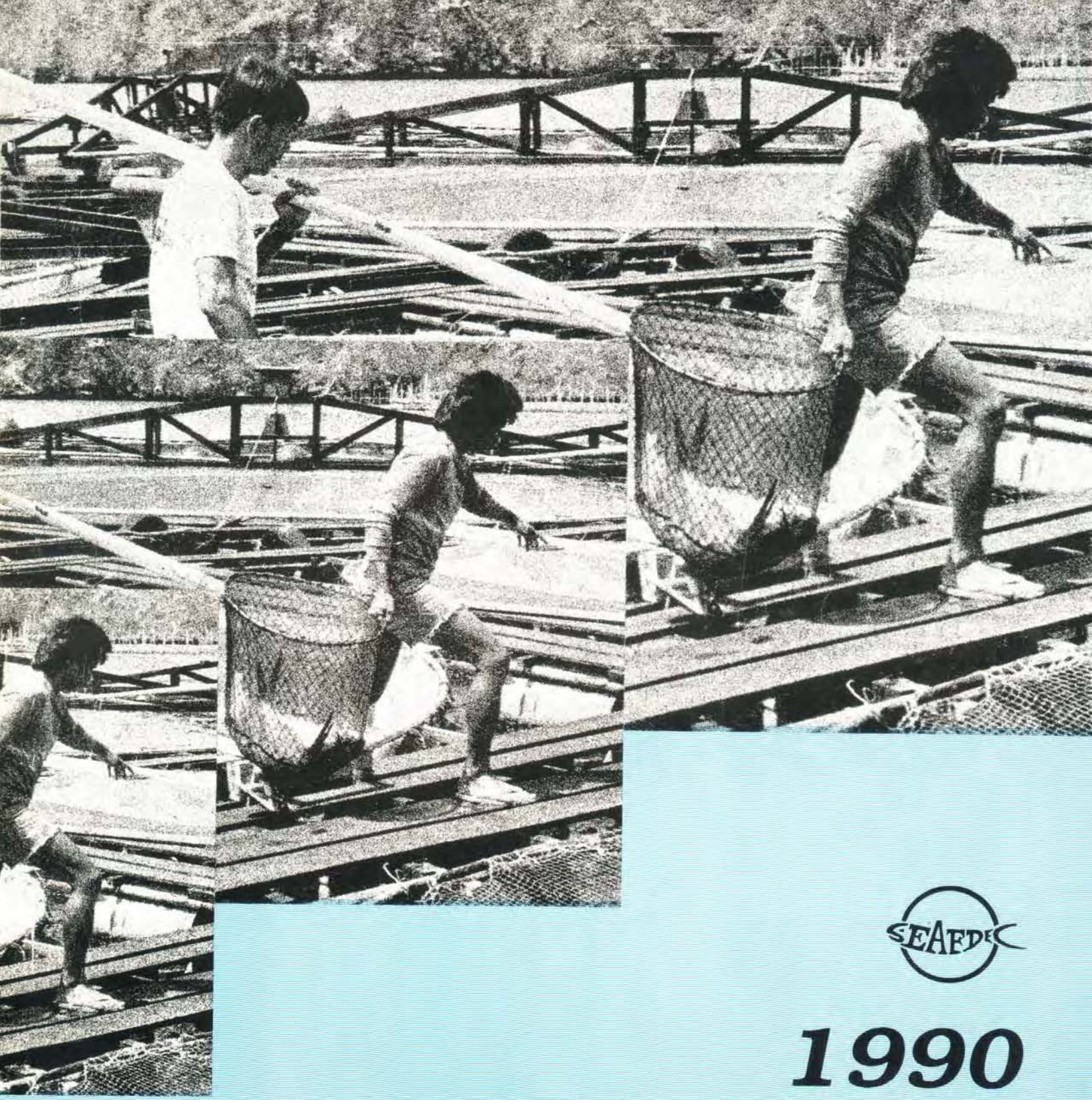

1990 Annual Report

Aquaculture Department
Southeast Asian Fisheries Development Center

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Southeast Asian Fisheries Development Center

The Southeast Asian Fisheries Development Center (SEAFDEC) is a regional treaty organization established in 1967. Its Member-Countries are Japan, Malaysia, the Philippines, Singapore, and Thailand.

Created to develop fishery potentials in the region in response to the global food crisis, SEAFDEC undertakes research on appropriate technologies, trains fisheries and aquaculture technicians, and collects, analyzes, and disseminates fisheries and aquaculture information. To pursue these objectives, the Center established three key departments - Marine Fisheries Training Department (Thailand), Marine Fisheries Research Department (Singapore), and the Aquaculture Department (Philippines). A fourth department, the Marine Fishery Resources Development and Management Department, was approved by the *Council of Directors* (SEAFDEC's policy-making body representing the Member-Countries) at its 23rd Meeting in December 1990 for establishment in Malaysia.

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ISSN 0116-712X

Published and Printed by:
Aquaculture Department
Southeast Asian Fisheries
Development Center
Tigbauan, Iloilo, Philippines 5021
June 1991

Production:
Audiovisual-Print Staff
Training & Information Division
Copy Editors: M.Castaños/J. Lagoc
Photographs: I. Tendencia/R. Elizon
Cover Design: R. Elizon

FOREWORD

This *1990 Annual Report* of the Aquaculture Department (AQD) of the Southeast Asian Fisheries Development Center (SEAFDEC) caps 17 years of existence. Through this Report, AQD's performance against goals and objectives may be gauged as encompassed by the following mandates:

- Promote and undertake aquaculture research relevant and appropriate for Southeast Asia;
- Develop human resources for aquaculture development in the region; and
- Disseminate and exchange information on aquaculture.

With the furtherance of these mandates, SEAFDEC/AQD built its stature in the international scientific community, attaining a level of respect unenjoyed before. This is due to the numerous scientific publications (the raw materials of technology development) the staff have produced, and the various awards these papers have won.

The activities summarized here followed the *Plan of Operation* and the *Program of Work of AQD* as approved by the SEAFDEC Council, its governing body, at its 22nd Meeting in Singapore on 21-24 November 1989. The year saw a redirection of AQD's research thrust to seafarming and searanching, with the closure of its Leganes Brackishwater Station. The ecological and social costs resulting from an overextended fishpond industry far outweigh the profits of fishpond operators and the short-term benefits for the country, hence the shift in AQD priorities. Research on seafarming and searanching, now in high gear, is expected to tap the great potential of the region's extensive coastline. Target beneficiaries are the poor coastal dwellers that comprise the bulk of the communities.

Despite a disastrous typhoon which damaged research facilities and the adverse economic and financial environment, AQD steadfastly pursued its mandates, thanks to the dedication of the staff and the trust and confidence of the SEAFDEC Council and the national and international organizations with which it has linkages.

F.J. Lacanilao
Chief
SEAFDEC Aquaculture Department

CONTENTS

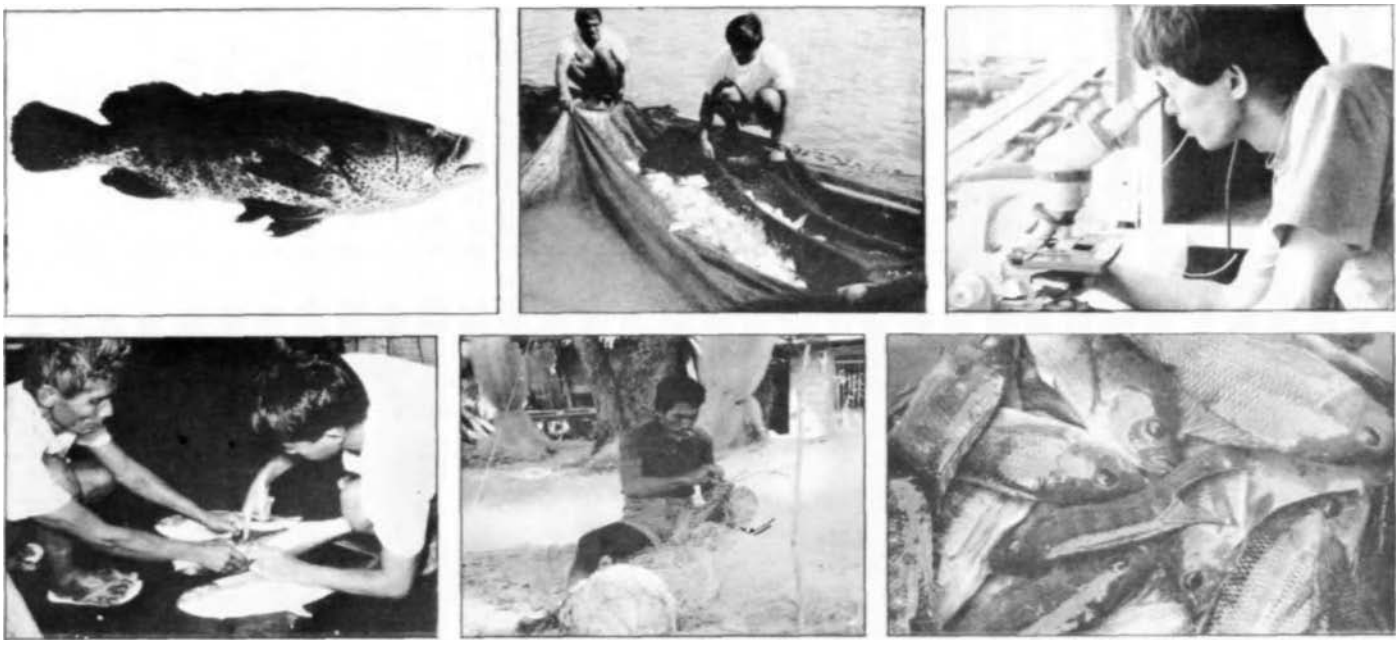
TAKING STRIDES INTO THE '90s

Over the years, the Aquaculture Department has grown to be the biggest arm of SEAFDEC when viewed alongside the Marine Fisheries Training Department in Thailand and the Marine Fisheries Research Department in Singapore. Manning its main station, two substations, and a liaison office in Manila is a personnel complement numbering 375 as of 31 December 1990.

With its accomplishment in the 1980s as groundwork, the Department took firm strides into aquaculture R & D in the new decade. Research studies focused on species of importance in Southeast Asia: the finfishes sea bass (*Lates calcarifer*), grouper (*Epinephelus* spp.), snapper (*Lutjanus* sp.), mullet (*Mugil cephalus*), rabbitfish (*Siganus guttatus*), milkfish (*Chanos chanos*). tilapia (*Oreochromis* spp.), catfish (*Clarias macrocephalus*), and bighead carp (*Aristichthys nobilis*); the crustaceans tiger shrimp (*Penaeus monodon*) and white shrimps (P. *indicus, P. merguiensis*); the molluscs (*Placuna placenta* and *P. sella*); and the seaweeds (*Gracilaria* spp.).

Highlighting 1990 are six significant events: (1) the establishment of a pilot Seafarming and Searanching Project in Antique, Panay Island, central Philippines: (2) the spontaneous spawning of captive grouper (*Epinephelus suillus*) in July; (3) natural spawning of milkfish in concrete tanks in September, the first in SEAFDEC/AQD milkfish research; (4) increased number of scientific papers (published, in press, submitted); (5) a SEAFDEC/AQD paper winning first prize in the socioeconomic category at a National Research Symposium in July; and (6) intensified dissemination of hatchery technologies.

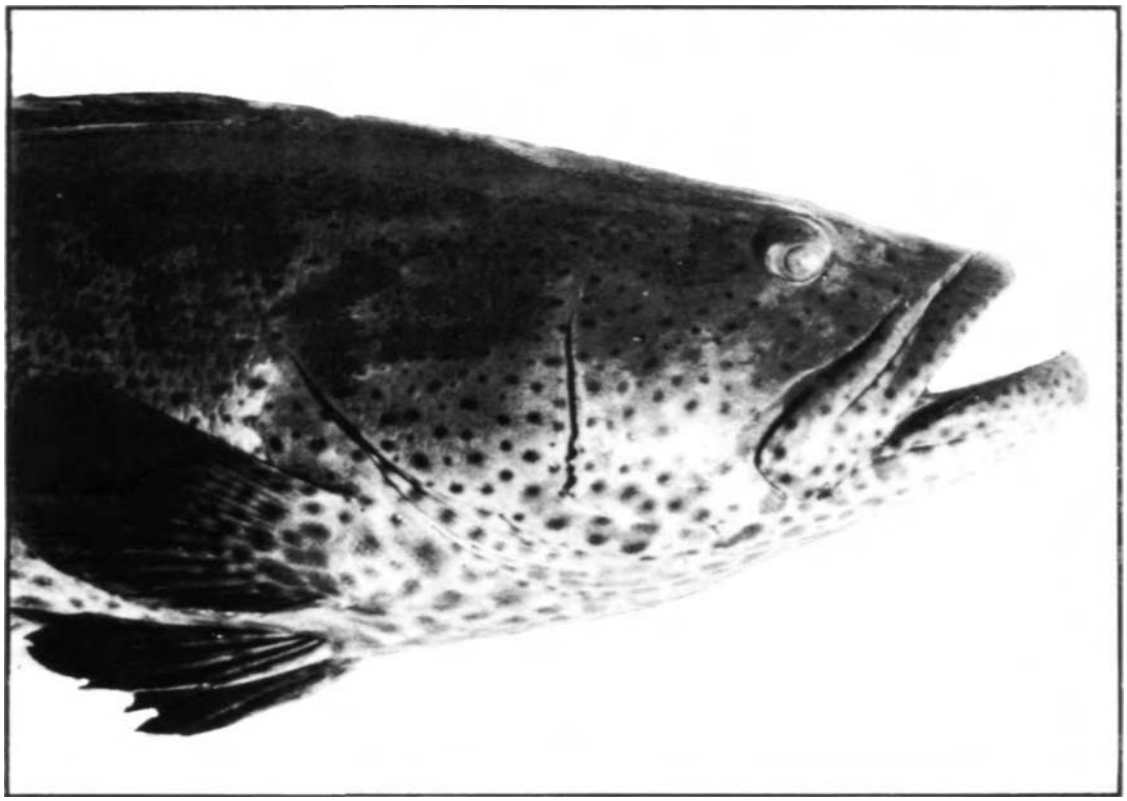
Fish and fishers, scientists and aquaculturists form a mosaic in the service of science.



"The seafarming studies (which Maralison fisherfolks above welcomed in their barangay) address the issues of degraded coastal habitats, diminishing fish stocks, failure in law enforcement, and the need for alternative job opportunities."
- Dr. F.J. Lacanilao

Seafarming/ searanching

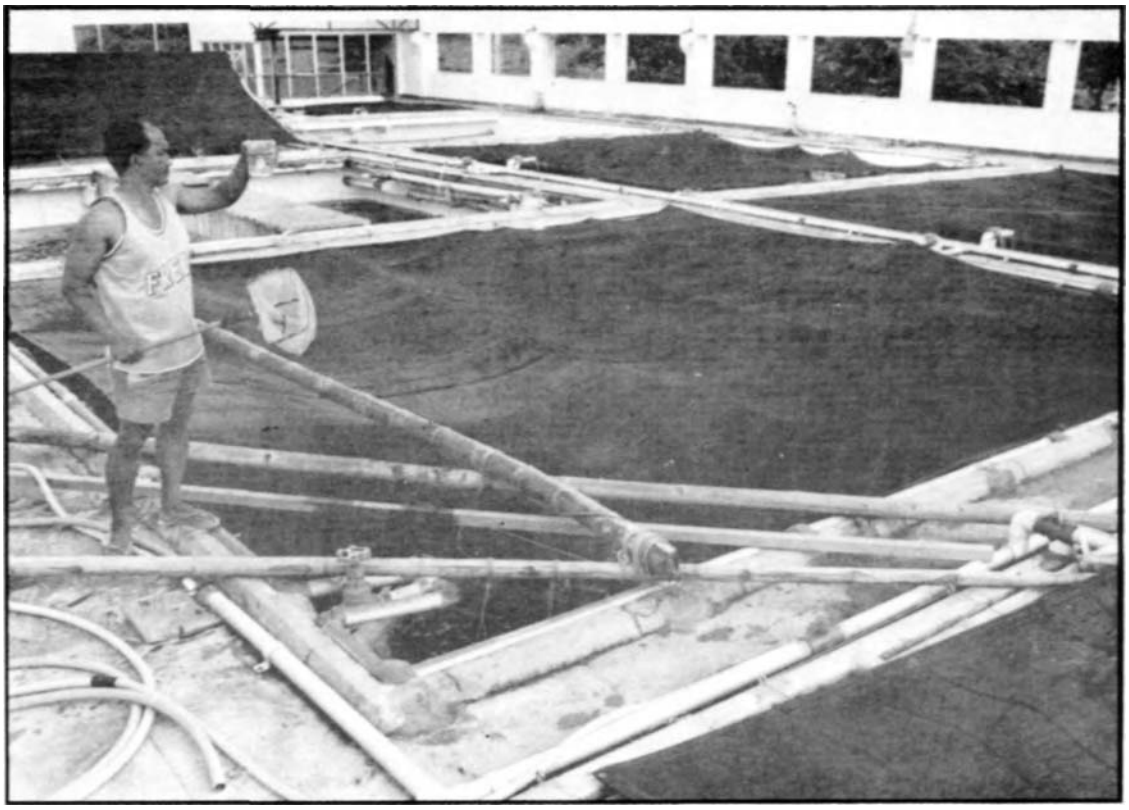
The Seafarming and Searanching Project in Maralison Island, Culasi, Antique aims to develop model marine hatchery-nursery systems of selected species for culture and release of juveniles; provide alternative livelihood through cultivation of appropriate finfishes, molluscs, crustaceans, and seaweeds; regenerate fish habitats such as coral reefs, seagrass beds, and mangrove swamps; and increase fish stocks by releasing juveniles of suitable species. The project takes on a holistic approach with the participation of small fish farmers/fishermen's association, the ultimate beneficiary, and support of non-government organizations in the community. With the grant of exclusive use rights to the association to control and manage their occupational area, the project is expected to serve as a model for other countries in Southeast Asia.



The spontaneous spawnings of grouper are expected to usher in the hatchery technology that will solve the problem of grouper fry supply. More significantly, the spawning success will in the long run establish grouper culture as another alternative to shrimp culture.

Grouper

Grouper (*Epinephelus suillus*), a delicious, highly priced food fish locally called "lapu-lapu," spawned and hatched successfully for the first time at the Department's main station in Tigbauan, Iloilo. The first spontaneous spawning occurred on 4 July from the broodstock maintained in 50-t concrete tank. Eggs collected from the first recorded spawning numbered 975 000 of which 97% hatched. The spawners consisted of 6 females weighing 3-5 kg taken from AQD's Igang Marine Substation in Guimaras Island. There were also 4 males weighing 7-12 kg with the females. Spawnings occurred for 12-21 consecutive days every month from July to December.

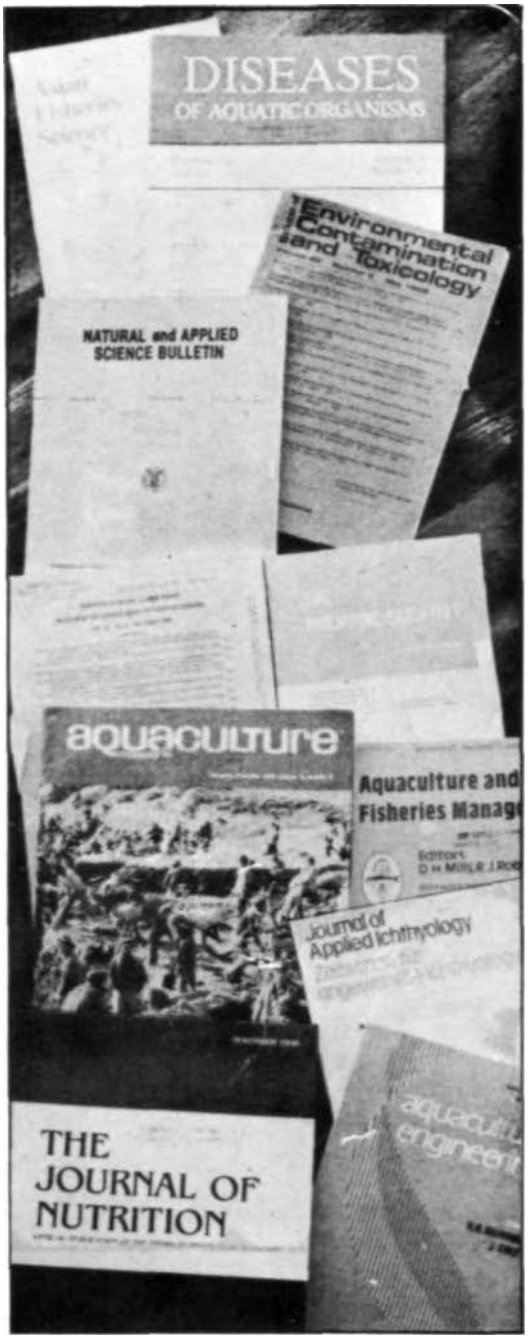


Spawning of milkfish in concrete tanks provides an alternative to egg production in floating cages particularly, where the site for floating cages may not be available.

Milkfish

The first documented natural spawning of milkfish in concrete tanks occurred 24 September-9 November at the Tigbauan Main Station. The results indicate that aside from 6-m and 10-m diameter (3-m deep) floating net cages (AQD's Igang Marine Substation), 25-m x 35-m fishponds (in Taiwan), 6-m diameter (2-m deep) canvas tanks (in Indonesia), captive milkfish could spontaneously spawn in another holding structure - the concrete tank. The total number of eggs collected were lower than those collected from floating cages and fishponds; hence, the researchers believe that the technique must be improved to enhance production and collection of milkfish eggs.

Although the cost of maintaining broodstock in net cages is lowest, construction of concrete tanks near the hatchery is deemed more advantageous in terms of closer monitoring and coordination of broodstock and hatchery operations. Year-round and off-season maturation and spawning is possible under controlled environmental conditions. This can only be achieved if broodstock are reared in concrete tanks where conditions such as photoperiod and temperature can be manipulated. With refinements in technique, spawning in concrete tanks is expected to ultimately provide off-season supply of milkfish fry.



Comparative number of SEAFDEC/AQD researchers and their publications in 1976-90, by 5-year period

	1976-80	1981-85	1986-90
Researchers (Ph.D. & M.Sc.)	41	69	60
Publications			
Journal	65	86	154
(International)*	(28 or 43%)	(50 or 58%)	(124 or 81%)
Proceedings	6	26	96
Total	71	112	250
Publications per researcher	1.73	1.62	4.18

* Refereed journal covered by *Current Contents*.

Comparative number of participants in SEAFDEC/AQD short-term training courses in 1976-90, by 5-year period

	1976-80	1981-85	1986-90	Total
Malaysia	63	33	45	141
Philippines	113	366	493	962
Thailand	56	36	54	146
Singapore	1	3	1	5
Indonesia	33	20	20	73
Other Countries	25	42	60	127
TOTAL	281	500	673	1454

Comparative number of SEAFDEC/AQD in-house publications in 1976-90, by 5-year period

	1976-80	1981-85	1986-90
Newsletters	225	213	299
Extension Manuals	5	4	14*
Extension Pamphlets & Leaflets	0	5	17
Abstracts	0	0	11
Proceedings & Compilations	5	4	4
Quarterly Research Reports**	16	4	0
Others	6	18	53
TOTAL	257	248	398

* Includes reprinting. ** Discontinued in 1982.

The number of scientific papers AQD researchers publish in different journals (collage) continuously grow in 17 years of the institution's existence (top table) . These are then translated into various training programs (middle table) and popular publications (bottom table) which correspondingly increase with the results of research.

Scientific papers

The vigor of the institution's research activity is reflected in the number of scientific papers produced during the year: published - 67. in press - 25, and submitted for publication - 50.

The Best Paper Award received by Research Associate Renato F. Agbayani and coworkers continues the yearly recognition given to AQD research since 1987 by *different institutions such as the International Center for Living Aquatic Resources Management (AQD won the 1987 Naga Award), Department of Science and Technology (1987, 1988 Dr. Elvira Tan O. Memorial Award for Fisheries Research), and the Department of Agriculture (1989 National Research Symposium).*

Best paper award

"An Economic Analysis of the Modular Pond System of Milkfish Production in the Philippines" by R.F. Agbayani, D. Baliao, N. Franco, R. Ticar, and N. Guanzon, Jr. was AQD's award-winning paper in the Department of Agriculture/Bureau of Agricultural Research (DA/BAR) National Research Symposium held 23-25 July.

