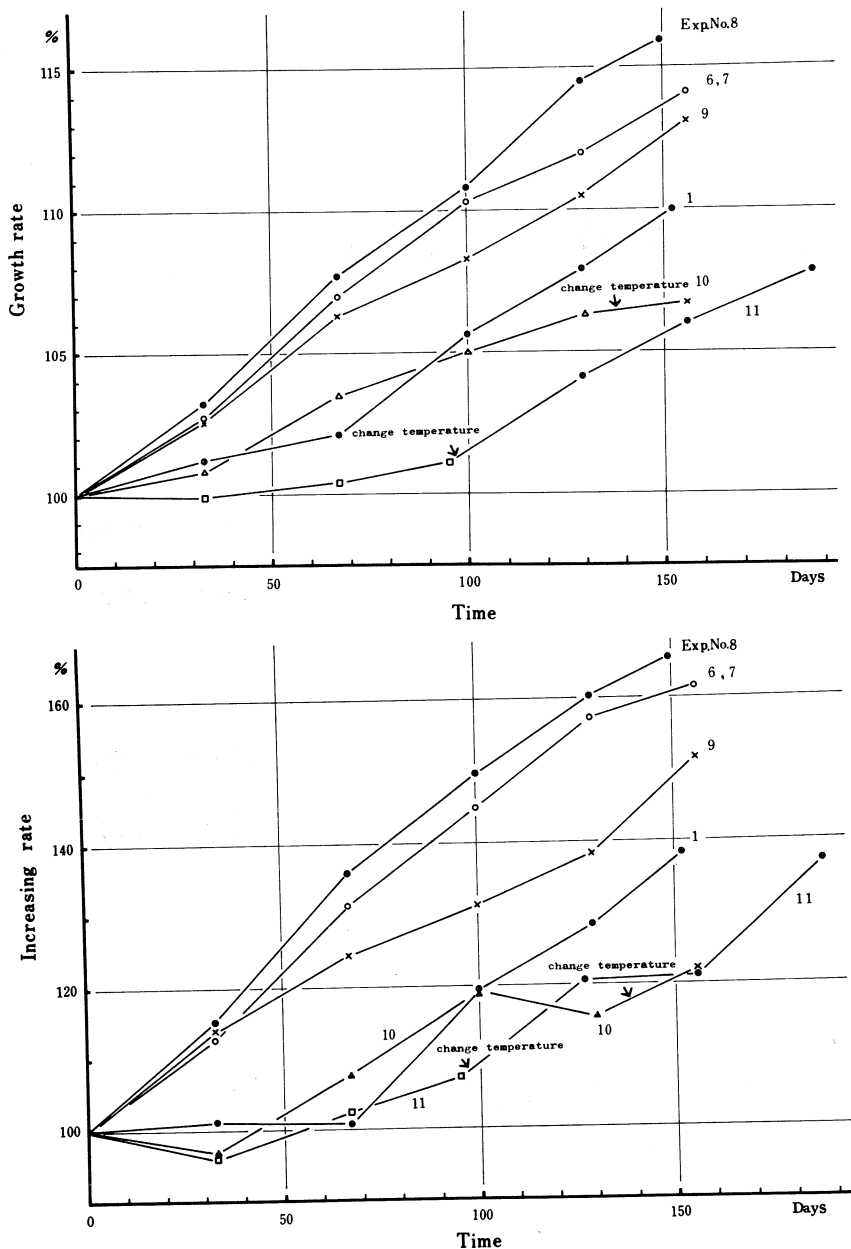


Figure 2. Growth in shell length (above) and in weight (below) of *Haliotis discus hannai* under six different water temperatures; Exp. 1 was at 22.4°C, 6 and 7 at 20.0°C, 8 at 17.1°C, 9 at 14.3°C, 10 at 11.5 and 14.7°C and 11 at 8.2 and 17.5°C, from Kikuchi and Uki. (Growth rate is expressed as percentage of size at start of experiment (57-92 mm shell length)).

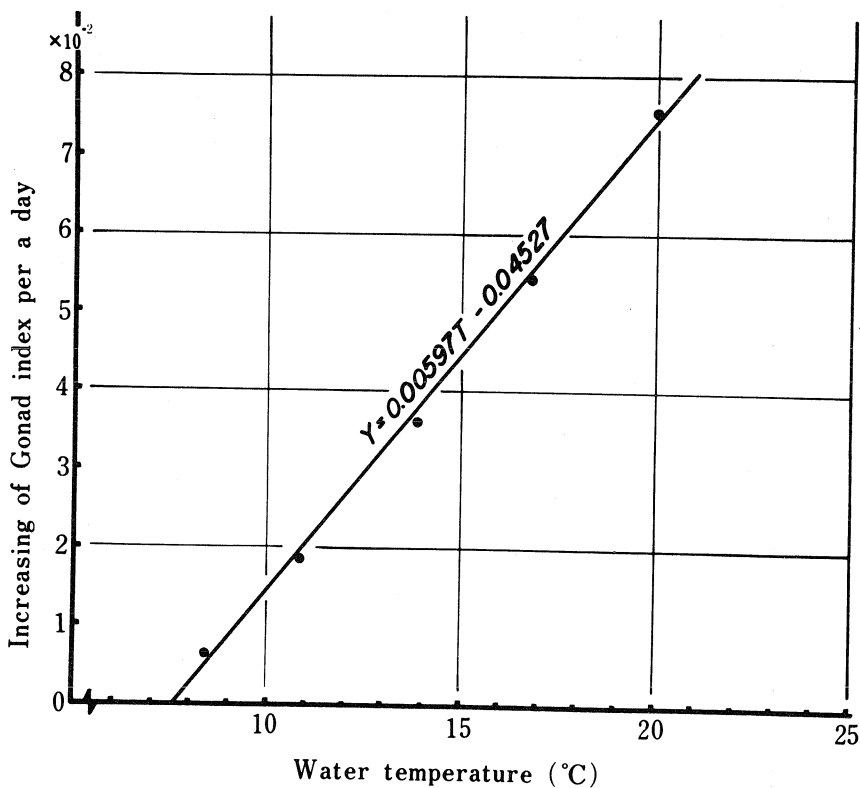


Figure 3. Relation between water temperature and maturity of *Haliotis discus hannai* from Kikuchi and Uki (1974).

maturation occurs is 7.6°C . The cumulative effects of the daily exposure to water temperature above 7.6°C defines the advance of sexual maturity more precisely than the "Gonad Index" which is determined by the naked eye. Above 1,500 Integral Water Temperature (where Integral Water Temperature = $\sum (\text{Daily Temp } (^{\circ}\text{C}) - 7.6^{\circ}\text{C}) \text{ No. Days Exposure}$), this species has reached sexual maturity and can be induced to spawn any time thereafter (Kikuchi and Uki, 1974a).

In commercial practice, the rearing of adults at 20°C , if begun in the winter season, will result in mature adults in approximately 80 days when they reach 1,500 Integral Water Temperature. Maturity can be maintained for at least three months and no spawning will occur in the rearing tank except in the event of unforeseen accidents, such as stoppage of the water circulation or temperature drift. The growth rate at

this time, if correlated to the feeding rate, can also serve as an indicator of the suitability of the rearing conditions for inducement of sexual maturation. Healthy abalone will feed at a rate of 10% body weight per day when fed ad libitum. A feeding rate of less than 5% is unsatisfactory and might indicate poor water quality or other problems. Aeration is also a prerequisite for suitable rearing conditions.

As shown in Figure 4, conditioning for sexual maturity in *H. discus*, a closely related, warm water species, is similarly related to cumulative water temperature, (Kikuchi and Uki, 1974c). In other species such as *H. gigantea*, however, some other variable, such as light conditioning or food supply, must stimulate sexual maturation since the Integral Water Temperature has no such effect (Kikuchi and Uki, in press).

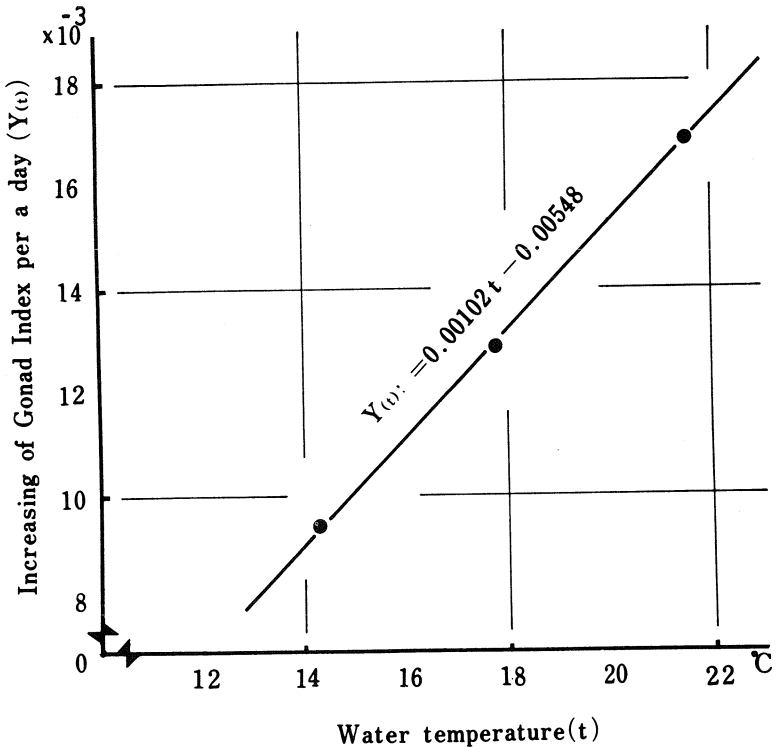


Figure 4. Relation between water temperature and maturity of *Haliotis discus* from Kikuchi and Uki (1974c).

ARTIFICIAL SPAWNING TECHNIQUES

In the past stimuli such as raising the water temperature and/or air drying the mature abalone provided only a limited and unreliable method of spawning induction. With the development of U.V. irradiated seawater treatment, however, a technique that could be applied consistently and effectively at the hatcheries had at last been found.

In accordance with the findings of Kikuchi and Uki (1974b), filtered seawater, when irradiated with U.V. light, 2,537 Å, is an effective inducer of spawning in *H. discus hannai*. The response, measured in terms of time elapsed from induction to spawning, occurs more rapidly upon increasing the irradiation of the given volume of water used. Eight hundred milliwatt hours per liter of U.V. irradiation was found to be sufficient. Spawning would occur in about 3 hours. No harm to the parents or offspring due to the use of irradiated seawater has been detected. Induction of spawning by this method has been successful with every species of *Haliotis* endemic to Japan, as well as with some scallops. Uncertainty still exists as to the underlying cause for this effect. It can only be postulated that some photochemical reaction results in the production of an inducer or elimination of a repressor of spawning in the water (Figure 5).

At the Oyster Research Institute abalone can be made to spawn at any time during the day. It has been substantiated that natural spawning of *H. discus hannai* occurs mainly at nightfall. Abalone cultured at 17°-20°C in rearing tanks are therefore maintained for several months at an artificial photoperiod with darkness beginning at 2:00 p.m. When spawning is desired, the abalone are removed at 11:00 a.m., measured, dried for a half hour, and then placed in separate 20-liter containers. U.V. irradiated seawater flows into these containers at a rate of 100-200 mls/min. Within 30 minutes water temperature is raised 3°C above that in the rearing tanks, maintained for one hour, and then brought down to the rearing tank temperature. This is done to ensure maximum spawning success, although it has been proved that the actual spawning stimulus is the U.V. irradiated seawater alone. Spawning occurs between 2:30 p.m. and 3:30 p.m., and success is greater than 90%. Regulation of the spawning time is very important in order to minimize later inconveniences during fertilization, washing of the zygotes, selection of the most suitable larvae, control of settlement, and so forth.

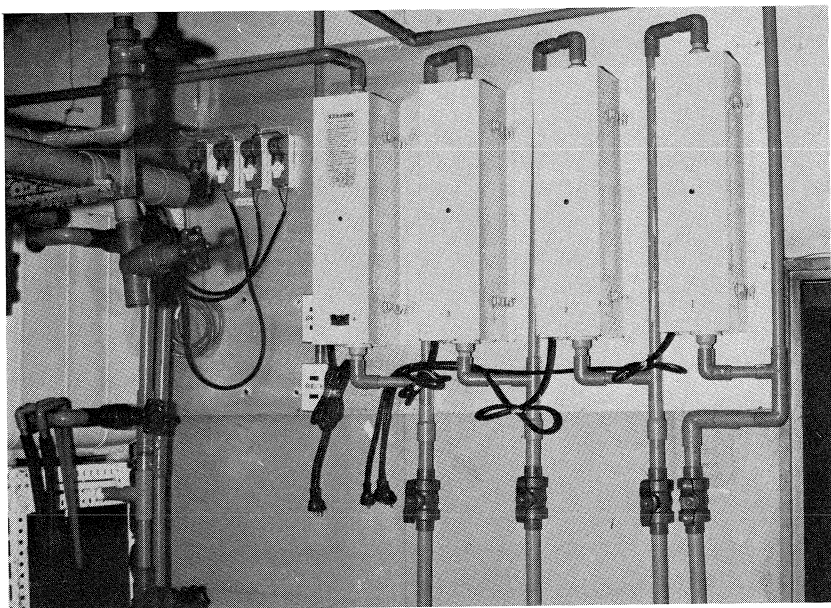
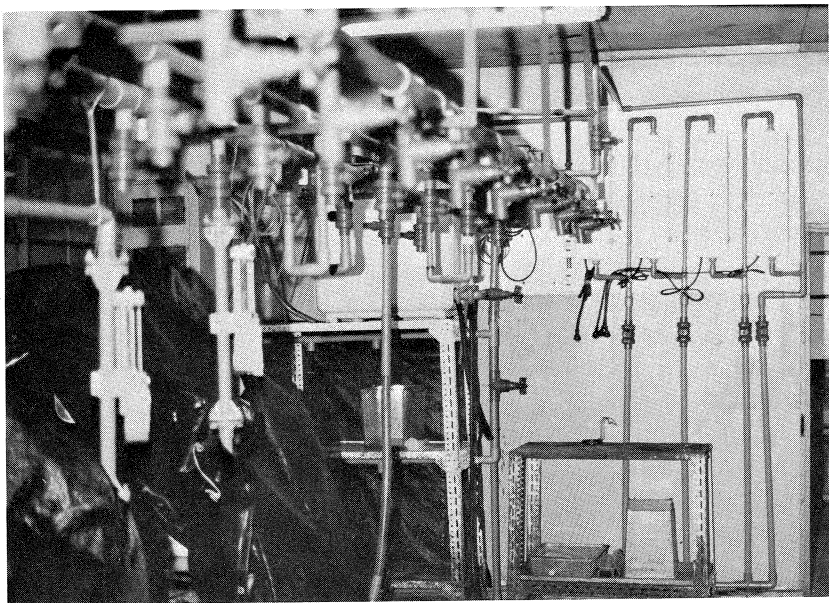


Figure 5. Operation room for spawning at Oyster Research Institute (above), and the U. V. irradiation equipment (below).

DIET DETERMINATION

The choice of proper food for the rearing of the metamorphosed larvae subsequent to settling, as well as for juveniles, is critical if one is to have a successful hatchery operation.

Certain marine algae were determined to be acceptable for inclusion in the diet of young *H. discus hannai* (22-28 mm in shell length), using as a standard the rate of growth achieved on a diet of *Eisenia byciclis*, a species found in abundance throughout the year along the coast of Japan (Kikuchi et al, 1967). Algae were fed ad libitum. Among the 20 algal species individually tested, there was considerable variation in the amount consumed by the juveniles. *Undaria* and *Eisenia* were the most suitable food for the increase of shell length and weight, followed by *Codium*, *Ulva*, *Grateloupia* and *Rhodymenia*, each of which gave good results. Diets of marine algae such as *Dictyopteris*, *Pachydictyon* and *Acrosorium* resulted in little growth. These results, expressed in *Eisenia* equivalents (Rate of growth on test species/Rate of growth on *Eisenia*), are shown in Table 1.

Benthic diatoms such as *Cocconeis* spp. and *Navicula* spp., which grow as a thin layer on the plates, were already recognized through hatchery experience to be an acceptable food for the metamorphosed larvae. Many other benthic diatom species are recognized in Japanese abalone hatcheries as suitable food for juveniles less than 20 mm in shell length.

As the preceding discussion illustrates, the four challenges described by Ino (1960) are being met with gratifying success. Increasing attention is being focused on the third problem, the prevention of mortality due to undernourishment during the juvenile stage on substrate such as vinyl plate. The solution seems to lie in the development of pure diatom cultures that can in turn be provided to the various age groups of abalone so that an optimal food species can be selected. Eventually a system could be designed that would continuously provide the young abalone with the preferred food.

AFFORESTATION IN THE FIELD

The northern Pacific coast of Japan, which possesses many rocky areas suitable for abalone, is also the location of many desert-like rocky areas that sustain no large algae growth. This condition has persisted for a long time, and has resulted in very limited growth of the standing abalone and sea urchin populations. In a nationwide project, afforestation techniques were developed and proved successful in the field. Our

TABLE 1

Eisenia equivalent of the marine algae from Kikuchi et al. 1967.

Algae	Eisenia equivalent
<u>Undaria pinnatifida</u>	1.02
<u>Eisenia bicyclis</u>	1.00
<u>Codium fragile</u> , <u>C. divaricatum</u>	0.72
<u>Grateloupia filician</u> , <u>G. turuturu</u>	0.61
<u>Ulva pertusa</u>	0.58
<u>Rhodymenia intricata</u>	0.58
<u>Pachymeniopsis</u> sp.	0.37
<u>Gelidium amansii</u>	0.35
<u>Sargassum ringgoldianum</u>	0.31
<u>Acrosorium flabellatum</u> , <u>A. yendoj</u> ,	0.24
<u>A. uncinatum</u>	
<u>Dictyopteris divaricata</u>	0.21
<u>Pachymeniopsis elliptica</u>	0.06
<u>Pachydictyon coriaceum</u>	0.05
<u>Plocamium telfairiae</u>	0.04
<u>Chrysomenia wrightii</u>	0.03
<u>Symphyocladia latiuscula</u>	0.01
<u>Pikea californica</u>	0.01
<u>Callophyllis crispata</u>	0.01
<u>Schizymenia dubyi</u>	0.00
Articulated corallines	0.00

initial hypothesis explaining the existence of such sea 'deserts' was that heavy grazing pressure by animals resulted in the eventual depletion of the standing stock of macroalgae. If this were the case, we reasoned, afforestation by means such as the long-line kelp culture method in conjunction with continuous population control of herbivores by fisheries activity could make the areas once more suitable for abalone culture.

The straightforward method was to remove the animals as their growth rate increased in response to the increase of macroalgae. In this way we could postulate a production rate of 40 tons of algae per ha per year as well as four tons of animals. The most serious competitor of abalone is the sea urchin, whose grazing pressure endangered many of the newly forested areas. Two hundred grams of sea urchin per square meter was sufficient to destroy the algal community. Fortunately the sea urchin's gonad which develops rapidly after the animal feeds only a

few months on the seaweed, is highly prized in Japan for its taste. Therefore an economic incentive exists for the fisherman to remove the sea urchins. The algal community recovers upon removal of the urchins, and abalone can increase their growth rate. Therefore, it has been proved that such areas of low production can be converted into areas supporting sizeable benthic fisheries, provided the composition and density of the animal community is controlled and afforestation is properly practiced.

CONCLUSION

With abalone aquaculture underway the scope of our investigation is increasing to include not only biological studies on the early life history of the abalone and related diatoms and algae, but also ecological studies to understand the complex dynamic relationships between these organisms in the field. Young and adult abalone eat macroalgae such as *Undaria*, *Eisenia*, and *Laminaria* for food. At the same time, however, they may be ensuring the reproductive success of the species as a whole. It has been observed at the Oyster Research Institute that metamorphosed larvae set on the mucus of algae were often destroyed. If this occurs in nature, afforested areas may produce large parent generations of abalone but few offspring. Such areas may therefore insure annual repopulation with juvenile abalone. Another hazard, at least under hatchery conditions, is depletion of oxygen at the surface of the culture plates and asphyxiation of the settled larvae due to the respiration of the benthic diatoms on the plate. In our experiments, this was corrected by continuous artificial illumination (Figure 6). Other interesting research presently underway concerns the possibility that abalone may secrete a mucus that induces their larvae to settle in areas suitable for them. The results of these investigations could greatly benefit the young abalone culture industry.

I would like to thank Dr. Kent Price for his help in preparing this presentation and Mr. Tim Ahern for his help in preparing the English manuscript.

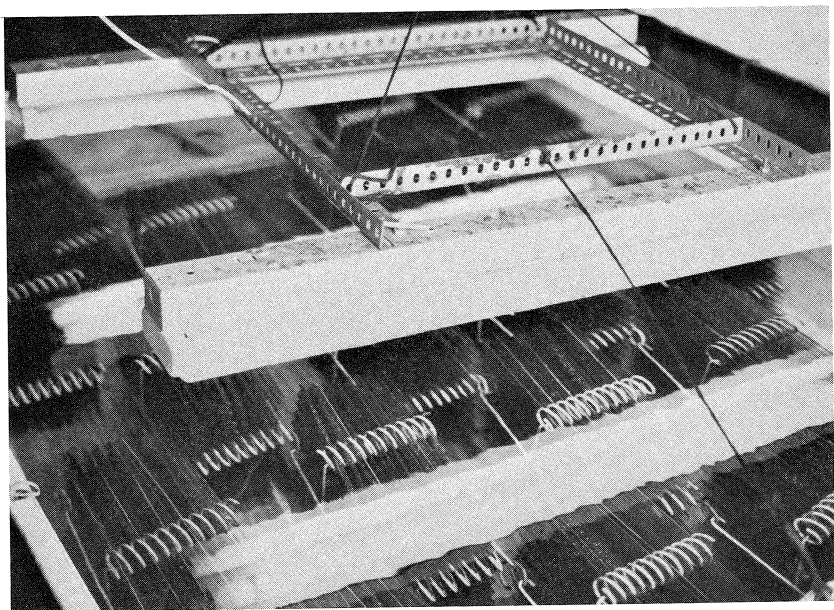


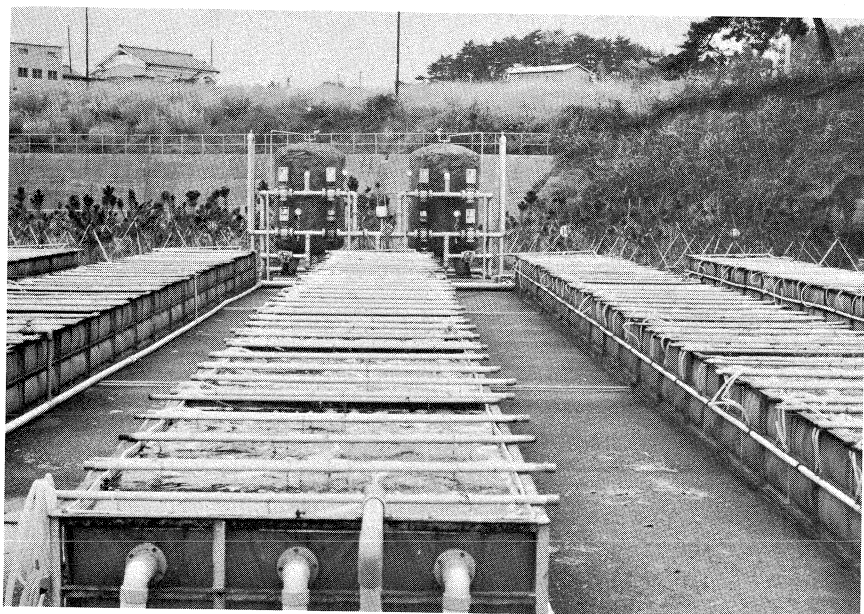
Figure 6. Illumination at settling of abalone larvae at Oyster Research Institute.



Figure 7. Culturing plates for the abalone juveniles in the waterways at Oyster Research Institute.



Figure 8. Floating waterway for the culture of juveniles at Oyster Research Institute (above), and waterway at Sendai power station (below).