The abstract of the paper published in *Aquaculture* 83 (1989):249-259 follows: "In 1980, the annual yield of milkfish ponds in the Philippines was 800 kg/ha while the potential yield is estimated to be 2000 kg/ha. The modular pond system analyzed in this study can largely close the gap between actual and potential yield through more efficient use of pond capacity to increase the number of croppings up to 7 times in a year. Pilot-scale production using the modular pond system was done at the Leganes Research Station (LRS), SEAFDEC, Iloilo, and at three cooperating commercial farms. Scale of operation ranged from 2.7 ha to 7.9 ha. From 2 to 7 production runs were recorded with per hectare outputs ranging from 278 kg to 341 kg per run. Input costs were based on actual figures and the ex-farm milkfish price of P21.00 (4 to 6 fish/kg). The average return on investment and payback period for all sites was 68.81% and 1.25 years, respectively."

In last year's DA/BAR Symposium, AQD also won first place in the fisheries and aquatic category with the paper entitled "Luminous Bacterial Disease of *Penaeus monodon* Larvae in the Philippines."

The Philippines as well as other countries in the region now uses SEAFDEC/AQD's *latest hatchery technologies. Unlike fry from the wild, hatchery-reared fry are of uniform size, are not mixed with predators, and are available in bulk.*

Hatchery technologies

During the year. AQD intensified the dissemination of its hatchery technologies. Upon request of the Department of Agriculture, AQD conducted two sessions of the *Training Course in Milkfish Hatchery,* with support from the International Development Research Centre of Canada, to provide a core group of trained hatchery technicians. With the planned regular offering of this course, designed to use shrimp hatchery facilities, the milkfish industry is expected to go great lengths.

AQD's newest hatchery technologies are contained in three extension manuals; the first two on milkfish and sea bass were released during the year while the manual on shrimp hatchery operations was being revised. These technologies, developed through over a decade of research at AQD, answer the seed supply problems of the milkfish, sea bass, and shrimp grow-out cultures. Hatchery, as an initial phase in finfish and crustacean culture, has become an indispensable step to meet the growing fry requirements of the industry.

Milkfish Hatchery Operations is intended for practicing shrimp hatchery operators who would like to diversify operations to include larval rearing of milkfish. Procedures important in the daily operations of a small-scale milkfish hatchery are described in detail. With these procedures, a minimum production of 60 000 fry per run of 21 days could be attained.

Sea Bass Hatchery Operations addresses the principles and procedures for rearing sea bass - from eggs to metamorphosis - as practiced by AQD. It also hopes to generate and enhance interest in sea bass as an experimental animal and as food fish to culture. With the sea bass hatchery techniques it has accumulated, AQD has included the *Training Course in Sea Bass Hatchery* for the first time in its training lineup for 1991.

The techniques described in the updated version of *Prawn Hatchery Operations* are not only applicable to the giant tiger shrimp *Penaeus monodon,* but also to other penaeid and metapenaeid species, e.g., "hipong puti" (*P. indicus* or *P. merguiensis*), "hipong bulik" (*P. japonicus*), *P. latisulcatus, P. semisulcatus,* and "suahe" (*Metapenaeus ensis*). The manual presents the underlying principles and step-by-step instructions on shrimp larval and post-larval rearing.

RESEARCH

Research management was expanded with the addition of Nursery as a new section, completing the three phases of aquaculture production as research sections: Breeding, Nursery, and Farming Systems. Feed Development and Fish Health are the two essential support sections.

Significant findings under the five sections follow.

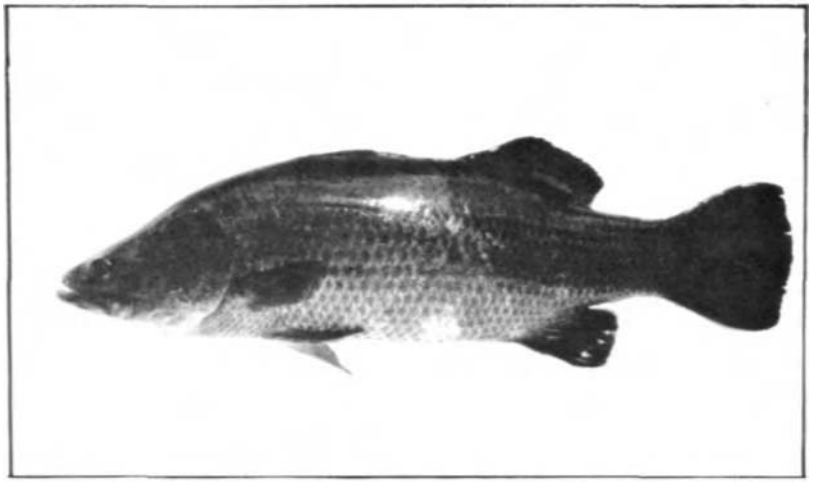
Studies

Sea Bass (*Lates calcarifer*)

- **Spawning response of sea bass to LHRHa stored under various conditions**

Injection of luteinizing hormone-releasing hormone analogue (LHRHa) stored for more than 90 days in a refrigerator (4- 10°C) or for more than 30 days at room temperature (28-30°C) significantly reduced the spawning response of sea bass (0-25%) injected once with these hormone preparations. Compared to those treated with a fresh hormone preparation, fish had similar spawning rates (75-100%) after injection of LHRHa previously frozen (-70°C or -4°C) for 50 days, subjected to 5 and 10 cycles of alternate freezing and thawing, and exposed to direct sunlight for 6 h. Sea bass implanted with pelleted LHRHa kept at room temperature for 120 days also had high spawning rates (75-100%). Only egg production levels of fish injected with LHRHa frozen for 50 days at -70°C and those which received an injection of the hormone exposed to direct sunlight were significantly different compared to fish treated with a fresh preparation of LHRHa.

The Asian sea bass, hates calcarifer



- **Spermiation response of sea bass to LHRHa and methyltestosterone** (Thesis Research)

Milt ($19.7x10^6$ spermatozoa/μl) hydration or decrease in sperm density in mature sea bass occurred after a single injection of LHRHa (20 or 80 μg/kg). Milt hydrated 12 to 50 h after LHRHa (80 μg/kg) injection alone or in combination with methyltestosterone (200 μg/kg). A lower LHRHa dose (20 μg/kg) also induced milt hydration 24 h after injection; milt returned to pre-injection levels 48 h after treatment. Milt hydration was not observed during 6 weekly samplings of mature sea bass receiving weekly injections of a high (200 μg/kg) or a low (100 μg/kg) MT dose. However, whether 80 μg/kg LHRHa was injected or not to fish on the 6th week, fertilization of spawned eggs occurred after the 6th weekly injection of a low MT dose.

- **Hormonal induction of off-season gonadal maturation and spawning of sea bass broodstock**

Five monthly implantations of pelleted LHRHa, 17 α-methyltestosterone and their combination (100 μg of each hormone/kg body weight) starting mid-November failed to stimulate vitellogenesis and spermiation in post-spawned sea bass. Implantation of these pelleted hormones (200 μg of each hormone/kg) at 45-day intervals from mid-November until February (1991)

did not advance sexual maturation among post-spawned sea bass.

- **Nursery of sea bass in floating cages - 1. Effects of varying stocking densities and supplemental feeding**

Sea bass fry [initial mean total length (TL), 1.9 cm] were reared for 42 days at stocking densities of 150, 300, 600, and 1600/m^3 in 1m x 1m x 1m cages. Cages were illuminated and fish were either fed minced trash fish or not. Fish stocked at 300/m^3 attained highest mean TL with (5.07 cm) or without (4.03 cm) supplemental feeding. Survival in all treatments did not vary significantly. Generally, growth and survival of fed groups were significantly higher than the unfed. The occurrence of shooters and mortality due to cannibalism were not related to different stocking densities.

- **Nursery rearing of sea bass in ponds - 1. Food and feeding**

Sea bass fry were stocked in 1 m^3 cages in a brackishwater pond in Silay, Negros Occ. These were fed artificial diet at 5, 10, and 20% biomass/day. Mean survival after 1 - and 2-week rearing were 28.7% and 13.5%, respectively. The experiment was terminated after the 2nd week due to poor survival.

- **Rearing of sea bass fry in tanks at different stocking densities and salinity levels**

After a month of culture, sea bass fry reared in nursery tanks provided with biofilters and stocked at different densities of 500, 1500. and 4500 fish/t showed mean (2 replicates) survival of 38.7, 52.7, and 34.5%, respectively. Mass mortality occurred in sea bass stocked at 1500 and 4500 fish/t and reared with 75% daily water replacement while sea bass stocked at 500 fish/t showed low survival (10.9%).

- **Use of pre-adult *Artemia* as feed for sea bass fry**

Pre-adult *Artemia* were fed to sea bass fry at 0.625, 1.25, 2.5, and 5.0 ind/ml. Ricebran-fed pre-adult *Artemia* gave better growth (final TL=2.0-2.5 cm) and survival (76-89%) compared to enriched pre-adult *Artemia.* Growth and survival were significantly better in treatments that received 2.5 and 5.0 pre-adult *Artemia*/ml.

- **Food consumption of sea bass in captivity - 11. Group feeding**

Preference of hatchery-reared sea bass over 2 prey choices (tilapia and sea bass) was determined under laboratory conditions using time series trials. Sea bass actively selected tilapia as food within the 1st and 2nd h as demonstrated by the large proportion of predators (60.4 and 46.4%, respectively) that fed only on tilapia. Among the fish that ate both prey at the same time, tilapia also represented a higher proportion of the total prey consumed. These results strongly indicate that sea bass predators were actively selecting tilapia over sibling prey.

- **Verification studies of hatchery production of sea bass**

In contrast with previous results, a 2nd trial on the use of highly unsaturated fatty acid (HUFA) emulsion showed that HUFA enrichment hastened metamorphosis but did not improve survival of fry. Since different batches of sea bass eggs from induced-spawned parents with low fertilization rates have been used, further trials will be done to confirm these results.

Biomass culture of Artemia in tanks using rice bran.

- **The influence of temperature and salinity on the oxygen consumption of sea bass larvae** (Thesis Research)

Day 0 sea bass larvae were reared in 16, 24, and 32 ppt water salinities. Day 7, 15, 23, and 31 larvae were subjected to 8 salinity levels of 0, 8, 16, 24, 32. 40, 48, and 56 ppt for 96 h. Results showed that there was a significant difference in survival and growth in length and weight among the different age groups. Acclimation salinity affected the salinity tolerance of the larvae ($P<0.05$). Moreover, an interaction between acclimation and test salinities on the survival of the larvae was observed ($P<0.01$).

- **Economic analysis of an integrated sea bass system**

A 10-year discounted cash flow projection of a floating cage sea bass broodstock farm revealed that the project is economically viable. The economic indicators used were internal rate of return (IRR), net present value (NPV), and benefit-cost ratio (B/C).

In progress:

- Photoperiodic and hormonal induction of off-season gonadal maturation of sea bass

Nutrition and feed development for sea bass - I. Requirement for sulfur amino acids by sea bass juveniles

Practical diet development for sea bass: dietary protein and energy ratio

Deferred:

- The use of various *Artemia* biomass preparations as feed for sea bass fry in nursery tanks

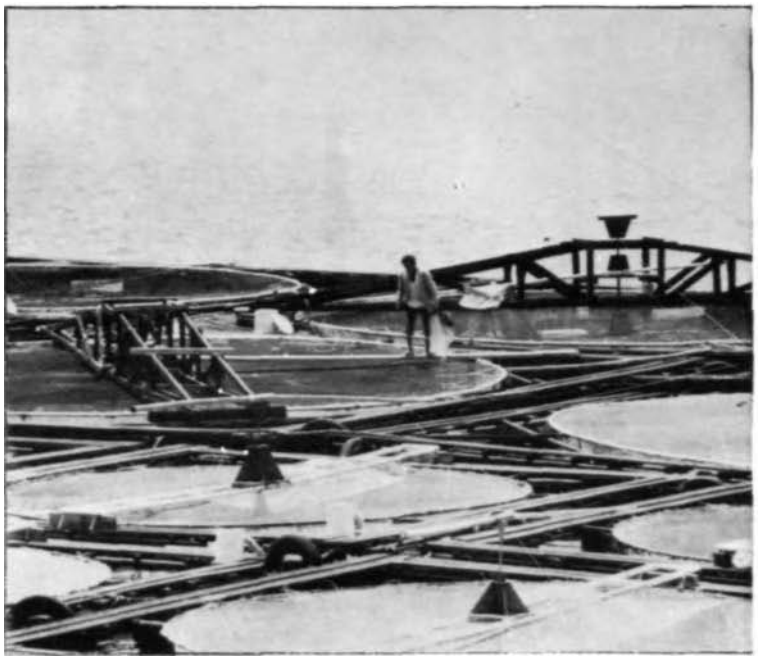
Grouper (*Epinephelus* spp.)

- **Induction of sex change in juvenile female grouper *Epinephelus malabaricus* by intraspecific inter-action**

Only 4% of juvenile female grouper possessed an ovotestis 11 wk after groups of fish of different sizes were stocked in communal tanks. Other fish sampled had gonads at an immature (54%), maturing (33%), or possibly sex transition (8%) stages. Similarly, fish had ovaries at various stages of sexual maturity (22 wk after initial stocking).

- **Broodstock development of *Epinephelus* sp. and *Lutjanus* sp. for seed production**

The Igang Marine Substation (left) is the center for most studies in marine finfish broodstock development like milkfish, sea bass, and grouper; the snapper, Lutjanus sp. (right).



Four mature males (7-12 kg) and 6 females (3-5 kg) reared in a 50-t concrete tank spawned 11-15x from July to Oct. Similarly, a mature female (5 kg) paired with 2 spermiating males (6 kg) in a 4m x 4m x 3m floating net cage spawned 5-8x from July to Oct. Daily egg collection varied: 22 500 - 3 240 000 eggs in the concrete tank and 45000-750 000 in the floating net cage. Fertilization rates of 71-81% and hatching rates of 50-76% were recorded in tanks whereas, fertilization and hatching rates in net cages ranged from 72-87% and 30-68%. respectively.

- **Development of grouper broodstock in floating net cages**

Twenty-four groupers from the wild were stocked in floating net cages at Igang Marine Substation.

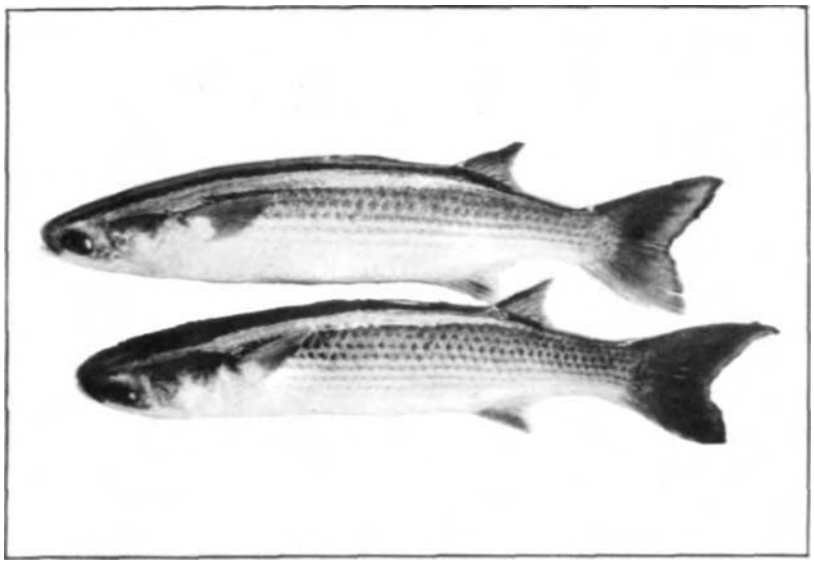
- **Induced sex control in juvenile grouper, *Epinephelus* sp.**

Gonad of juvenile grouper consists of a thin connective tissue with few germinal cells lying ventral to the urinary bladder. Preliminary histological observations failed to detect a significant alteration in germ cell morphology between fish fed a plain diet and fish fed a mibolerone-enriched diet.

- **Development of a dry food for carnivorous fish larvae and** fry **(grouper and seabream)**

Five formulated diets were tested as feed for grouper fry [0.3 g body weight (BW); 3.0 cm TL). The main attractant used in the diets were: (1) shrimp meal paste, (2) squid meal, (3) squid liver meal, (4) fermented soybean meal, and (5) a relatively equal ratio of the 4 attractants. Diet 4 gave higher survival (39%) for weaning to artificial diet. Moreover, diet 4 given in combination with minced fresh fish meat to 1.0 g BW fish gave high survival (92%). However, fish fed minced fresh fish meat alone gave highest survival (97%).

Survey of *Epinephelus* spp. fry in the northeastern coast of Panay Island



Mullet (Mugil cephalus) is one of the marine species being studied for propagation.

Epinephelus spp. fry [size, 2.2-5.1 cm SL (standard length); 0.2-4.8 g BW] started to appear in Aug. with a peak in Oct. Fry habitat seems to be corraline rocks and wood along sandy beach. Juveniles and adults exhibited distinct habitat preference, e.g., *E. megachir* in rocks close to open water and *E. tauvina* in logs along channels of inshore water. Salinity in the survey areas, 30-36 ppt; temperature, 26-29°C.

In progress:

- Grouper (*Epinephelus* sp.) culture in ponds given artificial diets

Snapper (*Lutjanus* sp.)

- **Species identification of snapper (Genus *Lutjanus*) in Panay Island**

Seven species of *Lutjanus* have been identified: *L. decussatus, L. fulviflamma, L. vitta, L. carponotatus. L. malabaricus, L. gibbus,* and *L. fulvus.* Fish were caught in the waters off Palawan, Guimaras, and Roxas.

Mullet (*Mugil cephalus*)

- **Mullet broodstock development for fish propagation**

Thirty wild, adult mullet were stocked in a 6-m diameter floating net cage in Apr. and fed fish pellet (24% protein) daily at 5% of their body weight until Oct. Thereafter, fish were fed crustacean pellet (36% protein) at the same ration. Sexually immature gonads were obtained in May-Oct. However, of the 23 fish sampled in Nov., 4 were maturing females (mean egg diameter, 0.24-0.50 mm) and 2, maturing males with thick milt.

In progress:

- Characterization of milt consistency in the grey mullet

Milkfish broodstock are held in 6- and 10m diameter floating cages in Igang.

Rabbitfish (*Siganus guttatus*)

- **Ovarian development of rabbitfish fed lipid-enriched diet**

Rabbitfish fed formulated diets (36% protein) enriched with cod-liver and soybean oils (12% lipid) produced mature oocytes for 5 consecutive months. However, fish fed a regular diet of lipid-poor pellets (36% protein and 4% lipid) intermittently produced mature eggs. In all monthly samplings, more mature females were observed among fish fed a lipid-rich diet compared to those fed a lipid-poor pelleted feed.

- **Application of thyroid hormones to mature female rabbitfish broodstock and its effect on larval survival**

Survival of fry from T_4 and T_3 injected and uninjected females were not significantly different.

Milkfish (*Chanos chanos*)

- **Hormonal induction of off-season maturation of milkfish**

There were no maturing or mature hormone-implanted (E_2, E_2+ LHRHa, or LHRHa) and control female milkfish sampled 12 wk after treatment while 11-67% were sexually maturing 19 wk following hormone implantation. Percentage of mature hormone-implanted (T, T+LHRHa, or LHRHa) and control males progressively decreased 12 and 19 wk after hormone administration in all treatments.

- **Determination of the maintenance rate of milkfish of varying body size at different rearing temperatures**

Experimental fish were fed 27% protein diet at 4 different feeding levels. Even the lowest feeding level yielded growth. A new diet and lower feeding levels will be tested.

- **Feeding biology of milkfish larvae: feeding pattern, digestion, and satiation time**

Milkfish larvae (4.97±0.42 mm; TL±SD) were initially fed rotifers at day 3.25. Mean number of rotifers in the gut was 3.6±3.1 with the amount ingested by the larvae positively correlated with larval size. Feeding (10% of larvae) started at 0500 H (14.2 lux) for day 7 larvae (TL=6.04±0.4 mm); at 0400 H (18.6 lux) for day 14 (TL=7.89±0.7 mm); and at 0600 H (0.85 lux) for day 21 (TL=8.87 ± 1.3 mm). Milkfish larvae showed diurnal feeding pattern with 3 feeding peaks for day 7 and day 21 larvae (1000, 1300, and 1600 H) and 4 for day 14 (0700, 1000, 1300, and 1600 H).

- **Verification of the larval rearing**

techniques for milkfish developed at SEAFDEC

Of the 61 larval rearing runs conducted for the entire spawning season, 17 gave positive results. Survival rates in concrete tanks averaged 18.4±4%, while runs in the canvas tanks gave negative results. Parallel runs conducted in a private hatchery using 10-t rectangular concrete tanks gave survival rates of 7-48%.

- **Mechanisms of recruitment and survival of milkfish larvae in coastal waters** (Thesis Research)

Preliminary results showed that feeding incidence of wild milkfish fry caught along the intertidal zone was very low (<10%). However, plankton collection showed that food organisms, phyto- and zooplankton, existed within this area. Catch per unit effort (CPUE) was relatively higher at night than during the day. Based on frequency distribution of total length, wild milkfish fry did not show a regular increase. Analysis of the number of otolith increments and gut content are needed to elucidate the mechanisms of recruitment and survival of milkfish fry in the intertidal zone.

- **Alternative feeding and rearing schemes in the larval rearing of milkfish**

Milkfish larvae fed a microparticulate feed (AP) in combination with *Brachionus* (Br) were significantly bigger than those fed AP or Br alone. Mass mortality on day 12 occurred among larvae fed AP. Mean survival at day 20 of AP+Br- and Br-fed larvae were 10.2 and 17.0%, respectively.

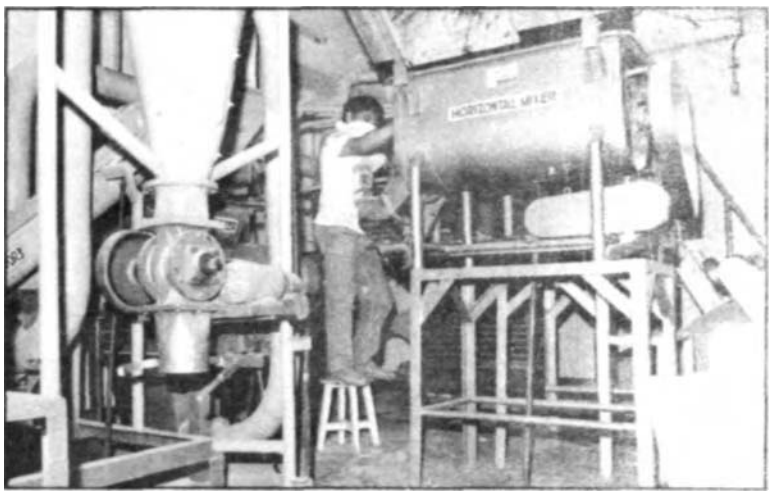
- **Development of supplemental diet for milkfish through assessment of natural food contribution to fish growth**

The growth and food consumption rates of natural food- and natural food+feed - dependent fish were compared. Growth rates for 96 culture days followed a linear model with higher slope in the fed fish. Difference in average fish weight (1.0-39 g) and daily ratios (224-425 gcal/fish/day) between treatments increased with time, suggesting that dietary input should be improved as natural food production decreases. These differences may provide a reliable estimate in the formulation of supplementary diet for different standing crops and pond productivity.

- **Effects of dietary fiber on growth and production of milkfish in brackishwater ponds**

Substitution of organic matter (protein, fat. carbohydrates, fiber) in the feed with rice straw compost up to 50% was possible without significantly affecting fish growth and yield. A significant reduction in growth was observed at 75% replacement level. Simple cost-benefit analysis showed that 50% replacement of feed with rice straw compost at a feeding rate of 1.75% body weight was the most profitable in culturing milkfish (690 kg/ha biomass).

Stock feeds for feeding experiments are processed in the newly installed feed mill.



Dietary requirement of milkfish juveniles for essential amino acids

Several feeding experiments were conducted using amino acid test diets (containing 40-45% crude protein) to determine the quantitative requirements of milkfish juveniles for essential amino acids. Optimum requirement levels for growth were determined for arginine, threonine, isoleucine, leucine, phenylalanine at tyrosine levels of 0.45 and 1.2%. Replacement value of tyrosine for phenylalanine was 44%.

- ***In vitro* determination of protein digestibility in milkfish** (Thesis Research)

Protease activity of extracts from milkfish intestine and pyloric caeca was determined using azocasein as substrate. Results confirmed previous published data that protease activity in pyloric caeca is 2.5x higher than in the intestine.

- **Prevention of *Aeromonas hydrophila* infection in milkfish by vaccination**

Aeromonas hydrophila from naturally infected milkfish were screened for strain variability. Flagellar H, somatic O, sonicated S, and crude lipopolysaccharide L antigens were prepared from a pool of 5 strains of A. *hydrophila.* Immunogenicity of the individual antigens was tested. Preliminary results showed that vaccinated milkfish did not develop protective immunity against the pathogen although humoral antibodies were detectable.