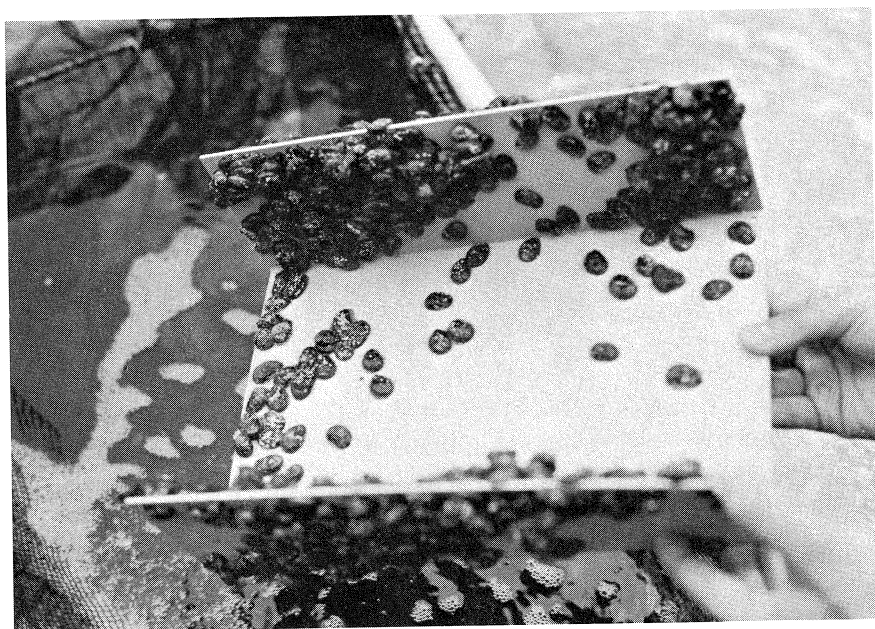


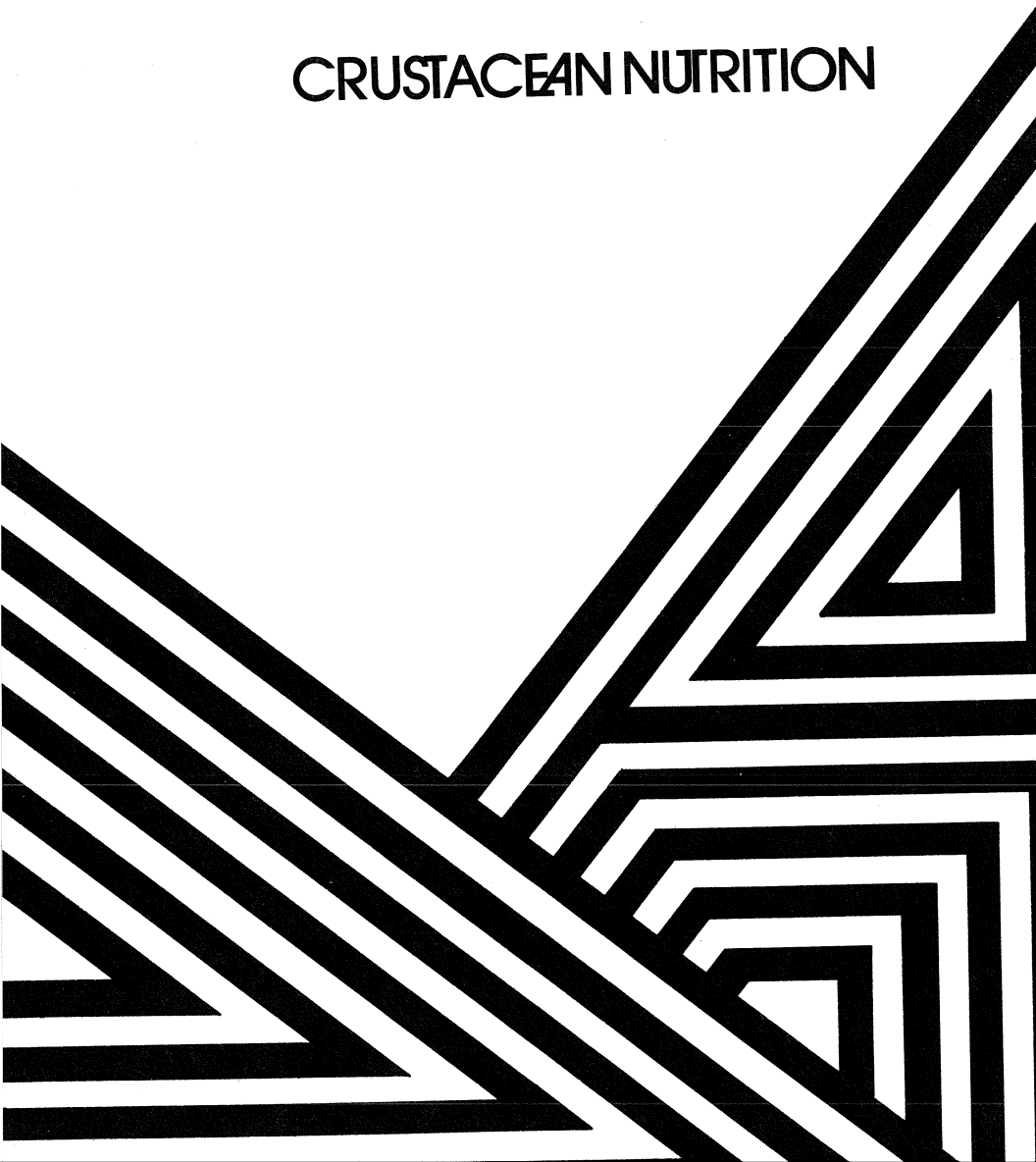
Figure 9. Abalone seed before releasing to the field.

LITERATURE CITED

- Bardach, J. E., J. H. Ryther and W. O. McLarney. 1972. Aquaculture. Wiley-Interscience, a division of John Wiley & Sons, New York, 868 pp.
- Imai, T. 1970. Recent status of invertebrate mariculture in Japan and its prospects for the future. Symposium at University of Washington.
- Ino, T. 1952. Biological studies on the propagation of Japanese abalone (Genus *Haliotis*). Bull. Tokai Reg. Fish. Res. Lab. (5), 1-102, plates XXXIV. (with English summary).
- Kikuchi, S., Y. Sakurai, M. Sasaki and T. Ito. 1967. Food value of certain marine algae for the growth of the young abalone, *Haliotis discus hannai*. Bull. Tohoku Reg. Fish. Res. Lab. (27), 93-100. (with English summary).
- Kikuchi, S. and N. Uki. 1974a. Technical study on artificial spawning of abalone, genus *Haliotis*. I. Relation between water temperature and advancing sexual maturity of *Haliotis discus hannai* Ino. Ibid. (33), 69-78 (with English summary).
- Kikuchi, S. and N. Uki. 1974b. Technical study on artificial spawning of abalone, genus *Haliotis*. II. Effect of irradiated seawater with ultraviolet rays on inducing to spawn. Ibid. (33), 79-86 (with English summary).
- Kikuchi, S. and N. Uki. 1974c. Technical study on artificial spawning of abalone, genus *Haliotis*. V. Relation between water temperature and advancing sexual maturity of *Haliotis discus* Reeve. Ibid. (34), 77-85 (with English summary).
- Kikuchi, S. and N. Uki (in press). Technical study on artificial spawning of abalone, genus *Haliotis*. VI. On sexual maturation of *Haliotis gigantea* Gmelin under artificial conditions.
- Leighton, D. L. 1972. Laboratory observations on the early growth of the abalone, *Haliotis sorenseni*, and settling success. Fish. Bull. 70 (2), 373-381.
- Loosanoff, V. L. 1969. Development of shellfish culture techniques. Proc. Conf. Artificial propagation of commercially valuable shellfish. Coll. Marine Studies, University of Delaware. 9-40.

Murayamam S. 1935. On the development of the Japanese abalone, *Haliotis gigantea*. Jour. Coll. Agr. Tokyo Imp. Univ. 13 (3), 227-231, plates XIV.

CRUSTACEAN NUTRITION



**Selected Studies of Protein and Amino Acid Requirements for
Macrobrachium rosenbergii Larvae Fed Neutral Density Formula Diets**

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INTRODUCTION

Successful rearing of the larval stages of several species of the Asian prawn *Macrobrachium rosenbergii* has been accomplished by using either live food or combinations of live and chopped organic foods. Ling (1969) first successfully reared the larval stages of *M. rosenbergii* using live zooplankton (e.g. rotifers, cyclops, copepods, and insect larvae) and chopped fish, shellfish, and steamed chicken egg. Minamizawa and Morizane (1970) were able to rear *M. rosenbergii*, *M. japonicus*, and *M. formosense* through all larval stages using combinations of live *Artemia salina* nauplii, chopped fish, and chopped short neck clam. Lewis (1962) succeeded in maintaining *M. carcinus* through the second moult using live protozoa and finely ground fish. The necessity of using green algae in larval culture has been debated, although Minamizawa and Morizane (1970) and Fujimura (1966) have reported successful larval culture using various species of marine single celled algae. However, phytoplankton has been reported to have no nutritional value for developing larvae (Sick and Beaty, 1974). Although larvae of many species of *Macrobrachium* larvae were successfully reared using the above foods, none of these studies attempted to quantify amount of food supplied or to match type of food used with the nutritional requirements of larval stages.

Greater efficiency of feeding, improved survival, and shortened duration of metamorphosis has been partially achieved by quantifying feeding procedures. Fujimura (1966), for example, gradually increased the size of chopped fish flesh from that passing through a 24 mesh per

centimeter screen for 1 to 3-day old larvae to fish flesh passing through an 8 mesh screen for 9-day and older larvae. He also quantified the amount of fish flesh and *Artemia salina* fed. Through calculation of several caloric budgets and execution of a series of growth studies, Sick and Beaty (1975) illustrated that both concentration and size of live *A. salina* nauplii are critical to rate of development and survival. Sick and Beaty (1974) reported metamorphosis of *Macrobrachium rosenbergii* larvae through all larval stages using only formula food. However, survival was very low and time of complete metamorphosis to postlarval stage was relatively long. The objective of this study was to both improve growth using formula diets and to use such food to study nutritional requirements of larval stages.

MATERIALS AND METHODS

A base fish, soybean, and shrimp meal diet was used to test several potential attractants (Table 1). Linseed oil was chosen as a lipid source since it has a high percentage of essential $\omega 3$ fatty acids (Sick and Andrews, 1974). Other unsaturated lipid sources, such as corn oil (high percentage of linoleic acid, $18:2\omega 6$) that have double bonds at the $\omega 6$ position are known to inhibit growth in some aquatic organisms (Stickney and Andrews, 1971; Sick and Andrews, 1974). Large molecular protein compounds, betaine, morin, ovomucin, avidin, ovoglobulin and ovalbumin were selectively added to the base diet (Table 1). The diets were manufactured into a porous, pliable gel and cut into cubes measuring approximately 3 mm on a side. Each diet was labeled with $25 \mu\text{Ci g}^{-1} {}^{65}\text{Zn}$ (added as ZnCl_2 with a specific activity of 5 Ci g^{-1}). Amount of each diet incorporated was determined by difference after measuring amount of activity remaining in uneaten food after a 12 hour feeding period. Each experimental unit was equipped with a fine spray of air bubbles which kept food in continuous suspension and was stocked with 25 stage-8 larvae at a concentration of $0.11 \text{ animals ml}^{-1}$. Food was maintained at a concentration of $0.0759 \text{ mg ml}^{-1}$ (adjusted every 2 hours) as previously suggested (Sick and Beaty, 1975). Temperature was $26.0 \pm 0.5^\circ\text{C}$, salinity was $10 \text{ }^\circ\text{oo}$, and light intensity was adjusted to 60 ft-c at the water surface. Amount of radioactivity remaining in uneaten food was determined with a solid iodide crystal well counter and activity corrected for dissolution. Amount of food eaten was converted to caloric equivalents as suggested by Sick (1975).

Rate of respiration was measured using the method of Sick et al. (1975). Briefly, the methods consisted of differentially using a Clark type polarographic electrode and disposable plastic tuberculin syringes

TABLE 1

Diets having several attractants added. Presented as percentage of total diet. Fish meal, soybean meal, and shrimp meal were each added to each respective diet at 10% of total diet.

Ingredients	DIET NUMBER						
	1	2	3	4	5	6	7
Avidin	15	0	0	0	0	0	0
Betaine	0	15	0	0	0	0	0
Freeze-dried <u>Artemia Salina</u>	0	0	36	0	0	0	0
Morin	0	0	0	15	0	0	0
Ovalbumin	0	0	0	0	15	0	0
Ovoglobulin	0	0	0	0	0	15	0
Ovomucin	0	0	0	0	0	0	15
Vitamin Mix ¹	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Mineral Mix ²	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Alginate ³	20	20	20	20	20	20	20
Linseed Oil	8	8	8	8	8	8	8
Cellulose	22	22	22	22	22	22	1
Calories mg ⁻¹	3.22	3.15	3.16	3.22	3.11	3.29	3.09

¹ Available from Nutritional Biochemical Co., Cleveland, Ohio. Standard vitamin diet fortification mixture in dextrose.

² Contains 30.0% K₂HPO₄, 9.4% KCl, 14.8% MgSO₄, 27.4% CaHPO₄ · 2H₂O, 1.4% FeCl₃, 0.2% MnSO₄ · 7H₂O, and 16.8% CaCO₃.

³ Available from Nutritional Biochemical Co., Cleveland, Ohio.

(5 ml) as respirometers. Electrode membranes were incorporated as one wall of a thermostated sample chamber having a volume of approximately 100 μ liter. Constant temperature baths were constructed to hold the syringe-respirometers vertically. A small square of bolting cloth was held over the sampling end of each syringe with a cut-off syringe end cap. Stage 7 or 8 *Macrobrachium rosenbergii* larvae were placed in the open (plunger) end and acclimated for 2 hours. Respiration experiments were initiated by refilling syringes sequentially with freshly oxygenated water, inverting the syringe, releasing trapped air bubbles, adjusting water volume and replacing the bolting cloth with parafilm.

The electrode was calibrated using seawater whose oxygen content was measured by standard Winkler titration. After incubation (1.5 to 2 hours), syringe water was injected into the sample chamber and

oxygen concentration recorded as percentage change. Three replicate measurements were made for each experiment using 1 larvae ml⁻¹. All readings were corrected for non-biological oxygen loss. Temperature, salinity, and light intensity were as described for ingestion rate experiments. All values were converted to caloric values correcting for increased respiration rate due to feeding (Sick, 1975).

A basal neutral density diet was modified to test effects of protein concentration and amino acid supplementation on larval metabolism (Tables 2 and 3, respectively). Total protein concentration in respective diets was 10, 15 and 20%. Amino acids supplemented into respective diets were arginine, glycine, proline, and taurine.

TABLE 2

Basal diet for protein studies of *Macrobrachium rosenbergii* larvae.¹ Sources for vitamin and mineral mixes and alginate were as given in Table 1.

Ingredients	DIET NUMBER						
	1	2	3	4	5	6	7
Fish Meal	7.5	11.3	15.0	6.5	6.5	6.5	6.5
Soybean Meal	7.5	11.3	15.0	6.5	6.5	6.5	6.5
Shrimp Meal	7.5	11.3	15.0	6.5	6.5	6.5	6.5
Ovalbumin	0	0	0	5.0	10.0	15.0	5.0
Freeze-dried <i>Artemia salina</i>	10.0	15.0	20.0	0	0	0	11.8
Vitamin Mix	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Mineral Mix	2.4	2.5	2.5	2.5	2.5	2.5	2.5
Alginate	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Linseed Oil	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Cellulose	53.5	37.3	21.0	69.6	64.6	54.6	49.7
Calories mg ⁻¹	2.15	2.38	2.91	2.19	2.38	2.68	2.49
Total Protein (%)	10	15	20	10	15	20	15

¹ Each value is expressed as a percentage of total diet.

Effects of protein concentration and amino acid supplementation on larval metabolism were analyzed after stages 4 to 8 had been fed respective diets for 10 days. Animals were maintained in 20 liter conical tanks (at a density of 0.40 liter⁻¹) (Figure 1). Culture media was continuously infused through nozzles constructed to create a gentle gyre throughout the water column and recirculated through a central charcoal, glass wool filter at a flow rate of 50 ml · min⁻¹. This culture system

TABLE 3

Diets used for studying supplementation of amino acids. Each value is expressed as percentage of total diet. Sources of vitamin and mineral mixes and alginate were as given in Table 1.

Diet	1	2	3	4	5	6
Fish Meal	15.0	15.0	15.0	15.0	15.0	15.0
Soybean Meal	15.0	15.0	15.0	15.0	15.0	15.0
Shrimp Meal	15.0	15.0	15.0	15.0	15.0	15.0
Ovalbumin	15.0	15.0	15.0	15.0	15.0	15.0
Vitamin Mix	2.5	2.5	2.5	2.5	2.5	2.5
Mineral Mix	2.5	2.5	2.5	2.5	2.5	2.5
Alginate	20.0	20.0	20.0	20.0	20.0	20.0
Linseed Oil	4.0	4.0	4.0	4.0	4.0	4.0
Arginine	0.5	0	0	0	0.5	0
Glycine	0	1.0	0	0	1.0	0
Proline	0	0	2.0	0	2.0	0
Taurine	0	0	0	0.5	0.5	0
Cellulose	10.5	10.0	9.0	10.5	7.0	11.0

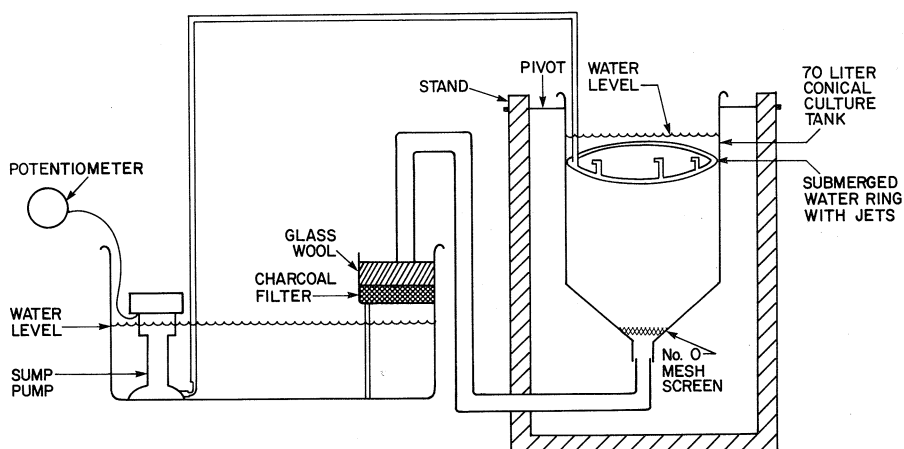


Figure 1. Re-circulating culture system used for growth studies with Macrobrachium rosenbergii larvae.

had the advantage of 1) stimulating animals to actively feed by simulating water movements found in nature; 2) maintaining food in continuous suspension and thereby optimizing availability; and 3) continually cleansing the culture system of fecal and other metabolic contamination.

Following the above feeding period, animals were removed from respective carboys and placed in 300 ml flasks at a density of 2 animals ml^{-1} . After a 6 hour period in filtered, UV, penicillin pre-tested medium, animals were removed and total ammonia and amino nitrogen were measured. Based on the method of Matheson and Tattrie (1964), total ninhydrin positive nitrogen was determined by ninhydrin reaction reading values at 570 nm on a spectrophotometer. Ammonia nitrogen was volatilized by rotary evaporation. Total amino acid nitrogen was determined by rereading at 570 nm, and ammonia values determined by difference.

RESULTS

Among attractants added to respective diets, larvae fed diets having ovalbumin added ingested significantly more calories than those fed diets having other attractants (Table 4, diet 5). Although betaine and morin, (diets 2 and 4, respectively) added to formula diets, were reported to stimulate feeding behavior in penaeid shrimp (Kanazawa, 1970), these attractants yielded significantly lower rates of ingestion (Duncan's multiple range test $P, < 0.05$) than other attractants tested. Although avidin and ovomucin (diets 1 and 7, respectively) were significantly better attractants than either betaine or morin, they were not as potent attractants as freeze-dried *Artemia salina* or ovalbumin (diets

TABLE 4

Relative rates of caloric ingestion [calories incorporated $\cdot (\text{mg animal dry wt})^{-1} \text{hr}^{-1}$]
 $\times 10^{-2}$ by stage 8 Macrobrachium rosenbergii larvae fed formula diets with
 several attractants (from Table 1). Values are means and standard
 deviations based on 3 replications.

Diets	Calories Ingested
1	5.349 $\pm$ 0.213
2	1.617 $\pm$ 0.149
3	7.829 $\pm$ 0.490
4	1.889 $\pm$ 0.178
5	11.223 $\pm$ 0.991
6	6.872 $\pm$ 0.417
7	4.116 $\pm$ 0.349

3 and 5, respectively). A diet supplemented with ovoglobulin (diet 6) was ingested at about the same rate as one supplemented with freeze-dried *A. salina*.

Larvae fed a diet supplemented with ovalbumin had a significantly higher growth efficiency than those fed a diet supplemented with freeze-dried *Artemia salina* (64% versus 42%, respectively) (Table 5). Although the efficiency for larvae fed an ovalbumin-supplemented diet was not as great as for larvae fed live *A. salina* (64% versus 95%, respectively), those fed an ovalbumin-supplemented diet had an efficiency comparable to that of other particulate feeders in field situations (67%, Ivlev, 1939).

Regardless of the amount of food ingested (i.e. attractant supplemented in each respective diet), the amount of total nitrogen excreted was inversely related to the total protein concentration of each respective diet (Table 6). Larvae fed diets with less than 10% total protein excreted significantly more nitrogen than animals fed diets with either 15 or 20% total nitrogen. Average total nitrogen excreted among all groups of larvae fed diets supplemented with freeze-dried *Artemia salina* meat was 43% larger than that excreted among groups fed diets supplemented with ovalbumin.

The ratio of oxygen consumed to total ninhydrin positive nitrogen excreted was directly proportional to total protein concentration of each respective diet (Table 6). There was no significant difference between O : N ratios obtained for groups of larvae fed diets with 15 and 20% total protein. However, those fed diets with 10% total protein had significantly lower O : N ratios (Duncan's multiple range test, $P < 0.05$, and AOV, $\cdot$ F test). Larvae fed ovalbumin-supplemented diets with 20% total protein had an O : N ratio not significantly different from larvae fed live *Artemia salina* nauplii.

In general, diets supplemented with selected amino acids (Table 3) caused a decrease in nitrogen excreted among respective groups of larvae (Table 7). Larvae fed a diet supplemented with glycine (diet 2) had a higher O : N ratio than larvae fed other amino acid supplemented diets. Similarly, a group of larvae fed a diet supplemented with taurine (diet 4) tended to have a relatively high O : N ratio. Larvae fed a diet having multiple amino acid supplementation (diet 5) had an O : N ratio 8% higher than the control. Total amino nitrogen excreted among larvae fed a glycine supplemented diet (diet 2), was 41% lower than that excreted by larvae fed a control diet (no amino acid supplementation), while larvae fed a taurine supplemented diet (diet 4) had a 46% lower excretion rate.

TABLE 5

Caloric energy budgets for Stage 8 Macrobrachium rosenbergii larvae. Comparison of diets having freeze-dried Artemia salina and ovalbumin added as attractants. Diets were Numbers 3 and 5 from Table 1. Values are expressed as means and standard deviations based on three replications.

Diet	[Calories Incorporated (mg an dry wt) ⁻¹ hr ⁻¹] X 10 ⁻²	Estimate of Calories Assimilated X 10 ⁻²	[Calories Respired (mg an dry wt) ⁻¹ hr ⁻¹] X 10 ⁻²	Efficiency (%/100)
Freeze-dried <u>Artemia salina</u>	7.084 ± 0.31	4.960	2.860 ± 0.11	42
Ovalbumin	11.223 ± 0.98	7.856	2.810 ± 0.19	64
Live <u>Artemia</u> <u>salina</u> nauplii	101.401 ± 12.38	70.980	2.951 ± 0.13	95

TABLE 6

Rate of oxygen consumed to total nitrogen excreted for Macrobrachium rosenbergii larvae (stage 8) fed several diets varying in attractant properties and total protein. Values are means and standard deviations based on 3 replications.

Diet Number	Respiration [$\mu\text{g-at O}_2$ (mg an dry wt) $^{-1}$ hr $^{-1}$]	Total N Excreted [$\mu\text{g-at N}$ (mg an dry wt) $^{-1}$ hr $^{-1}$]	Atomic Ratio O : N
1. 10% (Freeze-dried <u>Artemia salina</u>)	1.94 $\pm$ 0.010	0.579 $\pm$ 0.001	3.3
2. 15% (Freeze-dried <u>Artemia salina</u>)	1.92 $\pm$ 0.009	0.340 $\pm$ 0.001	5.7
3. 20% (Freeze-dried <u>Artemia salina</u>)	1.94 $\pm$ 0.008	0.320 $\pm$ 0.002	6.1
4. 10% (Ovalbumin)	1.93 $\pm$ 0.010	0.370 $\pm$ 0.001	5.2
5. 15% (Ovalbumin)	1.94 $\pm$ 0.009	0.273 $\pm$ 0.029	7.1
6. 20% (Ovalbumin)	1.91 $\pm$ 0.008	0.222 $\pm$ 0.031	7.6
7. 15% (Ovalbumin + Freeze-dried <u>Artemia salina</u>)	1.93 $\pm$ 0.009	0.261 $\pm$ 0.064	7.4
8. Live <u>Artemia salina</u> larvae	1.92 $\pm$ 0.010	0.214 $\pm$ 0.029	7.8

TABLE 7

Ratio of oxygen consumed to nitrogen excreted for *Macrobrachium rosenbergii* larvae (Stage 8) fed amino acid supplemented diets.

Values are means and standard deviations based on 3 replications.

Diets are from Table 3. Values are all expressed as:

$[\mu\text{g-at (mg an dry wt)}^{-1} \text{ hr}^{-1}]$.