In progress:

- Influence of broodstock ration size and cod-liver oil supplementation on quality of spawned eggs and larvae of milkfish
- Measurement of the critical oxygen tension of milkfish at different rearing temperatures and for different body mass groups

The Binangonan Freshwater Substation (left) is where many of the tilapia (right) genetic studies are conducted.

- Studies on the oxygen consumption of milkfish with different body mass and at different rearing temperatures
- Optimal level of cowpea in a practical diet for milkfish

Tilapia (*Oreochromis* spp.)

- **Development of a genetically stable reference population of red tilapia**

Based on electrophoretic analysis, a red tilapia strain from the Freshwater Aquaculture Center [FAC in Central Luzon State University (CLSU). Nueva Ecija] (% polymorphic loci, 13.3%; mean heterozygosity, 0.054) was less genetically variable than a SEAFDEC strain (% polymorphic loci. 26.7%; mean heterozygosity, 0.091). Genetic diversity of these 2 strains makes them suitable for the production of a reference population. Twenty breeding sets (10 sets of mated sibs/strain) were stocked at a female-male ratio of 3:1 in spawning tanks. Half of the breeders spawned and the majority were FAC strain.

- **Development of a high-yield red tilapia strain through introgressive hybridization**

Two hundred F_1 red tilapia breeders from an initial mating of 15 red tilapia females and 15 grey tilapia males (Chitralada strain) were mass spawned in 5m x 10m x 1m concrete tank. One

thousand one hundred thirty-four red fry and 454 grey fry were collected. Red fry will be used for selection and back-crossing experiments.

- **Growth, feed utilization, and body composition of young red tilapia given diets with varying protein and energy levels**

An 8-wk feeding trial was conducted on red tilapia fingerlings using diets with varying protein levels (25, 30, 35, and 40%) and varying protein-to-energy ratios (111, 100, and 80 mg/kcal). Weight gain, protein efficiency ratio (PER), and feed conversion ratio (FCR) were significantly affected by dietary protein level. Although fingerlings fed the 30, 35, and 40% protein diets had comparable growth and feed utilization, diets with a P/E ratio of 100 mg/kcal gave the best weight gain, PER, and FCR at each protein level. Body composition was significantly influenced by the dietary treatments.

- **Changes in plasma osmolality and chloride concentration during abrupt transfer and acclimation of tilapia strains to test salinities**

All genetic strains (mean body weight, 25 g) tested can tolerate abrupt transfer from freshwater to 15-20 ppt seawater. However, abrupt transfer to 32 ppt resulted in mass mortality of *Oreochromis niloticus* in 6 h and in 36 h for their F_1 hybrids. *O. mossambicus* can tolerate abrupt transfer from freshwater to 32 ppt seawater.

- **Development of genetic evaluation and selection criteria for tilapia broodstock: IV. Effect of restrictive and non-restrictive feeding on growth of *Oreochromis niloticus* strains**

A restrictive feeding regime retarded growth among Israel, NIFI (National Inland Fisheries Institute. Thailand), and CLSU tilapia test strains. However, the treatment effect was not significant. The Israel tilapia strain exhibited best growth (followed by CLSU and NIFI) in both restrictive and non-restrictive feeding regimes.

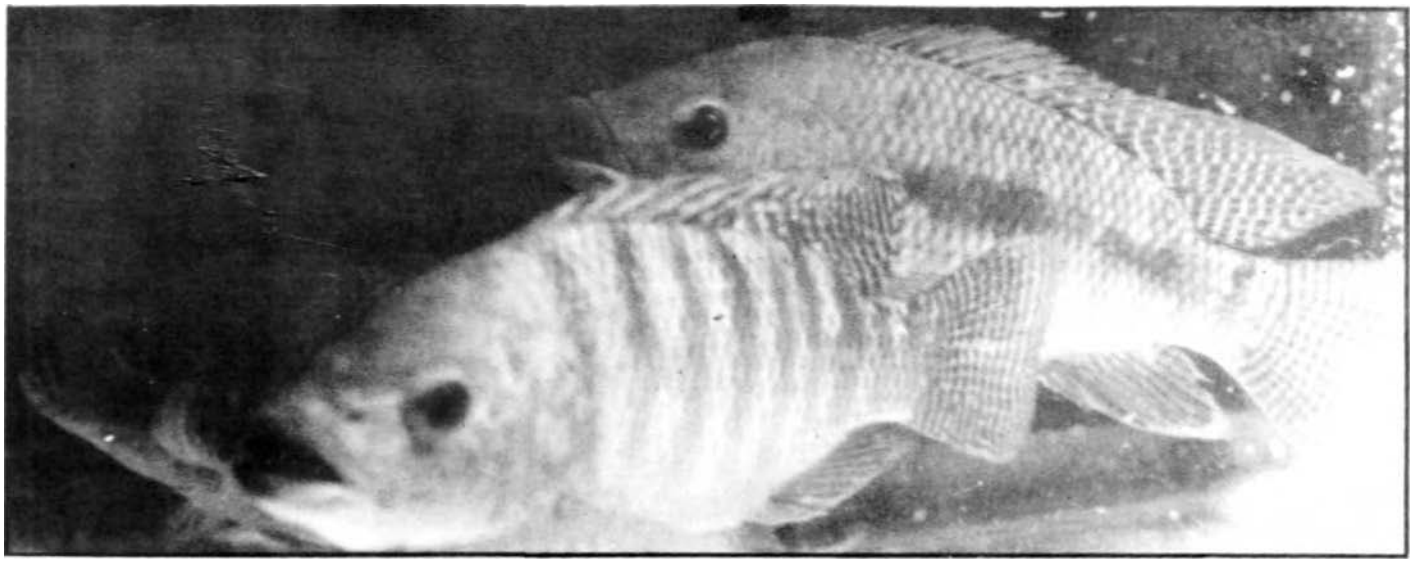
- **Performance evaluation of two *Oreochromis niloticus* strains in two lake environments**

NIFI strain on initial analysis gave better performance than the commercial strain in Laguna de Bay and Sampaloc Lake.

- **Effects of prolonged exposure to low level heavy metals on two Nile tilapia strains**

Fingerlings of *Oreochromis niloticus* strains (NIFI and CLSU) (mean body weight, 0.140 g; length, 15-16 mm) were exposed to a mixture of 5.0 mg/1 Zn, 0.5 mg/1 Cd, and 0.02 mg/1 Hg in 3 replicate aquaria. These heavy

A popular food fish, the hardy tilapia (especially Oreochromis niloticus) is the subject of intensive genetic studies.



A 3-kg bighead carp breeder.

metal concentrations were determined to be sublethal in previous single metal exposure experiments. The 3 metals appeared to have a synergistic effect, e.g., both strains exposed to metals showed significantly higher mortality from controls ($P<0.05$). Survival of NIFI strain was, however, significantly higher than CLSU strain ($P<0.05$) as early as the 4th day of exposure to the 10th day.

In progress:

- Practical methodology for genetic strain evaluation of tilapia in small- to medium-sized experimental facilities
- Effects of exposure to sublethal heavy metal mixtures on two generations of two Nile tilapia strains

Catfish (*Clarias macrocephalus*)

- **Broodstock development and induced spawning of catfish: induced spawning using LHRHa and pimozide**

Gravid catfish were induced to undergo final oocyte maturation and ovulation in Aug. and Sept. by simultaneous injection of a minimum dose of 0.05 µg LHRHa/g and 1 µg pimozide/g. Eggs stripped from fish 16-20 h after injection had comparable fertilization (75-99%), hatching (48-91%), and survival (68-99%) rates. However, female catfish given the same hormone dose failed to spawn spontaneously in the presence of mature males placed in 60-1 tanks with mud substrate and floating vegetation. Around 43 000 larvae were produced.

Bighead Carp (*Aristichthys nobilis*)

- **The influence of commonly used induced spawning agents on fertilization, hatching, and survival of bighead carp eggs and larvae**

Lake-reared bighead carp were induced to spawn by either HCG or LHRHa alone and LHRHa in combination with either HCG or reserpine. Eggs spawned by fish injected with LHRHa in combination with HCG had the highest mean fertilization rate (90%) whereas those from fish injected with only LHRHa had the lowest (32%). When eggs were incubated in hard water (300-500 mg/1 $CaCO_3$) at 27°C, 28% of eggs spawned by HCG - treated fish hatched while eggs from LHRHa+HCG - injected fish had the highest hatching rate (79%).

Giant Tiger Shrimp (*Penaeus monodon*)

- **Histological characterization of giant tiger shrimp testes**

Small-sized shrimps (<1 g) had poorly developed and undifferentiated testes. Testes of bigger-sized shrimps (>3 g) were more developed and showed signs of early spermatogenesis. Shrimps weighing 24 g (33-mm carapace length) had a fused petasma and translucent gonad. The vas deferens had 2 distinct lumen separated by an arc-shaped septum extending longitudinally.

- **Hormonal changes during vitellogenesis in tiger shrimp**

Immunoperoxidase staining method indicated that vitellogenin is localized in the ovary of the tiger shrimp. Primary oocytes can be observed among 2- to 3-month old shrimps.

- **Evaluation of suitability of different external tags for shrimp juveniles (artificial restocking of shrimps in Southern Panay)**

Compared with untagged shrimp juveniles and shrimps having small polyvinyl streamer tags (88.9%). all shrimps with large tags survived a week after tagging. In a 2nd study, untagged shrimps (91.7%) had significantly higher survival rates than tagged (68.3%) shrimps after 8 wk, although survival rates of tagged and untagged juveniles belonging to 2 size classes (small, 16-19 mm carapace length; large, 19-22 mm) were not different. In another study, increase in carapace length of small shrimp juveniles (4.6%) were significantly greater than large shrimps (3.8%); however, no difference in growth rates was observed between untagged (4.4%) and tagged (4.0%) shrimps.

- **Verification studies of SEAFDEC-developed hatchery techniques: larval rearing of giant tiger shrimp using chlorinated or UV-treated water**

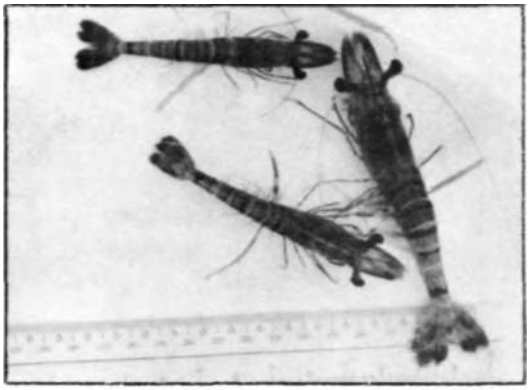
Mean percentage survival of *Penaeus monodon* larvae reared at a density of 50/1 in untreated (control), chlorinated, and UV-treated water were 17, 29, and 23%, respectively. Although initial total plate bacterial counts showed tenfold reduction for both chlorinated and UV-treated water as compared with untreated water, bacterial loads during hatchery run were extremely variable thus no significant difference was seen between treatments.

- **Abiotic and biotic changes in estuaries receiving effluents from shrimp culture ponds**

Twenty high-density shrimp farms in Western Visayas and Northern Mindanao surveyed from June to Oct. used at least 40 commercial products for various purposes: (a) pond preparation; (b) disinfectants to kill algae, fish, and other pests; (c) antibiotics for pathogenic bacteria and fungi; (d) feed additives, e.g., vitamins and minerals; (e) bioaugmentation products, e.g., bacterial enzymes to decompose organic matter; and (f) other products to induce plankton growth or molting and to harden the exoskeleton. Most of the products are Taiwanese; the rest local, U.S.. or U.K. products.

- **Evaluation of unprocessed feeds in semi-intensive shrimp pond culture**

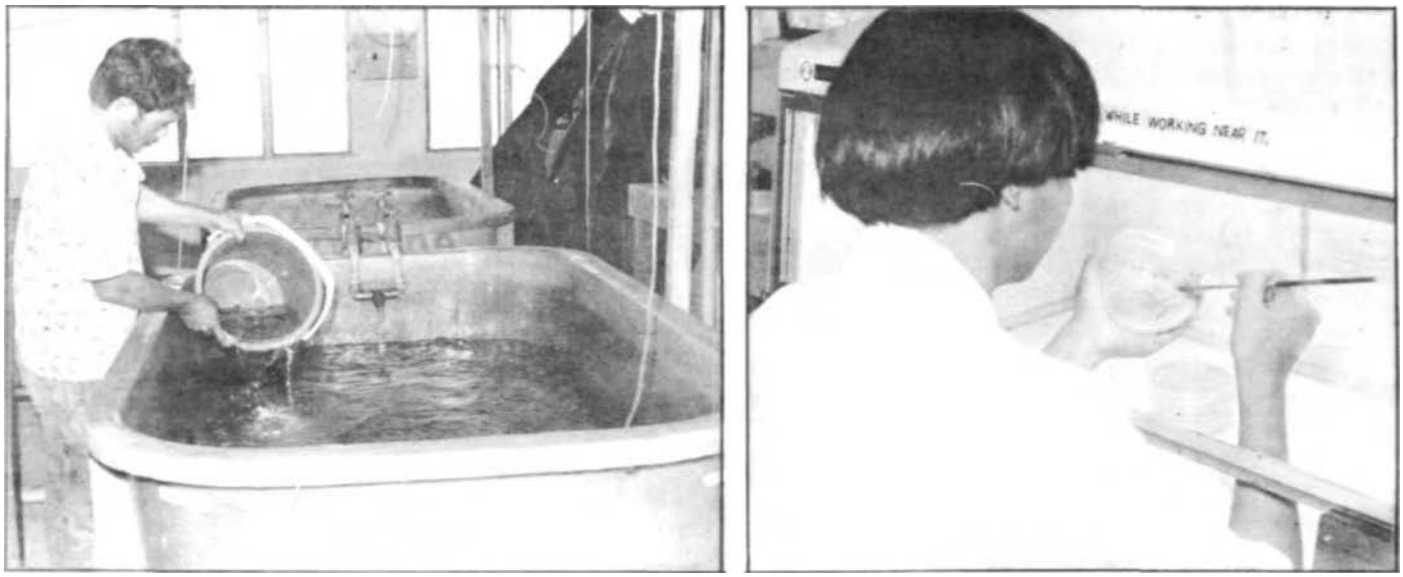
Growth and survival of shrimps given corn or cassava in combination with golden snails (ratio = 2:3) did not differ significantly ($P<0.5$) at stocking densities of 10 000 and 15 000. Survival was 45.1-60.9%; final mean body weights, 23.4-27.9 g.



Broodstock development of the giant tiger shrimp (left) in tanks (below) is a continuing study.

- **Effect of diet on the growth, survival, and gonadal development of ablated and unablated pond-reared shrimp broodstock**

Among the pond-reared *P. monodon* broodstock (initial average BW: 82.6 g females; 55.1 g males), only 4 attained stage II-IV gonadal maturity under the following conditions: water temperature, 22.5-28°C; dissolved oxygen , 4.0-12.0 ppm; and salinity, 34-40 ppt. Low survival appeared to be due to



Shrimps are sampled to determine the effect of diet on growth (left) while bacterial studies on shrimp continue (right).

predation, poor water quality, algal encrustations, and soft shelling. Stage IV shrimp failed to spawn and had low gonadosomatic index (4.5). Microscopic examination of samples revealed immature spermatozoa in male spermatophores and female thelycum.

- **Natural diet of various size classes of shrimp in pond**

Preliminary gut analysis showed that young shrimps (1-3 kg) ingested detritus, diatoms, green and blue-green algae, and animal remains. Generally, weight of foregut contents increased at night and decreased during the day.

Shrimp juvenile is injected with fixative for histological processing.

- **Amino acid requirement of shrimp: threonine, lysine, and valine**

Lysine requirement was determined for post-larval *P. monodon* using test diets containing graded levels of lysineat8.0, 11.5, 15.0, 18.5, 22.0, and 25.5 g/kg diet. Crude protein content of the diets was 37%. Highest growth rate was observed in shrimps given a diet containing 11.5 g lysine/kg diet or 3.2% of dietary protein. Survival rate of shrimp fed these diets ranged from 75 to 100%.

- **Effect of butylated hydroxy toluene on the quality of shrimp diets stored at different temperatures**

Practical diets with or without butylated hydroxytoluene (BHT) were stored at 4 different temperatures (10, 20, 30, and 40°C) for 10 wk. Higher 2-thiobarbituric acid (TBA) values were seen in shrimp diets without BHT stored at 10, 20, 30, and 40°C than those with BHT. TBA values started to increase after a week of storage.

Shrimp (mean body weight, 0.195 g) fed diets with or without BHT stored at lower temperatures (10 and 20°C) had better weight gain and survival than those at 30 or 40°C.

- **Effect of chemoattractant on the growth and feed efficiency of shrimp juveniles**

P. monodon juveniles reared individually in 4-compartment 50-1 fiberglass tanks were fed basal diet, basal diet + Finnstim (0.5, 1.0, 1.5, 2.0, 2.5% levels), reference diet, or commercial diet. Initial results showed shrimps fed basal diet + 1.0% Finnstim had the highest numerical survival, weight gain, and SGR, but the lowest feed conversion.

- **Evaluation of some local binding materials for shrimp diet**

Agar, *Kappaphycus,* com starch, cowpea, *Gracilaria,* and wheat gluten were evaluated as binders in a practical diet for shrimp. Water stability of diets with *Kappaphycus* and *Gracilaria* was 65% after 24 h while diets with other binders had water stabilities of 61-63%. Preliminary results showed high survival rate (52.5%) in shrimp fed diets with *Kappaphycus* and cowpea. However, shrimp fed diets containing agar and corn starch showed higher weight gain (1221.82% and 1017.67%) compared to shrimp fed diets with other binders (686.02%, 710.65%, 815.50%, and 732.96% for *Kappaphycus. Gracilaria,* cowpea, and wheat gluten, respectively).

- **Effect of dehulling on digestibility of various leguminous seeds as protein sources for tiger shrimp juveniles**

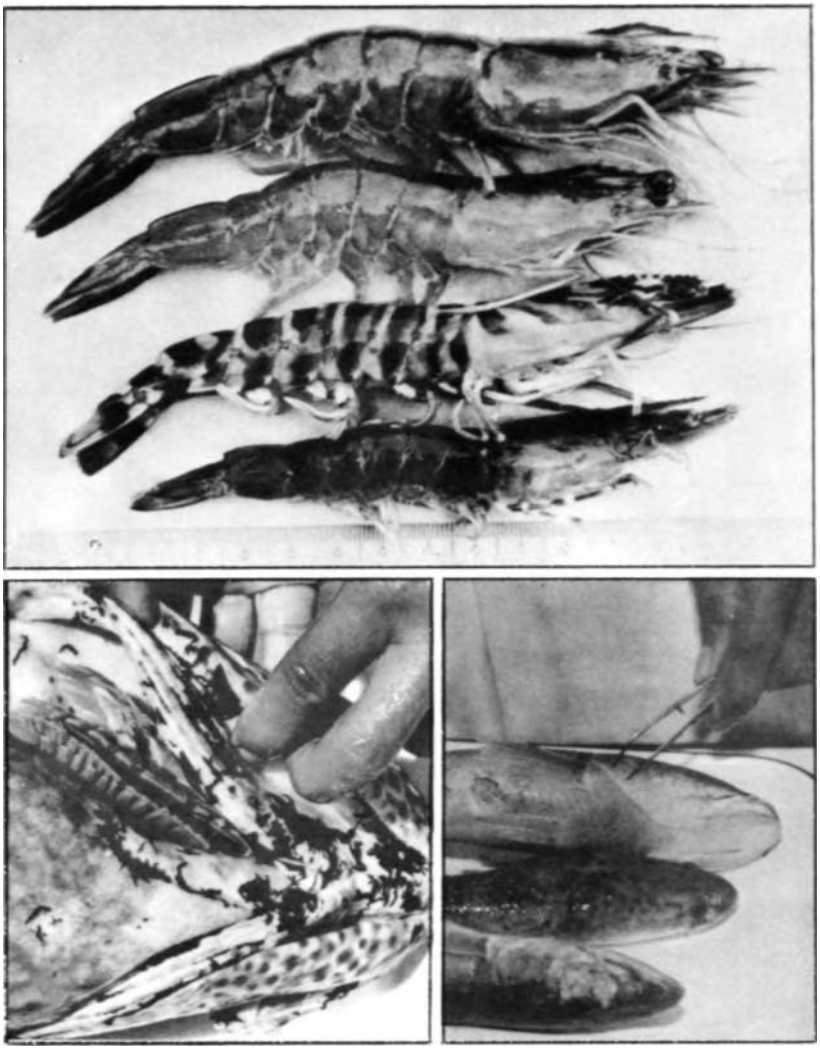
Dehulling significantly increased ($P<0.05$) the apparent protein digestibility of rice bean but not of cowpea. No significant difference ($P<0.05$) was observed among dietary treatments based on growth response and survival of the animals and no correlation existed between growth and apparent protein digestibility. *P. monodon* given dehulled cowpea diet performed best while those fed whole rice bean diet registered the poorest response. Results indicated that dehulling of legumes was partly responsible for the improvement in their nutritive value.

- **Detection of *Penaeus monodon* baculovirus (MBV) in hatchery systems**

Penaeus monodon samples from hatcheries and ponds were histologically examined for the presence of eosinophilic bodies in hypertrophied nuclei of the hepatopancreas cells which are indicative of MBV infection. The earliest stage where infection was found was postlarva 3. Infected shrimps from ponds had slow growth rates and generally had pale yellow to reddish brown hepatopancreas. Infection was also characterized by necrosis and degeneration of hepatopancreatic tubules with secondary bacterial invasion.

- **Investigations on the vertical and horizontal transfer mechanisms of the luminescent bacterium, *Vibrio harveyi***

Bacterial isolation showed that unspawned eggs of both wild and ablated *Penaeus monodon* females harbored no



Health is wealth just as well in shrimps (top) and other finfishes (bottom) as it is in humans. Importing countries demand high-quality products.

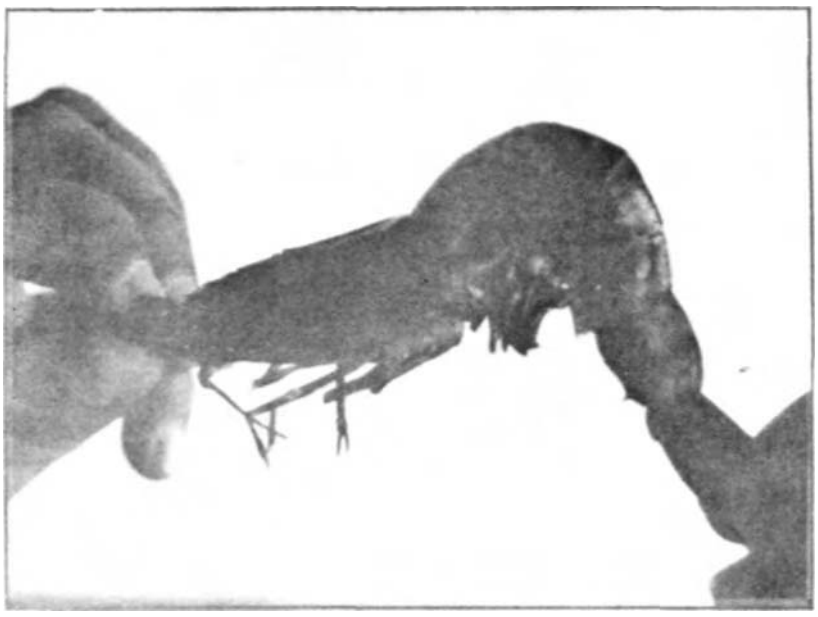
bacteria. However, the midgut contents of these spawners contained up to 10^9 bacterial cells/g, 30% of which was composed of *Vibrio harveyi.* The diatom *Chaetoceros calcitrans* did not harbor *V. harveyi* at any phase of its growth. Twenty-four h old *Artemia salina* nauplii appear to have no resident V. *harveyi,* but the culture water contained a small population.

Tolerance of *V. harveyi* to various salinity and pH levels was tested. The bacteria survived and grew in media with 0.5% to 7% salt, and pH levels from 6 to 9. In the absence of nutrients, *V. harveyi* had a narrower salinity tolerance (0.5%-4%).

• Vibriosis of shrimp in hatchery: epidemiology and control

The marine ciliate *Euplotes* is a potential biocontrol agent against the causal bacterium of luminiscent vibriosis in penaeid shrimps. *Vibrio* proved very hardy in seawater: daily cell counts for 7 days were invariable ($P<0.05$). But in *Euplotes-Vibrio* mixed in stationary cultures, Vibrio load from a high 2.8×10^7 cfu/ml decreased to nil levels as *Euplotes* increased by as much as 2833%. Near total elimination of *Vibrio* was possible even in 5 days with *Euplotes* at peak logarithmic growth. Daily population build-up of the ciliate was onefold without and threefolds with aeration.