Diet Number	O : N	Total Nitrogen	Total Amino Nitrogen
1	7.0	0.273 $\pm$ 0.011	0.133 $\pm$ 0.006
2	8.1	0.235 $\pm$ 0.019	0.091 $\pm$ 0.005
3	7.4	0.258 $\pm$ 0.021	0.127 $\pm$ 0.004
4	7.6	0.251 $\pm$ 0.009	0.101 $\pm$ 0.001
5	7.8	0.245 $\pm$ 0.007	0.130 $\pm$ 0.040
6	7.2	0.265 $\pm$ 0.012	0.129 $\pm$ 0.012

DISCUSSION

Although the chemical and behavioral processes associated with chemoreception are largely unknown among crustaceans, some proteinaceous substances have been advocated as potential attractants. Betaine (trimethylammonium hydroxide) is relatively soluble in water and is noted for its sweet taste. Morin, like betaine, has been used as an attractant in formula diets for penaeid shrimp (Kanazawa, 1970). It is an aromatic compound noted for its fragrant aroma. Egg white protein is known for its attractant properties among natantian larvae, particularly *Macrobrachium rosenbergii* (Ling, 1969). For example, ovomucin is a minor constituent of egg protein and is also related to the glycoprotein constituents of gastric juices in salivary enzymes. Avidin, another egg white protein, is a glycoprotein, primarily associated with manose. Ovalbumin, the largest protein component of egg white (75%) contains a large variety of amino acids and is associated with hexose.

Among potential attractants used, only those associated with egg protein fractions had any significant effect as an attractant. Perhaps the relatively high solubility of betaine and morin in an aqueous medium detracted from their effectiveness as chemo-effectors in food. Amino acids have long been advocated as chemo-effectors among invertebrates. Perhaps it was the relatively large variety of amino acids contained in ovalbumin that aided in increasing the rate of food ingestion.

Although the growth efficiency obtained using formula diets supplemented with ovalbumin compared favorably with efficiencies reported for field conditions (Table 5), an efficiency of 64% may not be adequate for *Macrobrachium rosenbergii* larvae. Many filter feeders have been reported to have higher growth efficiencies than the average value of 67% reported by Ivlev (1939). For example, *Artemia salina* nauplii have been reported to have an efficiency of 85 to 90% (Sick, 1975a) and several species of copepods averaged 72% (Conover, 1966). The fact that larvae fed live food had a relatively high growth efficiency (95%, Table 5), compared to ovalbumin-supplemented formula diet (64%) may have indicated that even ovalbumin supplemental diets were not adequate for optimal growth efficiency.

Higher total nitrogen excretion with relatively low concentrations of total protein in the diet (diets 1 and 4, Table 6) probably indicated metabolizing of body protein. Several authors have reported use of body protein among invertebrates during periods of low protein assimilation and general starvation (e.g. Conover and Corner, 1968; Snow and Williams, 1971). Mayzaud (1973) reported a significant increase in nitrogen excretion among *Meganyctiphanes norvegica* and *Acartic clausi* during periods of starvation or severely reduced food. Diets with only 10% content of total protein (diets 1 and 4, Table 6) probably did not provide sufficient sustenance to prevent at least a minor amount of metabolic disequilibria among *Macrobrachium rosenbergii* larvae.

Similarly, some degree of metabolic disequilibria is indicated by relatively low O : N ratios among most of the diets examined (Table 6). Ratios of 8 to 10 probably are representative of normal lipid-protein metabolism. Mayzaud reported values of 8.20 for populations of *Meganyctiphanes norvegica* during continuous feeding in the spring and 4.12 for populations under semi-starved situations during the winter months. With the exception of larvae fed the low protein diet (10%), all O : N values among the diets tested were between 4 and 8, indicating primarily protein metabolism and at least some state of metabolic disequilibria. Larvae fed a diet with a 20% total protein content and ovalbumin supplemental had an O : N (7.6, Table 6) possibly indicative of relatively normal lipid-protein metabolism.

Dietary amino acid supplementation may have supplemented metabolic loss of critical amino acids caused by dietary protein deficiency. Increases in rate of amino acid excretion because of physical stress have been reported in several zooplankton species (Johannes and Webb, 1965). Exposure to heavy metals is known to cause increases in rate of excretion of selected amino acids (Sick, 1975b), glycine being

excreted at a significantly higher rate than other amino acids analyzed. It is not inconceivable that increased rates of excretion of selected amino acids could occur as a result of low protein ingestion (i.e. starvation). For example, hydrolysis of body proteins would yield energy and free amino acids. In addition, transamination of components in carbohydrate metabolism (e.g. Kreb's cycle intermediates) could also be used for energy production and could yield free amino acids. Many of the free amino acids created by such conversions would probably be excreted. Excessively high glycine excretion, due to the nutritional stress of inadequate diet, has been reported in marine zooplankton (Sick and Baptist, 1975) and may occur in *Macrobrachium rosenbergii* larvae. Therefore, dietary supplementation of selected amino acids (e.g. glycine and taurine) may have served as energy sources and resulted in less conversion of body reserves (i.e. proteins and carbohydrates) and therefore lower rates of nitrogenous excretion.

LITERATURE CITED

- Conover, R. J., and E. D. S. Corner. 1968. Respiration and nitrogen excretion by some marine zooplankton in relation to their life cycle. J. Mar. Biol. Ass. U.K. 48: 49-75.
- Duncan, D. B. 1955. Multiple Range and Multiple F Tests. Biometrics 11: 1-42.
- Fujimura, T. 1966. Notes in the development of a practical mass culturing technique of the giant prawn *Macrobrachium rosenbergii*. Indo-Pacific Fisheries Council, F.A.A. Regional Office for Asia and the Far East, Bangkok, Thailand, 12th Session.
- Ivlev, V. S. 1939. Transformation of energy by aquatic animals. Internat. Rev. ges. Hydrobiol. μ Hydrogr. 38: 449-458.
- Johannes, R. E. and K. L. Webb. 1965. Release of dissolved amino acids by marine zooplankton. Science 150: 76-77.
- Kanazawa, A., M. Shimaya, M. Kawasaki, and K. Kashiwada. 1970. Nutritional requirements of Prawn-I. Feeding on Artificial Diet. Bull. Jap. Soc. Sci. Fish. 36 (9): 949-954.
- Lewis, J. B. 1962. Preliminary experiments on the rearing of the freshwater shrimp, *Macrobrachium carcinus*. University of Miami Institute of Marine Science, Gulf and Caribbean Fisheries Institute, Proceedings of the 14th Annual Session, Miami Beach, Florida, November, 1961.
- Ling, S. W. 1969. Methods of rearing and culturing *Macrobrachium rosenbergii* (de Man) F.A.O., Rep. (57)3: 589-606.
- Matheson, A. T. and B. L. Tattrie. 1964. A modified Temm and Cocking ninhydrin reagent for peptidase assay. Can. J. Biochem. 42: 95-103.
- Mayzaud, P. 1973. Respiration and nitrogen excretion of zooplankton II. Studies of the metabolic characteristics of starved animals. Mar. Biol. 21: 19-28.
- Minamizawa, A. and T. Morizane. 1970. Report on study about cultivation technique for fresh water shrimp. Ehime Prefecture Fishery Laboratory, Bureau of Commercial Fisheries, Office of Foreign Fisheries. U. S. Dept. Interior, Washington, D. C.

- Sick, L. V., and H. Beaty. 1974. Culture techniques and nutrition studies for larval stages of the giant prawn, *Macrobrachium rosenbergii*. University of Georgia Marine Science Center, Technical Report Series, 74-5.
- Sick L. V., and J. W. Andrews. 1974. The effect of selected dietary lipids, carbohydrates, and proteins on the growth, survival, and body composition of *Penaeus duorarum*. Proceedings of the World Mariculture Society, January, 1973, Monterrey, Mexico.
- Sick, L. V., and H. Beaty. 1975. Development of formula foods designed for *Macrobrachium rosenbergii* larval and juvenile shrimp. Proceedings of the World Mariculture Society. January, 1975.
- Sick, L. V. 1975a. Nutritional effect of five species of marine algae on the growth, development, and survival of the brine shrimp, *Artemia salina*. Mar. Biol. 31(4): 209-218.
- Sick, L. V. 1975b. The effect of prolonged exposure to cadmium on rate of amino acid excretion in the marine copepod *Pseudodiaptomus coronatus*. Submitted.
- Sick L. V., J. Hall, and G. J. Baptist. 1975. A comparison of methods for measuring respiration rates of micro-zooplankton. Submitted.
- Sick L. V., and G. J. Baptist. 1975. Effects of environmental concentrations of cadmium on rates of cadmium uptake and metabolism of the marine copepod *Pseudodiaptomus coronatus*. Submitted.
- Snow, N. B. and P. J. LeB. Williams. 1971. A simple method to determine the O : N ratio of small marine animals. J. Mar. Biol. Ass. U.K. 51: 105-109.
- Stickney, R. R., and J. W. Andrews. 1971. Combined effects of dietary lipids and environmental temperature on growth, metabolism, and body composition of channel catfish (*Ictalurus punctatus*). J. Nutrition 101(12): 1703-1710.

Studies On The Development Of Compounded Diets For Prawns

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INTRODUCTION

In reviewing the subject of prawn nutrition and the preparation of formulated feeds for their culture it is helpful initially to itemize the various aspects of diet formulation that must be considered if successful rations are to be produced (Table 1). The list is long and somewhat forbidding and I do not suggest that complete and definitive information on all these topics is necessary before attempts can be made to compound commercial rations for prawns. I do suggest, however, that at present there are sufficiently large gaps in our knowledge that make ration formulation for prawns a very empirical science.

The purpose of this paper is to review such available information on prawn nutrition in light of the list of factors summarized in Table 1 and where appropriate, to highlight the areas that I consider to be most critical for future work. There are, however, two points that I should like to make before embarking on such a discussion. First, my comments refer to the development of diets for prawns reared in intensive culture systems where there is no scope for supplemental nutrition. Second, in studies on prawn nutrition and in the whole field of prawn cultivation many different species have been used and there is a danger, particularly in nutritional work, of assuming that results which have been obtained for one species will automatically apply to others. This will probably be so in many cases but not in all, and this should constantly be borne in mind when interpreting the available data.

TABLE 1

Factors to be considered in diet development for prawns

Nutritional requirements	Amino acids, fats, carbohydrates, minerals, vitamins Protein level Protein/energy relationships Variations with age and moult cycle Growth factors
Available ingredients	Conventional food meals and con- centrates Novel proteins (SCP) 'Trash' products Artificial detritus
Diet evaluation	Experimental conditions Growth and gross conversion Nitrogen balance and NPU Digestibility - rate and efficiency Food intake - rate and efficiency Palatability, texture Quality of product
Husbandry considerations	Feeding level Environmental requirements Fecal matter Automatic feeding
Compounding considerations	Water stability Degree of milling Form of final product Ease of storage and handling
Cost	

NUTRITIONAL REQUIREMENTS

Definitive information is lacking on specific nutritional requirements of prawns. There have been two studies (Cowey and Forster, 1971 and Shewbart, Mies and Ludwig, 1973) using C^{14} tracer techniques that have indicated the qualitative amino acid requirements of prawns and these, not surprisingly, have shown that the same amino acids are likely to be essential for prawns as for other groups of animals

such as fish and mammals. However this technique does not provide information on the quantitative requirements for the essential amino acids — these remain to be determined.

Even less information is available on the requirements for other nutrients. A requirement for dietary sterols has been demonstrated in crustacea and Kanazawa et al. (1970) have shown that for *Penaeus japonicus* a level of 0.5 g cholesterol per 100 g of diet met this requirement. Shewbart, Mies and Ludwig (1973) have suggested there may be a requirement for linolenic acid in *Penaeus aztecus* and Deshimaru and Kuroki (1974) found that the best growth with *P. japonicus* fed a purified diet based on casein occurred when a very high level of mineral supplement (19.5%) was included.

For the precise definition of nutritional requirements the development of a good purified ration is necessary. Such a ration would allow specific nutrients to be excluded or included at limiting levels so that deficiency symptoms and hence quantitative requirements can be identified. Deshimaru and Kuroki (1974) have recently made some progress in this field with a diet based on casein which when fed on *P. japonicus* compared quite well with a control diet based on squid meal. With a diet such as this, work on the detailed identification of both quantitative and qualitative requirements for specific nutrients should be possible and should receive priority attention.

Some attempts have been made to short-cut this process by relating the biochemical composition of formulated feeds to that of prawns themselves. Deshimaru and Shigeno (1972) reported that rations which gave the best results most closely approximated the amino acid configuration of the prawn *P. japonicus*. In addition, they also showed that good natural foods such as short-necked clams also had an amino acid composition similar to that of squid and mysid shrimp meal which were the most satisfactory components in formulated rations. Interestingly, fish meal, conventionally regarded as a high quality protein source, gave poor results and in comparison with prawn flesh, was shown to be low in phenylalanine, histidine, arginine and lysine. It seems possible therefore, that prawns could have particularly high requirements for these. In the case of arginine some evidence is provided by Kitabayashi et al. 1971, who found that the quantity of squid meat extract in a diet could be reduced if 0.83% arginine was substituted. They also reported a growth promoting effect of methionine when this was added at a level of 0.52% to a standard squid-based diet.

From a summary of the very limited information on the requirements for specific nutrients, I would now like to consider the require-

ments of prawns for the different nutrient groups. Some of the best results in terms of overall growth have been obtained on diets with very high protein levels, i.e., 60% and above (Deshimaru and Shigeno 1972; Kitabayashi et al. 1971, see Table 2). In contrast other workers have reported much lower levels as being optimal (see Table 2) and have found growth inhibition as dietary protein was increased. However, since growth in the latter studies was generally poor the results may have been affected as much by inadequate dietary formulation as by the level of dietary protein. It should be pointed out, however, that the good growth results with high protein diets have been obtained with *P. japonicus* while the lower values have generally been obtained with

TABLE 2

Summary of data available on optimum dietary protein levels for prawns-

Author	Species	Main Diet Ingredient	Optimum Protein Level %
Andrews, Sick and Baptist 1972	<u>Penaeus setiferus</u>	Fish Meal	28 - 32
Balazs, Ross and Brooks 1973	<u>Penaeus japonicus</u>	Shrimp Meal	40
Deshimaru and Kuroki 1974a	<u>Penaeus japonicus</u>	Casein	54
Deshimaru and Shigeno	<u>Penaeus japonicus</u>	Squid Meal	60
Lee 1971	<u>Penaeus monodon</u>	Casein/Fish Meal	46
Forster and Beard 1973	<u>Palaemon serratus</u>	Shrimp Meal Fish Meal (freeze) (dried)	40 40
Shewbart, Mies and Ludwig 1973	<u>Penaeus aztecus</u>	?	22.5 - 35

other species. *P. japonicus* is known to be predominantly carnivorous in the wild while *P. setiferus* and *P. aztecus* together with many other penaeids are considered to be detritus feeders.

There could, therefore, be considerable interspecific differences in protein requirements and this illustrates the possible dangers of assuming that prawns are nutritionally a homogenous group. At the present time therefore it is not possible to generalize on the requirement for dietary protein although it is to be hoped and perhaps expected that levels above 60% will prove unnecessarily high — if not, feed costs could be prohibitive.

As with the other aspects of nutritional requirements, information on optimum levels of different dietary energy sources for prawns is limited. In the case of lipids the formulated feeds on which best growth has been obtained have contained relatively low levels, Deshimaru and Shigeno (1972), quote a level of 8.8% crude fat in their best diet and it seems unlikely that there is much to be gained by providing higher levels. Shudo et al. (1971) report that the addition of 4% squid liver oil added to their standard ration improved the growth of *P. japonicus*, but Forster and Beard (1973) and Andrews, Sick and Baptist (1972) both report inhibition of growth at levels of 15% and 10% supplementation respectively. In purified diets Sick and Andrews (1973) found 10% lipid gave better growth than none at all while Deshimaru and Kuroki (1974) found that a level of 6% gave better growth than none or 12%. Although the data is limited, it would appear that prawns do not require high levels of dietary lipid and that the optimum level is probably between 5-10%.

Even less attention has been paid to the effects of different levels of dietary carbohydrate although the indications are that prawns can use quite high levels and that starch rather than glucose or dextrin will give best results. Sick and Andrews (1973) report better performance of *P. duorarum* on a diet containing 40% corn starch than on diets containing 10% starch or 40% and 10% glucose. Similarly Forster and Beard (unpublished data) found that a standard laboratory diet to which 15% wheat starch had been added gave better growth (138.1% live-weight increase in 28 days) of *Palaemon serratus* than the same diet to which glucose, oyster glycogen, dextrin or no carbohydrate supplement was added — these giving 125.6, 105.6, 105.4 and 120.1% live-weight increase respectively. In this context it is interesting to note the observation of Kitabayashi et al. (1971) that glucose addition to their standard diet was only beneficial when added together with Vitamin C; the level of glucose supplementation was only 3.75% however.

Finally, in considering nutritional requirements, possible variations related to the moulting cycle must be borne in mind. For example, there are obviously considerable needs for the synthesis of chitin but it is not known whether these needs are cyclic or relatively constant. Various authors have added glucosamine, as a precursor of chitin, to their formulations but only Kitabayashi et al. (1971) report any beneficial effect when glucosamine was included at a level of 0.53% in their standard diet. It seems likely that given the necessary substrates prawns can synthesize glucosamine, and hence chitin, both quickly and efficiently and so dietary supplementation with glucosamine may be an unnecessary complication. Cowey and Forster (1971) found that *Palaemon serratus* injected with C¹⁴ labelled acetate rapidly incorporated a large proportion of this substrate into glucosamine which was isolated from the protein/chitin residue. Other special dietary requirements related to the moulting cycle, e.g., a possible high or fluctuating demand for calcium, are areas still in need of investigation.

AVAILABLE INGREDIENTS

Alongside an assessment of the nutritional requirements of prawns, consideration must be given to the range of available raw materials from which practical rations can be formulated.

First there is the whole range of dry food meals and concentrates that are used routinely in conventional animal husbandry. Many of these have already been tried in diets for prawns with varying degrees of success. Some initial conclusions may be drawn from these attempts. Perhaps the most conspicuous observation is the general failure of fish-meal based diets to perform well (Balazs, Ross and Brooks, 1973; Forster and Beard, 1973; Sick and Andrews, 1973). Reference has already been made to the work of Deshimaru and Shigeno (1972) and to their observation that in comparison with an analysis of prawn flesh and some successful formulated rations for *P. japonicus*, fish meal was low in phenylalanine, lysine, histidine and arginine. The available evidence suggests that fish meal, the basis of many formulated fish foods and a highly important component of diets for many other farmed animals, may not be of much use in formulated feeds for prawns.

In contrast a number of authors have reported good results with diets based on, or containing high levels of soya bean protein, (Balazs, Ross and Brooks, 1973; Deshimaru and Shigeno, 1972; Kanazawa et al. 1970; and Sick and Andrews, 1973). Similarly shrimp meal from various sources has been used in many studies and perhaps not sur-

prisingly has given good results (Balazs, Ross and Brooks, 1973; Forster and Beard, 1973; and Sick, Andrews and White, 1972). Of the various feedstuffs that are traditionally available soya bean meal and shrimp meal would seem to be the best prospects. In addition some very good results have been reported with squid meal (Deshimaru and Shigeno 1972 and Kitabayashi et al., 1971) although this can hardly be considered a conventional animal feedstuff.

Another group of protein rich feedstuffs which can be considered for use in prawn rations are the group of single cell proteins, or novel proteins, that are being produced by a number of different processes in different parts of the world (Table 3). These are derived from mass cultures of simple organisms such as bacteria, yeast, fungi and algae. As shown in Table 3 the projected production by 1980 is very significant.

TABLE 3

Potential world-wide production and pilot facilities for SCP by 1980

Presented at a symposium of the American Chemical Society Meeting, Philadelphia, 9 April 1975 by Jeremy Wells.

	Organism	Substrate	Estimated 1975-80 capacity (t/a)
Czechoslovakia			
Stovnaft Kojetin	Yeast	Ethanol	60,000
Stovnaft Kojetin	Yeast	n-Paraffins	100,000
Finland			
United Paper Mills	Fungi	Sulphite liquor	10,000
France			
British Petroleum, Lavern	Yeast	Gas oil	100,000
Group Francais des Proteines	Yeast	n-Paraffins	40,000
Germany			
Biological Carbon Research	Algae	Carbon dioxide	pilot
Italy			
Italproteine, Sardinia	Yeast	n-Paraffins	100,000
Liquilchimica, Calabria	Yeast	n-Paraffins	200,000
Mexico			
Soea Texcoco	Algas	Carbon dioxide	pilot

TABLE 3 (cont.)

	Organism	Substrate	Estimated 1975-80 capacity (t/a)
Romania			
Renipret	Yeast	n-Paraffins	60, 000
Republic of China	Yeast	n-Paraffins	1, 000
Switzerland			
Nestle	Bacteria	Ethanol	1, 000
Taiwan			
Chinese Petroleum	Yeast	n-Paraffins	100, 000
United Kingdom			
British Petroleum	Yeast	n-Paraffins	100, 000
ICI	Bacteria	Methanol	100, 000
Shell Chemical	Bacteria	Methane	1, 000
RHM Foods	Fungi	Carbohydrates	10, 000
Tate and Lyle	Fungi	Carbohydrates	4, 000
USSR	Yeast	n-Paraffins	200, 000
United States			
Ameco Foods Co.	Yeast	Ethanol	5, 000
LSU/Sechtol	Bacteria	Cellulose	10, 000
US Army	Bacteria	Cellulose	10, 000
Japan			
Mitsubishi Gas	Yeast	Methanol	100, 000
Sunimoto	Bacteria	Methanol	100, 000
Kyowa Hakko	Yeast	n-Paraffins	100, 000

It must be emphasized that each of these products is likely to differ substantially in its composition and therefore evaluation of the products as possible dietary ingredients for prawns is likely to be a lengthy process. Nevertheless such an investigation should be undertaken since these products will probably offer the long-term advantages of price stability and continuity of supply. The only product which has been investigated to date is petroleum yeast. Deshimaru and Shigeno (1972) report good growth of *P. japonicus* when that yeast was included in a diet at a level of 20%. Forster and Beard (1973), however, found an adverse effect on growth of *Palaemon serratus* when a petroleum yeast was sub-

stituted for fish meal at a level of 45% in a standard laboratory diet. Nevertheless, it was shown to be well assimilated (Forster and Gabbott, 1971).

Other possible ingredients which must be considered but which will not be discussed in this review are 'trash' products (for example various offals including shrimp waste), and artificial detritus for prawns cultured in pond farming systems. These topics have previously been discussed by Grajcer and Neal (1972) and Caillouet, Heinen and Tabb (1972).

DIET EVALUATION

I would now like to move on from a consideration of various dietary ingredients to a discussion of their evaluation, the ways in which this can and should be done, and some of the results that have been achieved in recent years with certain formulations. The first thing I wish to say concerns the nature and standardization of experiments to study diet development in prawns — here I would merely echo the comments of Zein-Eldin and Meyers (1973) who standardized procedures for undertaking experiments and reporting results. In many cases critical parts of these procedures have not been followed, e. g., the use of sand and gravel substrates in aquaria during feeding experiments, prevent meaningful interpretation of data.

The most obvious method of assessing the performance of a ration is by measuring growth. Where reasonable, growth that occurs on a particular diet should be supported by the determination of gross food conversion efficiency. Table 4 is a summary of some of the growth and conversion data available and you will see there are considerable differences. Some very satisfactory growth rates have been obtained for different species on formulated feeds and the rates are certainly fast enough to make their commercial culture attractive. There is much less information available on conversion efficiencies although the values quoted by Venkataramiah, Lakshmi and Gunter (1974) together with the recent information from the Conwy laboratory (Wickins in preparation) suggest that conversion rates of the order of 2 : 1 should be obtainable. This is further supported by conversion rates quoted for wet foods, e.g., Shigeno (1975) gives values which increase from 4.58:1 to 10.49:1 as the prawn *P. japonicus* fed with clam meat grew from 1.69 to 18.35g live-weight. When put on the basis of dry weight of food : live-weight gain of the prawns these values would be approximately 1.2 to 2.6:1. The significance of conversion efficiency is obviously related to the cost of the diet but its importance on a commercial scale cannot be overesti-

TABLE 4

Summary of the best available growth and conversion data for prawns fed on compounded diets

Author	Species	Main Diet Ingredient	Growth (g)	Experimental Time (Days)	Conversion (Dry Weight Food : Live Weight Prawn)
Balazs, Ross and Brooks (1973)	<u>Penaeus japonicus</u>	Shrimp Meal	1.5 - 6.44	25	--
Deshimaru and Kuroki (1974)	<u>Penaeus japonicus</u>	Casein	0.84 - 2.09	30	4.8
Deshimaru and Shigeno (1972)	<u>Penaeus japonicus</u>	Squid Meal	5.31 - 15.71	60	--
Forster (1972)	<u>Penaeus monodon</u>	Shrimp Meal	0.07 - 3.39	84	--
Kanazawa et al. (1970)	<u>Penaeus japonicus</u>	Soya Bean Protein	1.57 - 2.22	30	--
Lee (1971)	<u>Penaeus monodon</u>	Fish Meal/Casein	0.51 - 1.19	60	6.5
Sick, Andrews and White (1972)	<u>Penaeus aztecus</u>	Shrimp Meal	4.0 - 10.56	42	5.5
Venkataramiah, Lakshmi and Gunter (1974)	<u>Penaeus aztecus</u>	?	0.16 - 1.52	42	1.89
Wickins (in preparation)	<u>Penaeus merguensis</u>	?	0.40 - 5.9	56	2.0
Zein-Eldin and Meyers (1973)	<u>Penaeus aztecus</u>	Shrimp Meal	1.3 - 5.0	65	--

mated. Scope for improvement lies with both better formulations and improvement of husbandry procedures. The study of conversion efficiencies is an area which should receive more attention.

Other approaches to ration and ingredient evaluation include more detailed analysis of food conversion and especially studies on the use of protein such as nitrogen balance studies and estimates of Net Protein Utilization (NPU). These measurements are especially helpful in evaluating different high protein ingredients (Cowey and Sargent, 1972) and could therefore be of value in the field of prawn nutrition. The only reported values for NPU in prawns are those of Lee (1971) who gives four values including one of 1.04 for *P. monodon* fed on a casein/fish meal diet having a protein content of 45.75% (growth and gross conversion quoted in Table 4). In comparison with values for fish fed on various proteins (Cowey and Sargent, 1972) this is very low but since it is not clear from the data just how this value was derived, its validity must remain in doubt.

Another factor to be considered in evaluating formulated feeds or individual ingredients is digestibility. Several studies have been undertaken in this area (Nose, 1964; Lee, 1970; Forster and Gabbott, 1971 and Condrey, Gosselink and Bennett, 1972). These have all shown that several species of prawns can digest a variety of dietary ingredients very efficiently and therefore the ability of prawns to digest formulated feeds is unlikely to be a limiting factor in ration development. Table 5 sum-

TABLE 5

Protein Assimilation by Prawns from Diets Based on Fish Meal

Author	Species	Fish Meal in Diet %	Assimilation of Protein %
Lee (1970)	<u>Penaeus japonicus</u>	98	82.4
	<u>Penaeus monodon</u>	98	76.2
	<u>Penaeus semisulcatus</u>	98	80.7
	<u>Metapenaeus monoceros</u>	98	81.4
Forster and Gabbott	<u>Palaemon serratus</u>	60	90.5
	<u>Pandalus platyceros</u>	60	75.4
Nose (1964)	<u>Penaeus japonicus</u>	96.6	89.0
Condrey, Gosselink and Bennett (1972)	<u>Penaeus aztecus</u>	Principle ingredient, probably about 40%	86.0

marizes data on the assimilation of protein from diets based on fish meal and as can be seen the values are uniformly high.

In addition to assimilation efficiency, other aspects that have to be considered in conjunction are the efficiency of food ingestion and the rate of assimilation. These aspects are especially critical for prawns because of the way in which they feed and because of the structure and function of the anterior alimentary canal.

The mechanism of food ingestion has been described by several authors (Patwardhan, 1935a and b; Dall, 1968; Snow, 1969; and Con-drey, Gosselink and Bennett, 1972). It is basically a wasteful process since food is sorted and partially masticated by the mouthpieces outside the buccal cavity before ingestion. With formulated feeds, (essentially collections of small individual particles bound together) particles can become dislodged during this process and then swept away by the respiratory water current. Therefore in preparing diets for prawns, consideration must be given to producing rations which are ingested quickly with the minimum of wastage.

Once it has been ingested the food is then further fragmented and partly digested in the proventriculus. Before it can pass further along the alimentary canal it must first pass through a filtering apparatus in the posterior chamber of the proventriculus which only permits the passage of partly digested food and the finest food particles. The need for the food to be reduced in this way therefore acts as a mechanism which regulates food intake. Any delay in the food reduction process and its subsequent passage through the pyloric filter means that the pro-ventriculus remains full of food and cannot accept anymore. Thus in the evaluation of formulated feeds for prawns it is important to consider the rate at which the food, once ingested, is reduced sufficiently to pass through the proventriculus into the mid-gut. It would be especially interesting to look at the relationship between this and food particle size, the nature of different ingredients, and food texture.

An indication of the sort of effect which might occur is given by Snow (1969). He reported that total gut clearance of *Palaemonetes varians* after a detritus or algal (*Platymonas*) meal occurred in 5 to 7 hours, but took 27 hours for a meal of dried animal protein (pelleted shrimp). Intermediate clearance times were recorded for fresh animal tissue such as tubificid worms and crab hepatopancreas which were cleared in 12 to 13 hours. It can be seen therefore that observations such as these when related to the efficiency of ingestion, nutrient density in the diet and digestibility of the dietary ingredients, will help by enabling rations to be prepared that will allow efficient and fast

absorption of nutrients. Providing those nutrients occur in the right proportions, the general performance of the diet should then be maximized.

Finally under the heading of diet evaluation two other factors need consideration. First, the ration must be attractive and palatable to the prawns. This seems to present no great problem since prawns respond vigorously to a variety of animal meals and extracts and it is a comparatively simple matter to include low levels of such materials in formulated feeds. Some interesting work by Mackie (1973) has shown that a synthetic mixture of chemicals based on the composition of squid muscle extract is highly attractive to lobsters and it may therefore be possible to include synthetic attractants in prawn diets if any commercial advantage can be shown. Certainly a system of controlled release of attractant might have advantages. Second, the quality of the prawns once produced must be acceptable to the consumer. Once other aspects of compounding and formulation have been resolved this takes on fundamental importance since the objective of any prawn farming operation must be to produce a product which is at least as good as natural supplies. To date there are no published observations on this aspect.

HUSBANDRY CONSIDERATIONS

Another group of factors that must be considered in any general study of diet development and that can have highly important effects on diet performance are various husbandry procedures. These include, particularly, the level at which the food is given with respect to the live-weight of the prawns and the environmental requirements of the prawns for optimum performance. They also include the ease with which a particular ration can be fed automatically and the effect of the diet on the nature and quantity of fecal matter produced since in intensive culture operations, at least, this solid waste has to be removed from the system. In this present review I propose only to consider the first question, namely, feeding level.

In the production of trout it is well established that feeding the fish to satiation does not result in optimum performance in terms of the balance between maximum growth and optimum conversion efficiency. Accordingly various feeding tables and equations have been developed to enable the trout farmer to calculate the correct feeding level for his stock based on the water temperature and mean live weight of the fish. In the field of prawn nutrition we are a long way from such sophistication, but as successful diets for prawns are developed, it will

be necessary to obtain this information and in my view this is a priority for further study. Such data as is presently available in the literature on feeding levels for prawns is summarized in Table 6. The levels quoted are those on which fastest growth was obtained rather than those on which the best balance was achieved between growth and conversion. The values show, however, that feeding level will decrease with increasing size, as might be expected, and they provide a starting point for more definitive work in this area.

COMPOUNDING CONSIDERATIONS

The last aspect of diet preparation I wish to discuss I have termed 'Compounding considerations' in general. Included under this heading are the degree of milling required, the nature of the final product (in particular whether there is any need to prepare moist as opposed to dry diets), and water stability of the ration. The first and second of the aspects have been partly considered in the section 'Diet evaluation.' Research is required to establish whether there are any advantages in milling the ingredients especially fine or in preparing moist diets. If any advantages are found they will have to be balanced against the increased difficulties of compounding the diet.

The third point — the question of water stability — has received considerable attention (Forster, 1972; Meyers and Zein-Eldin, 1972; and Balazs, Ross and Brooks, 1973) and there are now a range of additives which can be used to increase the water stability of dry or moist feeds. Many rations have also been successfully bound by simply moistening the dry mix, extruding this through some form of die and oven drying. In this way the various naturally glutinous materials in the diet will confer an adequate degree of water stability. The need for such binding of the food ingredients derives from the prawns' slow feeding response and their habit of partially masticating their food outside the buccal cavity prior to ingestion.

Although methods for achieving the necessary degree of water stability present no great problems, the likely effects on production costs of preparing food pellets in an unconventional way has not yet been considered. There is much to be said for having a formulation that can be mixed and pelleted by conventional milling equipment since any requirement for unorthodox processing will increase costs and restrict the number of food compounders who are able to prepare the diet.

In this context it would be interesting to look at the technique of dry pellet expansion, used in various forms of food processing for both

TABLE 6

Summary of data on feeding levels for prawns which have given good growth

Author	Species	Main Diet Ingredient	Temperature (°C)	Size (g)	Food Fed Per Day (% of Body Weight)
Deshimaru and Shigeno 1972	<u>Penaeus japonicus</u>	Squid Meal	23 - 28	3.0	2 - 4
Liao 1969	<u>Penaeus japonicus</u>	Short-necked Clam (Wet)	25 - 30	1.0 8.0 18.0	34 10 7
Sick, Andrews and White 1972	<u>Penaeus aztecus</u>	Shrimp Meal	25 - 28	4.0 - 10.6	15
Venkataramiah, Lakshmi and Gunter 1974	<u>Penaeus aztecus</u>	? Dry Diet	26	0.16 1.52	40 8.1

animal and human foods (the same process is sometimes called cooking extrusion). Equipment for this process is now commonly available and one of the trout foods currently used in the United Kingdom employs this technique. The pellets produced are hard, easy to handle, extremely water stable and are made at little extra cost over traditional feed pellets.

Finally under the heading of 'Compounding considerations' must be added the ease of storage and handling. On a large scale any prepared food which is easily and inexpensively stored and conveniently handled offers considerable advantages. Any requirements for chilled storage or on-site preparation, such as mixing of moist diets, will inevitably increase overall production costs.

COST

Finally and by way of a summary, I would like to discuss the likely cost of prawn diets. It is tempting to suggest that these might be of the same order as the costs of some of the higher quality fish foods such as those for rainbow trout. However, it seems to me when looking down the list of factors in Table 1 and considering the information on prawn nutrition presently available, that overall feed costs for prawns are likely to be somewhat higher than for rainbow trout.

The available evidence suggests that protein requirements for prawns will probably be as high if not higher than for trout and some fairly costly ingredients such as shrimp meal or even squid meal may be required. Also the necessity for relatively water stable feeds for prawns will almost certainly involve some additional compounding costs. In addition it seems unlikely that conversion efficiencies in prawns will ever be as low as those achieved in trout farming. This is partly because of wastage implicit in the prawn habit of moulting and also because of the likelihood of food wastage during the initial feeding process. The limited data presently available suggest a food conversion ration of about 2:1 as being a reasonable target.

The general impression therefore is that feeding costs for prawns may initially be as much as 50% higher than for rainbow trout and although costs might be reduced through further research and development, there are intrinsic problems in prawn cultivation which will always ensure there is some differential.

REFERENCES

- Andrews, J. W., L. V. Sick, and G. J. Baptist. 1972. The influence of dietary protein and energy levels on growth and survival of penaeid shrimp. *Aquaculture* 1, p. 341-347.
- Balazs, G. H., E. Ross, and C. C. Brooks. 1973. Preliminary studies on the preparation and feeding of crustacean diets. *Aquaculture* 2, p. 369-377.
- Caillouet, C. W., J. M. Heinen, and D. C. Tabb. 1972. Pink shrimp feeding experiments with wheat bran. Proc. 3rd Ann. Wkshop Wld Maricult. Soc. St. Petersburg, Florida, USA. Jan. 20-28, p. 415-459.
- Condrey, R. E., J. G. Gosselink, and H. J. Bennett. 1972. Comparison of the assimilation of different diets by *Penaeus setiferus* and *P. aztecus*. *Fishery Bull.* 70 (4) p. 1281-1292.
- Cowey, C. G., J. R. M. Forster. 1971. The essential amino-acid requirements of the prawn, *Palaemon serratus*. The growth of prawns on diets containing proteins of different amino-acid compositions. *Mar. Biol.* 10, (1), p. 77-81.
- Cowey, C. B. and J. R. Sargent. 1972. Fish Nutrition. *Advances Mar. Biol.* 10, p. 383-492.
- Dall, W. 1968. Food and feeding of some Australian penaeid shrimps. *FAO Fish. Rep.*, 57 (2), p. 251-258.
- Deshimaru, O., and Kuroki. 1974. Studies on a purified diet for prawn - 1. Basal composition of diet. *Bull. Jap. Soc. Sci. Fish.*, 40 p. 413-419.
- Deshimaru, O., and K. Shigeno. 1972. Introduction to the artificial diet for prawn *Penaeus japonicus*. *Aquaculture* 1, p. 115-133.
- Forster, J. R. M. 1971. Some methods for binding prawn diets and their effects on growth and assimilation. *J. Cons. perm. int. Expln. Mar.* Vol. 34, No. 2, pp. 40-57.
- Forster, J. R. M. 1972. Studies on compounded diets for prawns. Proc. 3rd Ann. Wkshop Wld. Maricult. Soc., St. Petersburg, Florida, USA. Jan. 26-28, p. 389-402.

- Forster, J. R. M., and T. W. Beard. 1973. Growth experiments with the prawn *Palaemon serratus* Pennant fed with fresh and compounded foods. Fishery Invest., London., Ser. 2, 27, (7), 16 pp.
- Forster, J. R. M., and P. A. Gabbott. 1971. The assimilation of nutrients from compounded diets by the prawns, *Palaemon serratus* and *Pandalus platyoeros*. J. Mar. Biol. Ass. U. K. Vol. 51, No. 4, pp. 943-961.
- Grajcer, D. and R. Neal. 1972. Growth of hatchery reared *Penaeus aztecus* on experimental diets. Proc. 3rd Ann. Wkshop Wld Maricult. Soc. St. Petersburg, Florida, USA. Jan. 20-28, p. 461-470.
- Kanazawa, A., M. Shimaya, M. Kowasaki, and K. Kashiwada. 1970. Nutritional requirements of prawn - 1. Feeding on artificial diet. Bull. Jap. Soc. Sci. Fish. 36, (9), p. 949-954.
- Kitabayashi, K., H. Kurata, K. Shudo, K. Nakamura, and S. Ishikawa. 1971. Studies on formula feed for Kuruma prawn - 1. On the relationship among glucosamine, phosphorus and calcium. Bull. Tokai, Reg. Fish. Res. Lab. 65, p. 91-107.
- Kitabayashi, K., K. Shudo, K. Nakamura, and S. Ishikawa. 1971. Studies on formula feed for Kuruma prawn - II. On the utilization values of glucose. Bull. Tokai Reg. Fish. Res. Lab. 65, p. 109-118.
- Kitabayashi, K., K. Shudo, K. Nakamura, and S. Ishikawa. 1971. Studies on formula feed for Kuruma prawn - III. On the growth promoting effects of both arginine and methionine. Bull. Tokai Reg. Fish. Res. Lab. 65, p. 119-127.
- Lee, D. 1970. Study on digestion and absorption of protein in artificial feeds by four species of shrimps. China Fish. Monthly, 208, p. 2-4.
- Lee, D. 1971. Studies on the protein utilization related to growth of *Penaeus monodon* Fabricius. Aquiculture 1, (4), p. 1-13.
- Mackie, A. M. 1973. The chemical basis of food detection in the lobster *Homarus gammarus*. Mar. Biol. 21, p. 103-108.
- Meyers, S. P. and Z. P. Zein-Eldin. 1972. Binders and pellet stability in development of crustacean diets. Proc. 3rd Ann. Wkshop Wld Maricult. Soc., St. Petersburg, Florida, USA. Jan. 26-28, p. 361-364.

- Nose, T. 1964. Protein digestibility of several test diets in cray and prawn fish. Bull. Freshwater Fish. Res. Lab. Vol. 14, No. 1, p. 23-28.
- Patwardhan, S. S. 1935a. On the structure and mechanism of the gastric mill in Decapoda v. The structure of the gastric mill in Natantous Macrura - Caridea. Proc. Indian Acad. Sci., Ser. B 1, p. 693-704.
- Patwardhan, S. S. 1935b. On the structure and mechanism of the gastric mill in Decapoda. vi. The structure of the gastric mill in Natantous Macrura - Penaeidea and stenopidea; conclusion. Proc. Indian Acad. Sci., Ser. B 2, p. 155-174.
- Shewbart, K. L., W. L. Mies, and P. D. Ludwig. 1973. Nutritional requirements of brown shrimp *Penaeus aztecus*. Texas A&M Sea Grant Program. TAMU - SG - 73-205, 53 pp.
- Shigeno, K. 1975. Shrimp Culture in Japan. Publ. Association for International Technical Promotion, Tokyo 153 pp.
- Shudo, K., K. Nakamura, S. Ishikawa, and K. Kitabayashi. 1971. Studies on formula feed for Karuma prawn - IV. On growth - promoting effects of both squid liver oil and cholesterol. Bull. Tokai Reg. Fish. Res. Lab. 65, p. 129-137.
- Sick, L. V. and J. W. Andrews. 1973. The effect of selected dietary lipids, carbohydrates and proteins on the growth, survival and body composition of *Penaeus duorarum*. Proc. 4th Ann. Wkshop Wild Maricult. Soc. Monterrey, Mexico. Jan. 23-26, p. 263-276.
- Sick, L. V., S. W. Andrews, and D. B. White. 1972. Preliminary studies of selected environmental and nutritional requirements for the culture of penaeid shrimp. Fish. Bull. 70(1): 101-109.
- Snow, N. B. 1969. Studies on the nutrition, metabolism and energetics of the prawn *Palaemonetes varians* (Leach). Ph.D. Thesis. The University of Southampton, December 1969, pp. 220.
- Venkataramiah, A., G. J. Lakshmi, and P. Gunter. 1974. Studies on the effects of salinity and temperature on the commercial shrimp *Penaeus aztecus* Ives, with special regard to survival limits, growth, oxygen consumption and ionic regulation. US Army Engineer Waterways, Exp. Sta. Contract H.74.2, 130 pp.

Wickins, J. F. 197-. Prawn culture. *Oceanography and Marine Biology: An Annual Review*. In preparation.

Zein-Eldin, Z. P., and S. P. Meyers. 1973. General consideration of problems in shrimp nutrition. *Proc. 4th Ann. Wkshop Wld Maricult. Soc., Monterrey, Mexico Jan. 23-26*, p. 299-317.

Food and Growth of Penaeid Shrimp

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PROBLEMS IN POSTLARVAL PENAID SHRIMP PRODUCTION

The larvae of penaeid shrimp species hatch out as nauplii after the eggs have been spawned into seawater and metamorphose into zoea, mysis, and postlarval stages. The nauplius stage is strong because for nutrition it depends on the yolk carried in the body. The zoea ingests microscopic phytoplankton food, therefore, rearing zoea is a difficult problem. The mysis can take planktonic animals as well as phytoplankton, therefore, rearing larvae after mysis stage becomes easier.

Hudinaga succeeded in rearing a number of zoea of Kuruma shrimp, *Penaeus japonicus*, by supplying the cultured marine diatom, *Skeletonema costatum*, in 1938. The survival rates of eggs and larvae reported by Hudinaga were as follows: 70.6% at egg stage, 92.7% at nauplius stage, 27.9% at zoea stage, 86.5% at mysis stage, and 84.5% at postlarva (P₁-P₁₂) stage.

Thus, Hudinaga showed the feasibility of culturing Kuruma shrimp. However, the research was suspended because of World War II. After the war, Hudinaga continued the research and developed a method to produce postlarval penaeid shrimp using a tank (dimensions: 2m x 2m x 1m deep) with aeration. Cultured *Skeletonema costatum*, brine shrimp, *Artemia salina*, and baby neck clam meat were used as food for the zoea stage, the mysis and early postlarva stage, and the advanced postlarva stage, respectively.

Based on this method, the Kuruma Shrimp Farming Company was established at the site of an abolished salt field at Takamatsu, Japan in 1960. The facilities of the hatchery included 70 indoor concrete tanks for culturing *Skeletonema* (dimensions: 4m x 1m x 1m deep or 4m x

2m x 1m deep), 140 indoor wooden tanks for hatching (dimensions: 2m x 1m x 1m deep), and 104 outdoor concrete tanks for rearing advanced postlarva (dimensions: 3m x 1.5m x 0.5m deep).

The total number of baby shrimp produced annually increased from two million in 1960 to four million in 1961, and six million in 1962. However, the number of baby shrimp produced per female shrimp decreased from 1,260 in 1960 to 1,110 in 1961, and 630 in 1962. This indicates that the method of production (conventional method) is inadequate for supplying the increased demand for postlarval shrimp.

A serious problem was the difficulty in culturing *Skeletonema* in the summer season. Furthermore, even when an excellent culture of *Skeletonema* was introduced into the hatching tank, the physiological condition of *Skeletonema* became worse and the water quality of the tank deteriorated. This resulted in a serious mortality of larvae. Accordingly the decision was made to find other foods effective for the zoea stage. Another problem was the unexpected poor survival rate in the early postlarval stage, although *Artemia salina*, which is the best food for the stage, was used. Therefore, a decision was made to establish a better rearing method for postlarvae.

SURVIVAL AND GROWTH OF PENAEID SHRIMP LARVAE FED VARIOUS FOODS

Using various foods, we conducted a series of feeding experiments on the larvae of Kuruma shrimp in 1960, 1961, and 1962. The results are shown in Table 1.

It was shown that there are many effective foods. It was widely believed that zoea larvae depend on vegetable food such as diatoms for nutrition. However a better survival rate was shown for the zoea larvae fed oyster eggs compared to those fed cultured *Skeletonema costatum*. Most bivalve eggs and larvae may be usable from a nutritional viewpoint. Oyster eggs are particularly suitable for practical culture because they are available in quantity during the spawning season of Kuruma shrimp. However, when the spawning of Kuruma shrimp is concentrated over a relatively short period, it is difficult to supply the necessary amount of oyster eggs to the larvae.

The idea of using frozen oyster eggs was put into practice in 1961. However, the survival rate of zoea and mysis fed frozen oyster eggs was poor compared to that of larvae fed live oyster eggs because of a decrease in both nutritional value and buoyancy.

TABLE 1

Relation between developmental stages of Penaeus japonicus
and food suitable for culture

Kind	Zoea	Mysis	Stage of larvae	
			Early Postlarva	Advanced Postlarva
(Vegetable matter)				
Cultured <u>Skeletonema costatum</u>	S	+		
Cultured <u>Chaetoceros</u> sp.	-	-		
Planctonic diatoms	+	-		
Benthic diatoms	+	+	-	+
Cultured <u>Chlamydomonas</u> sp.	--			
Juice of <u>Ulva</u> sp. or eelgrass	--			
(Animal matter)				
Eggs of oyster	+	+		
Eggs of <u>Mytilus</u> sp.	+	+		
Veliger larvae of oyster	+	+		
Sperm of oyster	--			
Frozen eggs of oyster	+	-		
Cultured brackish rotifer	+	+	+	-
Emerged <u>Artemia salina</u>		S	S	
Cultured <u>Moina</u> sp.			-	
Copepoda			+	+
Frozen copepoda			-	
Gammarus				-
Frozen <u>Gammarus</u> sp.				+
<u>Balanus</u> sp.			+	+
Nematoda			--	
Annelida			-	+
Baby neck clam meat			+	S
Oyster meat				--
Trash shrimp meat				+
(Others)				
Artificial food				+
Yeast	--			
Fungi	--			
Fermentation waste	--			
Milk, Egg yolk	--			

Note

+: better than standard

S: standard food

-: poorer than standard, but usable

--: not effective

(From Hudinaga and Kittaka, 1966)

At that time a growing experiment was conducted using a pond of double bottom structure. Benthic diatoms satisfactory for promoting rapid larval growth were propagated in great quantities in the pond water because of the abundant nutrients and sunlight. The processing of benthic diatoms for feeding is as follows: Large concentrations of benthic diatoms floating on the surface were scooped, put in a container, vigorously stirred with seawater, and then filtered through a 100 to 180 mesh synthetic fiber netting.

The survival rate of zoea and mysis fed cultured Rotifer *Brachionus plicatilis* was poor, although in the experiment using small aquaria, cultured *Brachionus* was found to be an excellent food for zoea, mysis, and early postlarvae. Three culture tanks of *B. plicatilis* were provided for one shrimp larvae tank. This fact indicates that *B. plicatilis* is an unsatisfactory food unless mass culture of it is achieved.

The survival rate of early stage postlarvae was extremely poor when fed various foods, such as benthic diatoms, copepoda, nematoda, and annelida beginning at P₁ or P₂ stage. However, the survival rate was unsatisfactory (average 60%) even when fed a diet of *Artemia salina*. This is primarily because of the deterioration of water quality. The problem remained unsolved until the large scale production method was established in 1964 by Kittaka.

The food suitable for penaeid shrimp (*P. japonicus*) culture is shown diagrammatically in Figure 1.

DEVELOPMENT OF LARGE SCALE PRODUCTION

In our early studies, 1960 to 1963, we aimed to establish techniques for rearing Kuruma shrimp larvae under complete control. The hatching method for this goal was developed based on the following biological view:

- (1) The use of filtered seawater in order to avoid introduction and propagation of any marine organisms in the tank;
- (2) Installing the indoor tank and controlling the illumination of the tank;
- (3) The use of pure cultured *Skeletonema costatum* for zoea stage larvae.