P. monodon juvenile with body cramp.



In progress:

- Semi-intensive culture of shrimp given pellet feeds and supplemented with natural food
- Quality assessment of shrimp feeds and feed ingredients: thiobarbituric acid value, aflatoxin level, and urease activity and the biological effects on tiger shrimp

Deferred:

- Growth performance of shrimp fed formulated diet without vitamin and mineral supplement

White Shrimps (*Penaeus indicus/P. merguiensis*)

• Changes in the hemolymph and tissue proteins during the different stages of maturity in *Penaeus indicus*

Vitellogenin in hemolymph was detected in shrimp with immature (4.5 μg/ml) and vitellogenic (23.3 μg/ml) ovaries, gradually increasing when shrimps developed cortical rod-stage ovaries (48.3 μg/ml) and decreasing in spent shrimps (22.86 μg/ml). Ovarian protein levels increased at the onset of vitellogenesis but dropped to their lowest levels during the cortical rod-stage of ovarian development. Protein levels in the hepatopancreas increased among vitellogenic shrimps but decreased subsequently. Amino acid levels were higher in hepatopancreas than in ovaries.

• Larval rearing of white shrimps (*Penaeus indicus/merguiensis*): water management techniques

Larvae reared in UV- or chlorine-disinfected seawater had high survival rates during initial metamorphosis to mysis-1. Mysis larvae reared in UV-disinfected seawater showed comparable survival with those reared in chlorinated and antibiotic-treated water. At initial PL, molting until population was entirely postlarvae, survival in UV-disinfected seawater remained comparable with those reared in antibiotic-treated and chlorinated seawater.

- **Ingestion rates and food selectivity of *Penaeus merguiensis* larvae**

Ingestion rate of *Penaeus merguiensis* on *Chaetoceros calcitrans* was determined. Protozoea 1, 2, and 3 showed a maximum ingestion rate of 8250- 10 670, 8750-12 575, and 14 975 - 21 500 cells/larva/h, respectively. Apparent incipient limiting levels were 2.5-3.0, 2.5-3.0, and 4.0-5.0 x 10^4 cells/ml. No distinct trend was observed when *C. calcitrans* was fed to mysis stages.

- **Development and evaluation of larval diets for *Penaeus indicus/P. merguiensis***

Several formulated diets in combination with natural food were fed to *Penaeus indicus* larvae reared at 2 temperature ranges (29-30°C and 26-28°C). The rearing period from zoea 1 to postlarva 1 varied from 12-14 days for the larvae reared at 26-28°C and 10-11 days for those reared at 29-30°C. Survival rate almost doubled (64%) in larvae reared at 29-30°C and fed a combination of C-MBD (carrageenan-microbound diet) and *Skeletonema* .

In progress:

- Selective breeding of penaeid shrimps I. Relation of reproductive performance and morphometric traits with body size/tail weight in *Penaeus indicus*

Molluscs (*Placuna placenta/ P. sella*)

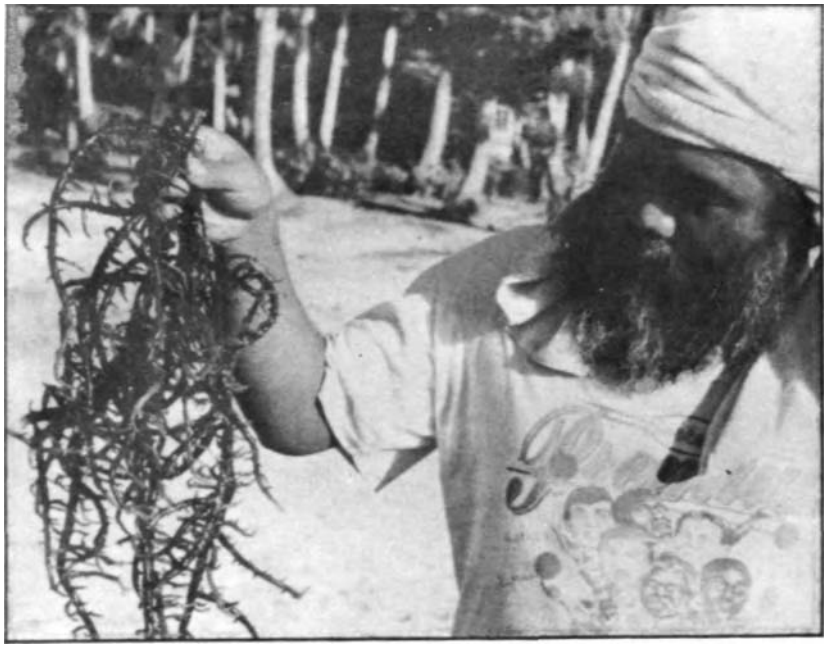
- **Algal food preference and growth of *Placuna placenta***

Based on ingestion rates, *Isochrysis galbana, Chaetoceros calcitrans. Chlorella virginica,* and *Tetraselmis tetrahele* were preferred by P. *placenta* (70 mm, sexually immature) over *Skeletonema tropicum.* Feeding P. *placenta* with /. *galbana* showed best growth while the combination of *I. galbana* and *T. tetrahele* resulted in highest survival, condition index, and gonad development after 5 months. Poorest growth, survival, and gonad development were shown by *P. placenta* fed *T. tetrahele.*

- **Induced spawning, larval and post-larval rearing of window-pane oyster, *Placuna placenta***

Water flow manipulation to induce spawning in *P. placenta* was not successful. Other methods such as heat shock, salinity shock, and addition of gametes were tried but with limited success. Spawners conditioned by feeding *Isochrysis galbana* released gametes. Fertilized eggs, however, did not survive. In another trial, eggs and sperms were not released simultaneously, thus, no fertilization occurred. Spent spawners restocked at Igang cove rematured (60% late active to ripe gonadal stage) 2 months after restocking.

Propagation of Placuna placenta or window pane oyster is vigorously pursued. Aside from being a cheap source of food, products from its capiz shells are exported worldwide.



Research on Gracilaria spp. gains prominence with seaweeds having become one of the country's sunshine industries.

- **Transplantation of window-pane oyster, *Placuna placenta* in Igang, Guimaras**

Growth of transplanted *P. placenta* after 5 months was higher in muddy area (16.16 mm) than in sandy area (-8.95 mm). Survival rates were 46% (muddy), 0% (sandy-muddy), and 0.8% (sandy).

- **Reproductive cycle and condition monitoring of *Placuna sella***

Majority of P. *sella* were on the early active to late active gonadal stages in Apr. and May, ripe to spent in June and July, early active to late active in Aug., spent in Sept., and early active to late active in Oct. and Nov. High meat condition index were observed in Apr., Aug., Oct., and Dec.

- **Assessment of the state-of-the-art and determination of gaps in mollusc research in the Philippines**

Of 17 bivalve species under 10 genera, extensive basic information are available for *Crassostrea iredalei,Perna viridis,* and *Placuna placenta.* In spite of the studies undertaken for *C. iredalei* and *P. viridis,* major gaps include mortality from spat to market size, fertility and survival of trochophore to spat stage, standard methodology for spatfall forecasting, effects of foulers/epibionts on growth rate, determination of productive level of water column, and hatchery technology.

In progress:

- Evaluation and restocking of depleted *Placuna placenta* beds

Seaweeds (*Gracilaria* spp.)

- **Assessment of the natural bed of Gracilaria sp. at Jaro, Iloilo City** (Thesis Research)

From Mar.- Dec., the highest *Gracilaria* biomass (30.44 g/m^2) was obtained in June which coincided with the highest rainfall (602.2 mm) and lowest water movement (3.14 cm/h). Tetrasporophytes and carposporophytes (fertile plants) were abundant in Apr. (64%) and Jan. 1991 (48%), respectively.

- **Production, marketing practices, and economic analysis of seaweeds industry in Western Visayas**

The seaweed industry in Western Visayas is focused on 2 commercial genera: *Kappaphycus* and *Gracilaria.* Stake-monoline (bottom) and raft monoline methods are used to culture *Kappaphycus* in the sea while a "rice-planting" method is used for *Gracilaria* both in canals and ponds. Stake-monoline method yields an average of 9 t/ha/crop while raft monoline produces 8 t/ha/crop. Culture of *Gracilaria* in canals and ponds using the "rice-planting" method yields 14 t/ha/crop and 2-4 t/ha/crop, respectively. Marketing outlet follows this pattern: producers-traders-exporters/processors.

- **Polyculture of *Gracilaria* (Gigartinales, Rhodophyceae) and *Lates calcarifer* Bloch in floating net cages**

Decreasing growth rate of *Gracilaria* cultured at increasing water depth (25, 50, 100 cm) was observed from Apr. to Oct. whether or not sea bass was present in the cage. After 6 months of culture, growth rate of sea

bass was 2.4-21.8 g/day; mean weight gained/cage, 3670-5027 g; production, 5230-7457 g; survival rate, 90-100%.

- **Characterization of agar extracted from different species of *Gracilaria***

G. blodgetti, G. coronopifolia, G. "verrucosa", and G. sp. were collected in Guimbal, Buenavista, Concepcion, and Leganes, Iloilo from Jan. to June 1989 to determine agar yield, gel strength, gelling and melting temperatures, and moisture and ash contents. Highest agar yield was obtained from *G. "verrucosa"* (36.9%), the lowest from G. *coronopifolia* (16.75%); highest gel strength was recorded in G. *blodgetti* (558 g cm^2), the lowest from G. *coronopifolia* (170 g cm^2). Gelling and melting temperatures were 38.5-40.0°C and 80.5-85.0°C, respectively; moisture and ash contents, 8.0-15.2% and 4.3-4.9%, respectively.

- **Production of *Gracilaria* spp. using different culture systems**

Bimonthly sampling of *Gracilaria* sp. 2 from Jan. to Feb. showed that stocking density of 250 g/hapa net gave the highest specific growth rate (SGR) for both floating cage (3.45%/day) and pond (1.7%/day) culture systems. At 350 *g/hapa* net, SGR was lowest at 1.75%/day and 0.64%/day for floating cage and pond, respectively.

In progress:

- Biology and phenology of *Gracilaria:* managing a *Gracilaria* bed
- Biology and phenology of *Gracilaria:* seasonal variation on the quality and quantity of agar gel

Others

- **Effect of water hardness on the hatching of eggs, growth, and survival of the larvae of indigenous freshwater giant prawn (*Macrobrachium* sp.)** (Thesis Research)

The effect of water hardness levels (75, 150, 600, 1200, and 2400 ppm for the initial run and 500, 1000, 1500, 2000, 2500, and 3000 ppm for the final run) on the growth and survival of *Macrobrachium* sp. larvae was tested. There was no significant difference in mean total length and dry weight of the larvae reared in water with different levels of hardness. However, survival rates were significantly higher in 1200 ppm during the 1st run and in 1500 and 2000 ppm in the 2nd run.

- **Optimal growth conditions of the common phytoplankton species used in shrimp/finflsh hatcheries in large outdoor continuous culture**

Batch cultures of *Isochrysis galbana* at 147.06 µM nitrate concentration and *Skeletonema tropicum* at 13.79 µM nitrate concentration showed specific growth rates (SGR) of 0.66±0.03 and 0.09±0.01 divisions/day, respectively. SGR (k) was computed using the formula: k=3.22b; where b is the estimated slope of the regression line derived from 11-day count for J. *galbana* and 6-day count for S. *tropicum.* Results showed that the turnover rate to be used in the chemostat study should be 66-91 and 7.38-14.77 1/day for the respective species.

- **Evaluation of credit needs and its availability in five selected fishing villages in Panay**

Of the 350 fishermen surveyed, 83% availed themselves of credit for their fishing operations, food, and other household expenses from institutional sources. Informal and non-institutional sources such as friends (36%), relatives (33%), local stores (13%), traders of fish (11%), and employers (4%) were the major sources of credit for 97% of the fishermen. Amount of credit availed of per fisherman ranged from P800 to P6000 (institutional sources) and P20 to P20 000 (non-institutional sources). Informal credit was preferred over formal sources due to: accessibility (51%), fast credit extension (31%), source known to borrower (18%), and liberal terms (16%). Repayment problems were encountered by 19% of the borrowers.

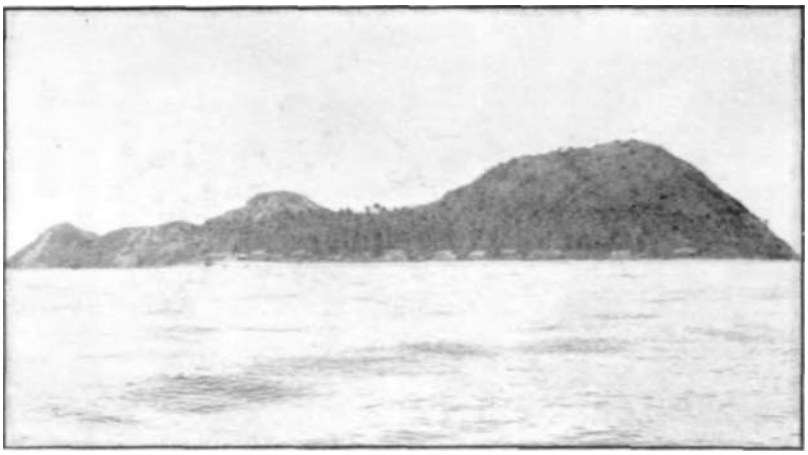
- **Factors affecting the acceptability of Territorial Use Rights in Fisheries (TURFs) among fisherfolks in five fishing villages in Panay** (Thesis Research)

One hundred fifteen fishermen and 96 women were surveyed using a pre-tested interview schedule translated into the local dialect. According to 82% of the respondents, the association has the right to establish rules among the fishers using a certain fishery. Eighty-five percent stated that granting TURFs to fisherfolk associations is beneficial while 80% said that they will benefit if the association in their village is granted TURFs. Ninety percent responded that they will cooperate with the association in regulating fishing activity while 63% are sure that other fisherfolks in their village will cooperate.

- **Social and economic considerations in the implementation of an integrated seafarming program**

A socioeconomic survey showed that the most favorable site for a pilot seafarming and searanching project is Culasi, Antique based on the following socioeconomic features: fishing as a major occupation, fishing income below poverty level, regulation of fishing practices, credit use, alternate livelihood, membership in associations, and awareness of NGOs.

- **Assessment of the marine communities of five candidate sites in Panay for a pilot seafarming project**



Maralison Island in Culasi, Antique is the site of SEAFDEC/AQD's pilot project in Integrated Seafarming and Searanching.

Five candidate sites in Panay were assessed based on the following physico-biological parameters: live coral, seagrasses and mangroves, hard bottom substrate, area, 10-30-m depth, water transparency, river run-off, and protection from southwest monsoon. Culasi, Antique registered the highest overall mean percentage (52%) and was chosen to be the pilot seafarming and searanching project site. The other sites registered the following percentages: Nueva Valencia (50%), Concepcion (45%), San Jose (28%), and San Dionisio (27%).

- **Economic analysis of algae (*Chaetoceros calcitrans* and *Skeletonema costatum*) production for shrimp hatcheries**

Production of microalgae, *Cnaetoceros calcitrans,* was conducted at SEAFDEC/AQD Phycology Laboratory. Cost analysis for algal starter production and for mass culture considered operating costs and fixed costs. Preliminary results showed that the average cost of producing algal starter was P33.00/1 while for outdoor mass culture, P1 218.00/t.

- **Effect of certain chemotherapeutants on survival and growth of brine shrimp (*Artemia* sp.) nauplii**

Several 24-h bioassay tests were conducted to determine the tolerance level of *Artemia* nauplii to Treflan-R oxytetracycline, furazolidone, and erythromycin. Results showed that instar I was less sensitive to the chemotherapeutants than instar II and III; instar II and III had similar sensitivity. Although instar II and III exhibited very high tolerance to the chemotherapeutants, their growth was affected.

- **Luminiscent bacteria and their bacteriophages from coastal waters** (Thesis Research)

Isolation of bacteriophages for the luminous bacteria (*Vibrio harveyi* was done. Experiments were conducted to determine the effectiveness of the phage preparations in the control/ prevention of luminous bacterial infection on the

larvae and postlarvae of *Penaeus monodon.* Preliminary results showed that within a 24-h period, a higher ratio of phage to bacteria (100:1) was more effective in wiping out bacterial population than a 10:1 ratio.

- **Histopathology of epizootic ulcerative syndrome in some freshwater fishes of Laguna de Bay** (Thesis Research)

Monthly sampling of catfish from West Bay of Laguna Lake for histological and hematological profile of naturally EUS-infected fish continued. However, no catfish samples were collected in Mar. Healthy snakehead from areas with no EUS history were collected and are now being maintained for histological and co-habitation studies in an EUS-affected area.

- **Biotechnology in aquaculture: use of microbes in larval rearing of shrimps and finfishes**

Axenic cultures of the yeast *Saccharomyces cerevisiae* and spores of the filamentous mold *Aspergillus flavipes* were used for the monoculture of *Moina macrocopa,* an alternative natural food for marine fish and shrimp larvae. Newborn *Moina* molted twice and released its first brood in 24 h; daily thereafter until death. The following are mean data for the species based en single cultures through 5 generations sustained *ad libitum* with S. *cerevisiae* vis-a-vis *A. flavipes:* life span = 7.9, 8.6 days; number of brood - 7.0, 8.4; interval between broods - 15.8,8.8. For mass culture, the estimated percentage distribution of the 3 size classes in a population grown for 8 days with *S. cerevisiae* is as follows: adult, 5%; subadult. 12%; young, 83%.

- **Viral and bacterial etiology of the epizootic ulcerative syndrome (EUS)**

Filtrates of EUS-affected snakehead and catfish were assayed for virus after inoculation into various cell lines. CPE-like (myopathic effects) changes on epithelial cells of carp and fathead minnow cell lines were observed with filtrates from 2 specimens, but these were gradually lost after prolonged incubation. Establishment of primary cell lines from snakehead and catfish was not successful.

Skin and muscles of snakehead and catfish were sampled for bacteria. Total bacterial counts/g tissue were $1.22x10^3$. $1.40x10^5$. $5.31x10^5$, and $1.14x10^7$ in apparently normal, slightly lesioned, moderately lesioned, and severely lesioned snakehead samples, respectively. In catfish, mean cfu/g tissue were $4.30x10^4$ and $2.00x10^5$ in apparently normal and slightly lesioned specimens. Bacteria were also isolated from the kidneys. Isolated bacteria were predominantly *Aeromonas hydrophila* occurring in 90% of sampled snakeheads and 33% of catfish specimens. Initial infection experiments induced dermomuscular necrotic lesions.

Deferred:

- Phytoplankton as a natural source of antibiotics in aquaculture

In the researcher's cubicle, nature's puzzles are painstakingly being unravelled that mankind may live a better quality of life.

Abstracts of research publications

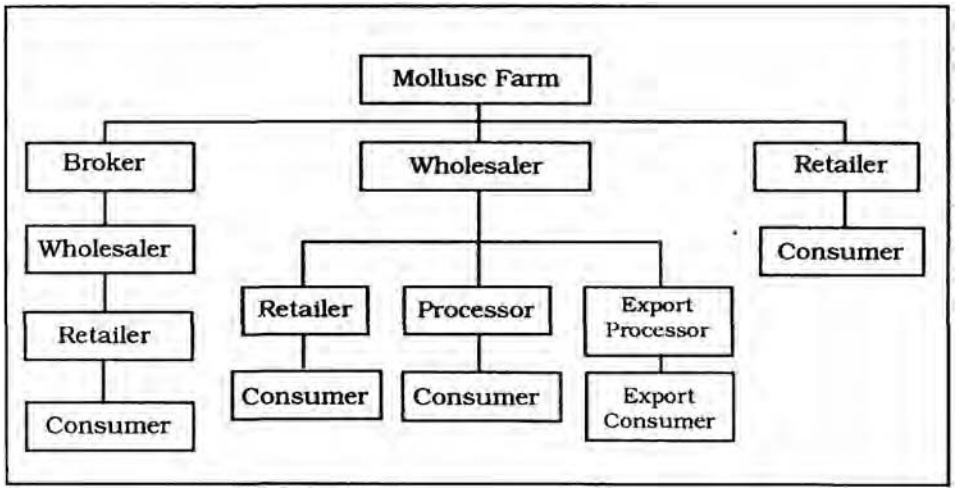
• **Agbayani RF. 1990. Economics of milkfish culture in the Philippines. Tanaka H, Uwate KR, Juario JV, Lee CS, eds. Proceedings of the Regional Workshop on Milkfish Culture Development in the South Pacific; 21-25 November 1988; Tarawa, Kiribati. Suva, Fiji: South Pacific Aquaculture Development Project/FAO; 101-108.**

The Philippines is both a producing and consuming country of milkfish. In 1984. statistics from the Bureau of Fisheries and Aquatic Resources (BFAR) revealed that there were about 207,000 ha of brackishwater ponds in the Philippines, 90% of which are devoted to milkfish culture. In 1980, the per capita consumption of fish was 20 kg per year (Smith and Chang, 1984) or 54% of per capita animal protein consumption. Milkfish is acceptable to all the socio-economic strata of the Filipino society.

Agbayani RF, Abella FF. 1989. Status of sanitation and marketing of mollusc in the Philippines. *In* Report of the Workshop and Study Tour on Mollusc Sanitation and Marketing; 15-28 October 1989; France. Bangkok, Thailand: Regional Seafarming Development and Demonstration Project, Network of Aquaculture Centres in Asia; 98-110.

The mollusc industry of the Philippines has contributed to the economic development of the country in terms of food production and dollar earning. The industry has continuously provided cheap but nutritious shellfishes. Production was 22,528 MT in 1981 which increased to 41,700 MT in 1985. Shellfish and shellcraft products exported in 1987 earned an estimated US$ 26.55 million.

Production of mollusc for food has not been a fully exploited activity area. Areas suitable for oyster and mussel farming have been underutilized. One reason for the underutilization of potential farming areas is the lack of efficient marketing and processing facilities and appropriate strategies that will encourage more consumption of shellfish. Specifically, the quality of shellfish must be improved through better harvesting, post-harvest/processing techniques and an efficient distribution system.



Distribution channels of shellfish in the Philippines (After Agbayani & Abella, Report of the Workshop and Study Tour on Mollusc Sanitation and Marketing: 102. 1989)

• **Agbayani RF, Baliao DD, Samonte GPB, Tumaliuan RE, Caturao RD. 1990. Economic feasibility analysis of the monoculture of mudcrab (Scylla *serrata* Forsskal). *Aquaculture* 91:223-231.**

Mudcrabs, *Scylla serrata* Forsskal, were monocultured at different stocking densities: 5000, 10 000, 15 000 and 20 000/ha for 90 days. Highest mean weight, survival and relative growth increment ($P<0.05$) were obtained from a stocking density of 5000/ha. Best feed conversion ratio of 1.72 and corresponding gross production of 1019 kg/ha per crop were attained at the same stocking density. The economic indicators, i.e., return on investment and return on equity, were also highest at 5000/ha stocking density and the payback period was shortest. Partial budgeting showed that no incremental benefit accrued from stocking beyond 5000/ha. Sensitivity analysis showed that even if the value of mudcrab were to decrease by 28%, mudcrab monoculture would still be economically viable.

- **Ayson FG, Parazo MM, Reyes DM Jr. 1990. Survival of young rabbitfish (*Siganus guttatus* Bloch) under simulated transport conditions. *J. Appl. Ichthyol.* 6:161-166.**

The effect of loading density, transport duration, water temperature, and salinity on survival of young rabbitfish (*Siganus guttatus* Bloch) under simulated transport conditions was investigated. The rocking motion of transport was simulated by an electric shaker. At ambient temperature and salinity (28°C/32 ppt S) increasing loading density and transport duration resulted in decreasing fish survival rates. When both temperature and salinity (20°C/ 20 ppt S) were lowered survival improved significantly. Dissolved oxygen, water temperature, and salinity were critical factors affecting survival of young rabbitfish during simulated transport. Based on the results, a density of 100 fish/L can be packed for 8 hours of transport and 300 fish/L for 2 hours at 28°C and 32 ppt S, and about 200 fish/ L for 4 hours at 20°C and 20 ppt S.