However, through many experiments and experiences, the initial views were modified accordingly:

- (1) The use of raw seawater filtered through a fine mesh synthetic fiber netting;

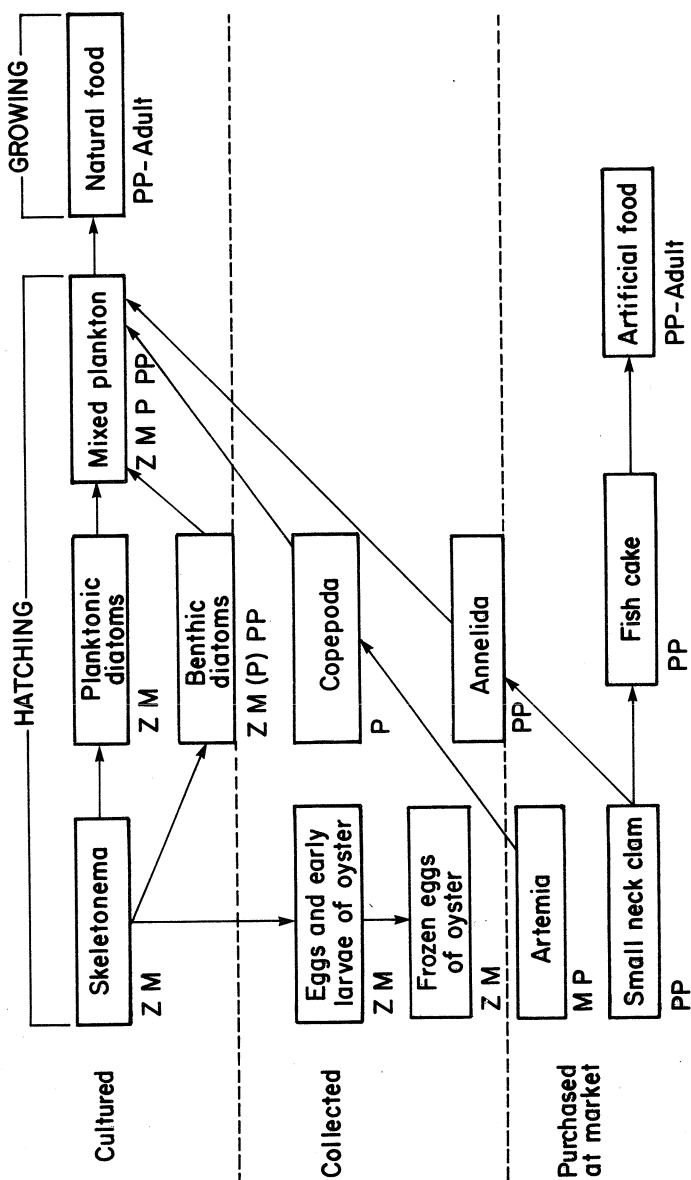


Figure 1. Diagram showing the relation between the developmental stages of *Penaeus japonicus* and the food suitable for practical fish culture. Methods of obtaining the food material are given on the left.

Abbreviations:

Z: Zoea stage

M: Mysis stage

P: Early stage of post larva

PP: Advanced stage of post larva

- (2) Propagating natural foods, such as diatoms, copepods, polychaetes, etc. by fertilizing;
- (3) The use of outdoor tanks, which have direct sunlight.

There were many salt concentration tanks at the abolished salt field in Yamaguchi Prefecture, Japan. The dimensions of the tank were 10m x 10m x 2m deep. Based on the above mentioned ideas, a hatching experiment was conducted during the period July to September, 1964, using 12 tanks with one biologist and three associates. Total number of larval shrimp produced was 13.5 million. Survival rate from nauplius to larval shrimp was 17.4%. The number of larval shrimp produced per female shrimp was 8,100.

In 1962 using the conventional method and employing seven biologists and 10 associates, the total number of larval shrimp produced was six million. Survival rate from nauplius to zoea was only 2.17%. The number of larval shrimp produced per female shrimp was only 630 (See Table 2).

An important aspect of the new method (the large scale method) was the use of fertilization in the tank water to promote plankton growth. Larval shrimp production using this method is diagrammatically compared with the conventional method in Figure 2.

The large scale production method introduces a maximal use of natural conditions. Results of hatching by this method are to some extent affected by local and seasonal conditions. Therefore, some improvement may be necessary depending on the conditions of the hatchery site.

Since 1965 the large scale method has become popular in foreign countries as well as in Japan. *Penaeus monodon*, *P. indicus*, and *Metapenaeus monoceros* were successfully hatched in Southeast Asian countries by this method. Brown shrimp, *Penaeus aztecus*, white shrimp, *P. setiferus*, and pink shrimp, *P. duorarum* have been also cultured since 1970 at a commercial hatchery along the Gulf of Mexico coast. Using basically the same method the hatchery has been able to produce a sufficient number of larval shrimp. Thirty-two tanks were used for the production. Survival rate of larvae and number of baby shrimp produced per tank, and growth rate of postlarval shrimp are shown in Tables 2 and 3, respectively.

Both a better survival rate and higher productivity were found in penaeid shrimp from the Gulf of Mexico than those found in the Kuruma shrimp of Japan. Furthermore, both the survival rate and growth rate

TABLE 2
Survival rate and number of larval shrimp per female shrimp

Site	Year	Kind	No. of females	No. of nauplius (million)	Survival rate (%)	No. of larvae per female	No. of larval shrimp (million)
Takamatsu	1960	Kuruma	1,774	197	1.13	1,260	2.24
Takamatsu	1961	Kuruma	3,604	538	0.74	1,110	4.00
Takamatsu	1962	Kuruma	9,364	754	0.78	630	5.92
Aio	1964	Kuruma	1,669	77	17.4	8,120	13.55
Panama City	1972	White	18,215	283	64.0	10,400	181.23
Panama City	1972	Pink	5,551	379	45.0	33,100	170.75
Panama City	1972	Brown	6,317	116	14.0	3,400	16.32

(Compiled by Kittaka)

TABLE 3
Growth rate of postlarval shrimp (Average, mg)

Stage	Kuruma (<u>P. japonicus</u>)	White (<u>P. setiferus</u>)	Pink (<u>P. duorarum</u>)	Brown (<u>P. aztecus</u>)
P10	2.5	3.2	3.2	2.7
P12		3.6	3.6	3.2
P14		5.6	4.3	3.6
P16	4.6	7.0	5.6	5.0
P17		7.2	5.8	6.4
P19	6.5		6.5	10.0
Total No. of larval shrimp (million)	1.2	3.3	2.0	2.1
Food	Baby neck clam	D-3	D-3	D-3

(From Kittaka, unpublished)

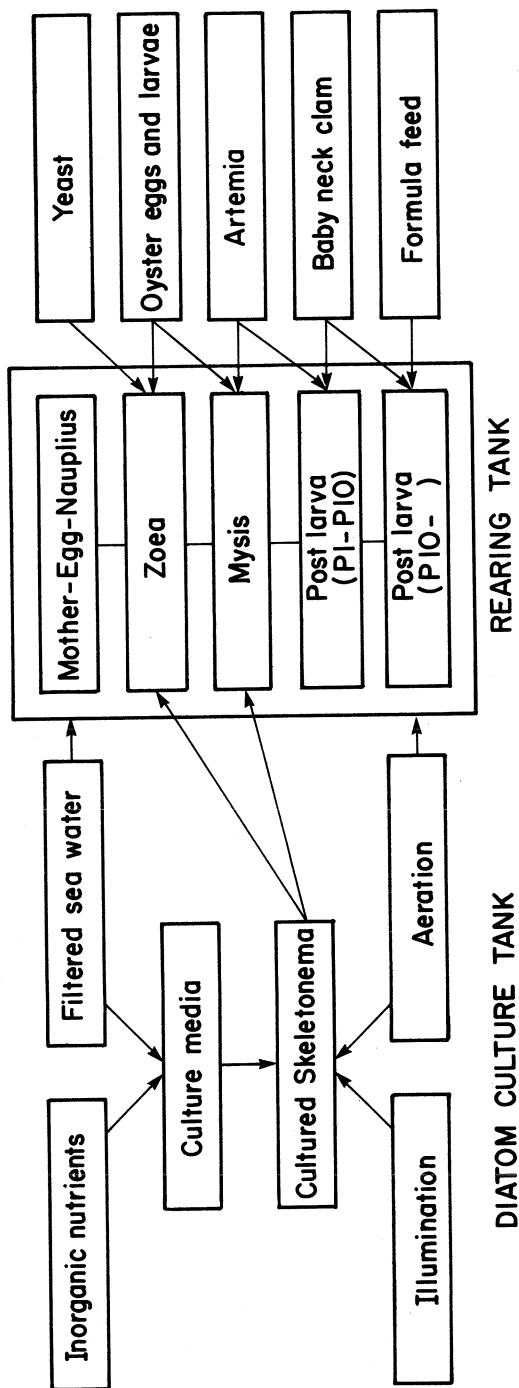


Figure 2-1. Diagram showing the conventional production method of penaeid shrimp larvae.

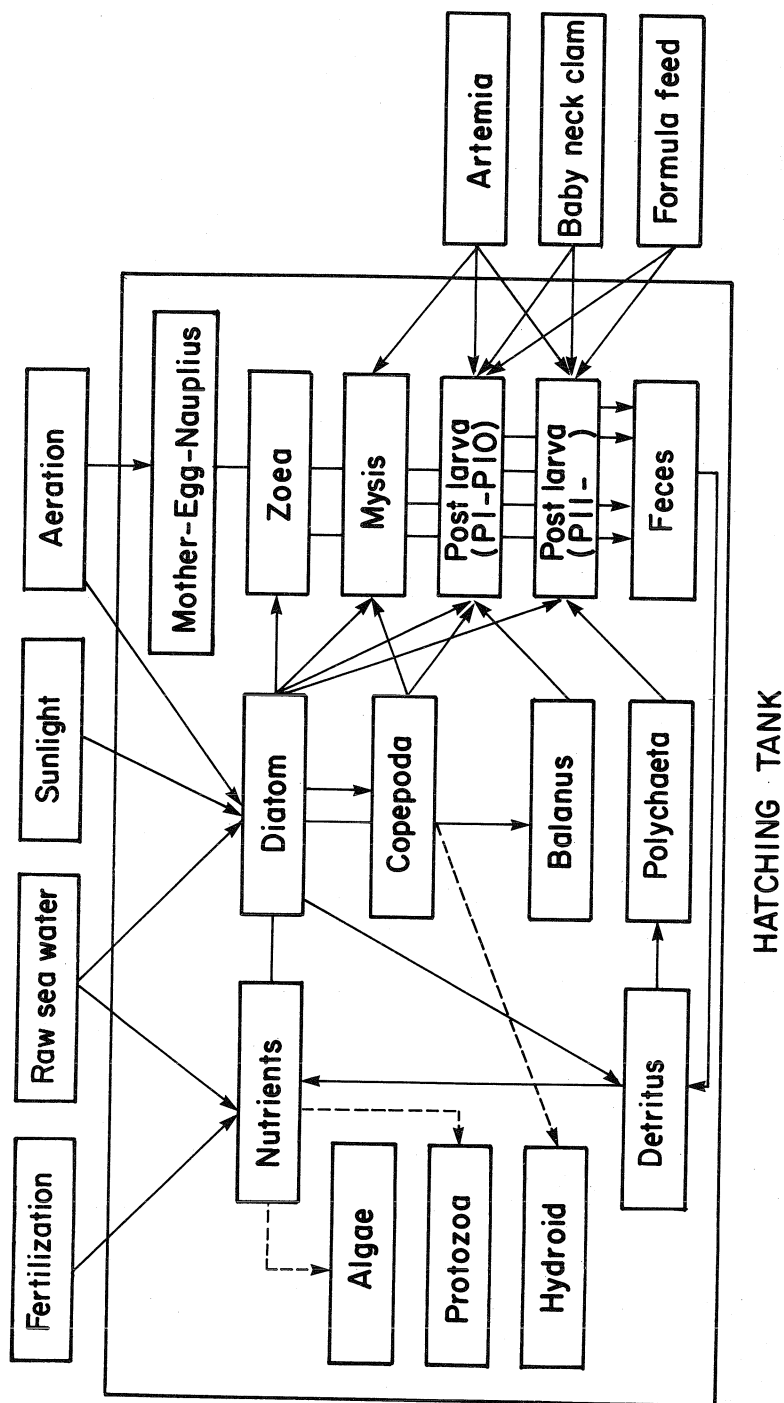


Figure 2-2. Diagram showing the large scale production method of penaeid shrimp larvae.

of white shrimp, *P. setiferus*, were better compared to those of brown shrimp, *P. aztecus*, and pink shrimp, *P. duorarum*. This fact is valuable in the selection of suitable species for practical penaeid shrimp culture.

PROBLEMS IN GROWING PENAEID SHRIMP

Penaeid shrimp farming has been practiced extensively in most coastal countries in Southeast Asia for a very long time, using juvenile shrimp collected from the adjacent sea. Attempts to culture penaeid shrimp intensively have been made in Japan since 1960, using hatchery-raised postlarval shrimp. In spite of the outstanding success in larval shrimp production, progress in growing shrimp is very slow. The production figures are shown in Table 4.

In the case of intensive culture, productivities of 1.4 and 2.5 kg/m² were shown for 1965 and 1966 using tanks (dimensions: 10m x 10m x 2m deep), designed for larval shrimp production. Recently, a large circular tank (area 400 m² x 2.5m deep) was constructed for growing shrimp exclusively. However, the productivity showed no significant increase in this tank compared to that shown in the hatching tank of standard size.

In the case of extensive culture the productivity was 0.25 kg/m² in the tidal ponds in Japan (area: 30,000 to 100,000 m²), 0.070 kg/m² in the ponds constructed in marsh areas in the U.S.A. (area: about 1,200,000 m²), and 0.028 kg/m² in a netted-off portion of a bay in the U.S.A. (area: 4,000,000 - 6,800,000 m²).

It is apparent that the productivity of penaeid shrimp is significantly lower when compared to productivity in fish culture. Furthermore, most cultured fish show a better growth in captivity with feeding compared to the growth found in nature. However, in the case of penaeid shrimp a poorer growth rate was shown in captivity, regardless of feeding, compared to that found in nature. This fact indicates that both the environmental and nutritional conditions in captivity are unsuitable for growth of penaeid shrimp.

KIND OF FOOD AND GROWTH OF KURUMA SHRIMP, *P. JAPONICUS*

Kuruma shrimp seem to eat various kinds of benthic animals in nature, but in growing ponds where the densities of shrimp are much higher than in nature it is necessary to supplement the feed. Kuruma shrimp will eat any kind of food that is of animal origin or that has an animal taste (non-animal, but soaked in an animal substance-rlm). They

TABLE 4

Site	Year	Acreage (m ²)	Total No.	Harvest Total wt. (ton)	No./m ²	g/m ²	Growing Method
(Extensive culture)							
Takamatsu, Japan	1962	1,250	16,200	0.14	13.0	114.0	Pumping
"	"	1,250	13,800	0.11	11.0	93.8	
Aio, Japan	1963	30,000	529,000	6.66	17.6	222.0	
"	1964	35,000	440,000	5.14	12.6	146.9	Tidal Pond
"	"	30,000	718,000	8.82	16.0	294.0	
CP, U.S.A.	1971	1,360,000	5,050,000	43.96	3.7	32.3	Pumping
"	1972	"	4,310,000	40.90	3.2	30.1	
"	1973	"	19,210,000	95.04	14.1	69.9	
NP, U.S.A.	1971	1,160,000	4,820,000	32.30	4.2	27.8	Pumping
"	1972	"	5,530,000	44.17	4.8	38.1	
"	1973	"	13,774,000	46.35	11.9	40.0	
WB U.S.A.	1971	4,056,000	13,170,000	112.46	3.2	27.7	Netting off a bay
"	1972	6,800,000	4,100,000	42.55	0.6	6.2	
"	1973	5,400,000	8,260,000	45.83	1.5	8.5	
(Intensive culture)							
Takamatsu, Japan	1962	552	18,200	0.183	33	333	Double bottom
Aio, Japan	1965	100	8,000	0.144	80	1,444	Hatching tank
"	1966	100	14,800	0.250	148	2,500	
Kagoshima, Japan	1970	400	74,400	1.060	186	2,650	Circular tank

Species of shrimp: P. japonicus in Japan, P. setiferus, P. aztecus, and P. duorarum in U.S.A.

Site: CP (Central Pond) and NP (North Pond), near St. Andrews Bay, Florida

WB (West Bay), a netted-off portion of St. Andrews Bay, Florida

are especially fond of baby-neck clams, *Mytilus sp.*, etc., but from the viewpoint of preparation of necessary foods in commercial growing we must use frozen or artificial food rather than bivalves. The problem of food must be looked at basically from the point of view of the nutritional requirements of penaeid shrimp, but at present these are poorly understood. Thus, this paper will discuss food value from a practical view, based on results obtained from feeding experiments in tanks 2m x 1m x 1m deep.

Fresh food and frozen food. Table 5 shows the results of feeding experiments in which *Mytilus sp.* crushed just prior to feeding or frozen trash shrimp were fed at a daily rate of about 20% of the body weight of the shrimp.

When rearing conditions were the same, both the growth rate and survival rate were better in tanks where fresh food rather than frozen food was fed. When water temperatures were lower, the difference in growth and survival rates between shrimp fed the two foods was not so noticeable. However, when water temperatures were about 30° both a remarkable mortality and retarded growth were observed one month after the start of the experiments among shrimp fed the frozen food. Accordingly, it is not advisable to feed only frozen food in seasons of high water temperature.

Table 6 shows the results of feeding experiments in which baby shrimp were fed fresh food (*Mytilus* crushed just prior to feeding) and frozen food (frozen Mysidicae) at water temperatures of 26°-29°.

The growth of shrimp fed fresh food was from 0.16g (initial size) to 5.6 -10.8g during a 2.5 month period. The growth of those fed frozen food was to 2.9 -3.3g in the same period.

Fresh food and frozen food in combination. The reason for the retarded growth of Kuruma shrimp fed frozen food is considered to be either a nutritional imbalance in trash shrimp and Mysidicae, or a change in the quality of the food during storage. If shrimp are fed frozen food and fresh food in combination, the poor results obtained from only frozen food are improved, which is very advantageous in practice.

The results of feeding experiments using fresh and frozen food in combination are shown in Table 7.

The growth rate of shrimp fed less than 75% frozen food and more than 25% fresh food is not significantly different from those fed 100% fresh food. When the rate of frozen food is increased, that is, frozen food 90% and fresh food 10%, growth becomes a little slower. Accordingly, the unfavorable results from shrimp fed frozen food only

TABLE 5

Growth rate of Kuruma shrimp fed fresh food and frozen food separately

No. of experiment	Food	Experimental period (days)	Avg. body wt. (g) Initial	Avg. body wt. (g) Final	Growth rate (%)	Specific growth rate (%)	No. of shrimp Initial	Survival rate (%)
1	Fresh	32	4.80	10.25	113.5	100	200	59
	Frozen	32	4.65	8.00	72.0	63	200	43
2	Fresh	32	4.70	12.76	171.5	100	200	79.5
	Frozen	32	4.75	7.00	47.3	28	200	19
3	Fresh	60	4.55	9.89	117.3	100	200	85
	Frozen	60	4.40	7.93	80.2	68	200	75
4	Fresh	50	5.70	13.47	136.3	100	200	36
	Frozen	50	5.70	8.50	49.1	28	200	5

Experimental conditions:

No. of exp.	Tank structure	WT (°C)	O ₂ (cc/e)	Rate of changing water
1	Standard	30	3 - 4	1/4
2	Double bottom	30	3 - 4	1/48
3	"	25 - 30	3 - 4	1/48
4	"	30	2 - 3	1/48

Tank dimensions: 2m x 1m x depth 0.5m.

Fresh food: *Mytilus* sp.Frozen food: Small trash shrimp
(From Kittaka, 1971)

TABLE 6
Growth rate of larval Kuruma shrimp fed fresh food and frozen food separately

No. of experiment	Food	Experimental period (days)	Avg. body weight (g) Initial	Final	Growth rate (%)	Specific growth rate (%)	No. of shrimp Initial	Survival rate (%)
1	Fresh	29	0.16	1.56	875	100	600	82.0
	Frozen	29	0.16	0.63	294	34	600	26.5
2	Fresh	73	0.16	8.95	5494	100	300	60.3
	Frozen	73	0.16	2.85	1681	31	300	54.9
3	Fresh	73	0.16	10.78	6638	100	150	59.3
	Frozen	73	0.16	3.33	1981	29	150	12.0

Tank structure: Double bottom
 Tank dimensions: 2m x 1m x depth 0.5m
 Water temperature: 26 - 29°C
 Rate of changing water per hour: 1/48
 Fresh food: Mytilus sp.
 Frozen food: mysids
 (From Kittaka, 1971)

TABLE 7

Growth rate of Kuruma shrimp fed fresh food and frozen food separately and in combination

No. of experiment	Food (%)		Experimental period (days)	Avg. body weight (g)		Growth rate (%)	Specific growth rate (%)	No. of shrimp		Survival rate (%)
	Fresh	Frozen		Initial	Final			Initial		
1	100	0	32	8.70	14.28	64.1	100	100		70
	75	25	32	9.30	13.68	47.1	73	100		57
	50	50	32	9.20	14.74	60.4	94	100		79
	25	75	32	8.90	14.30	60.6	94	100		80
	0	100	32	8.80	10.00	13.6	21	100		22
2	20	80	49	5.70	10.11	77.4	100	100		89
	10	90	49	5.50	8.61	56.4	73	100		76
3	75	25	51	11.38	17.43	53.1	100	80		90.0
	50	50	51	11.93	19.12	60.3	114	70		80.1
	25	75	51	11.85	17.91	51.1	96	81		88.9

Tank structure: Double bottom

Tank dimensions: 2m x 1m x depth 0.7m

Water temperature: 26 - 29°C (Exp. No. 1), 29°C (Exp. No. 2 and 3)

Rate of changing water: 1/48

Fresh food: *Mytilus* sp. (Exp. No. 1), Baby neck clam (Exp. No. 2 and 3)Frozen food: *Mysids* (Exp. No. 1), Small trash shrimp (Exp. No. 2 and 3)
(From Kittaka, 1971)

seem to be improved by feeding at a rate of more than 25% fresh food and less than 75% frozen food.

Artificial food- Squid soluble. As mentioned above, bivalves have excellent characteristics as food for Kuruma shrimp, but if we depend only on bivalves it may be difficult to expand the scale of growing because of the restriction of keeping food. Therefore, it is desirable to develop other kinds of food, especially pelletized food.

The growth of Kuruma shrimp fed pelletized food¹ in which the major component was fish meal was not very good as shown in Table 8.

There was no difference in the effect on body weight increase even if the pellet was made more attractive by immersing it in the juice of baby-neck clam meat. However, growth became better when shrimp were fed pelletized food and baby-neck clam in combination (pellet 75%, clam 25%). The value of the food conversion ratio, however, was much poorer in comparison to the food conversion ratio for fish culture where this type of pellet is normally used. So it is necessary to improve the components and form of the pellet for practical use.

Thus the growth of Kuruma shrimp is poorer when the shrimp are fed pelletized food made from fish meal.

Although the digestive process² of Kuruma shrimp has not yet been determined, an artificial food which is composed mainly of marine fish solubles from the viscera of fish may be more digestible.

In 1963, a kind of artificial food³ was manufactured, composed mainly of solubles from the viscera of squid (30%). Added to this was a kind of binder (gluten and poly phosphate), nutritional additives (amino acid and squid extract), an expander (bentnite), and gelatin in order to coagulate it. The results of feeding experiments using this food are shown in Table 9. Good results were obtained experimentally.

Kuruma shrimp have the ability to acclimate to lower water temperature when as a seasonal phenomenon it becomes gradually lower, and are able to withstand down to 5°C before there is mortality. The power to resist lower water temperatures varies depending on food. We have found much more tolerance to lower temperatures in tanks where shrimp were fed both this artificial food and baby-neck clam meat compared to the tanks where they were fed only artificial food or only baby-neck clam (see Table 10).

However, this artificial food tended to dissolve in higher temperature waters in a short time because of the gelatin. It is therefore not practical for use in growing ponds.

When Kuruma shrimp feed, the degree to which the food disperses is greater than it is in the case of fin fish because shrimp feed by using

TABLE 8

Growth rate of Kuruma shrimp fed a formula feed for trout fry

No. of experiment	Food (%)		Avg. body weight (g)		Growth rate (%)	Specific growth rate (%)	No. of shrimp Initial	Survival rate (%)	Conversion factor ***
	Formula feed	Baby neck clam	Initial	Final					
1	100	0	7.86	9.45	20.2	82	145	100	7.12
2	100	*	8.59	10.34	20.4	82	149	99	7.05
3	75	25	7.86	11.14	41.8	169	150	99	3.15
4	0	100	7.79	9.72	24.8	100	145	97	2.01

Experimental period: 18 days

Tank structure: Double bottom

Tank dimensions: 2m x 1m x depth 0.7m Water temperature: 25°C

Total feeding amount (g): 1,568 (No. 1), 1,762 (No. 2), 1,211 + 301** (No. 3), 484** (No. 4)

Total weight increase (g): 22/ (No. 1), 250 (No. 2), 480 (No. 3), 240 (No. 4)

* Immersed in the juice of baby neck clam meat

** Dry weight of baby neck clam meat

*** Conversion factor was calculated at dry weight.

(From Kittaka, 1971)

TABLE 9
Growth rate of Kuruma shrimp fed an artificial food

Food (%)	Baby neck clam	Experimental period (days)	Avg. body weight (g)		Growth rate (%)	Specific growth rate (%)	No. of shrimp Initial	Survival rate (%)	Total feeding amount (g)	Total weight increase (g)	Food Conversion ratio
Artificial food *			Initial	Final							
100	0	77	1.60	8.16	410	98	102	96	4,269	640	6.7
75	25	77	1.50	9.73	547	130	100	97	4,803	705	6.8
50	50	77	1.55	12.20	687	163	100	97	5,189	1,030	5.0
0	100	77	1.55	8.06	420	100	100	93	4,417	595	7.4

Tank structure: Double bottom

Tank dimensions: 2m x 1m x depth 0.7m

Water temperature: 27°C

*Composition: Squid soluble 30%, Bentrinite 15%, amino acid 0.5%, gluten 2%, Gelatine 7%, Polyphosphate 3%, Squid extract 0.5%, and Water 42%

TABLE 10

Survival rate of Kuruma shrimp fed an artificial food during wintering

No. of experiment	Food (%)		No. of shrimp Initial	Mortality (%)	Avg. body weight (g)
	Artificial	Baby neck clam			
1*	100	0	48	35.4	13.2
	75	25	122	23.8	15.0
	50	50	128	21.2	14.3
	0	100	90	32.2	13.4
2**	100	0	48	12.5	14.6
	75	25	49	6.1	16.2
	50	50	49	12.1	16.2
	0	100	45	11.1	14.0

Experimental period: Dec. 28, 1963 – Feb. 18, 1964.

Tank structure: Standard

Tank dimensions: 2m x 1m x depth 0.7m

*Shrimp were reared in the double bottom tank at higher density (75/m²) prior to the experiment**Shrimp were reared in the standard tank at lower density (25/m²) prior to the experiment

many appendages. So it seems that the key to food development in the future is the selection of binder materials.

Natural food. In order to determine the food value of natural food, shrimp in the feeding experiments were fed live natural food. The foods used were restricted to crustacea, such as *Gammarus* sp. *Caprella* sp., etc.. The results are shown in Table 11.

The growth rate was better in the tank where shrimp were fed frozen food than in the tank where shrimp were fed natural food. This seemed to be due to an insufficient amount of food, because the natural food was collected from a restricted area of growing pond drainage ditches in order to find a relation between the area necessary for natural food production and the number of shrimp cultured. It is an important fact that even with the feeding of these restricted kinds of natural foods, body weight increase was almost the same as with the feeding of frozen foods.

KIND OF FOOD AND GROWTH OF BROWN SHRIMP, *P. AZTECUS*, WHITE SHRIMP, *P. SETIFERUS*, AND PINK SHRIMP, *P. DUORARUM*

Marine invertebrates, such as baby neck clam and squid prove to be more effective food for Kuruma shrimp, *P. japonicus*, in Japan. How-

TABLE 11
Growth rate of Kuruma shrimp fed natural food in comparison with frozen food

No. of experiment	Food	Experimental period (days)	Avg. body weight (g)		Growth rate (%)	Specific growth rate (%)	No. of shrimp Initial	Survival rate (%)
1	Natural	64	2.12	7.32	245	89	160	89
	Frozen	64	2.19	8.23	275	100	160	84
2	Natural	64	2.12	5.32	151	87	160	58
	Frozen	64	2.09	5.72	174	100	160	70

Tank structure: Double bottom (Exp. No. 1), Standard (Exp. No. 2)

Tank dimensions: 3m x 1.5m x depth 0.4m

Natural food: Gammarus, Caprella

Frozen food: small trash shrimp

ever, these marine invertebrates are scarcely distributed in some areas. In order to promote penaeid shrimp culture in these areas, it is necessary to find another effective food. Toward this goal, a series of feeding experiments was conducted, using concrete tanks (dimensions: 2.4m x 1.2m x 1m deep) located at a Panama City, Florida shrimp farm in 1970 and 1971.

Squid in comparison with fish. Foods used for the experiment are as follows: trash fish caught in adjacent seas (T.F.); squid obtained at fish stores (S.Q.); pelletized food formulated by the Taiyo Fishery Co. in Japan (Taiyo PLT); pelletized food formulated by the Florida State University (FSU PLT); fish cake formulated by the Kyokuyo Co. in Japan (fish cake); and *Artemia* (Ar).

Major ingredients of Taiyo PLT and Fish cake are squid meal and *Fuhausia* sp. The composition of FSU PLT ingredients was fish meal 77.5%, stickwater 20%, and vitamins 2.5% (C. B. Subrahmanyam and C. H. Oppenheimer, 1970). Brown shrimp *P. aztecus*, was used in this experiment. The growth rate of brown shrimp fed various kinds of food separately and in combination during the 12-week period are shown in Table 12 and Figure 3.

It can be seen from Table 12 and Figure 3 that growth is best in tanks #1 (FSU PLT + SQ + *Artemia salina*) and #7 (TYO PLT + SQ + T.F.), and poorest in tanks #11 (FSU PLT), #10 (T.F. + SQ, Feeding rate 12.5%), and #9 (T.F.). This indicates that the body weight increase is affected by both the nutritional value and the feeding amount of food. FSU PLT contains mainly fish meal. The nutritional deficiency of a formulated diet composed of fish meal was also shown in the previous feeding experiment (See Table 8).

Artemia salina as a booster for the growth of penaeid shrimp. Shrimp fed only Trash Fish (#9) showed the lowest growth rate during the first 4-week period (See Figure 3). The poor growth rate was improved rapidly by the feeding of a constant amount of *A. salina* (0.1 g) per penaeid shrimp per day.

The effectiveness of *A. salina* was also observed in tank #1 fed FSU PLT and Squid in combination. *A. salina* eggs were added directly in tank #1 during the first two weeks and from the fourth to eighth week. The shrimp showed a better growth rate during the experimental period except during the weeks without supplemental *A. salina*.

These facts indicate that *A. salina* is one of the most effective foods not only for the postlarval stage but also for the juvenile stage. The relation between amount of *A. salina* supplemented and growth rate of shrimp is shown in Table 13.

TABLE 12

Growth rate of brown shrimp fed different kinds of food

No. of tank	Food	Avg. body weight (g) Initial	12 weeks	Growth rate (%)	Specific growth rate (%)	No. of shrimp Initial	Survival rate (%)
1	SQ* + Ar + FSU PLT	0.78	8.85	1,035	239	50	92
2	Fish cake + SQ*	0.85	6.08	615	142	50	86
3	Taiyo PLT	1.39	8.02	477	110	50	68
4	T.F + SQ (Feeding rate: 37.5%)	1.40	7.84	460	106	50	66
5	SQ	1.44	7.67	433	100	50	80
6	TF + SQ (Feeding rate: 25%)	1.42	6.09	329	76	50	76
7	T.F + SQ + Taiyo PLT	2.51	10.56	321	74	44	77
8	Fish cake	1.12	4.64	314	72	50	88
9	T.F + Ar**	1.46	5.46	274	63	50	44
10	T.F + SQ (Feeding rate: 12.5%)	1.48	5.19	251	58	50	50
11	FSU PLT	1.39	4.17	200	46	50	92

Experimental period: June 22, 1970 — Sept. 14, 1970

Tank structure: Standard

Tank dimensions: 2.4m x 1.2m x depth 0.8m

* T.F was used from 8th to 12th week

** Ar was supplemented from 5th to 10th week

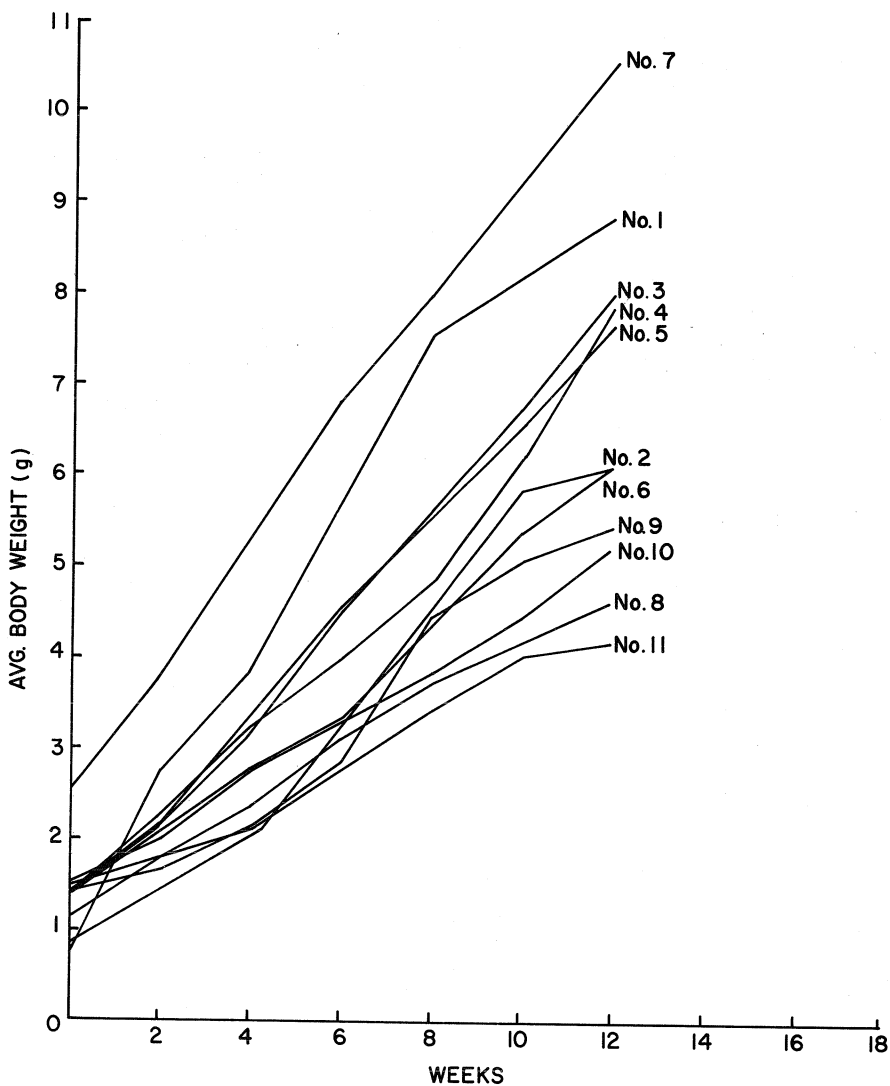


Figure 3. Growth rate of brown shrimp fed various food.

TABLE 13

Relation between amount of *Artemia salina* supplemented and growth rate of brown shrimp

No. of tank	Period (weeks)	Food (g)			Avg. body weight (g)		Growth rate (%)
		T.F	SQ	FSU	Initial	Final	
1*	0-2		88	21	0.78	2.73	251.8
	2-4		224	45	2.73	3.84	40.7
	4-6		358	72	3.84	5.75	49.6
	6-8		555	112	5.75	7.55	31.3
	8-10	620		124	7.55	8.22	8.9
9*	0-2	262		0	1.46	1.81	23.9
	2-4	356		0	1.81	2.11	16.5
	4-6	203		27	2.11	2.86	35.6
	6-8	290		52	2.86	4.44	55.1
	8-10	399		52	4.44	5.07	17.3

* See Table 12

Mysids as a natural food. Although *Artemia salina* is the most excellent live food for the growth of penaeid shrimp, it is never found in the natural environment of shrimp farms. The live food most similar to *A. salina* is considered to be Mysids, because they have a relatively short life cycle and feed preferably on bottom detritus. In order to confirm the food value of mysids a feeding experiment was conducted using brown shrimp, *P. aztecus*, white shrimp, *P. setiferus*, and pink shrimp, *P. duorarum*.

The combination of food used for the experiment was as follows:

- (1) Abundant mysids and Trash Fish
- (2) Abundant mysids and Trash Fish with Taiyo PLT
- (3) Scarce mysids and Trash Fish with Taiyo PLT

The results of the experiment during a 10-week period are shown in Table 14.

A better growth rate was shown in the case with abundant mysids regardless of the feeding of Taiyo PLT. This means that natural food, such as mysids, is more important for the growth of penaeid shrimp.

Artificial food - Shrimp Meal. The preceding experiments indicate that marine invertebrates generally show excellent food value for the growth of penaeid shrimp. However, there is a problem with the availability of marine invertebrates.

The inedible portions of shrimp are usually discarded in processing and can be turned into shrimp meal, which can be considered a possible raw material for an artificial food formula. The following elements were added to the shrimp meal: fish meal, gelatin, corn stick water, vitamins, and minerals. This feed was called MF-3, and its components are shown in Table 15. Results of the feeding experiment for pink shrimp, *P. duorarum*, fed MF-3 are shown in Table 16. The growth rate of shrimp fed MF-3 was 61% of the control fed Taiyo PLT, whereas the growth rate of those fed trash fish was only 18% of the control.

SUPPLEMENTAL FEED – D-3

Attempts have been made to release larval shrimp into natural sea areas, to grow them using natural food and then to harvest adult shrimp at a suitable time. In order to increase the production of shrimp, it is recommended that the natural foods be supplemented by some other feeds. For this reason MF-3 was modified to a more economical feed. The new feed was called D-3, and the ingredients are shown in Table 15.

Food value of a supplemental feed (D-3) for young penaeid shrimp. The growth rate of brown shrimp fed D-3 is shown in Table 17. In com-

TABLE 14

Growth rate of brown, pink and white shrimp fed various food with and without natural food

Kind	Natural food	Food	Avg. body weight (G) Initial	Final	Growth rate (%)	Specific growth rate (%)	Survival rate (%)
Brown	cc	T.F + PLT	0.28	4.15	1,382	152	96.6
	cc	T.F	0.30	3.03	910	100	96.6
	r	T.F + PLT	0.28	2.23	696	76	78.3
Pink	cc	T.F + PLT	0.30	2.66	787	134	90.0
	cc	T.F	0.31	2.12	584	100	83.3
	r	T.F + PLT	0.30	1.62	440	75	91.7
White	cc	T.F + PLT	1.16	4.96	328	149	85.0
	cc	T.F	1.17	3.73	219	100	91.7
	r	T.F + PLT	1.09	3.51	222	99	83.3

Experimental period: 10 weeks for brown and pink, 8 weeks for white

PLT: Taiyo PLT

Natural food: Mysids

cc: 46 - 58 mysids per shrimp per day

r: 4 - 5 mysids per shrimp per day

TABLE 15

Ingredients and composition of shrimp feeds

Ingredients	MF-3	D-3
	(in percentage)	
Shrimp meal	25	33
Squid meal	2	--
Fish meal	49	56
Whey	--	8
Gelatine	10	--
Corn stick water	8	--
Vitamin mixture	5	3
Mineral mixture	1	--
Total	100	100

(Kittaka, unpublished)

parison with the case of shrimp fed Taiyo PLT and trash fish in combination or separately, a good growth rate was shown with D-3. In comparison with the results of the preceding experiment of brown shrimp fed Taiyo PLT (See Table 12 and Figure 3), a better growth rate was also confirmed with the brown shrimp fed D-3 as shown in Table 17.

Food value of a supplemental feed (D-3) for postlarval penaeid shrimp. A further evaluation of the food value of D-3 for postlarval shrimp is needed because the use of the minced baby-neck clam meat is not practical from both nutritional and economical viewpoints. The growth and survival rates of postlarval brown shrimp (*P. aztecus*) fed crushed D-3, crushed Taiyo PLT, and trash fish, separately, during a 4-week period are shown in Table 18.

The body weight increase of postlarvae fed D-3 was about 75% of that fed Taiyo PLT and about 2.5 times of that fed trash fish. A better survival rate was shown in the postlarvae fed D-3, due to the fact that D-3 crumbles relatively rapidly in the water and that postlarvae prefer to feed on crumbled D-3.

Based on the results of this experiment, D-3 has been used as food for postlarval shrimp in hatchery production. Pelletized D-3 was crushed and separated into small and large particles using 16" mesh net. Postlarvae were fed small ones during the early stage (P2-P10) and large ones during the advanced state (P10-on). The amount of food fed to produce one million baby shrimp is shown in Table 19.

TABLE 16

Growth rate of pink shrimp

Food	Avg. body weight (g) Initial	8 weeks	Growth rate (%)	Specific growth rate (%)	Survival rate (%)	Total weight inc. (g)	Feeding amount (g)	Conversion factor
MF-3	0.474	1.952	311	61	88.3	75.01	303.4	4.04
Taiyo PLT	0.389	2.367	508	100	81.7	92.65	208.0	2.24
T.F	0.281	0.639	127	25	95.0	19.57	708.8	36.22

Experimental period: Nov. 30, 1970 — Jan. 25, 1971

Tank structure: Standard

Tank dimensions: 2.4m x 1.2m x depth 0.8m

No. of shrimp at the beginning: 60

TABLE 17

Growth rate of brown shrimp fed D-3

No. of experiment	Food	Avg. body weight (g) Initial	Avg. body weight (g) 6 weeks	Growth rate (%)	Specific growth rate (%)	No. of shrimp Initial	Survival rate (%)	Conversion factor
1	D-3	1.90	5.36	182.0	104	30	83.3	3.68
	Taiyo PLT	1.82	5.14	181.7	100	30	100.0	2.10
	T. F	1.69	2.54	50.7	26	29	93.3	33.0
2	D-3	3.48	6.57	88.6	102	56	88.6	--
	D-3 + T.F	3.69	6.52	76.5	93	43	76.5	--
	Taiyo PLT + T.F	3.04	6.07	99.8	100	57	99.8	--
3	D-3	1.05	5.36	413	135	30	83.3	3.51
4	Taiyo PLT	1.39	5.61	305	100	50	82.0	2.02

Experimental period: Feb. 12 - Mar. 25, 1971 (No. 1)
 Feb. 13 - Mar. 29, 1971 (No. 2)
 Jan. 29 - Mar. 25, 1971 (No. 3)
 June 22 - Aug. 17, 1970 (No. 4)

TABLE 18

Growth rate and survival rate of postlarval Brown shrimp fed D-3

Food	Avg. body weight (mg) Initial	4 weeks	Growth rate (%)	Specific growth rate (%)	Survival rate (%)
D-3	3	46	1,433	77	83.1
Taiyo PLT	3	59	1,866	100	47.8
T.F.	3	20	566	30	64.7

Experimental period: Mar. 31 — Apr. 25, 1971

Tank dimensions: 2.4m x 1.2m x depth 0.8m

Initial No. of shrimp: 2,000

TABLE 19

Necessary amount of food to produce 1 million baby shrimp

Kind	Kuruma	White & Brown	Pink
<u>Artemia salina</u>	5	5	5
Baby neck clam	12*	--	--
D-3	--	11.8	6.3
Survival rate (%)	25	64	45

*Dry weight

(Compiled by Kittaka)

Amount of D-3 per one million larval shrimp was 11.8 kg for white and brown shrimp and 5.7 kg for pink shrimp, whereas amount of baby neck clam meat per one million larval Kuruma shrimp was 16 kg (as dry weight).

Better survival rates were found in white and brown shrimp and pink shrimp compared to Kuruma shrimp. This means that there is a difference in survival rate between penaeid species.

Growing penaeid shrimp with a supplemental feed (D-3). Pelletized food has been used as a supplemental feed in two 300-acre ponds and a 2500-acre netted-off portion of a bay near Panama City, Florida since 1971 (Figure 4). The production of each area is shown in Table 20.

The food conversion ratio of D-3 was 2.27 in the pond and 3.75 in the bay. The recovery rate of shrimp was 45.3% in the former and 13.0% in the latter. The growth rate of penaeid shrimp is depressed in the pond due to the presence of feed competitors, such as sheepshead minnows, grass shrimp, and blue crabs. The survival rate of penaeid shrimp is depressed in the bay due to the escape of shrimp through netting enclosures. When these situations improve, a better food conversion ratio will be shown for penaeid shrimp fed D-3.

THE SIGNIFICANCE OF THE MICRO-ORGANISMS FOR THE GROWTH OF PENAEID SHRIMP

The growth rate of postlarval white shrimp in nature is shown in Figure 5. White shrimp showed a steady growth in both ponds in 1973, although no food supplement was given during the initial 14 days. Apparently the growth of shrimp depended on natural food such as diatoms, copepods, and polychaetes which were propagated in the ponds by fertilizing prior to the release of postlarval shrimp.

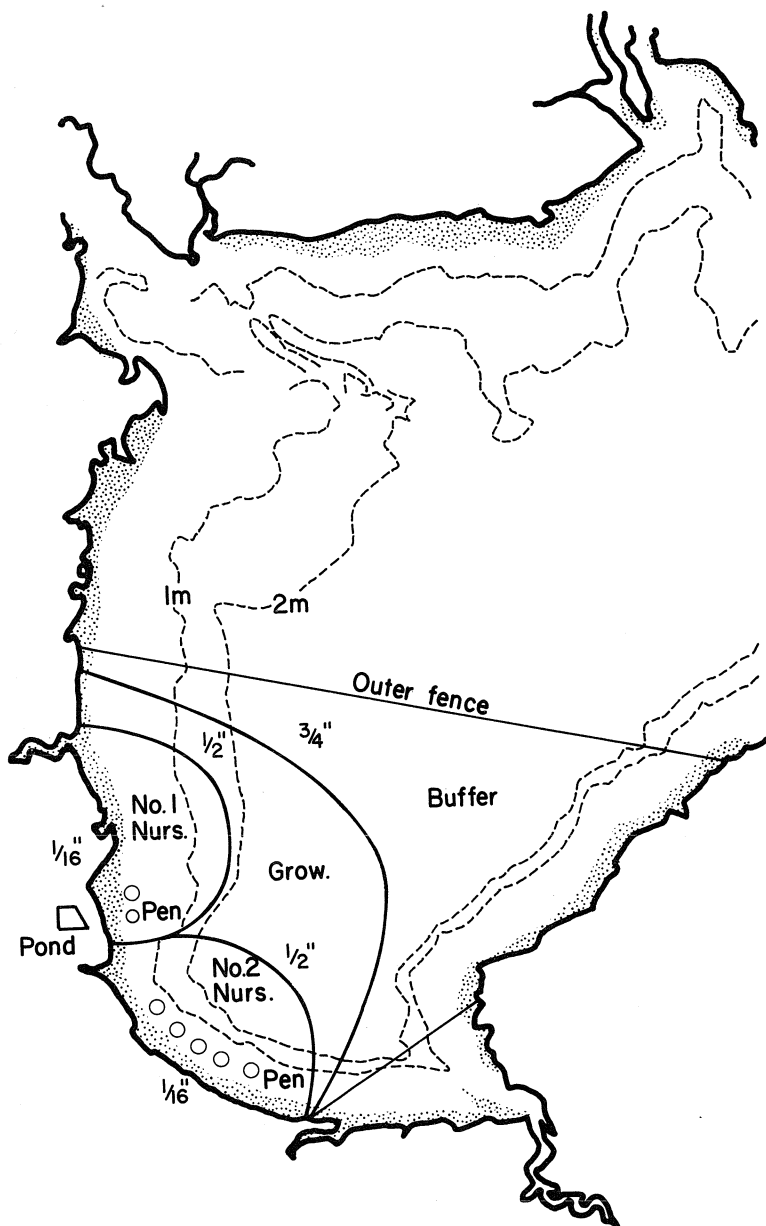


Figure 4. Map showing growing area of West Bay.

TABLE 20

Results of shrimp farming

Area	Year	Kind	No. of shrimp released (million)	No of shrimp harvest (million)	Recovery rate (%)	Total harvest (lbs.)	Amount of D-3 (lbs.)	Conversion factor
CP	1971	Brown	24.05	5.05	21.2	97,750	769,274	7.87
	1972	White	15.70	4.31	27.4	90,880	273,243	3.01
	1973	White	43.50	19.22	44.2	211,200	--	--
NP	1971	Brown	16.85	3.06	18.1	23,970		
	1971	White	3.40	1.76	51.7	47,940	366,432	5.10
	1972	White	12.20	5.53	45.3	98,160	222,567	2.27
	1973	White	43.50	13.77	31.7	103,000	--	
WB	1971	Brown	24.95	0	0	0		
	1971	White	101.40	13.17	13.0	250,000	936,631	3.75
	1971	Pink	203.65	--	--	--		
	1972	Brown	16.32	1.51	9.25	35,200		
	1972	White	137.37	2.56	1.86	58,160		
	1972	Pink	159.40	0.05	0.03	1,400		
	1973	Brown	52.1	0.83	1.59	19,850		
	1973	White	105.8	7.38	6.97	80,600		

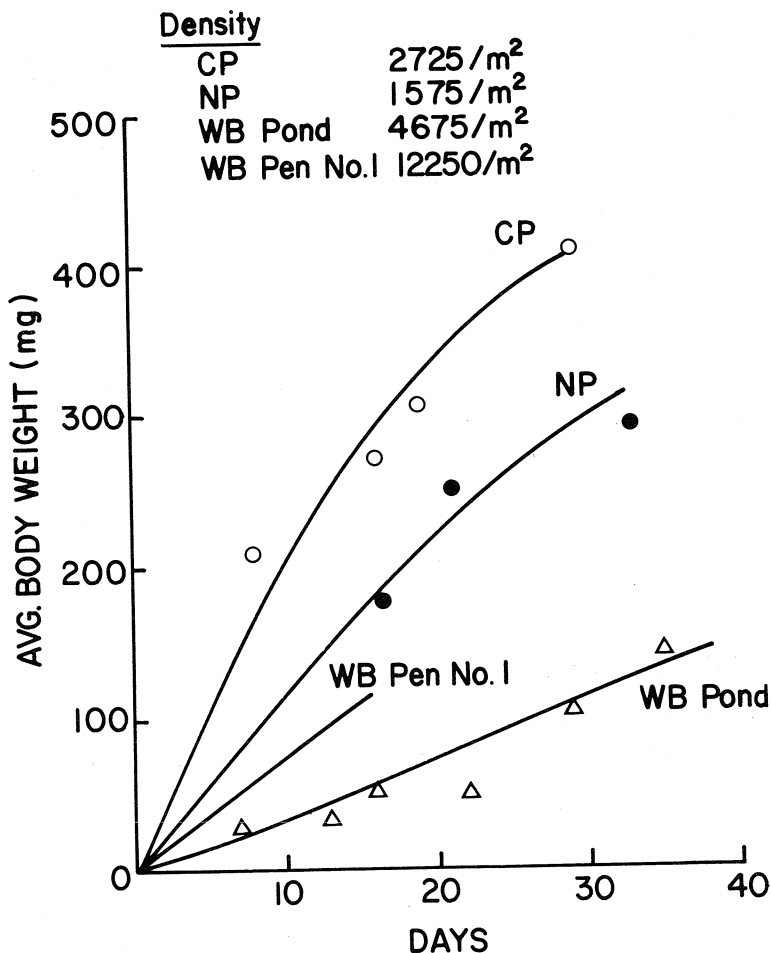


Figure 5. Growth rate of baby white shrimp.