Survival (%) of young rabbitfish after 4 hours of simulated transport at a loading density of 300 fish/L and at two temperature and salinity combinations (*After* Ayson et al., *J. Appl. Ichthyol.* 6:163. 1990)[1]

Temperature (°C)	Salinity (ppt) 32	20
28	49±14.3[b]	21±5.3[c]
20	38±4.2[bc]	84±6.7[a]

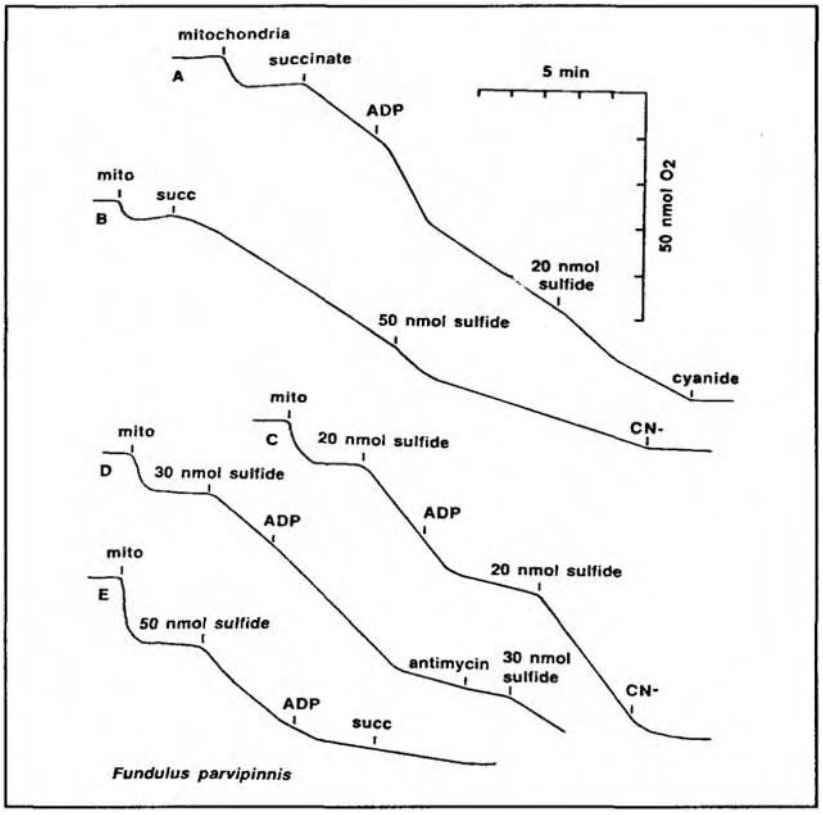
[1]Values are means ± SD of 3 replicates; Values with different superscripts are significantly different (P<0.05)

- **Bagarinao T, Vetter RD. 1989. Sulfide tolerance and detoxification in shallow-water marine fishes. *Mar. Biol.* 103:291-302.**

Hydrogen sulfide is a potent inhibitor of aerobic respiration. Sulfide is produced in sediments, and many species of fish live in association with the bottom. Tolerance tests, enzyme assays, and chromatography of sulfur compounds in thirteen species of shallow-water marine fishes (collected in San Diego, California, USA in 1987-1988) indicate adaptations to sulfide that vary with habitat and lifestyle. Tidal-marsh inhabitants, like *Gillichthys mirabilis* and *Fundulus parvipinnis,* have higher tolerance to sulfide (96 h LC_{50} at 525 to 700 μM) relative to outer-bay and open-coast inhabitants (surviving < 12 h at much lower concentrations). The cytochrome c oxidase of all species shows high activity and susceptibility to sulfide poisoning with 50% inhibition at 30 to 500 nM in various tissues. The two marsh species are able to survive at sulfide concentrations already inhibitory to their cytochrome c oxidase and fatal to other species. All species detoxify sulfide by oxidizing it to thiosulfate. All have sulfide-oxidizing activity in the blood, spleen, kidney, liver and gills, which correlates significantly with heme content. Thiosulfate appears in the tissues of sulfide-exposed fish and builds up to high concentrations (up to 2 mM) with stronger and longer exposure. Unexposed fish contain little or no thiosulfate. Sulfide is barely detectable in the tissues, even in high-sulfide exposure tests. We suggest that fish blood, in having high sulfide-oxidizing activity and no cytochrome c oxidase, can act as a short-term first line of defense against sulfide, and thus minimize the amount that reaches the vital organs. The results of this study indicate that sulfide is a significant environmental factor influencing the ecological distribution of marine fishes.

- **Bagarinao TU, Vetter RD. 1990. Oxidative detoxification of sulfide by mitochondria of the California killifish *Fundulus parvipinnis* and the speckled sanddab *Citharichtys stigmaeus*. *J. Comp. Physiol.* 160B:519-527.**

Earlier whole-animal experiments have shown that the California killifish (*Fundulus parvipinnis*) from tidal marshes is highly tolerant to sulfide while the speckled sanddab (*Citharichtys stigmaeus*) from the open coast is intolerant to sulfide. In the present paper, we demonstrate that the liver mitochondria of the California killifish detoxify sulfide by oxidizing it to thiosulfate and produce ATP in the process.



California killifish mitochondrial oxygen consumption: A. Succinate with 20 µM sulfide; B. Succinate with 50 µM sulfide; C. With 20 µM sulfide; D. With 30 µM sulfide; E. With 50 µM sulfide. Mitochondrial protein, 0.5 mg in A, B; 0.9 mg in C, D, E (After Bagarinao & Vetter, J. Comp. Physiol. 160B:521. 1990)

Sulfide oxidation is obligately and stoic - hiometrically linked to mitochondrial electron transport to oxygen. Concentrations up to 20 µ*M* sulfide stimulate mitochondrial respiration while 50 µ*M* sulfide causes half-inhibition. Sulfide oxidation by mitochondria is adversely affected at pH<7.4. ATP production is maximal at 10 µ*M* sulfide. The finding of sulfide oxidation coupled to ATP production by killifish is unprecedented among vertebrates. In comparison, mitochondria of the speckled sanddab oxidize sulfide at a much lower rate. This is the first demonstration of biochemical adaptation to sulfide among coastal marine fishes.

- **Basiao ZU. 1988. Effects of initial stocking size in the growth of Nile tilapia fingerlings in cages without supplemental feed in Laguna Lake, Philippines. *Nat. Appl. Sci. Bull.* 40:171-175.**

Oreochromis niloticus fingerlings were stocked at three different initial size ranges of 1-3, 7-12 and 20-30 g in fixed net cages in Laguna Lake, Philippines. These were reared without supplemental feed for 120 days. Fingerlings with the biggest initial size at stocking were the most efficient in terms of average weight gain, average final fish weight and total fish production.

- **Basiao ZU, Doyle RW. 1990. Interaction between test and reference populations when tilapia strains are compared by the "internal control" technique. *Aquaculture* 85:207-214.**

Several strains of *Oreochromis niloticus, O. mossambicus* and their hybrids occur in the Philippines and others are likely to be introduced or developed locally in the near future. Our objective was to study biological interaction. The paper reports on an experimental design in which "reference" fish are included in each replicate to provide internal statistical control.

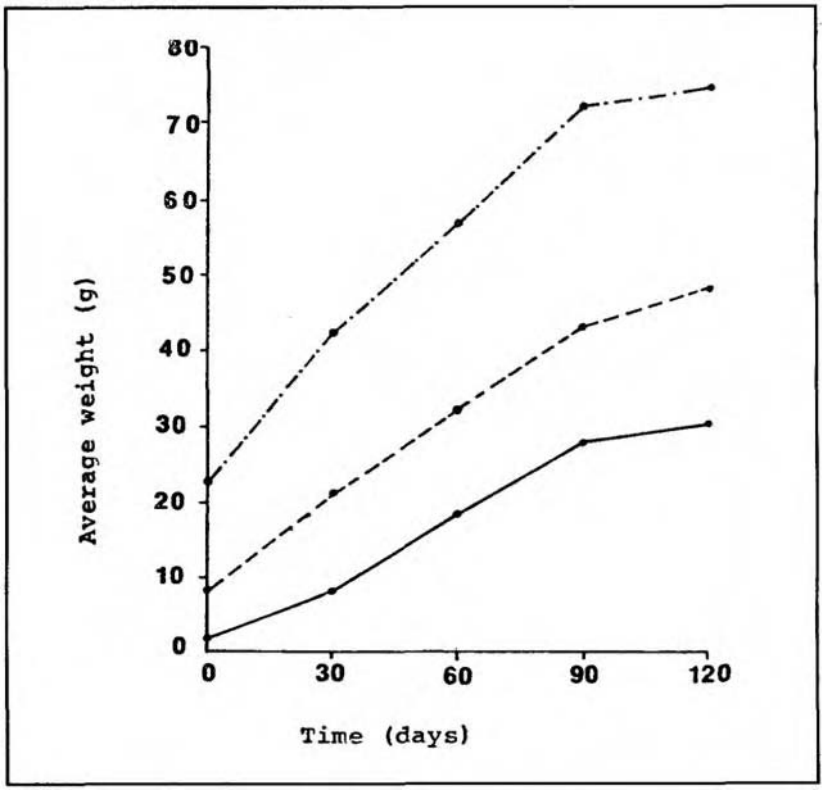
Growth of 10 full-sib families from each of two domestic strains was compared. A third strain of red tilapia was mass spawned to provide reference fish. Thirty equal-sized fry from each family were matched with 30 red tilapia fry and reared for 8 weeks in laboratory aquaria. The fish were deliberately crowded to provide a "worst-case scenario" for the application of the reference-strain technique. The objective was to see whether behavioural interaction causes statistical or genotype X environment interactions that create problems in the analysis.

The test strains interacted biologically with the reference strain in different ways: the growth (change in length) of strain 1 only was negatively correlated with reference growth. Statistical interaction did occur in this extreme situation. We speculate that the reference-fish technique will be more useful in experiments in ponds or cages where variable environmental factors induce positive, rather than negative, correlations between reference and test strains.

- **Basiao ZU, Doyle RW. 1990. Use of internal reference population for growth rate comparison of tilapia strains. I. In a crowded environment. Hirano R, Hanyu I, eds.**

Proceedings of the 2nd Asian Fisheries Forum; 17-22 April 1989; Tokyo, Japan. Manila, Philippines: Asian Fisheries Society; 503-504.

Our objective was to develop an efficient procedure for comparing species and strains. This paper reports an experimental design in which reference fish are included in each replicate to provide internal statistical control over environmental variation. Growth of 10 full-sib families from each of three strains of *O. niloticus* were compared in a crowded environment. A third strain of red tilapia was mass-spawned to provide reference fish. From each family, 25 equal-sized tilapia fry were matched with 25 equal-sized red tilapia fry and reared for three weeks in small floating cages (10 x 10 x 12 inches) inside a bigger tank. Growth of test strains were positively correlated with growth of the reference population. The three strains differed significantly in their growth rates. The reference fish technique is useful in experiments in ponds or cages, where variable environmental factors induce positive correlations between reference and test strains.



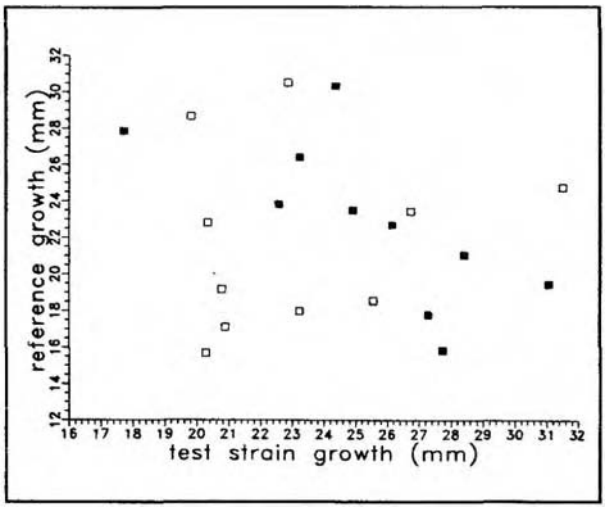
Growth curves of O. niloticus stocked at different initial sizes (—, 1-3; - - -, 7-12; —·—·—, 20-30 g initial weight) (After Basiao, Nat. Appl. Sci. Bull. 40: 174. 1988)

- **Bautista MN, Baticados MCL. 1990. Dietary manipulation to control the chronic soft-shell syndrome in tiger prawn, *Penaeus monodon* Fabricius. Hirano R, Hanyu I, eds. Proceedings of the 2nd Asian Fisheries Forum; 17-22 April 1989; Tokyo, Japan. Manila: Asian Fisheries Society; 341-344.**

Prawn (17.0-20.0 g) collected from brackishwater ponds, were induced to become soft-shelled in the laboratory for 3-4 weeks. Eight isocaloric and isonitrogenous diets containing 0:0; 0:1; 1:0; 1:0.2; 1:1; 1:2; 0.2:1; and 2:1 ratios of Ca to P were fed to the soft-shelled prawn. Two runs of 31-day experiments with 5 and 4 replications for the first and second runs, respectively, were done. Shell quality, survival and growth of prawn were observed biweekly. The hepatopancreas, exoskeleton and residual tissues were analyzed to determine the levels of Ca:P.

Prawn fed a diet with 1:1 Ca to P ratio (Diet 5) gave the best response in terms of weight gain (62%) and recovery from soft-shelling (89%). Soft-shelled prawn fed Ca-P-deficient diet did not improve in shell quality. Survival ranged from 50 to 100%, and was significantly low with prawn fed the Ca- or P-deficient diets.

The highest levels of both Ca and P occurred in the exoskeleton of normal prawn. The increased levels of these minerals in the hepatopancreas and depressed levels in the exoskeleton in soft-shelled prawn indicated impaired mobilization of these mineral elements



Mean growth of test fish plotted against the mean growth of reference fish in the same aquaria. Open squares=strain 1; solid squares=strain 2 (After Basiao & Doyle. Aquaculture 85:210.1990)

from the former to the latter for use in formation and hardening of shell.

- **Baticados MCL, Lavilla-Pitogo CR, Cruz-Lacierda ER, de la Peña LD, Suñaz NA. Studies on the chemical control of luminous bacteria *Vibrio harveyi* and *V. splendidus* isolated from diseased *Penaeus monodon* larvae. *Dis. Aquat. Org.* 9:133-139.**

The minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of 24 drugs for luminous bacteria *Vibrio harveyi* and *V. splendidus* were determined. Only chloramphenicol, sodium nifurstyrenate and the nitrofurans (furazolidone, nitrofurazone, nitrofurantoin and Prefuran) showed relatively low MICs and MBCs (<24µg ml^{1}). The bacteria showed varied responses to chloramphenicol and Prefuran, and low sensitivity to oxytetracycline. Chloramphenicol, oxytetracycline and Prefuran are commonly used in shrimp hatcheries. Shrimp larvae showed high survival rates and active swimming movement after 24 h exposure to in vivo bactericidal doses of chloramphenicol, Furacin, nitrofurantoin (protozoea only), oxytetracycline (nauplius only), Prefuran (mysis only) and sodium nifurstyrenate, but the drugs caused deformities in the carapace, rostrum, and setae. Chemical control of luminous vibriosis among shrimp larvae appears limited based on the efficacy of existing and readily available drugs, because of the possible development of resistant strains of bacteria and the limited tolerance of the shrimp larvae to the drugs.

- **Benitez LV. 1989. Milkfish Nutrition: A Review. Fortes RD, Darvin LC, De Guzman DL, eds. Fish and Crustacean Feeds and Nutrition: Proceedings of the Seminar-Workshop on Fish and Crustacean Feeds and Nutrition; 25-26 February 1985; University of the Philippines in the Visayas, Iloilo City. Los Baños, Philippines: PCAMRD; 31-34.**

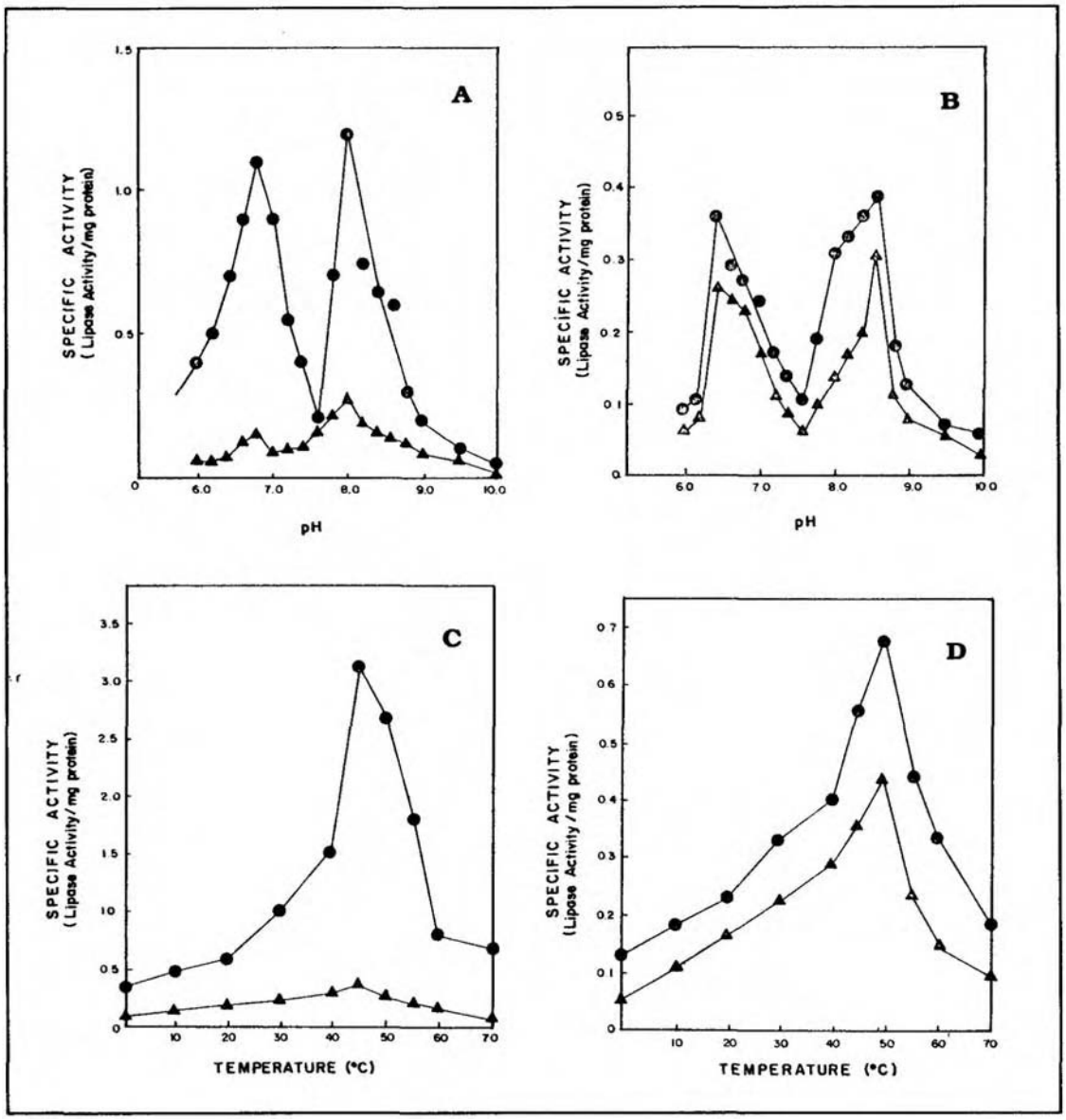
This paper reviews recent work on milkfish nutrition. Substantial progress had been made towards understanding the digestive physiology of milkfish. Major enzymes involved in the digestion of carbohydrates, protein and lipids had been detected in the pyloric caeca, intestines and pancreas of milkfish. The most active carbohydrates were involved in the hydrolysis of α-glucosidic bonds. Intestinal amylase activity consistently reached the peak at about noon when milkfish gut was full. This confirms that milkfish is a daytime feeder. No cellulase activity was detected in any region of the digestive tract although the fish relies heavily on algae and other plant sources for food.

Trypsin, chymotrypsin and general proteases were also detected in milkfish digestive tract. A powerful milkfish trypsin inhibitor was detected in the filamentous algae, *Chaetomorpha brachygona* which is the predominant species in lumut. Lipases in the pancreas and intestines had two pH optima, suggesting a physiologic versatility for lipid digestion in milkfish.

There is limited information on the nutrient requirement of milkfish. Most studies showed that milkfish fry has a dietary requirement of 40% protein, and 7-10% lipid. Studies on the protein-energy requirement of the fingerlings suggested that 30-40% protein, 10% fat and 25% carbohydrates are required. Subsequent studies showed an optimum protein energy to total metabolizable energy ratio of 44.4%. Amino acid test diets for milkfish had been formulated to contain white fish meal, gelatin and appropriate amino acid mix. These diets were recently used to determine tryptophan requirement of milkfish.

- **Borlongan IG. 1990. Studies on the digestive lipases of milkfish *Chanos chanos*. *Aquaculture* 89:315-325.**

Milkfish grown on two natural foods were examined to determine the distribution pattern of the digestive lipases along the digestive tract and to identify the optimum condition for lipase activity. One food consisted of a biological complex of unicellular algae

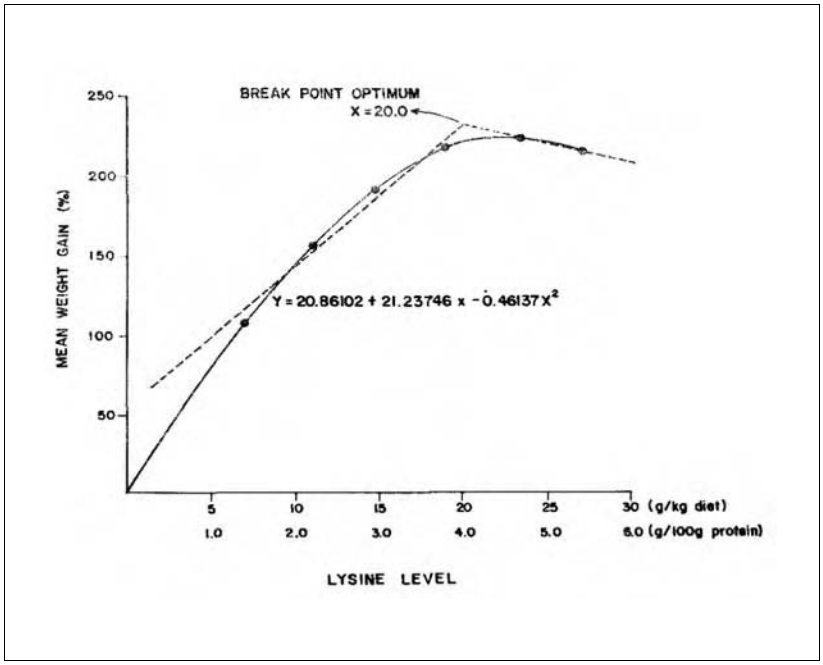


Digestive lipases of milkfish: pH activity profiles of the anterior intestinal (A) and pancreatic (B) lipases; temperature-activity profiles of the anterior intestinal (C) and pancreatic (D) lipases. •, Food A; ▲, Food B (After Borlongan, Aquaculture 89: 320-321. 1990)

and diatoms (Food A) and the other consisted of fibrous filamentous green algae, predominantly *Chaetomorpha brachygona* (Food B). The major sites of lipase secretion in milkfish digestive tract were the intestines, pancreas and pyloric caeca. Lipase activity was somewhat higher for fish grown on Food A than those grown on Food B. Intestinal lipase activity was observed to be maximal at 45°C and at pH 6.8 and 8.0. Activity of pancreatic lipase was observed to be maximal at 50°C and at pH 6.4 and 8.6. The detection of two well-defined pH optima, one at slightly acidic and the other at alkaline pH for both the intestinal and pancreatic lipases suggests a physiological versatility for lipid digestion in milkfish.

- **Borlongan IG, Benitez LV. 1990. Quantitative lysine requirement of milkfish (*Chanos chanos*) juveniles. *Aquaculture* 87:341-348.**

A feeding experiment was conducted to determine the quantitative dietary requirement of milkfish juveniles for lysine. Milkfish (*Chanos chanos* Forsskal) of mean weight 5.92±0.14 g were fed diets containing 7.0, 11.0, 15.0, 19.0, 23.0 and 27.0 g lysine/kg dry diet for 12 weeks. The amino acid test diets contained white fish meal and zein supplemented with crystalline amino acids to provide an amino acid profile similar to milkfish proteins except for lysine. Each of the six diets was fed to four replicate groups of 25 fish in a completely randomized



Mean weight gain of milkfish juveniles given graded levels of lysine (After Borlongan & Benitez, Aquaculture 87:345. 1990)

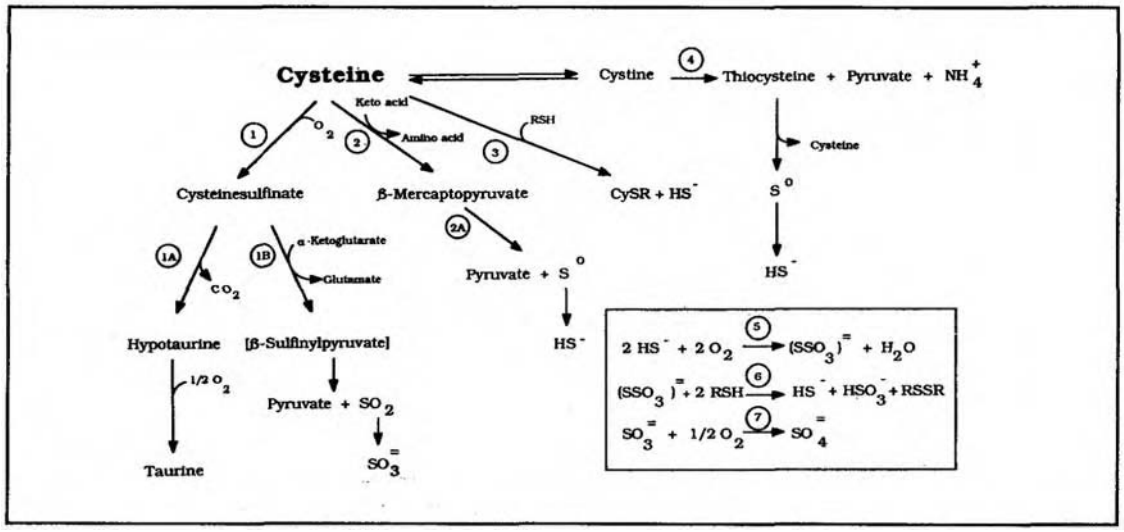
design and at a feeding rate of 5% of the fish body weight per day. On the basis of the growth response, lysine requirement of juvenile milkfish was found to be 20g/kg diet. This value corresponds to 4.0% when expressed as a percentage of the dietary protein. Survival (94-97%) was consistently high in all treatments. Except for loss of appetite resulting in low food intake and depressed growth, no nutritional deficiency signs were observed in fish given the lysine-deficient diets.

- **Coloso RM, Stipanuk MH. 1989. Metabolism of cyst(e)ine in rat enterocytes. *J. Nutr.* 119:1914-1924.**

Cyst(e)ine was metabolized by rat enterocytes to pyruvate and inorganic sulfur but not to taurine. Cystine was the major extracellular form of cyst(e)ine present during the incubation, and addition of bathocuproine disulfonate, a copper chelator that maintained 60% of the total cyst(e)ine in the sulfhydryl form, had no effect on total sulfur release from cyst(e)ine. Oxidation of cyst(e)ine to $^{35}SO_4^{2-}$ or $^{14}CO_2$ was reduced by about 50% when unlabeled cysteinesulfinate was added to incubations of enterocytes with labeled cyst(e)ine. Thus, about one half of cyst(e)ine metabolism appeared to involve its oxidation to cysteinesulfinate and the transamination of cysteinesulfinate to the putative intermediate sulfinylpyruvate, which decomposes to yield sulfite and pyruvate. The remainder of cyst(e)ine catabolism in enterocytes appeared to involve release of sulfur from cyst(e)ine prior to its oxidation. Inhibition of γ-cystathionase by propargylglycine, although incomplete, resulted in substantial inhibition of cyst(e)ine catabolism. The accumulation of cysteinethiosulfonate, which forms nonenzymatically upon incubation of cyst(e)ine with thiosulfate, and the inhibition of cysteinethiosulfonate formation by propargylglycine demonstrated the catabolism of cyst(e)ine by β-cleavage catalyzed by γ-cystathionase. Sulfide released from cyst(e)ine in this reaction appeared to be oxidized to thiosulfate before it was further oxidized to sulfite and sulfate. In addition to being oxidized to sulfate, some of the sulfite formed by enterocytes reacted with cyst(e)ine in the incubation medium to form sulfocysteine. Activities of enzymes of cyst(e)ine catabolism in rat enterocytes corresponded with the observed metabolism of cyst(e)ine by various pathways.

- **Coloso RM, Drake MR, Stipanuk MH. 1990. Effect of bathocuproine disulfonate, a copper chelator, on cyst(e)ine metabolism by freshly isolated rat hepatocytes. *Amer. J. Physiol* 259:E443-E450.**

The metabolism of L-cysteine was studied in freshly isolated rat hepatocytes. Because cysteine is rapidly oxidized in oxygenated incubation medium at neutral pH, the effect of bathocuproine disulfonate, a copper-specific chelator, was investigated. Addition of bathocuproine disulfonate resulted in a higher extracellular cysteine-to-half-cystine ratio in incubations of hepatocytes with cysteine. Bathocuproine disulfonate also increased the total uptake and metabolism of cysteine plus cystine [cyst(e)ine] by hepatocytes, which is consistent with the more efficient transport of cysteine than of cystine by freshly isolated rat hepatocytes. The partitioning of cysteine between cysteinesulfinate -dependent and cysteinesulfinate-independent path-



Pathways of cyst(e)ine catabolism in mammalian cells. Enzymes that catalyzed numbered reactions are 1) cysteine dioxygenase, 1A) cysteinesulfinate decor oxydase, 1B) aspartate aminotransferase, 2) aminotransferase(s), 2A) mercaptopyruvate sulfurtransferase, 3) cystathionine β-synthase, and 4) γ-cystathionase [cyst(e)ine desulfhydrase]. Inset illustrates further oxidation of inorganic sulfur by 5) sulfide oxidase, 6) thiosulfate sulfurtransferase or glutathione (GSH)-dependent thiosulfate reductase, and 7) sulfite oxidase (After Coloso et al., Am. J. Physiol. 259: 444. 1990).

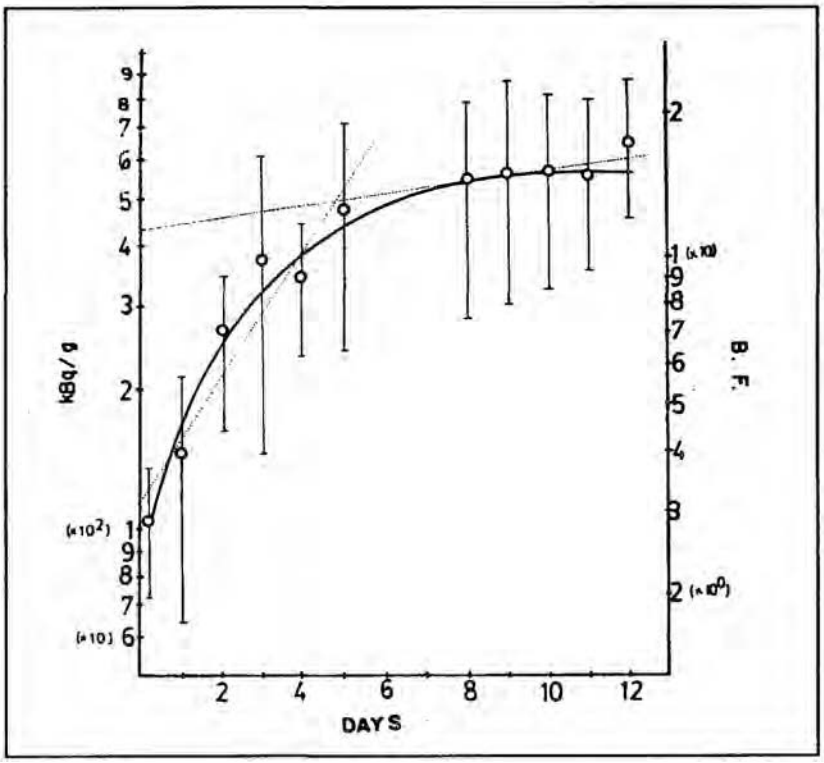
ways of catabolism was also altered by the addition of bathocuproine disulfonate; the percentage of total catabolic flux that resulted in taurine plus hypotaurine formation was greater, and the percentage of total catabolic flux that occurred by the β-cleavage of cystine in a reaction catalyzed by γ-cystathionase was less in incubations that contained bathocuproine disulfonate. Thus addition of bathocuproine disulfonate to maintain a higher extracellular thiol-to-disulfide ratio favored cysteinesulfinate-dependent catabolism of cysteine in rat hepatocytes.

- **Cuvin MLA, Umaly RC. 1988. Uptake and elimination of iodine-131 by the freshwater clam *Corbicula manilensis* Philippi from water. *Nat. Appl. Sci. Bull* 40:141-158.**

Whole-body uptake of Iodine-131 by the freshwater clam, *Corbicula manilensis,* from contaminated water was followed using NaI scintillation counter. The bioaccumulation factor (BF) exclusive of shell was 5.44. The degree of bioaccumulation of I-131 by the different tissues is as follows: visceral remains > gills > gut > gonads > mantle > muscle > foot. The specific activities of the different tissues corresponded with their BF values. The relative distribution of I-131 in the different tissues was generally proportional to the weight ratio of each tissue. Elimination studies gave the effective half-life, $Te_{0.5}$ of 4.5 days. Estimation of $Te_{0.5}$ in the different tissues gave the following values: 12 d (mantle), 3.9 d (gonad and muscle), 3.6 d (gut), 3.4 d (gills), 2.4 d (foot) and 1.9 d (visceral remains).

- **Cuvin-Aralar MLA. 1990. Mercury levels in the sediment, water and selected finfishes of Laguna Lake, the Philippines. *Aquaculture* 84:277-288.**

Monthly samples of sediment, water and commercially important species of fish, primarily *Oreochromis niloticus* and *Chanos chanos,* plus a few other species, were collected from the West Bay area of Laguna Lake, The Philippines from January to December 1987. Mercury levels were determined in all samples by cold vapor atomic absorption spectrophotometry. Results showed that sediment samples contained mercury levels ranging from 26.7 to 177 ppb. Mercury levels in water samples were low, ranging from below detectable to 0.5670 ppb. The mercury levels in the water were negatively correlated with conductivity and dissolved oxygen and positively correlated with turbidity. The mercury burden of the fish species samples was below the maximum permissible level set by the WHO and USFDA of 0.05 ppm. No direct correlation was observed between mercury levels in sediment and water, water and fish and sediment and fish.

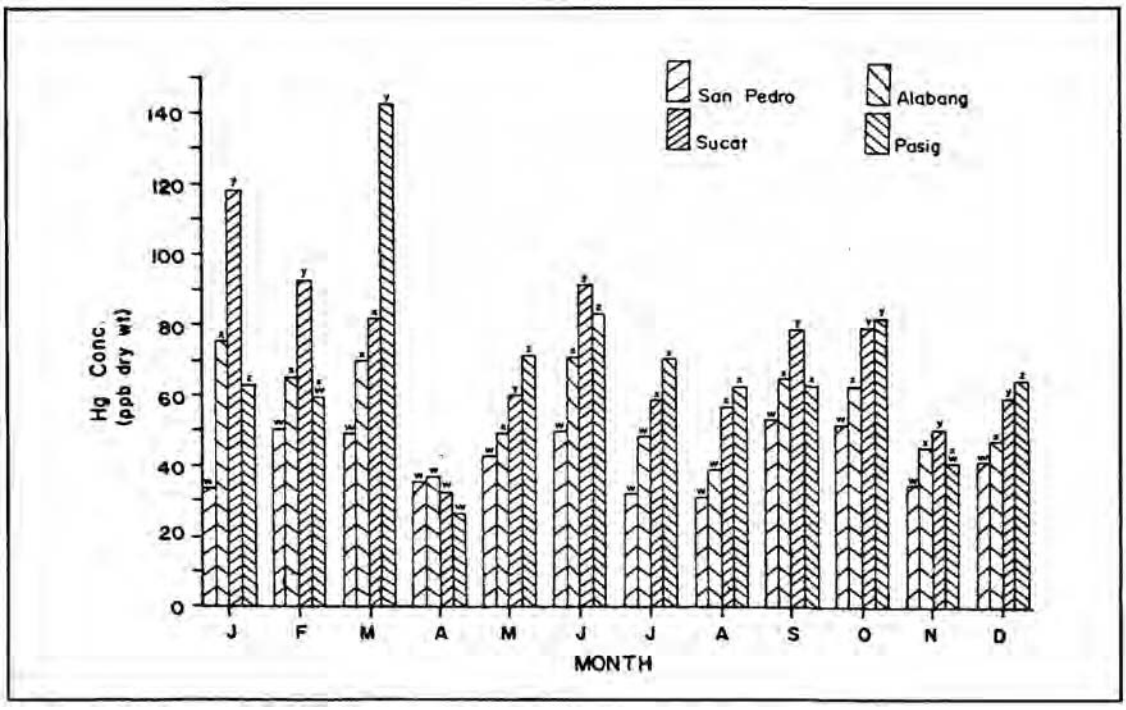


Whole-body uptake (kBq/g) and bioaccumulation factor (BF) of I-131 by clams from water. Dotted lines represent regression for the first 5 days of exposure (ln y = ln 119.15 + 0.301x, r = 0.949) and for the last 5 days (ln y = ln 415.86+0.035x. r = 0.854) (After Cuvin & Umaly, Nat Appl. Sci. Bull. 40: 145. 1988)

- **Cuvin-Aralar MLA, Furness RW. 1990. Tissue distribution of mercury and selenium in minnows, *Phoxinus phoxinus*. *Bull. Environ. Contam. Toxicol.* 45:775-782.**

The protective effect of selenium against mercury toxicity has been extensively demonstrated in a number of studies (Burke et al. 1977; Kasuya 1976). Since mercury uptake is not always diminished by the presence of selenium (Kim et al. 1977) and neither does selenium enhance the elimination of mercury (Lucu and Skreblin 1981; Cuvin and Furness 1988), these findings indicate that the mechanism for the observed protective action of selenium against mercury toxicity lie along different lines. It is believed that the rechanelling of mercury from one organ or tissue to another is one of the general mechanisms involved in the protective action of selenium against mercury toxicity. This is supported by the fact that one of the observed effects of selenium treatment on mercury-intoxicated animals is the apparent modification of the distribution pattern of mercury in the different organs and tissues. Decreased mercury levels in the kidney after selenium treatment has been demonstrated in rats by Chen et al. (1974) and Potter and Matrone (1974).

The following study aims to determine the effect of selenium on the distribution pattern of mercury in a common freshwater fish, the minnow *Phoxinus phoxinus* (Order Cypriniformes; Family Cyprinidae). Conversely, the effect of mercury on the tissue distribution of selenium will also be studied.



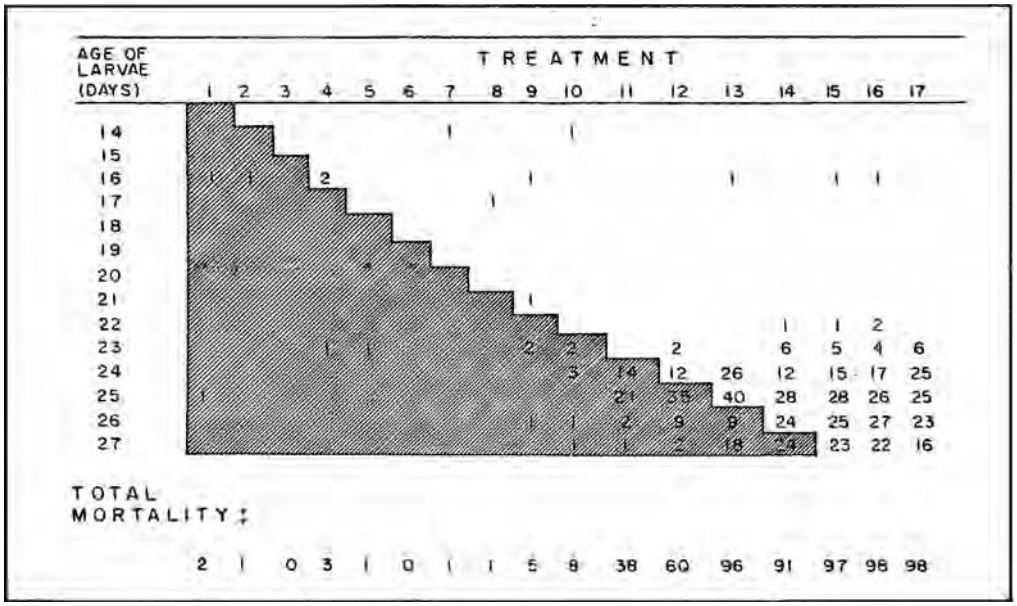
Comparison of mercury levels in sediments from 4 sampling stations. Bars with the same letter are not significant, P<0.05 (After Cuvin-Aralar, Aquaculture 84:281. 1990)

- **Dhert P, Duray M, Lavens P, Sorgeloos P. 1990. Optimized feeding strategies in the larviculture of the Asian sea bass *Lates calcarifer*. Hirano R, Hanyu I, eds. Proceedings of the 2nd Asian Fisheries Forum; 17-22 April 1989; Tokyo, Japan. Manila, Philippines: Asian Fisheries Society; 319-324.**

This paper reports on the progress made at the Tigbauan hatchery of SEAFDEC in the Philippines, with the larviculture of the Asian seabass *Lates calcarifer* when using w3-HUFA enriched *Brachionus* and *Artemia.*

In order to optimize the transitions of the different prey types, larval mouth size was analyzed as a function of larval development: San Francisco Bay (SFB) type *Artemia* nauplii can be ingested from days 8 to 10 onwards, and Great Salt Lake (GSL) nauplii from days 10 to 15 (with some variations depending on growth differences between culture trials). In view of the high correlation between mouth size and total larval length, the feeding of different size classes of *Artemia* can be better programmed, i.e., SFB nauplii can be fed when fish larvae measure 4 mm in length, and 24 hr enriched GSL can be given to 7 mm larvae. The incorporation of the HUFA's 20:5w3 and 22:6w3 in the live prey *Artemia,* and possibly *Brachionus,* greatly improved larval ability to metamorphose, although it did not affect growth nor survival until day 21. However, when 21 day old fry were subjected to a stress test, much higher survival rates were obtained in HUFA-enriched seabass larvae, illustrating their superior physiological condition over fry cultured with non-enriched *Brachionus* and *Artemia.*

These findings were used to propose an improved feeding strategy for the larviculture of *Lates calcarifer.*



Daily mortality (%) for sea bass larvae in relation to HUFA feeding (shaded area, feeding of HUFA-enriched Artemia, e.g., treatment 1 received the HUFA diet from day 14 onwards, treatment 2 from, day 15 onwards, etc.) (After Dhert et al., Aquaculture 90: 69. 1990)

Dhert P, Lavens P, Duray M, Sorgeloos P. 1990. Improved larval survival at metamorphosis of Asian seabass (*Lates calcarifer*) using ω3-HUFA-enriched live food. *Aquaculture* 90:63-74.

Asian seabass (*Lates calcarifer*) larvae were fed *Brachionus* cultured on *Chlorella* and as soon as ingestion was possible, different types of *Artemia,* i.e., nauplii of the San Francisco Bay (SFB) strain, Great Salt Lake (GSL) strain or GSL nauplii that had been bioencapsulated with an emulsion containing high levels of the ω3-HUFAs (highly unsaturated fatty acids) 20:5 and 22:6. San Francisco Bay *Artemia* with a good natural fatty acid profile and small body size could be offered earlier than the larger but HUFA-poor Great Salt Lake strain. The poor nutritional quality of the latter, however, could be corrected by enriching the nauplii with an ω3-HUFA emulsion for 24 h, after which time high levels of the ω3-HUFAs 20:5 and 22:6 were obtained. When the *Artemia* diet offered before metamorphosis included natural or supplemented essential fatty acids, no significant differences in dry weight, length or survival of the fish were noticed, as compared to fish fed the naturally deficient GSL *Artemia.* Onset of metamorphosis and physiological condition after metamorphosis, however, were influenced by the HUFA content of the ingested prey. Seabass larvae fed SFB or enriched GSL *Artemia* started metamorphosis on day 19, while those in the non-enriched series never

achieved metamorphosis and died of a nutritional deficiency syndrome by day 27. An indication of the physiological condition of the larvae and the early detection of the syndrome was possible by subjecting 21 - and 25-day old larvae to a stress test: abrupt exposure of the larvae to 65-ppt saline water resulted in abundant and early mortality in HUFA-deficient fish larvae. Fry receiving ω3-HUFA-fortified *Artemia* had a superior physiological condition which was reflected by significantly lower mortality figures in the stress test.

- **Doyle RW, Field CA, Basiao Z. 1990. A statistical procedure for using 'reference fish' to compare the growth of genetic strains in aquaculture. Hirano R, Hanyu I, eds. Proceedings of the 2nd Asian Fisheries Forum; 17-22 April 1989; Tokyo, Japan. Manila, Philippines: Asian Fisheries Society; 499-502.**

No simple, well-accepted procedures exist for comparing the relative performance of genetic strains of fish in aquaculture environments. The major technical problem is providing adequate statistical replication of ponds or cages. Inclusion of an "internal reference" population of standard fish in each pond can reduce replicate error caused by variation in the physical environment, food supply and population density.

This statistical procedure is feasible only if there is no biological interaction (interference competition) between the strain and the reference fish. We describe an experimental design in which the replicates contain different proportions of reference fish relative to the test strains. This permits the effects of interstrain interference to be estimated. Data from growth of tilapia *Oreochromis mossambicus* in crowded aquaria are used to illustrate the effect of interference and other nonlinearities in the experimental design.

- **Dy-Peñaflorida V, Millamena OM. 1990. Variation in the biochemical composition of *Penaeus monodon* tissues during the reproductive cycle. *Israeli J. Aquacult.-Bamidgeh* 42:84-90.**

The gonadosomatic index (GSI) and the hepatosomatic index (HSI) were determined for wild-caught, eyestalk ablated *Penaeus monodon* at five reproductive stages (I to V). Tissues of the muscle, ovary and hepatopancreas in each stage were analyzed for crude protein and amino acid composition.

Results showed that the GSI increased from stage I (immature) to IV (fully mature) but declined at stage V (spent) while the HSI did not change significantly ($P<0.05$). Muscle protein content did not vary markedly from stage I to V except for a slight lowering at stage III (late maturing). Ovarian protein increased from stage I to IV and decreased at stage V while from stage II, the reverse was true for hepatopancreas.

Generally, few significant differences in amino acid content were found. Glutamic acid content signifi-

Maturity index (±SE) of *Penaeus monodon* broodstock, n=6 *(After* Dy- Penaflorida & Millamena, *Israeli J. Aquacult.-Bamidgeh* 42:85. 1990)

Stage	Gonadosomatic Index[1]	Hepatosomatic Index[2]
I (Immature)	2.25 ± 0.55[a]	2.44 ± 0.23[a]
II (Early maturing)	5.78 ± 0.83[b]	2.06 ± 0.02[a]
III (Late maturing)	7.07 ± 0.27[b]	2.15 ± 0.19[a]
IV (Fully mature)	10.41 ± 0.03[c]	2.05 ± 0.52[a]
V (Spent)	1.88 ± 0.03[a]	2.13 ± 0.03[a]

Means in columns with the same superscript are not significantly different ($p<0.05$).
[1]GSI = (ovary weight/body weight) x 100.
[2]HSI = (hepatopancreas weight/body weight) x 100.

Crude protein content (±SE) of *Penaeus monodon* broodstock, n=2 (*After* Dy-Penaflorida & Millamena, *Israeli J. Aquacult.-Bamidgeh* 42:86. 1990)

Stage	g protein/100 g dry matter		
	Muscle	Ovary	Hepatopancreas
I (Immature)	90.67 ± 0.01[b]	68.16± 0.13[a]	48.61 ± 0.20
II (Early maturing)	90.68 ± 0.06[b]	72.20 ± 0.07[c]	56.05 ± 0.41
III (Late maturing)	87.05 ± 0.27*	71.72 ± 0.42[c]	52.80 ± 0.18
IV (Fully mature)	89.44± 0.18[b]	73.34 ±0.18[c]	50.53 ± 0.17
V (Spent)	92.62 ± 0.04[b]	70.41 ± 0.29[b]	58.47 ± 0.02

Means in columns with the same superscript are not significantly different ($p<0.05$).

cantly differed among stages in all three tissues. In addition, phenylalanine in the muscle, histidine and lysine in the ovary and arginine and leucine in the hepatopancreas differed among stages. The muscle had slightly higher arginine and glutamic acid contents than the ovary and hepatopancreas but was lower in threonine and valine. The ovary had the lowest glycine content.

Results showed similar amino acid levels among tissues and maturation stages, suggesting that amino acids are fixed and not affected by much variation.