The significance of natural food such as small crustaceans has been discussed in this paper. The importance of diatoms should also be emphasized because there is much evidence that points toward increased growth for penaeid shrimp under conditions of abundant diatom propagation. A typical example is seen in the case of intensive culture with double bottom structure (See Table 4). When the experiment was taken indoors, the growth of penaeid shrimp was usually very poor (See Table 5). Therefore the production was carried on using outdoor tanks without roofs, so that the benthic diatoms propagate abundantly in the

tanks. Micro-animals, such as copepods and polychaetes, also propagate with benthic diatoms. It is observed that penaeid shrimp ingest micro-organisms continually regardless of the presence of high food concentrations.

Recently it has been shown that crustaceans are incapable of biosynthesizing cholesterol, and that they require sterol as a diet. Cholesterol is a precursor of a molting hormone in crustaceans, which are capable of converting photosterol to cholesterol. From these facts it will be assumed that the micro-organisms, such as benthic diatoms, copepods, and polychaetes grown on the sand play an important role nutritionally in the growth of penaeid shrimp. Improving formula feed is, of course, important. But the advantage derived from this is restricted in relatively small scale production. From a practical viewpoint it is more desirable to release larval shrimp into natural sea areas at a suitable density, to grow them entirely on natural food, and to harvest. In order to enhance the effect of releasing, it will be significant to promote propagation of natural food by applying artificial procedures such as fertilizing, controlling competitors, and improving bottom conditions.

ACKNOWLEDGEMENT

Deep gratitude is expressed to the late Dr. Motosaku Fujinaga for his valuable advice and encouragement throughout the work.

Footnotes

- 1 Pellet food for rainbow trout fry.
- 2 Some data on this has been furnished by Nose.
- 3 The details may be published by Yamashita.

REFERENCES

- Hudinaga, M. 1942. Jap. J. Zool., 10, 305-393.
- Hudinaga, M., and J. Kittaka. 1966. Inform. Bull. Plankto. Japan, 13, 83-94.
- Hudinaga, M., and J. Kittaka. 1967. Inform. Bull. Plankto. Japan, Commemoration number of Dr. Y. MATSUE, 35-46.
- Hudinaga, M., and J. Kittaka. 1975. Bull. Jap. Soc. Sci. Fish., 41, 843-854.
- Kittaka, J. 1972. In "Kanzen Senkai Yoshoku (Thorough Mari-culture)" (T. IMAI and M. Hudinaga et al., ed.), Koseisha Koseikaku, Tokyo, 344-408.
- Subrahmanyam, C. B. and C. H. Oppenheimer, 1970.

Nutritional Studies of Lobsters (*Homarus Americanus*)

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INTRODUCTION

The lobster (*Homarus americanus*) is similar in many respects to other crustaceans which have demonstrated aquaculture potential. However, the need for individual compartments in order to prevent cannibalism coupled with the use of elevated water temperatures to accelerate growth rates has led to an increasingly industrialized approach. Thus, research relating to lobster culture is distinguished by the movement toward highly mechanized, fully controlled, intensive culture systems such as those envisioned by Schuur and co-workers (1974). Development of a suitable feed is, of course, one of the primary elements necessary for the actualization of these systems. If the popular comparison between aquaculture and agriculture is used, this type of aquaculture is analogous to only the most advanced systems of the broiler industry. Available knowledge of the nutritional physiology of the lobster, however, is nonexistent compared to that which exists for poultry. Hopefully, this review of the preliminary work which has been completed along with an outline of areas still to be investigated will be useful in providing an overall perspective.

Natural food sources will be absent in the controlled environment systems, therefore, all nutrient requirements (macro, micro, and trace) will have to be supplied by the formulated rations. In addition, the physical properties of these rations must match the requirements imposed by use of the industrialized approach. The ideal ration properties described in detail by Shleser and Gallagher (1974), can be condensed into four main points.

- *provides all essential nutrients*

- *promotes rapid growth*
- *economical*
- *adaptable to commercial practices*

Considering all the factors involved, for lobster aquaculture, these criteria would best be met by a dry, extruded pellet in which optimal nutrient levels are supplied by variable feedstuffs depending on their cost. Unfortunately, lobster nutritionists are a long way from producing the ideal diet — indeed we are still struggling to define essential nutrient requirements.

WORK TO DATE

Zein-Eldin and Meyers (1973) in discussing problems of shrimp nutrition stated that "the choice of environmental conditions that are standard, reproducible, and optimal is the first problem of the nutritionist." Presently, lobster research is dependent on experimental animals hatched from females taken from nature. Upon hatching, the pelagic larvae are moved to a larval rearing tank (Hughes et al., 1974) and fed live brine shrimp. After 10-14 days, at a water temperature of 20°C, the larvae have molted into fourth stage lobsters with the characteristic benthic morphology. Fourth stage lobsters are then moved into individual compartments.

At the Bodega Marine Laboratory animals were held in 10 cm square compartments with 1 mm screened bottoms. The compartments were contained within water tables connected to a semi-recirculating seawater system. Central to the system is a 500 liter sump which was fed with fresh seawater, the total water volume of the system being maintained by a standpipe drain. The seawater in the system was maintained at a constant temperature by immersion heaters or a heat exchange loop. The seawater was pumped from the sump through a series of filters (sand and anthracite column filter, 25 and 5 μ m disposable cartridge filters), U.V.-irradiated, and then distributed to the water tables. Table overflow was returned to the sump for recirculation. Although other investigators have developed systems which are adapted to the particular requirements of their individual laboratories, all of the systems have the common goal of reducing the effect of non-nutritional variables to a minimum.

Lobster growth was measured in terms of carapace length, (the distance from the rear of the eye socket to the end of the carapace in a line parallel to the midline of the body), or in terms of wet weight or both. In order to take advantage of the relatively higher molting frequency of

juvenile animals, the majority of feeding experiments have been conducted for a 90-120 day period following the fourth molt. The juvenile lobsters were typically fed *ad libitum* once or twice a day. The uneaten food from the previous day is removed by siphoning.

The first artificial diets used in evaluating nutritional parameters of lobsters were primarily those which had been developed for fish and other crustaceans (Shleser and Gallagher, 1974). Although none of the diets were satisfactory in terms of growth and survival, it was found that lobster would readily consume a wide variety of artificial diets. Therefore, efforts were undertaken to examine nutritional requirements using these diets.

Generally, two standard forms of diet, a moist-type and a dry-type were used. The moist diet, typified by the experimental diets developed by Castell and Budson (1974) used a gelling agent for a binder. Diet ingredients were mixed with hot water and the gelling agent (either agar or gelatin), then solidifies upon cooling of the mixture. The moist food was then formed or cut into small pellets and frozen until fed to the lobsters. Dry diets primarily depend on the use of wheat gluten as a binder. The use of high-gluten wheat flour was originally suggested for use with aquatic crustaceans by Balazs and co-workers (1973). Purified wheat gluten is typically used in our laboratory as a binder for extruded diets (Conklin et al., 1975). Finely ground diet ingredients including wheat gluten are mixed with hot water and kneaded until an elastic dough is formed. Pellets were formed using a laboratory macaroni extruder and then oven-dried.

Very little is known about the nutritional requirements of lobsters. The primary problem is the lack of a satisfactory artificial diet with which to proceed. In comparison to a diet of live, adult brine shrimp, artificial diets were about half as effective in terms of normalized biomass (Conklin et al., 1975). Juvenile lobsters fed the brine shrimp diet, in our system, for a three-month period exhibited a carapace length increase of about .08 mm/day. Survival of lobsters on the brine shrimp diet is regularly 100%. One of the reliable artificial diets for lobsters, the standard shrimp diet (Zein-Eldin and Meyers, 1973), only produces an average growth rate of .046 mm per day with 96% survival. Experiments designed to elucidate nutritional requirements quite often yield ambiguous results because of other nutritional deficiencies and imbalances. Early attempts to define optimal protein levels have been frustrated in this way.

Experiments varying the protein level for juvenile lobster have only been able to demonstrate that 10% protein is not adequate

(Gallagher, personal communication). Castell and Budson (1974), using adult animals, found that decreasing the dietary protein level below 60% resulted in an increasing loss in body weight. It is probable, however, that this apparent high protein requirement was due to a limiting essential amino acid or other nutritional imbalance in the diet. Gallagher and Brown (1975a) found that adult brine shrimp, which promotes rapid growth of juvenile lobsters, contains about 40% protein on a dry weight basis.

Lobsters appear to have the same qualitative essential amino acid requirements as other animals which lack a urea cycle (for example, chickens) (Gallagher and Brown, 1975b) but quantitative requirements have yet to be worked out.

Although vitamin mixtures are routinely added to the artificial diets, the role of specific vitamins in the diet has yet to be defined. In fact, Castell and co-workers (1975) removed the entire vitamin mixture except for alpha-tocopherol and choline chloride from a well-defined artificial diet and were unable to detect any significant difference in growth or survival. Again, this was probably due to a lack of other nutrients which were limiting. In our recent work we found that a vitamin mixture is useful in the replacement of rice bran in the diet and that choline chloride produces a definite increase in growth (unpublished results).

Lipid requirements for the lobster have recently been suggested by Castell and Covey (1975). The lipid requirement of lobsters was best satisfied by cod liver oil when compared to corn oil or hydrogenated coconut oil. It was suggested by the authors that this is probably due to an essential fatty acid requirement for linolenic acid. Castell and Covey (1975) were also able to show a cholesterol requirement, with the optimum dietary level being around 0.5% of the dry weight of the diet.

In examining lobsters grown on artificial diets in comparison to those reared on brine shrimp, one is struck by three obvious differences. Those fed present purified formulations are of a pale bluish to white color, have a thinner more flexible shell, and of course, are much smaller. Preliminary unpublished data from a variety of sources indicate that lobsters are incapable of producing astaxanthin from beta-carotene although they require astaxanthin for normal coloration. Various crustacean meals or extracts seem to serve as a usable dietary source of astaxanthin but it is not clear whether the function of these supplements is limited entirely to pigmentation.

Weiss (1971) noted a change to a high calcium diet at the time of molt in the adult lobster. Use of eggshell meal seemed to reduce the

incidence of soft shell in juvenile lobsters (Conklin et al., 1975) but no clear difference could be seen in growth and survival. Similar lack of difference was noted by Castell et al. (1975) upon deletion of the mineral mix (73.5% calcium phosphate) from an artificial diet. Work is in progress (Gallagher, personal communication) to further examine the effect of calcium and phosphate in the diet. Although the work that has been done is only a bare beginning and a multitude of problems are unanswered, there are several encouraging aspects. One promising area is the high level of informal cooperation which is developing among lobster nutritionists. An overall view of the experimental work now in progress indicates that a reliable maintenance diet that will support a reasonable growth rate and prevent mortality due to malnutrition will be available within a year. The availability of such a diet should allow for the rapid and definitive elucidation of both qualitative and quantitative nutritional requirements.

FUTURE WORK

Even though reliable laboratory diets will be available in the near future, it should be made clear that this achievement has little direct significance in terms of a lobster farming industry. Not only are there major areas of research still untouched but, in addition, new techniques need to be created before the rational exploration of these areas can begin.

The major technical problem limiting further diet development is the inability to differentiate between qualitative and quantitative growth responses to a particular diet. For example, an improved growth rate with a specific diet that is consumed in large amounts may not indicate better nutrient quality if the control diet is only moderately consumed. Knowledge of the amount consumed will be essential to the determination of both essential nutrient requirements and cost-effective diets with respect to feed conversion. The difficulty in recovering the uneaten food portion from an aqueous environment makes it infeasible to measure consumption during *ad libitum* feeding trials. Controlled feeding techniques, where the amount of feed offered is below the *ad libitum* consumption level, are difficult to carry out because of changes in appetite. In nutritional studies with other animals a classical early deficiency symptom has been loss of appetite. Variation in consumption associated with the molting process of crustaceans further complicates this problem. Nevertheless, controlled feeding techniques must be developed so that the effect of consumption on growth can be evaluated in conjunction with nutrient quality. Defining the influence of environ-

mental factors on consumption and determining satiety control mechanisms will be necessary for the rational development of these new feeding techniques.

This kind of definitive qualitative research is needed in three major areas before a realistic attempt can be made in formulating least-cost lobster diets from commonly available feed ingredients.

- *Definition of any changes in nutrient requirements throughout the life cycle of the lobster.*

In electing to use juvenile lobsters because of the higher molting frequency, the hopeful assumption has been that data collected with juveniles will be valid for larger animals. Although this may be true in general, work with other animals would indicate that there is a strong possibility of significant differences in optimum nutrient levels required during the lobster's lifespan. Preliminary observations by Carlberg (personal communications) indicate changes do occur in the relative efficiency of artificial diets when experiments are continued over a period of a year compared to the usual three-month period. One can easily foresee the eventual development of diets which are specific for the particular stage of growth and development.

- *Definition of minimal qualitative requirements along with delineation of optimal quantities of both essential and growth promoting nutrients.*

Some of the ingredients typical of present crustacean diets may be superfluous and could be replaced by small amounts of specific nutrients. Our present difficulty in trying to understand the use of shrimp meal in the lobster diet helps illustrate this point. Unpublished work which we have just completed indicates that while shrimp meal is useful in promoting growth and survival of lobsters, this effect has little to do with chitin, the major component of shrimp meal. Again, because of the lack of consumption values, it is not known if the shrimp meal merely improves palatability or if there is a specific nutrient in shrimp meal which is essential. Since specific vitamin and mineral requirements are unknown, "shotgun" mixtures have been used extensively in attempting to meet all possible requirements. With the anticipated development of purified artificial diets, it is expected that specific vitamin and mineral requirements will be elucidated rapidly. Knowledge of essential vitamin and mineral requirements should alleviate the present difficulties in defining optimal protein levels.

- *Translation of results derived with purified constituents into a form useful to the commercial feed industry.*

Once nutritional parameters have been defined it will be necessary to relate this data to the wide variety of complex feedstuffs used in commercial feeds. Coefficients of digestibility, usable nutrients, and energy content of feedstuffs have been compiled for a number of farm animals (N.A.S., 1971). The necessary flexibility of least-cost diet formulation is dependent on being able to match the nutritional requirements of the animal with the percentage of these feedstuffs that are available for use. Until similar coefficients have been defined for the lobster, practical diets will be limited to a small number of "tried and true" recipes. It is probable that much of the information available for farm animals, especially poultry, can be used for crustaceans once similarities and differences in food use have been defined. Adoption of the terminology commonly used in vertebrate animal studies (Harris et al., 1968) will facilitate this process.

Aside from the development of a practical commercial diet, another area which has yet to be explored is the interaction between diet and environment. For example, work in progress at several laboratories indicates lobsters consume more and have a faster growth rate when reared in the dark. Possible interactions associated with temperature and space may be of major importance in determining the economic feasibility of differing culture systems. Almost all of the nutritional work has been carried out at a temperature of 20-21°C, however, it is quite possible that optimal nutrient ratios change significantly with temperature. Space limitation of lobster growth as shown by Shleser (1974) raises some interesting nutritional questions. A space-limited animal presented with *ad libitum* food must be either reducing its intake or modifying its use of the food. An understanding of these and other interactions opens the possibility of reducing the overall cost of rearing lobsters.

SUMMARY

Development of a lobster aquaculture industry depends to a large extent on the availability of nutritionally complete rations. The inability to differentiate between qualitative and quantitative growth responses using *ad libitum* feeding techniques is seen as a major stumbling block in the elucidation of nutritional requirements. However, definition of essential nutrient requirements is only one of several areas which have to be investigated before practical commercial rations can be formulated.

Optimal levels of both essential and growth promoting nutrients should be identified for all phases of the lobster life cycle. Once nutritional parameters have been defined, the final task of relating these parameters to the wide variety of feedstuffs used in conventional animal feeds can begin.

ACKNOWLEDGEMENT

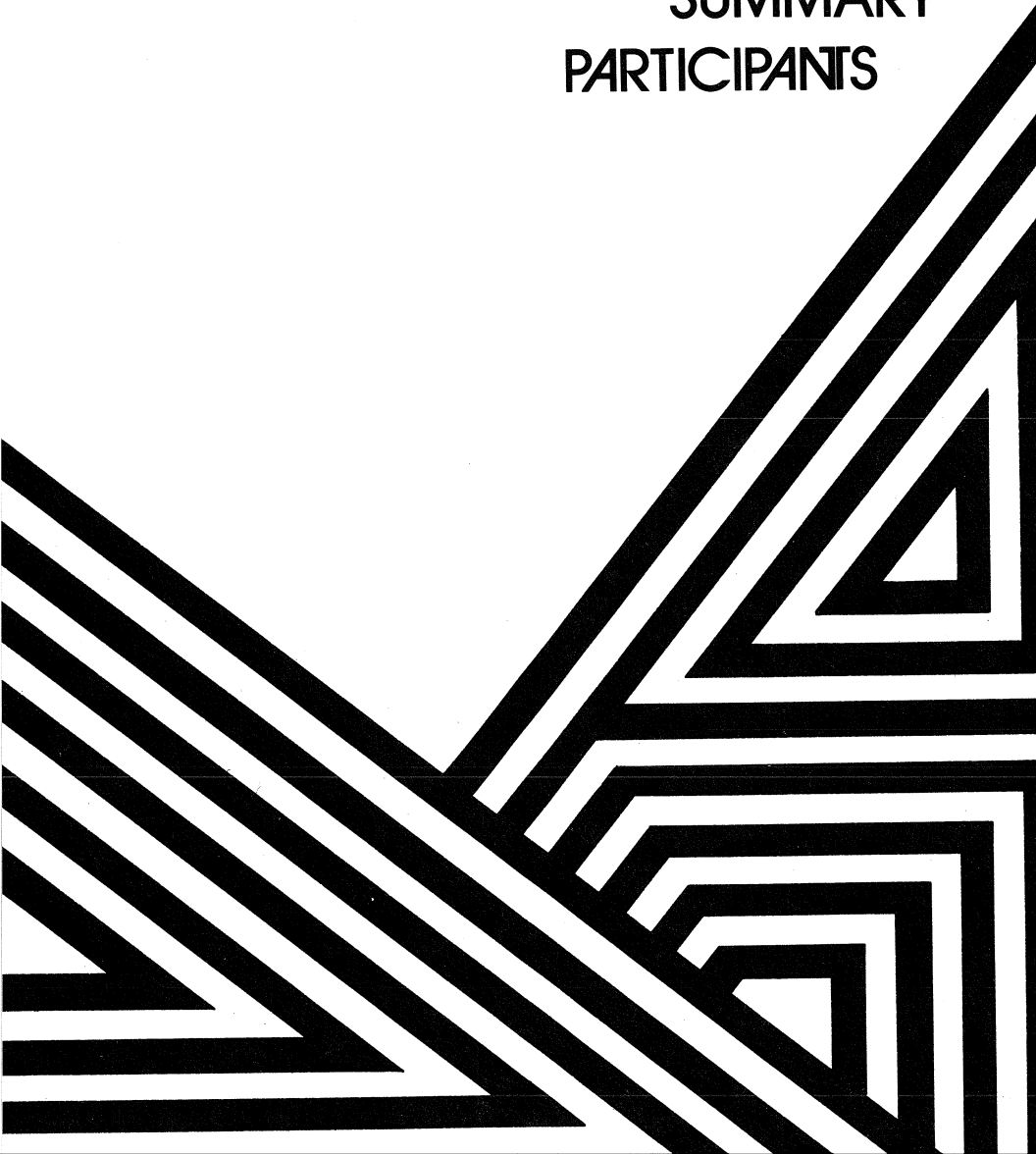
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LITERATURE CITED

- Balazs, G., E. Ross and C. C. Brooks. 1973. Preliminary studies on the preparation and feeding of crustacean diets. *Aquaculture*. 2:369-377.
- Castell, J. D. and S. D. Budson. 1974. Lobster nutrition: the effect on *Homarus americanus* of dietary protein levels. *J. Fish. Res. Board Can.* 31:1363-1370.
- Castell, J. D. and J. F. Covey. 1975. The role of lipids in the nutrition of lobsters (*Homarus americanus*). Proceedings 6th Annual Workshop World Mariculture Society. (In press)
- Castell, J. D., E. G. Mason and J. F. Covey. 1975. Nutritional requirements of juvenile lobsters (*Homarus americanus*); cholesterol. *J. Fish. Res. Board Can.* (In press)
- Conklin, D. E., K. Devers and R. A. Shleser. 1975. Initial development of artificial diets for the lobster, *Homarus americanus*. Proceedings 6th Annual Workshop World Mariculture Society. (In press)
- Gallagher, M. and D. W. Brown. 1975a. The composition of San Francisco Bay brine shrimp (*Artemia salina*). *J. Agric. Food Chem.* 23: 630-632.
- . 1975b. Amino acid requirements of the lobster (*Homarus americanus*). *Federation Proc.* 34:880 (abstract).
- Harris, L. E., J. M. Asplund, E. W. Crampton. 1968. An international feed nomenclature and methods for summarizing and using feed data to calculate diets. Bull. 479, Agriculture Experiment Station, Utah State University, Logan, Utah.
- Hughes, J. T., R. A. Shleser and G. Tchobanoglous. 1974. A rearing tank for lobster larvae and other aquatic species. *Progressive Fish Culturist*. 36:129-133.
- National Academy of Sciences. 1971. Atlas of nutritional data on United States and Canadian Feeds. National Academy of Science, Washington.
- Shleser, R. A. 1974. Studies of the effects of feeding frequency and space on the growth of the American lobster, *Homarus americanus*. Proceeding 5th Annual Workshop World Mariculture Society. 5: 149-155.

- Shleser, R. A. and M. L. Gallagher. 1974. Formulation of rations for the American lobster, *Homarus americanus*. Proceedings 5th Annual Workshop World Mariculture Society. 5:157-164.
- Schuur, A. M., R. G. Allen and L. W. Botsford. 1974. An analysis of three facilities for the commercial production of *Homarus americanus*. Paper No. 74-5517, presented at the winter meeting of the American Society of Agriculture Engineers, Chicago. December 10, 1974.
- Weiss, H. M. 1971. The diet and feeding behaviour of the lobster, *Homarus americanus* in Long Island Sound. University Microfilms 71-16, 057. Ph.D. thesis, University of Connecticut.
- Zein-Eldin, A. P. and S. P. Meyers. 1973. General considerations of problems in shrimp nutrition. Proceedings of the World Mariculture Society. 4:299-317.

**SUMMARY
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Summary Statement

Harold L. Goodwin, Rapporteur

My task is to try to summarize what we have heard during the two days of this conference. What I have tried to do is to seek those elements which are common to the problems we all face: to define the nutritional requirements, and then to create or find foods needed for the culture of the organisms with which we work.

The task would be hard enough even if we were dealing with uniformity within a given species. The papers we have heard appear to assume that such uniformity exists. But nature has produced enormous variation and diversity even within species, and populations tend to differ in nutritional requirements and in responses to the environments. I suggest that we keep in mind that what applies to one population of cultured animals does not necessarily apply *in toto* to all other populations.

THE AQUATIC MEDIUM

Culture of any living organisms begins with the environment and the aquatic environment is one of daunting complexity. The aquatic animals we want to culture are, to a considerable extent, an extension of their environment. They are immersed in it, it flows through their gills, it either supports or stresses them, depending on its physical and chemical characteristics and their physiological response. For purposes of comparison and to relate one experiment to another, we do need a standard, as has been mentioned, but to be sure the standard really represents seawater as the animal knows it takes some chemistry that is specifically directed to the problem.

There is evidence in the literature that the presence or absence of a given biological molecule may make the difference in survival or health of an organism, including the availability of food on which the organism

normally feeds. Field biologists have noted that two bays, apparently identical and adjacent, have different dominant species. We don't understand the reasons for this. So the aquatic medium is primary, and seawater presents problems we haven't really faced.

In determining the nutritional requirements and feeds for cultured animals, our tendency is to focus on feeds the culturist provides. Yet, the natural aquatic environment itself nourishes some animals through tissue absorption of nutrients. As was noted, this is true in the case of mollusks, although the quantitative contribution is unclear. Keeping in mind that all animals are immersed in waters containing dissolved nutrients, is there not a good possibility that animals other than mollusks might derive some aspects of nourishment from the water itself?