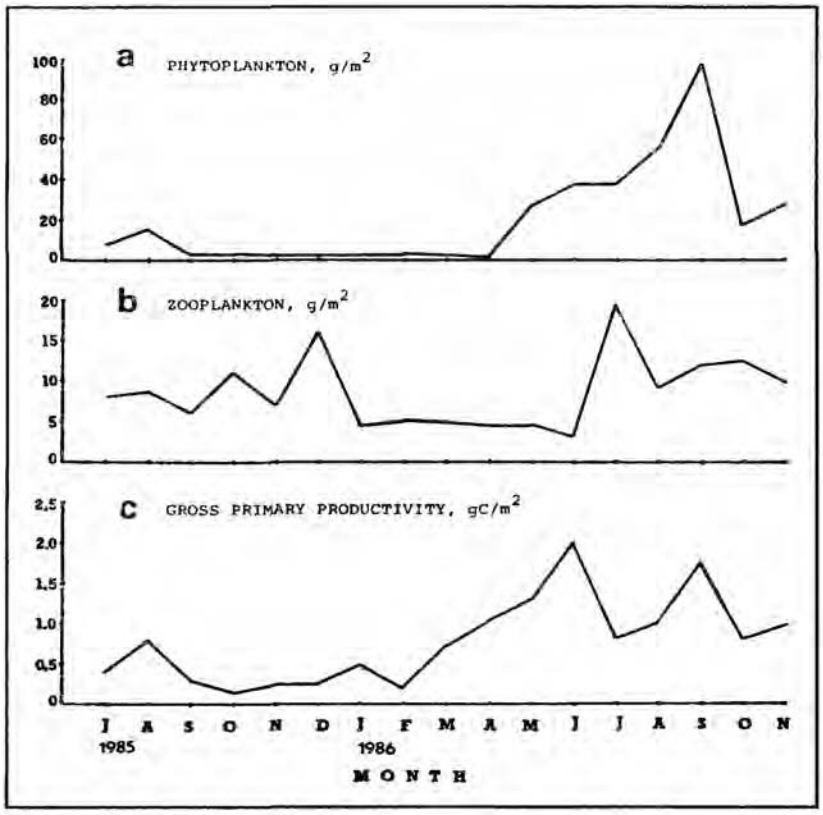
- **Ferriols-Pavico JM, Gonzal AC, Aralar EV. 1990. Practical water chemistry for fishfarmers. I. Teaching strategies. Hirano R, Hanyu I, eds. Proceedings of the 2nd Asian Fisheries Forum; 17-22 April 1989; Tokyo, Japan. Manila, Philippines: Asian Fisheries Society; 789-792.**

The understanding of water chemistry is vital in proper management and maintenance of the aquatic environment. Years of ambitious yet seemingly futile efforts by the authors in making fishfarmers comprehend and appreciate chemical reactions the way chemists do. paved the way for the formulation of teaching strategies which communicate water chemistry in a light, friendly but informative manner. Lectures made use of eye-catching cartoons which gave life and romance to rigid chemical equations for photosynthesis, acid-base neutralization and reaction of soap to hardwater. Theoretical concepts, being well understood, served as a springboard for farmers in translating abstract ideas into field situations.

Laboratory practicals become less intimidating as standard laboratory glasswares and material were replaced by day to day material within the farmers' easy reach, such as nursing bottle for graduated cylinder, disposable syringe for pipettes and so on. Extension workers can find these strategies very helpful when explaining water chemistry and management to fishfarmers.

- **Fermin AC, Reyes DM Jr. 1989. HCG and LHRH-A induced spawning in bighead carp reared in floating cages in Laguna de Bay. *Philipp. Sci.* 26:21-28.**

Hormone-induction of spawning in bighead carp *Aristichthys nobilis* Rich. by single or double intraperitoneal injection with varying combined dosages of HCG and LHRH-A was conducted. Fish were spawned successfully following a single or double injection with 1800 to 2000 IU HCG in combination with 10, 15 or 20 μg LHRH-A per kg body weight. Fish injected with lower dosages of HCG at 1200 or 1500 IU/kg body weight plus 20 μg/kg LHRH-A did not spawn. Ovulation response time in fish given two injections of combined HCG and LHRH-A varied from 7.7±1.7 to 9.0±1.6 hours and did not differ significantly ($P>0.05$). Fish given a single injection ovulated after 12.0±0.1 hours. No significant difference was found in the total number of eggs spawned per fish among the injection



Seasonal patterns *in phytoplankton biomass (a), zooplankton biomass lb), and gross primary productivity (c) at west cove, Tapao Point, Laguna de Bay from July 1985 to November 1986 (After Fermin, J. Appl. Ichthyol. 6:132.1990)*

protocols (P>0.05). However, lower fertilization and hatching rates of eggs were observed in fish that spawned spontaneously in the tank as compared to those fish whose eggs were stripped and dry-fertilized (P<0.05).

- **Fermin AC. 1990. Year-round sexual maturation of bighead carp in Laguna de Bay (Philippines). *J. Appl. Ichthyol.* 6:129-135.**

Sexual maturation in bighead carp *Aristichthys nobilis* reared without supplemental feeding in floating cages in Laguna de Bay was observed year-round . Percent maturation ranged from 3 to 94% in females and 0 to 82% in males from July 1985 to July 1986. High maturation rates in both sexes occurring in March 1986 were positively correlated with high inorganic turbidity in the lake. Low natural food productivity (phyto- and zooplankton) during high turbidity did not negatively affect fish growth. Fish may have depended partly on suspended particles as additional food sources when production of more suitable food (zooplankton) was low. Increased production of natural food as a result of water clearing by seawater intrusion during May to September did not enhance growth and maturation of the fish. There was a low negative correlation between chlorinity and percent maturation of both sexes.

- **Garcia, LMaB. 1989. Spawning response of mature female sea bass, *Lates calcarifer* (Bloch), to a single injection of luteinizing hormone-releasing hormone analogue: effect of dose and initial oocyte size. *J. Appl. Ichthyol.* 5:177-184.**

The effect of various doses of luteinizing hormone-releasing hormone analogue (LHRHa) ranging from 1 to 100 μg/kg body weight on the spawning response of mature female sea bass, *Lates calcarifer* (Bloch) was tested. A single intramuscular injection of LHRHa resulted in a dose-related increase in the spawning rate (number of spawnings of each fish over four consecutive days) of mature fish. An LHRHa dose of 5 μg/kg and less induced low spawning rates of 16.7% to 37.5% or at least one spawning every four days. However, mature sea bass spawned more than once (43.8-58.3%) in four days at dose levels of 10 μg/kg and above. Hormone treatment within the dose range tested did not influence the number, fertilization and hatching rates of spawned eggs.

The influence of initial oocyte size on the LHRHa-induced spawning response of mature sea bass was also examined. Sea bass with an initial oocyte diameter of 0.30-0.39 mm did not respond to the single injection of 100 μg LHRHa/kg. In contrast, LHRHa induced spawning among sea bass with an initial egg size of 0.40-0.49 mm, although two of four sea bass of the same stage of ovarian maturity spawned spontaneously. Fish having an initial oocyte size of 0.50-0.55 mm spawned with and without LHRHa treatment. Spontaneous spawning among saline-injected sea bass occurred at a later time (24-58 h post-injection) compared to fish induced to spawn by a single injection of LHRHa (8-36 h post-injection). The initial spawning response time interval for fish with an initial egg size of 0.50 mm or greater was further

reduced to 8-9 h by LHRHa. These results indicate that LHRHa can successfully induce spawning in mature female sea bass which have attained a critical oocyte diameter and that the spawning response interval is reduced with a further increase in egg size beyond the critical oocyte diameter limit.

- **Garcia LMaB. 1990. Advancement of sexual maturation and spawning of sea bass, *Lates calcarifer* (Bloch), using pelleted luteinizing hormone-releasing hormone analogue and 17 α-methyltestosterone. *Aquaculture* 86:333-345.**

The ability of luteinizing hormone-releasing hormone analogue ID-Ala^6, Pro^9-NEt)LHRH], 17 α-methyltestosterone (MT), and their combination to advance gonadal maturation and spawning in captive sea bass (*Lates calcarifer* Bloch) broodstock was tested. Several hormonal therapies were tested including monthly implantation of a low dose (100 μg/kg body weight) of each hormone and a high dose (200 (μg/kg) implanted to sexually-quiescent sea bass at 45-day intervals. A high dose of LHRHa alone or. in combination with MT induced a significant number of mature females (43-71%) in April, 45 days after a single implantation in early March. A lower dose of pelleted LHRHa and LHRHa plus MT was found effective (78-80%) only in May following three monthly implantations. Two and three monthly implantations of a low dose of MT failed to stimulate oocyte growth in April and May whereas a less frequent mode of application of a high dose of the androgen inhibited ovarian growth in April only. A low dose of MT alone significantly increased the number of mature males in April (90%) and May (100%) after two and three monthly implantations. All male sea bass which received three monthly implantations of a low dose of LHRHa and LHRHa plus MT were sexually mature in May. Sexually mature sea bass obtained from these experiments spawned in early May after a single intramuscular injection of LHRHa. These results demonstrate the potential use of pelleted LHRHa and MT to significantly advance gonadal development and spawning in sea bass earlier than the annual breeding season.

LHRHa-induced spawning response of sea bass as a function of initial oocyte diameter (*After* Garcia, *J. Appl Ichthyol.* 5: 181. 1989)

Initial Oocyte Diameter Range (mm)	Treatment	N	% Spawned[1]	Time to 1st Spawning (h)	Initial Position of Germinal Vesicle
0.3O-0.39	Control	4	0	–	Central
	LHRHa	4	0	-	Central
0.40-0.49	Control	4	50	36-58	Central
	LHRHa	10	100	30-36	Central
0.50-0.55	Control	2	100	24-26	Central to Slightly off-center
	LHRHa	2	100	8-9	Central to Slightly off-center

[1]Expressed as a percentage of the total number of fish (N) injected once intramuscularly with either saline (control) or 100 μg LHRHa/kg body weight.

- **Garcia LMaB. 1990. Fishery biology of milkfish (*Chanos chanos* Forsskal). Tanaka H, Uwate KR, Juario JV, Lee CS, eds. Proceedings of the Regional Workshop on Milkfish Culture Development in the South Pacific; 21-25 November 1988; Tarawa, Kiribati. Suva, Fiji: South Pacific Aquaculture Development Project/FAO; 66-76.**

Milkfish (*Chanos chanos* Forsskal) is one of the most important food fish species in the world. In Indonesia, Taiwan and the Philippines, more than a quarter of a million tonnes of milkfish are harvested annually in brackish ponds, contributing roughly 60% of the total fish production from aquaculture in Southeast Asia. This tremendous level of production from a single fish commodity is projected to further increase in the coming years to meet the dietary protein needs of an ever-growing population in Southeast Asia. To address vital research gaps afflicting the milkfish industry, research has correspondingly intensified over the past 15 years particularly in the Philippines, Taiwan and Hawaii. Results of such research projects have widespread application not only among Southeast Asian nations but also among many untapped areas in the Pacific, the Middle East, Africa and Central America where

milkfish culture is feasible.

A sound approach to initiate a milkfish aquaculture project is to have an adequate knowledge of the basic biology of this species. Several researchers have presented in great technical detail some of these biological aspects at numerous symposia (Juario et al., 1984, Lee, Gordon and Watanabe, 1986). This paper will therefore summarize in moderate detail some recent additional information on several aspects of milkfish biology: taxonomy, distribution, life history and habitat, food and feeding habits, growth, reproduction and tolerance to environmental conditions. Aside from increasing our understanding of milkfish, it is hoped that this short review will goad others to undertake further scientific research on many unknown aspects of the species, thus contributing to both the quality and the quantity of milkfish served on our dinner tables.

- **Garcia LMaB. 1990. Spawning response latency and egg production capacity of LHRHa-injected mature female sea bass, *Lates calcarifer* Bloch. *J. Appl. Ichthyol.* 6:167-172.**

Mature female sea bass (*Lates calcarifer* Bloch) were injected once intramuscularly with 20 μg luteinizing hormone-releasing hormone analogue (D-Ala^6, Pro^9-LHRH-ethylamide) per kg body weight (BW) at different times of the day. Following hormone injection, the incidence of initial spawning and the number of eggs spawned by each fish were recorded. Saline-injected fish did not spawn. Fish spawned at dawn or 33.7-40 h after an LHRHa injection at either 11.00 h or 17.00 h. When LHRHa was administered at 23.00 h or 05.00 h, sea bass spawned during the day or at 38-47.3 h post-injection. Mean egg production levels of 26.8-34.4x10^4 per kg BW were higher for fish which spawned at dawn. Low mean egg production levels (6.2- 19.9x10^4 eggs per kg BW) were observed when LHRHa-injected fish spawned during the day. These results demonstrate that the time of initial occurrence of spawning varied with the time of day that LHRHa was administered and that the number of eggs shed was influenced by the time of day that hormone-induced sea bass spawned.

- **Hurtado-Ponce AQ. 1990. Vertical rope cultivation of *Gracilaria* (Rhodophyta) using vegetative fragments. *Bot. Mar.* 33:477-481.**

Preliminary field culture of *Gracilaria* using vegetative fragments inserted between braids of ropes suspended vertically inside a floating cage was undertaken to assess the daily growth rate and monthly yield as influenced by three different spacing intervals.

Daily growth rate of cuttings at 10 cm intervals ranged from 0.6 to 7.2% with yields of 11 to 415 g m^{-1} line^{-1}, those at 15 cm from 1.4 to 9.1% with yields of 18 to 502 g m^{-1} line^{-1}, and at 20 cm from 1.7 to 10.5% and with yields of 20 to 379 g m^{-1} line^{-1}. Both growth and yield were minimum in December at all spacing intervals but maximum in April at 10 and 15 cm and in February 20 cm.

Results of the analysis of variance (ANOVA) showed a non-significant interaction between spacing interval and culture month on daily growth rate and monthly yield of *Gracilaria.* This indicates that the effect of spacing interval on the daily growth rate and monthly yield was not significantly influenced by the culture month; likewise the effect of culture month did not differ significantly with the intervals used. The main effects, however, of spacing interval and culture month to daily growth rate were significant. Yield was significantly affected by the culture month but not by spacing interval.

- **Juario JV. 1990. Milkfish culture in the Philippines. Tanaka H, Uwate KR, Juario JV, Lee CS, eds. Proceedings of the Regional Workshop on Milkfish Culture Development in the South Pacific; 21-25 November 1988; Tarawa, Kiribati. Suva, Fiji: South Pacific Aquaculture Development Project/FAO; 88-97.**

Milkfish culture was introduced into the Philippines probably more than 300 years ago from Indone-

Average monthly yield of *Gracilaria* [expressed as mean fresh weight (g m^{-1} $line^{1}$)] at 3 different spacing intervals (5=3 ± SE) (*After* Hurtado-Ponce, Bot. *Mar.* 33:479. 1990)

Months	Spacing intervals (cm)		
	10	15	20
December	11[a] ±2.9	18- ± 2.5	20[a] ± 7.2
January	26" ± 8.2	52[ab] ± 12.3	61" ± 44.9
February	254[b] ± 70.9	291[c] ± 17.1	379[b] ± 5.7
March	352[bc] ± 47.7	269[c] ±67.9	284[b] ± 32.1
April	415[c] ± 76.5	502[d] ± 27.4	337[b] ± 82.4
May	83" ± 31.5	87[b] ± 5.2	99" ± 38.9

Means in a column having the same superscript are not significantly different (P<0.05).

sia or China (Herre and Mendoza, 1929). Since then, it has been cultured in brackishwater ponds. In the early 1970s, milkfish culture in pens began in Laguna de Bay, a freshwater eutrophic lake (Delmendo and Gedney, 1974). This was found to be very successful and commercially viable.

- **Kohno H, Duray M, Gallego A, Taki Y. 1990. Survival of larval milkfish, *Chanos chanos,* during changover from endogenous to exogenous energy resources. Hirano R, Hanyu I, eds. Proceedings of the 2nd Asian Fisheries Forum; 17-22 April 1989; Tokyo, Japan. Manila, Philippines: Asian Fisheries Society; 437-441.**

Survival of laboratory-reared larvae of milkfish, *Chanos chanos,* during transition from the prelarval to postlarval stages (from hatching to 178 hours after hatching) was examined in relation to the changeover of energy sources. On the basis of the growth in total length, yolk resorption and feeding of the larvae, the prelarval and early postlarval stages of the fish can be divided into five phases as follows: 1) rapid growth corresponding to rapid yolk resorption; 2) less rapid growth with less rapid yolk resorption; 3) stagnant growth with rapid yolk resorption, yolk being still the only nutrient for the larvae; 4) stagnant growth based on both yolk and exogenous food, from the onset of feeding to the complete exhaustion of yolk; and 5) rapid growth based solely on exogenous food (beyond 120 hours after hatching). The survival rate decreased when the larvae depended solely on yolk (phases 1 to 3), then leveled off when they had both endogenous and exogenous energy sources (phase 4), and again declined when they came to depend totally on exogenous food (phase 5). Feeding during the period from the onset of feeding to complete yolk resorption seems important for the successful survival of milkfish larvae thereafter.

- **Kohno H, Gerochi D, Trino A, Duray M. 1989. Effects of feeding frequency and amount of feeding on growth of the grouper, *Epinephelus malabaricus. Phil. J. Sci.* 118:89-100.**

The effects of feeding frequency and amount of food on the growth of juvenile groupers (*Epinephelus malabaricus*) were investigated for 12 weeks of rearing in net-cages set in an earthen

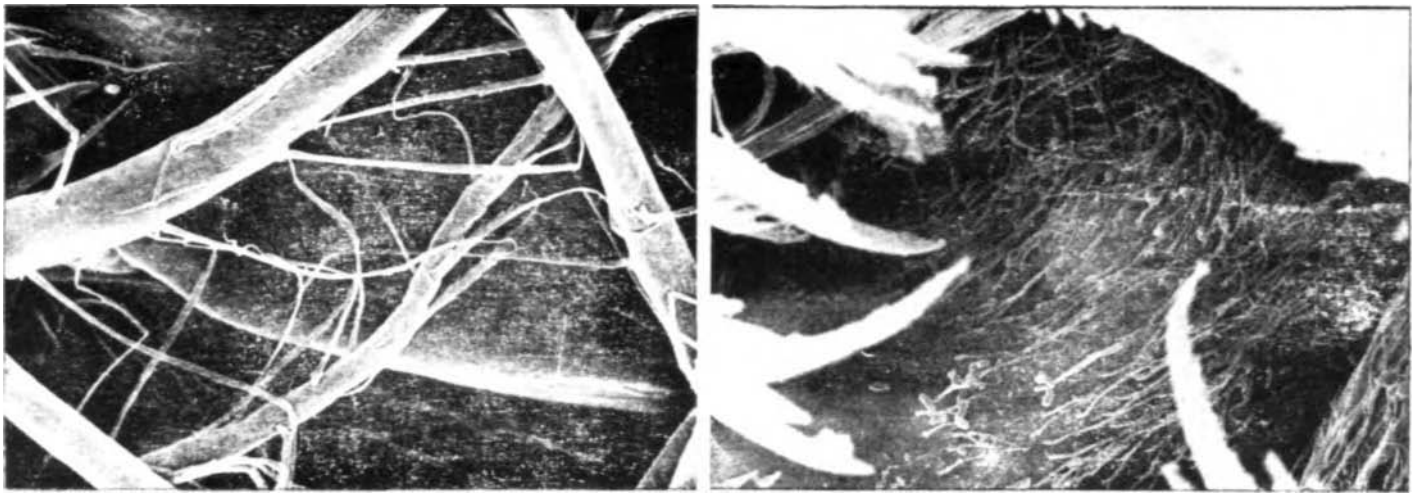
pond. The juveniles (110-130 g in body weight, BW) attained marketable size (500 g) in 12 weeks. Feeding to satiation levels once a day gave the best growth (mean BW = 509.4 ± 56.5 g) and relatively good food conversion ratio (4.78) compared to the other treatments (twice a day, once in two days and once in every three days). Concerning feeding levels, in which feed was given twice a day at 15, 10, 5 and 1% of total fish biomass per day, the best fish growth (426.6 ± 54.0 g) and a reasonable food conversion ratio (4.53) were obtained at 5% fish biomass. There was a positive correlation between the growth of fish and water temperature in both experiments.

- **Lavilla-Pitogo CR, Baticados MCL, Cruz-Lacierda ER, de la Peña LD. 1990. Occurrence of luminous bacterial disease of *Penaeus monodon* larvae in the Philippines. *Aquaculture* 91:1-13.**

Larval mortalities associated with luminiscence have been observed in epizootic proportions in black tiger prawn (*Penaeus monodon*) hatcheries in Panay Island, Philippines. Luminescent vibrios, identified as *Vibrio harveyi* and *V. splendidus,* were isolated from infected larvae but not from uninfected ones. These bacteria were also recovered readily from seawater samples from nearshore areas, the main source of hatchery rearing water. Thus, it is possible that the nearshore seawater is one major source of infection. Pathogenicity tests resulted in significant mortalities of larvae and postlarvae of *P. monodon* within 48 h of immersion challenge. Scanning electron microscopic observations show that colonization by the bacteria occurred specifically on the feeding apparatus and oral cavity of the larvae, suggesting an oral route of entry for the initiation of infection.

- **Lio-Po GD, Lavilla-Pitogo CR. 1990. Bacterial exoskeleton lesions of the tiger prawn *Penaeus monodon*. Hirano R, Hanyu I, eds. Proceedings of the 2nd Asian Fisheries Forum; 17-22 April 1989; Tokyo, Japan. Manila, Philippines: Asian Fisheries Society; 701-705.**

Tank- and pond-reared *Penaeus monodon* with exoskeletal lesions were examined. The incidence rate was up to 36% for broodstock in concrete tanks and to 20% for pond-reared prawns. The increase in disease incidence was related to prawn age or duration of culture. Symptoms consisted of focal melanization of the ventrolateral border of the carapace, melanized fissures at the dorsolateral area of the abdominal segments, melanized erosion of the telson or uropod. and blisters on the carapace. Bacterial isolation yielded mostly *Vibrio* spp. Pathogenicity was tested on healthy P. *monodon* juveniles by a combination of injury and exposure to the test bacteria. Cumulative mortality was 60% within 72 hours in stabbed prawns and 20-



Scanning electron micrograph of a Penaeus monodon zoea 1 feeding apparatus (without bacteria attached) (left) and oral region with V. harveyi after 24 h exposure to the bacteria (right) (After Lavilla-Pitogo et al., Aquaculture 91:8-9. 1990)

40% after 96 hours for superficially-cut prawns. Growth of the bacteria in culture was active in 0.5-8% NaCl and at 12-40°C. In-vitro, test isolates were sensitive to chloramphenicol, furazolidone, nitrofurantoin, oxytetracycline and sulfamethoxazole trimethoprim; and resistant to erythromycin, furanace, kanamycin and streptomycin.

- **Macaranas JM, Pante MJR, Benitez LV. 1990. Heterogeneity in Philippine milkfish populations. Hirano R, Hanyu I, eds. Proceedings of the 2nd Asian Fisheries Forum; 17-22 April 1989; Tokyo, Japan. Manila, Philippines: Asian Fisheries Society; 477-483.**

Twelve milkfish populations from six coastal areas in the Philippines, collected at the fry stage and reared until fingerling size, were analyzed for electrophoretic variation at 28 presumptive loci. Seven loci were polymorphic at the 0.99 level (5 at the 0.95 level) while 21 were monomorphic. Significant departures from the expected Hardy-Weinberg genotype distributions were observed only at the *Pgm* locus in samples 843 and 862. The mean Nei's genetic distance among populations was .0008±.00006; the distances were generally small among samples collected during the early fry season [Mean D = .00057±.00026 (n=55)] but large between the late fry season sample 853 and all other samples [Mean D = .0017±.00053 (n=11)]. G-tests for heterogeneity revealed significant differences in genotype distributions at the Est-1, *Gpi-1, Gpi-2, Mp-2* and *Pgm* loci within each year of sampling, within one location at different sampling years, and within two geographical areas east and west of the Philippines. No significant cline in any of the heterogeneous loci was observed. Based on the small genetic distances observed among the early fry season samples, an explanation posed for this heterogeneity is that the samples might have been derived from small numbers of spawns rather than from different subpopulations. On the other hand, the temporal isolation of sample 853 and its high divergence from the rest of the samples suggests that it might be derived from a different subpopulation.

The results of this study suggest the existence of at least two subpopulations of milkfish in Philippine waters and provide a biochemical explanation for the discreteness of fry occurrence peaks observed by Kumagai (1983).

- **Marte CL. 1990. Hormone-induced spawning of cultured tropical finfishes. In Advances in Tropical Aquaculture; 20 February-4 March 1989; Tahiti, French Polynesia. France: IFREMER; 519-540.**

Commercially important tropical freshwater and marine finfishes are commonly spawned with pituitary homogenate. human chorionic gonadotropin (HCG) and semi-purified fish gonadotropins. These preparations are often administered in two doses, a lower priming dose followed a few hours later by a higher resolving dose. Interval between the first and second injections may vary from 3-24 hours depending on the species. Variable doses are used even for the same species and may be due to variable potencies of the gonadotropin preparations.