NUTRITION VS. FEED RESEARCH

Dr. Provasoli commented in his keynote address on the shotgun approach to creating rations with no real understanding of the nutritional needs of the animal. Of course he is right. But on the other hand, the urgency of developing useful feeds for animals in commercial production means that we must go in for empirical structuring of feeds. At the very least, cut-and-try methods have told us some of the questions to ask about the essential nutrition of the organism. There are few animals grown by human society for which we have perfect diets. Perfection always is a goal to be sought, but in commercial terms it may not be necessary. In many animals, diet deficiencies of some kinds may not show up in physiological deterioration until the animal is past its prime. Since we harvest the animals at or before they reach their primes, such deficiencies are unimportant. I suggest that we need to pay attention only to those deficiencies that interfere with growth to marketable size, or to the important organoleptic qualities of the product.

Dr. Provasoli also made the point, again an important one, that most of the on-going research is not really nutrition research but feed development. I think it's apparent to most aquaculturists that while the quality of nutrition research may be very good, the quantity is not sufficient to the need. Among the members of this conference are representatives of most groups engaged in nutrition research. If there were enough of you to meet the need, this conference would be several times the size. Please don't ask me where the funds will come from for more nutrition research. I can only say that, if the job is to be done adequately in quality and in quantity, the funds must come from somewhere.

COMPARISON OF RESULTS

A constant difficulty is the ability to compare results of feeding and nutrition experiments. One reason mentioned by Dr. Koganezawa is lack of standards. There is a lack of standard procedures even with experimenting with the same genera or species. So, when Lowell Sick presents a paper on prawn nutrition, and Douglas Conklin presents one on growth vs. diet for the same or a related animal, it is difficult if not impossible to make a one-for-one comparison of results because their experimental protocols have been quite different. It doesn't matter how informative the papers were (and both were very informative).

As we look for common elements across all of aquaculture, we do find such elements. For example, results obtained in nutrition research or in feeding experiments are species-specific. They are also specific to the animal's stage of development. These specificities apply whether the animals are Dr. Halver's trout or Dr. Sick's prawns.

POINTS TO REMEMBER

Among the quantities of data and results that we have heard were some nuggets of wisdom with universal — or near-universal applicability. I noted these as points we should all remember, regardless of the species we're trying to grow.

- At some stages in the development of all animals, and at all stages in the development of some animals, we must be aware of the effect of residence time of food in the gut. The best of all diets will do no good unless it stays in the gut long enough for assimilation of the nutrients and energy that the animal needs. To cite one example not mentioned at this meeting, Reuben Lasker of the National Marine Fisheries La Jolla laboratory found that one species of anchovy with which he was working consumed brine shrimp as fast as he could put the shrimp in the tank. Yet, the fish were starving. He found the reason was that the particular species had to reach a certain stage of development before a kink developed in the gut, a kink that allowed retention of the brine shrimp long enough so the animal could digest it.
- The question of nutrient leaching was mentioned several times. I think there is a good bit of observational evidence that leaching has been so rapid in some of the diets we have formulated that the nutrients simply were not available to the animal in the amounts that the experimenter planned. With better binders and better physical structure of rations such leaching can be eliminated or reduced. But, while we're

watching for undesirable leaching, let's be careful not to structure the feed so that nutrients get tied up so tightly in unassimilable binders that the animal can't use them.

- Regardless of species, it takes healthy parents to produce healthy offspring. Special attention should be paid to brood stock. Conditioning of brood animals pays off in stronger, and even more numerous progeny. Perhaps one of the finest examples of brood stock handling is Lauren Donaldson's rainbow trout at the University of Washington. Maintenance of the best possible environment, and food of a quality many humans cannot afford, have resulted in breeding rainbows of extraordinary quality and fecundity.

- It is clear from several papers presented here, and from the experience of other researchers, that nutrition plays a vital role in maturation of species. Just how maturation is affected is a question to be answered by a combination of physiological and nutritional research. In some species, Penaeid shrimp for instance, nutrition may be the key to maturation.

- We can also conclude as a generality that nutrition and disease are related. A healthy animal is obviously more disease-resistant. It is becoming more apparent as we gain experience in aquaculture, that many diseases that plague the culturist are related to nutritional stress.

- For all cultured animals, the problem of feeding includes feeding rate; that is, quantity, frequency, and timing. Dr. Kanno reported that he fed his animals promptly by the clock, on a "United Airlines schedule." Feeding, of course, must relate to the behavior of the animals and their normal feeding patterns. Sometimes the patterns can be changed advantageously, and sometimes change may be adverse. Ideally, all commercial culturists would like to produce a modified kind of feed lot routine, growing and fattening their animals to market size in the optimum way. But overfeeding can produce its own kind of stress — not to mention the damage to the environmental system caused by deterioration of unused food. We also have such questions as whether nocturnal feeders should be fed by day, at the convenience of the culturist, or whether a better product is produced in some cases by keeping the culturist up all night. Finally, feeding rate includes such considerations as temperature, pH, salinity, and the rate of water exchange in the system.

- As a general statement, we seem to have the least experience with animals in their larger stages. Among the crustaceans, for example, there has been much more work on larval development than in grow-out systems. Yet, for commercial culture, the costliest part of feeding is during grow-out.

- Both Douglas Conklin and John Forster stressed changes in quantities to be fed at different stages of the animal's growth. Quantity is related to food structure, with particle size of importance for some animals. And, as the total quantity changes, changes may also be necessary in the relative quantities of components.

- In changing components, experiments have shown that, even for essential materials, too much may result in toxicity just as too little results in deficiency. Some of the essential amino acids and lipids become toxic when the quantity is too great.

- Availability of protein was perhaps not stressed enough. Not only must there be good protein in the diet, but it must be available to the animal. Some protein products may assay very high in protein, yet have much of the protein locked up. This was true of early fish protein concentrate — 80% pure animal protein — of which only a part was assimilable.

- John Forster brought out a very important point to remember — that fecal production from a given ration is important. It is not only necessary to keep the system clean, to keep down carbon loading, that provides a good substrate for bacteria that may include pathogens, but it is also necessary to dispose of residues. A good ration meets the animals' needs, but a better ration also reduces fecal output. Dr. Forster also added that large scale production means automated feeding — hand feeding is too costly. So the best ration suits the animal, reduces fecal production, and is structured for automatic feeding.

- Minerals were mentioned, but I don't think enough was said about their importance beyond meeting the animal's physical needs. It isn't enough to produce a healthy animal, it should also be one that tastes good. The right minerals in the right quantity may make the difference. I can't imagine *Homarus americanus*, the American lobster, without the flavor imparted by iodine.

SPECIFIC FOODS FOR SPECIFIC CREATURES

The first day's session emphasized that finfish are not alike, that what Dr. Halver feeds his salmon cannot be fed with the same results to Dr. Yone's bream. We also had an interesting comparison of production with an artificial ration and wet fish — Dr. Dupree reported 15 years of catfish production on manufactured feed, with an increase in production from 400 acres to 75,000 acres, and an increase in quantity from .3 million pounds to 112.5 million pounds. Market value in those 15 years went from a tenth of a million to 40 million dollars. Dr.

Furukawa described the production of yellowtail in Japan with wet fish as the primary feed, with 88,483 tons of yellowtail produced from 669,559 tons of wet fish and an additional 10,000 tons of artificial ration. If we reduced the wet fish weight to dry weight equivalent, the conversion would probably not be too different from the conversion ratios for many other fish.

One reason for the wide interest in aquaculture, apart from profit potential, is that it may help materially to alleviate the world protein shortage. The shortage is already so acute in many countries that wet fish may have to be used to feed humans, with large scale aquaculture based entirely on artificial feeds. It's possible that any animal that cannot consume artificial rations, and is therefore competing with humans for natural foods, may have to be eliminated from culture. It's possible, but not probable. A well-designed artificial ration with structure, attractant, texture, and taste that appeals to the animal probably will be an acceptable feed for any creature we really want to grow.

Among the crustaceans, brine shrimp looms as a prime necessity. I wonder if it isn't considered necessary because it's so convenient. There are substitutes. *Moina* is widely used in Asia, and American experimenters have had excellent results with *Daphnia*. There are others that may serve, too, even though they may not be as handy as *Artemia*. The shortage of high quality brine shrimp eggs may force more research into substitutes.

We have not heard much about detritus. Many of the animals we like, including shrimp, are detritus feeders, in part. We don't know enough about what the detritus offers, and it certainly varies from place to place. Experiments at Louisiana State University have looked at what shrimp consume in the marshes, and it turns out to be a rather large variety of proteins, vitamins, lipids, and minerals. I haven't heard anyone suggest that we need an artificial detritus — but perhaps that is what some of the feeds turn out to be.

At this point in time, some of our cultured animals still depend on natural productivity in the systems in which they're grown. Artificial rations, when fed in an essentially sterile system produce, in Dr. Kanno's phrase, "too much skinny." In pond culture of *Macrobrachium*, as a prime example we don't yet know how much of the food we put into the pond is consumed directly by the prawns and how much simply fertilizes natural productivity. If we accept the assumption that aquaculture should be based on systems that require a minimum energy subsidy to achieve results, we'd better understand the relationship between natural productivity and artificial feed.

PROBLEMS OF SCALE

Dr. Mel Carriker spoke of the problem faced by the University of Delaware. How does one increase an order of magnitude in the production of algae to feed mollusks? The particular species of algae best suited to oyster growth do well in small systems, but what happens when the algae must be produced in quantities that will result in millions of pounds of oyster meats each year? No one has yet grown selected species of algae on such a scale. Until scaling up is tried, problem definition will be mostly guesswork.

Dr. William MacGrath of Ralston Purina introduced a fact of life that didn't make the conferees very happy. For industry to produce structured rations for sale to culturists, the market must be large enough to warrant industry investment and operations. The quantity of interest to a feed company is larger than the foreseeable aquaculture market. If the demand grows enough, the feed companies will find it profitable to respond. But we're discussing individually designed rations for each species — or at least for each genera. It may be that the culturist will have to be his own feed producer, depending on the species he is culturing, buying a basic ration and adding his own vitamin packets, and lipid and protein supplements, and perhaps minerals as well. Related to large-scale feed production is the availability of materials at prices the culturist can afford. Can we envision competition between fish meal and soybean? What's the outlook on any given day for oils, alginates, gums, and glutens? The culturist may end up following the commodities market the way he'd like to follow his stock on the Exchange. We don't know because we're not there yet, except in catfish and trout.

THE POTENTIAL OF NATURAL FOODS

John Glude described activities using natural foods. He mentioned a mimosa-type wild plant, called koa haole in Hawaii, which has a pretty good amino acid profile, but also contains an alkaloid that causes problems to some animals. Among mammals, it causes cows to lose hair, but pigs do well on it. Alain Michel at the Centre Oceanologique du Pacifique in Tahiti is feeding the *Leucocephala* to *Macrobrachium*, and the prawns appear to be doing well. The sharp-nosed mollie, *Poecilia*, also eats the plant. So perhaps it is a hopeful source of feed for many aquatic animals.

Coconut meal was discussed in Glude's paper. Another use of coconut, apart from direct use, is as a substrate for producing insect larvae. Copra fly larvae are one possibility that look good from preliminary

experiments. Sea Grant supported a project at Woods Hole and the New Alchemy Institute to optimize production of midge larvae as aquaculture food, with good results.

We need alot more experimentation with the use of natural products, both as direct foods and as substrates for production of foods. A principal reason is that current feeds are the result of high energy consumption. Increasing energy costs will mean higher feed costs.

Dr. Harold Webber and I have had a continuing discussion of alternate ways to produce aquaculture feeds. Fermentation of such wastes as plant residues, conversion by bacterial and fungal processes, and using residues and metabolites from the prime culture to produce a fodder animal in the same system are all quite feasible ideas that need work.

Perhaps it's natural that culturists working in tropical areas are pushed toward using whatever is available, because the more sophisticated feeds must be imported and cost too much. In Fiji, brewery mash has been used as a resource for tilapia and rabbitfish culture. The culturists tried just dumping the mash into the ponds, letting it dry and then dumping it, and leaving it to decay and develop a good population of insect larvae, worms, and other organisms before putting it into the ponds. The animals thrived on it. In Puerto Rico, researchers at the Mayaguez campus of the University are trying rum mash, and there are experiments with fermentation mash from antibiotic production. New Caledonia culturists noticed that breakage always resulted when flour was delivered, and they swept the flour up and fed it to their shrimp. The shrimp did well.

What other waste products are there which might be either a food or an organic fertilizer, or perhaps a substrate for growing other foods or for conversion through natural and well-known processes? It's worth thinking about and investigating.

I might also remind you that we really know little about natural productivity in extensive systems — ponds, earthen raceways, and so on. We know there is natural productivity, and we know it contributes to growth and health of some cultured animals. But there's no good quantification, and we judge the richness of a pond by the animals it produces without knowing whether production is optimum, whether it can be improved by management, or whether it can be sustained.

CONCLUSIONS

Dr. Charles Epifanio of the University of Delaware has been growing his oyster stock for five years, and he tells us he doesn't know much about them. Dr. John Halver of Sport Fish and Wildlife has a century of salmon culture behind his work, and he says doesn't know much about salmon. I'm told there's a lot of ignorance behind the culture of trout, and we have a lot to learn about catfish. We have even more to learn about the species more recently brought into experimental or pilot scale culture. Yet, just about all oyster harvests are based on at least a minimal kind of aquaculture, even if it's only replacing cultch — in a lot of places it's much more than that. And we find oysters in markets and restaurants. Trout account for 85% of the institutional market for that fine fish, with the rest from imports and natural fishery. Salmon culture is spreading, and salmon hatcheries are increasingly important to the natural fishery — which really isn't natural anymore in many places because it depends on hatcheries. The catfish industry has grown tremendously in only 15 years, and the commercial acreage devoted to freshwater prawns increases yearly.

In spite of our vast ignorance, we're obviously doing something right. But no one will argue against the fact that we need to do it far better than we're doing it now, and nutrition and feeds are vital to viable commercial culture.

What we suffer from in nutrition and feed research, as in all our aquaculture efforts, is a lack of strong, long-range funding commitments. I don't know how we're going to get such a commitment, short of electing a struggling aquaculturist as President. Meanwhile, I suggest that we continue to combine empiricism with definitive nutritional and physiological research. But don't carry empiricism too far. Remember the tale of the farmer whose hay costs got so high that he decided to teach his mule to get along without food. As he explained, "'Twould've worked, but just as he was learnin' he up and died."

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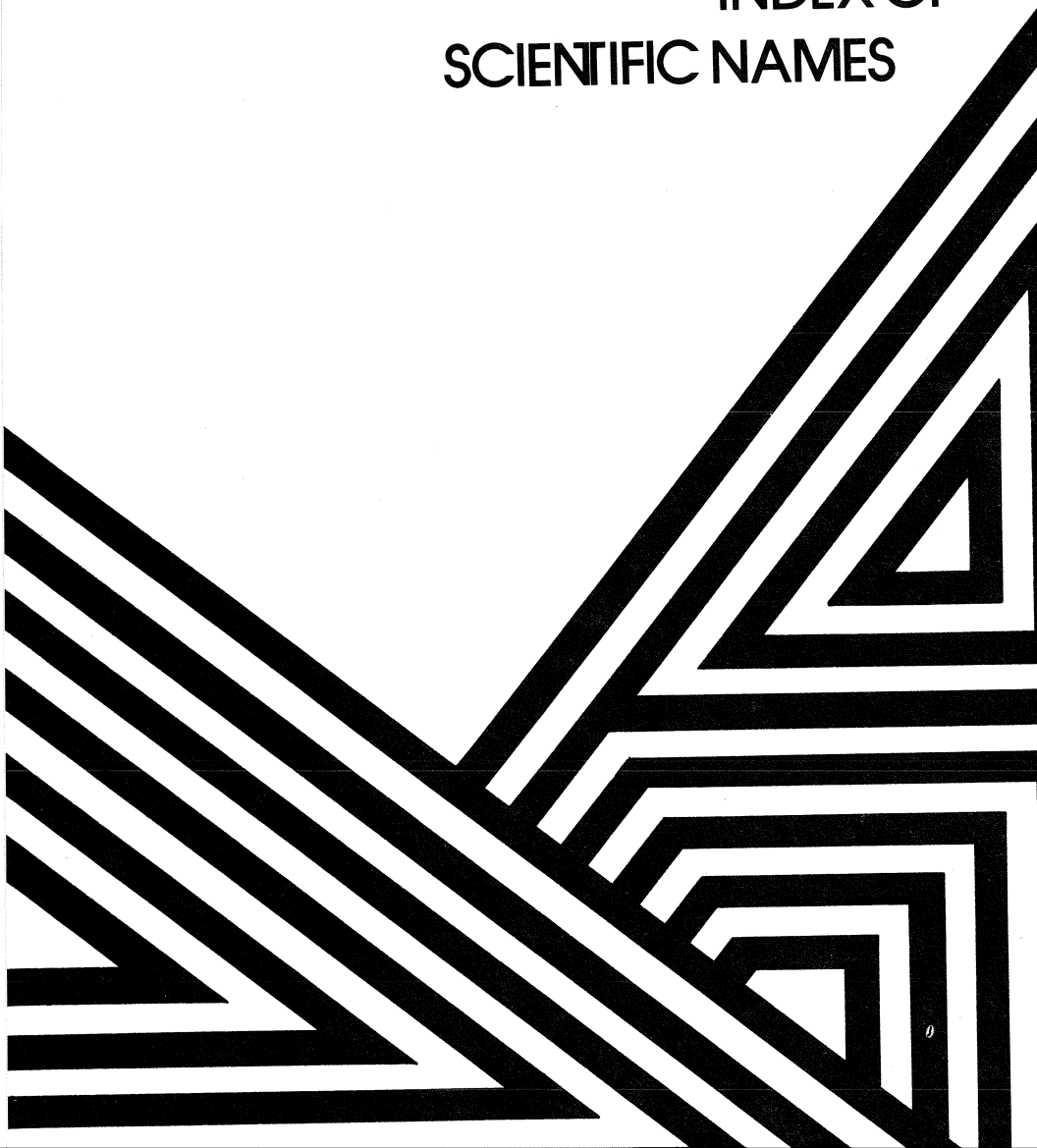
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INDEX OF
SCIENTIFIC NAMES



INDEX OF SCIENTIFIC NAMES

- Acartic clausi* 225
Acrosorium 204
Acrosorium flabellatum 205
Acrosorium uncinatum 205
Acrosorium yendoii 205
Aequipecten (Argopecten) irradians 128, 144
Ammodytes personatus 86
Amphirora 144
Amphora 144
Anadara broughtonii 164, 167-8
Anadara subcrenata 164
Apogon 107
Artemia 14-16, 18, 19, 138, 270, 305
Artemia salina 13, 215-18, 220-3, 249-52, 270, 273-4, 280
Artina pectinata 164, 167-8
Astacus astacus 18
- Bacillariophyceae* 176
Ballanus 251
Bellerophon 178
Birgus latro 107
Brachidontes recurvus 128
Brachiomonas sub-marina 176
Brachionus plicatilis 252
- Calanus helgolandicus* 136
Callophyllis crispata 205
Caprella 268-9
Carteria chuii 178, 180-1
Chaetoceros 251
Chaetoceros calcitrans 141, 165, 167-8, 176-7
Chaetoceros gracilis 141, 167
Chaetoceros simplex 141, 167, 178
Chanos 107
Chlamydomonas 142, 167, 175, 251

Chlamydomonas coccoides 176-7
Chlamys nobilis 164, 167-8
Chlorella 138, 142, 167-8
Chlorella autotrophica 176-7
Chlorella stigmatophora 176-7
Chlorococcum 142
Chlorophyceae 176
Chromulina pleiades 141
Chromulina pusilla 176
Chroomonas salina 175, 178, 180-1
Chrysophyceae 176
Chrysomenia wrightii 205
Cocconeis 204
Codium 204
Codium divaricatum 205
Codium fragile 205
Coryphaena hippurus 108
Crassostrea gigas 128, 180-1, 187-8
Crassostrea virginica 128, 135, 138, 140-4, 178-9, 181, 184-8
Cricosphaera carterae 176-7
Cryptomonas 175-7
Cryptophyceae 176
Cyclotella nana 141

Daphnia magna 13, 14, 16, 19, 305
Dicrateria 142
Dicrateria inornata 141-2, 176-7
Dictyopteris 204
Dictyopteris divaricata 205
Dunaliella 142
Dunaliella euchlora 138, 176-7
Dunaliella primolecta 142
Dunaliella tertiolecta 140, 176-7

Eisenia 204, 206
Eisenia byciclis 204-5

Engraulis japonica 86

Enteromorpha 113

Fucus 138-9

Fuhausia 270

Fulvia mutica 164, 167-8

Gammarus 251, 268-9

Gelidium amansii 205

Grateloupia 204

Grateloupia filician 205

Grateloupia turuturu 205

Haliotis 202

Haliotis discus 196, 201

Haliotis discus hannai 197-202, 204

Haliotis gigantea 196, 201-2

Hapthophyceae 176

Hemiselmis rufescens 141

Homarus americanus 287, 304

Isochrysis galbana 129, 138, 140-3, 176-8, 180-1

Laminaria 138, 197, 206

Leucaena 110, 114

Leucaena glauca 108, 110

Leucaena insularum 114

Leucaena leucocephala 108-11, 113, 115, 306

Macrobrachium 19, 107, 215, 305-6

Macrobrachium carcinus 215

Macrobrachium formosense 215

Macrobrachium japonicus 215

Macrobrachium rosenbergii 113-5, 215-20, 222-226

Mactra sulcataria 164

Meganyctiphanes norvegica 225

Mercenaria campechiensis 178

Mercenaria mercenaria 128, 135, 137-8, 140-1, 177-81, 184-6

Meretrix lamarckii 164, 167-8
Meretrix lusoria 164
Metapenaeus monoceros 239, 254
Micromonas pusilla 176-7
Micromonas squamata 176
Minchinia nelsoni 8
Moina 251
Moina macrocopa 13-19
Monas 132, 139, 167
Monochrysis lutheri 129, 138-42, 165, 167-8, 176-7, 188
Mugil 107
Mya arenaria 128
Mysidicae 261
Mytilus 251, 261-4
Mytilus edulis 128, 140, 144, 181, 185-6

Nannochloris 142
Nannochloris atomus 176-7
Navicula 204
Nitzschia closterium 142, 167

Olisthodiscus 176-7
Oncorhyncus kisutch 27
Oncorhyncus tshawytscha 28
Ostrea edulis 128, 130, 138-42, 177-8, 180-1, 186
Ostrea lurida 128, 135
Oxyrrhis 140

Pachydictyon 204
Pachydictyon coriaceum 205
Pachymeniopsis 205
Pachymeniopsis elliptica 205
Palaemonetes varians 240
Palaemon serratus 18, 232-4, 236, 239
Pandalus platyceros 239
Patinopecten yessoensis 164, 167-8

Pavlova gyrans 176-7
Pecten albicans 164, 167-8
Pecten maximus 128, 141-2
 Penaeidae 107, 113
Penaeus aztecus 231-3, 238-9, 243, 254, 256, 259-60, 268, 270, 274, 276
Penaeus duorarum 233, 254, 256, 259-60, 268, 274
Penaeus indicus 254
Penaeus japonicus 15, 17, 231-4, 236-9, 243, 249, 251-3, 256, 259-60, 268
Penaeus merguensis 238
Penaeus monodon 232, 238-9, 254
Penaeus semisulcatus 239
Penaeus setiferus 232-3, 254, 256, 259-60, 268, 274
Phaeocystis 132
Phaeodactylum tricornutum 132, 140-1, 144, 167-8, 176-8, 180-1, 186
Pikea californica 205
Pinctada maximus 128
Placopecten magellanicus 128
Platymonas 240
Plocamium telfairiae 205
Poecilia 107, 115, 306
Poecilia mexicana 108, 112
Poecilia vittata 112
Polydactylus sexfilis 107, 112, 115
 Prasinophyceae 176
Prosopsis 114
Prosopsis insularum 114
Prymnesium parvum 142, 175
Pyramimonas 142
Pyramimonas grossi 142, 176-7
Pyramimonas obnata 141

Rhodomonas ovalis 167
Rhodymenia 204
Rhodymenia intricata 205

Salmo gairdnerii 25

Salvelinus fontinalis 33
Sargassum ringgoldianum 205
Saxidomas giganteus 128, 178
Scenedesmus obliquus 138
Schizothaerus keenae 164, 167-8
Schizymenia dubyi 205
Scylla serrata 107
Seriola quinqueradiata 85
Siganus 107, 113
Siganus argenteus 113
Siganus canaliculatus 113, 115
Siganus spinus 113
Skeletonema costatum 141, 174-7, 186, 249-52
Spisula sachalinensis 164, 167-8
Stichococcus 142
Symphycladia latiuscula 205

Tapes semidecussata 180-1
Tetraselmis 130, 141
Tetraselmis chuii 176, 178
Tetraselmis inconspicua 176
Tetraselmis suecica 141, 176-7, 186
Tetraselmis tetrahele 176
Thalassiosira 141
Thalassiosira fluviatilis 176
Thalassiosira pseudonana 178, 180-2, 188
Tigropus 140
Trachurus japonicus 86
Tresus capax 128

Ulva 138, 204, 251
Ulva pertusa 205
Undaria 197, 204, 206
Undaria pinnatifida 205
Urophycis chuss 109

Venerupis phillippinarum 17

Vibrio 132

Xanthophyceae 176

Zostera 136