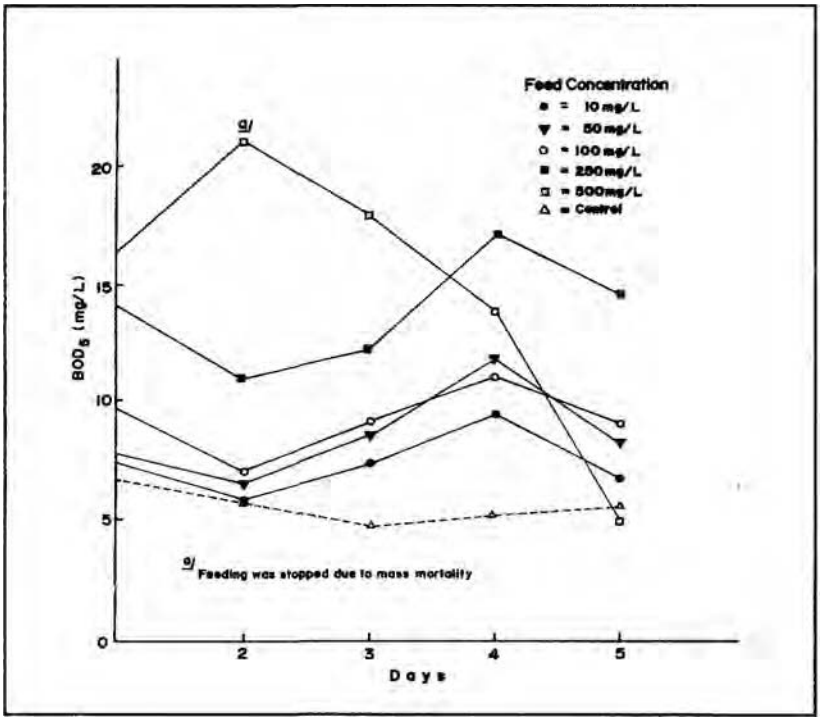
Synthetic analogues of luteinizing hormone-releasing hormone (LHRHa) are becoming widely used for inducing ovulation and spawning in a variety of teleosts. For marine species such as milkfish. mullet, sea bass, and rabbitfish, a single LHRHa injection or pellet implant appears to be effective. Multiple spawnings of sea bass have also been obtained following a single injection or pellet implant of a high dose of LHRHa. In a number of freshwater fishes such as the cyprinids, LHRHa alone however has limited efficacy. Standardized methods using LHRHa together with the dopamine antagonists pimozide, domperidone and reserpine have been developed for various species of carps. The technique may also be applicable for spawning marine teleosts that may not respond to LHRHa alone or where a high dose of the peptide is required.

Although natural spawning is the preferred method for breeding cultivated fish, induced spawning may be

necessary to control timing and synchrony of egg production for practical reasons.

- **Millamena OM. 1990. Organic pollution resulting from excess feed and metabolite build-up: Effect on *Penaeus monodon* postlarvae. *Aquacult. Eng.* 9:143-150.**

Penaeus monodon postlarvae, PL, with mean weight and mean total length of 0.57 mg and 6.5 mm, respectively, were stocked in aerated aquaria at 20 litre^{-1} of seawater. They were fed minced shrimp meat at rates of 10, 50, 100, 250, and 500 mg litre^{1}. Aquaria with diatoms as feed served as control. Growth and survival rates were examined at each feeding level. Water quality parameters measured were dissolved oxygen (DO), ammonia (NH_4-N) and nitrite (NO_2-N) nitrogen concentrations and 5-day biochemical oxygen demand (BOD_5). Growth of postlarvae was similar at all feeding levels and greater than the controls. Survival rate at the lowest feeding level was better but not significantly higher (P>0.05) than the control group and decreased as feed concentration increased. A feeding level of up to 50 mg litre^{1} proved to be satisfactory for both growth and survival. Beyond this level, accumulation of organics, low DO, and build-up of metabolites caused a rapid deterioration in water quality. Even at sub-lethal levels a combination of adverse environmental conditions imposed stress upon the cultured organisms and decreased survival rates.



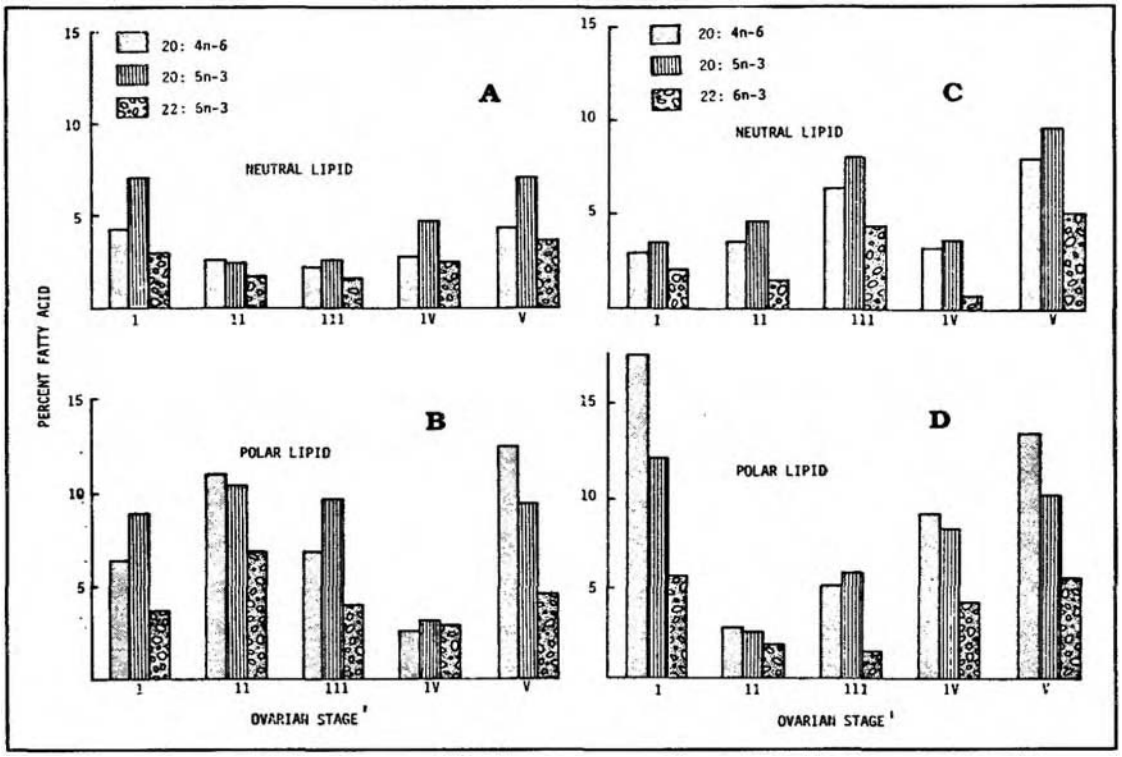
BOD_5 variations at different feed concentrations in culture water for P. monodon postlarvae (After Millamena, Aquacult. Eng. 9:148. 1990)

- **Millamena OM, Pascual FP. 1990. The tissue lipid content and fatty acid composition of *Penaeus monodon* broodstock from the wild. *J. World Aquacult. Soc.* 21:116-121.**

Tissues (hepatopancreas, muscle, gonad) of wild *Penaeus monodon* females with ovaries in different stages of development and males were analyzed for lipid content and fatty acid composition. Hepatopancreatic tissue had the highest mean levels of lipid at 15.72-25.20% in females and averaged 46.20±1.53% in males. Levels of lipid in the muscle were low with no marked variation throughout the maturation stages. Ovarian lipid levels increased significantly at stage II (early maturing ovaries) and remained high through stage IV (fully mature ovaries). Peak lipid level was found at stage III (late maturing ovaries) and coincided with a decline in hepatopancreas lipid. Ovarian and hepatopancreatic lipids declined drastically in spent females. Profiles of fatty acid in the tissues consistently showed the presence of PUFA: 20:4n-6, 20:5n-3 and 22:6n-3, with high levels in the polar lipid fraction of vitellogenic ovaries. These values were reflected in the male testis and spawned eggs. The findings suggest storage and utilization of lipids for maturation and spawning processes and that the phospholipids were mainly responsible for increases in ovarian lipids. The type of fatty acids present in maturing prawn ovaries is indicative of their importance in reproductive physiology.

Millamena OM, Peñaflorida VD, Subosa PF. 1990. The macronutrient composition of natural food organisms mass cultured as larval feed for fish and prawns. Israeli *J. Aquacult.-Bamidgeh* 42:77-83.

The macronutrient composi-



Relative concentrations of major polyunsaturated fatty acids in the neutral (A) and polar (B) lipids of the ovary and in the neutral (C) and polar (D) lipids of the hepatopancreas during ovarian maturation of P. monodon (After Millamena & Pascual J. World Aquacult. Soc. 21: 120. 1990)

tion of natural food organisms that are mass cultured as feed for the larval stages of fish and prawns in the SEAFDEC Aquaculture Department was determined by chemical analysis. The food organisms included five species of marine phytoplankton (algae): *Chaetoceroscalcitrans, Skeletonema costatum, Tetraselmis chui, Chlorella vulgaris* and *Isochrysis galbana,* and two zooplankton: *Artemia* sp. nauplii (San Francisco Bay strain) and *Brachionus plicatilis.* The algal species were grown in batches on Guillard and Ryther media and harvested during the exponential phase of growth using a procedure which preserved cellular integrity and prevented cell lysis. The zooplankton were cultured using standard techniques adopted at the SEAFDEC Larval Food Laboratory. Each species was analyzed for proximate composition (protein, fat, fiber and ash) and for mineral content (calcium and phosphorous). Nitrogen-free extract (NFE) was determined by difference. For the five algal species, the protein, fat and NFE contents varied from 22% to 48%, 2% to 16% and 14% to 24%, respectively. The zooplankton had higher protein and fat contents than any of the phytoplankton species except *I. galbana* which had the highest fat content. On the other hand, the phytoplankton, particularly the diatoms which have a siliceous cell wall, contained significantly higher quantities of inorganic matter (ash). C. *vulgaris* had the highest fiber content which may be attributed to its cellulosic cell wall.

- **Pantastico JB. 1989. Finfish nutrition in Asia: A Review. Fortes RD, Darvin LC, De Guzman DL, eds. Fish and Crustacean Feeds and Nutrition: Proceedings of the Seminar-Workshop on Fish and Crustacean Feeds and Nutrition; 25-26 February 1985; University of the Philippines in the Visayas, Iloilo City. Los Baños, Philippines: PCAMRD; 14-20.**

This review paper highlights country reports presented at the Workshop on Asian Finfish Nutrition sponsored by the International Development Research Centre of Canada in 1983. Results of researches on the amino acid, lipid, mineral and vitamin requirements of some cultured finfishes in the larval, juvenile and broodstock stages are discussed.

Complete essential amino acid

requirements have been determined for common carp; only three have been quantified for tilapia. Many researches were conducted to replace trash fish and fish meal as protein sources in fish diet. For *O. niloticus,* soybean meal can replace 50% of the fish meal in the grow-out diet.

Essential fatty acids influence the dietary value of live food organisms for fish larvae. Rotifers cultured with yeast were low in W_3 highly unsaturated fatty acids while those grown in *Chlorella* were high in 20:5W_3. Similarly, "lablab" showed higher W_3/W_6 ratio than "lumut."

On the other hand, chemical analyses of *Brachionus* cultured in baker's yeast or in *Chlorella,* and *Artemia* from two sources showed that mineral is not the principal factor in the dietary value of natural feeds.

Overall, fundamental knowledge of the nutritional requirements of finfishes in the region is lacking.

- **Pantastico JB, Baldia JP, Espegadera CC, Reyes DM Jr. 1990. Algal production and utilization relevant to aquaculture in the Philippines. Dogma IJ Jr, Trono GC Jr, Tabbada RA, eds. Culture and Use of Algae in Southeast Asia: Proceedings of the Symposium on Culture and Utilization of Algae in Southeast Asia; 8-11 December 1981; Tigbauan, Iloilo. Iloilo: SEAFDEC/ AQD; 99-111.**

Phycological researches in support of aquaculture are a recent development in the Philippines. Progress in this area gained momentum with increased efforts to expand fish farming in the Philippines as a means of producing more animal protein. The Aquaculture Department (AQD) of SEAFDEC, having taken the lead in larval rearing of penaeids and economically important fish species, intensified its search for promising algal species as natural feed. Imported and indigenous algal species were screened and tested for use in hatchery and nursery operations. The vital role of microalgae to sustain growth of larvae during critical stages of development was demonstrated.

- **Parado-Estepa FD. 1989. Larval rearing of *Penaeus monodon:* feeds and feeding techniques. Fortes RD, Darvin LC, De Guzman DL, eds. Fish and Crustacean Feeds and Nutrition: Proceedings of the Seminar-Workshop on Fish and Crustacean Feeds and Nutrition; 25-26 February 1985; University of the Philippines in the Visayas, Iloilo City. Los Banos, Philippines: PCAMRD; 21-23.**

Larval rearing of *P. monodon* requires mass culture of selected phytoplankton and zooplankton species. In the protozoeal stage, *Skeletonema, Chaetoceros* or *Tetraselmis* is given singly or in combination. The rotifer *Brachionus* and/or the brine shrimp *Artemia* are added to the diet as larvae metamorphose to the mysis stage. Attempts to replace these organisms with other organisms or with artificial diets are discussed.

The use of microparticulate diets as supplement to algal food or *Artemia* increases larval survival to 30-70%. The advantages of using this type of diet are enumerated.

- **Parazo MM. 1990. Effect of dietary protein and energy level on growth, protein utilization and carcass composition of rabbitfish, *Siganus guttatus. Aquaculture* 86:41-49.**

Six semipurified diets comprising three levels of protein (25, 35, 45% of dry matter) each at two levels of estimated energy (3161, 3832 kcal/kg) were fed to fry for 8 weeks in 250-l tanks at a stocking density of 80 fish/tank. Growth increased with increasing dietary protein ($P<0.01$) and energy ($P<0.05$). Within isocaloric diets, a positive correlation was found between growth and dietary protein-to-energy (P/E) ratio ($P<0.05$). Protein productive value (PPV) and protein efficiency ratio (PER) were negatively correlated with dietary P/E ratio. The equations describing this trend were: PPV=50.16-0.19X and PER=2.83-0.12X, where X is P/E. Based on responses for growth rate and efficiency of protein utilization, a diet with 35% protein and 3832 kcal/kg energy was found to be best for rabbitfish fry. Carcass fat levels in-

creased in those fish fed diets with 3832 kcal/kg energy. Carcass protein and ash percentages remained essentially constant and independent of dietary treatment.

- **Piedad-Pascual F. 1989. Prawn nutrition, feed development and feeding techniques for grow-out and broodstock. Fortes RD, Darvin LC, De Guzman DL, eds. Fish and Crustacean Feeds and Nutrition: Proceedings of the Seminar-Workshop on Fish and Crustacean Feeds and Nutrition; 25-26 February 1985; University of the Philippines in the Visayas. Iloilo City. Los Baños, Philippines: PCAMRD; 24-29.**

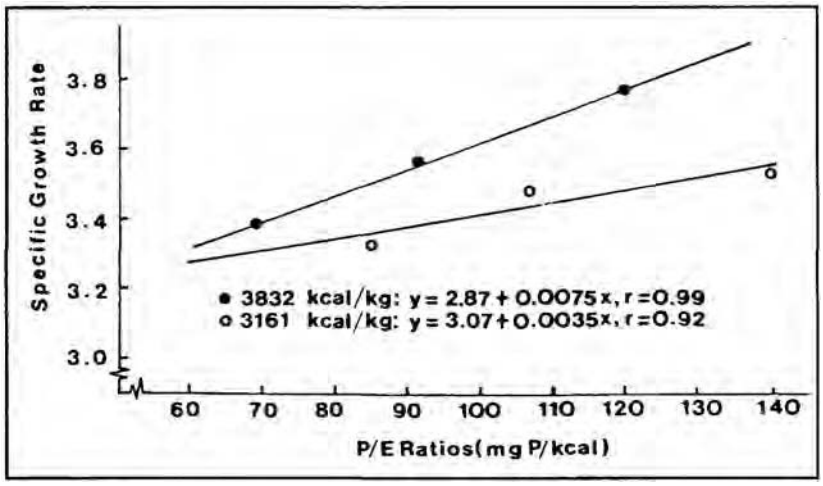
Knowledge of nutritional requirements of prawn juveniles and broodstock is scanty. However, relatively more information is now available with regard to requirements of juveniles compared to that of broodstock.

Many studies suggest the storage and utilization of lipids for maturation and spawning of *P. monodon* broodstock. Ovarian lipid increases more than twice at the start of maturation (Stage II) and reaches maximum at full maturity (Stage IV).

Prawn juveniles require 40-45% protein, 5-10% lipid, and 20-25% carbohydrates. A protein-energy ratio of 120 mg prot/Kcal results in better growth and survival. The quality of protein and lipid is very important for both stages of the prawn. Eleven amino acids are essential but quantitative requirements have not been established. Likewise, amounts of essential fatty acids such as arachidonic, docosapentaenoic and eicosahexaenoic acids have yet to be defined. Cholesterol and lecithin are also necessary.

Preliminary results of a 35-day feeding experiment showed that a vitamin free semipurified diet, choline-free and inositol-free diets significantly suppressed growth while niacin-free and pyridoxine-free diets provide for similar growth as that of a diet with all the vitamins present. Mineral needs have not been studied for *P. monodon.*

Feedstuffs of good nutritive value in prawn grow-out and broodstock diets are: fish meal, shrimp head meal, shrimp meal, mussel meat, *Acetes* sp. or "alamang," soybean meal, squid meal, earthworm meal, and annelids. In addition, cod liver oil, fish liver oil, soybean oil, and beef tallow have been used in varying proportions as sources of some essential fatty acids in practical diet development. Although there are practical diets that can be recommended, improvement of these diets will continue as new nutrient requirements are established.



Specific growth rate of S. guttatus fry as a Junction of dietary P/E ratio (After Parazo, Aquaculture 86: 45. 1990)

- **Piedad-Pascual F. 1989. An overview of the nutrition, feed and feeding of prawn penaeid/shrimps. Fortes RD, Darvin LC, De Guzman DL, eds. Fish and Crustacean Feeds and Nutrition: Proceedings of the Seminar-Workshop on Fish and Crustacean Feeds and Nutrition; 25-26 February 1985; University of the Philippines in the Visayas, Iloilo City. Los Baños, Philippines: PCAMRD; 4-9.**

This paper echoes what transpired during the First International Conference of Penaeid Prawns/Shrimps held in Iloilo City on December 4-7, 1984, particularly on the session on Nutrition and Feed Development. Around 25 papers were presented during the conference.

The nutrient requirements of *P. japonicus* and to some extent, *P. monodon* have been studied quite extensively compared to other penaeid species. Requirements for protein, carbohydrates, fats, amino acids and es-

sential fatty acids for juveniles and larvae have been defined compared to those of the broodstock.

Optimum protein levels for prawn juveniles vary from 28-38% for *P. kerathurus,* 40-46% for *P. monodon,* 43% for *P. indicus* and 50-54% for *P. japonicus.* Dissacharides like sucrose and trehalose have been found to be good sources of carbohydrates at 20-25% in the diet. Crustacean diets require around 0.5% cholesterol. There are few studies on vitamin and mineral requirements.

There are artificial diets for juveniles and microencapsulated diets that can completely replace live organisms as larval feed. Microencapsulated diets have been field-tested for *P. vannamei, P. stylirostris, P. monodon,* P. *indicus* and *P. merguiensis* in Ecuador, Taiwan, Malaysia, Thailand and the Philippines.

When a commercial diet for the broodstock becomes available there will be an artificial diet for the life cycle of the prawn.

- **Piedad-Pascual F. 1990. Mineral requirement of Penaeids. *In* Advances in Tropical Aquaculture: 20 February-4 March 1989; Tahiti, French Polynesia. France: IFREMER; 309-318.**

Marine shrimps absorb minerals from their aquatic environment aside from the minerals that come from the food they eat. Thus, the dietary requirement of shrimps for certain minerals will depend on the amounts and availability of these minerals in the aquatic environment. Dietary sources for growth may be necessary due to losses during moltings.

Most of the dietary studies for mineral requirements have been done under laboratory conditions with purified or semi-purified diets and hardly any information is available under practical culture conditions. Most published data for mineral requirements are for juvenile *Penaeus japonicus.* There are few data for *P. monodon, P. californiensis, P. merguiensis, P. aztecus.*

Calcium and phosphorus are the minerals that have been studied the most. These two have been found to be related to problems of soft-shelling in *P. monodon.* Apparently calcium and phosphorus requirements are within the range of 1 to 2%. The ratio of calcium to phosphorus in the diet is also an important factor in the efficient utilization of both minerals. It seems that a 1:1 ratio provides for good growth. Phosphorus deficiency results in reduced growth while lack of magnesium brings about decreased growth, poor survival and reduced feed efficiency in *P. japonicus.* Iron toxicity has also been observed in *P. japonicus.*

It might not be necessary to include some minerals in the diet of penaeids.

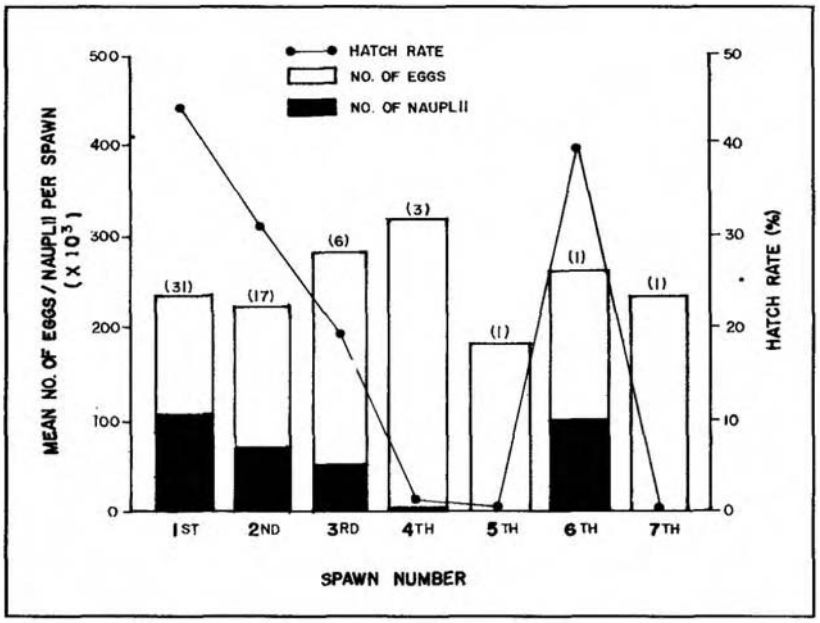
- **Piedad-Pascual F, Cruz EM, Sumalangcay A Jr. 1990. Supplemental feeding of *Penaeus monodon* juveniles with diets containing various levels of defatted soybean meal. *Aquaculture* 89:183-191.**

Varying levels of defatted soybean meal DSM (15, 25, 35, 45 and 55%) in supplemental diets with approximately 40% crude protein and 10% crude fat were fed to tiger prawn juveniles in lm x lm x lm net cages set on the bottom of a 1-ha earthen pond. Prawns were stocked at 10 or 20 per m^2 and fed the various diets for 3 months. Prawns were also stocked outside the cages at a stocking density of 0.5/m^2. Weight gains were significantly affected by supplemental feeding and stocking rate. Prawns that were stocked outside the cages weighed 13.2 g upon termination whereas those stocked at 10/m^2 and 20/m^2 were twice as heavy. Higher weight gains were recorded for prawns stocked at 10/m^2 compared to those stocked at 20/m^2. There were no significant differences in weight gains of prawns fed varying levels of soybean meal at stocking densities of 10 or 20/m^2, indicating that soybean meal can be incorporated into the ration at high levels. At both stocking rates, survival rates were relatively high and were not significantly different. Although no significant differences in growth were observed due to the different levels of defatted soybean meal in the diets, the diet with 35% DSM and 16% Peruvian

fish meal gave the best yield.

- **Piedad-Pascual F, Catacutan M. 1990. Defatted soybean meal and *Leucaena* leaf meal as protein sources in diets for *Penaeus monodon* juveniles. Hirano R, Hanyu I, eds. Proceedings of the 2nd Asian Fisheries Forum; 17-22 April 1989; Tokyo, Japan. Manila, Philippines: Asian Fisheries Society; 345-348.**

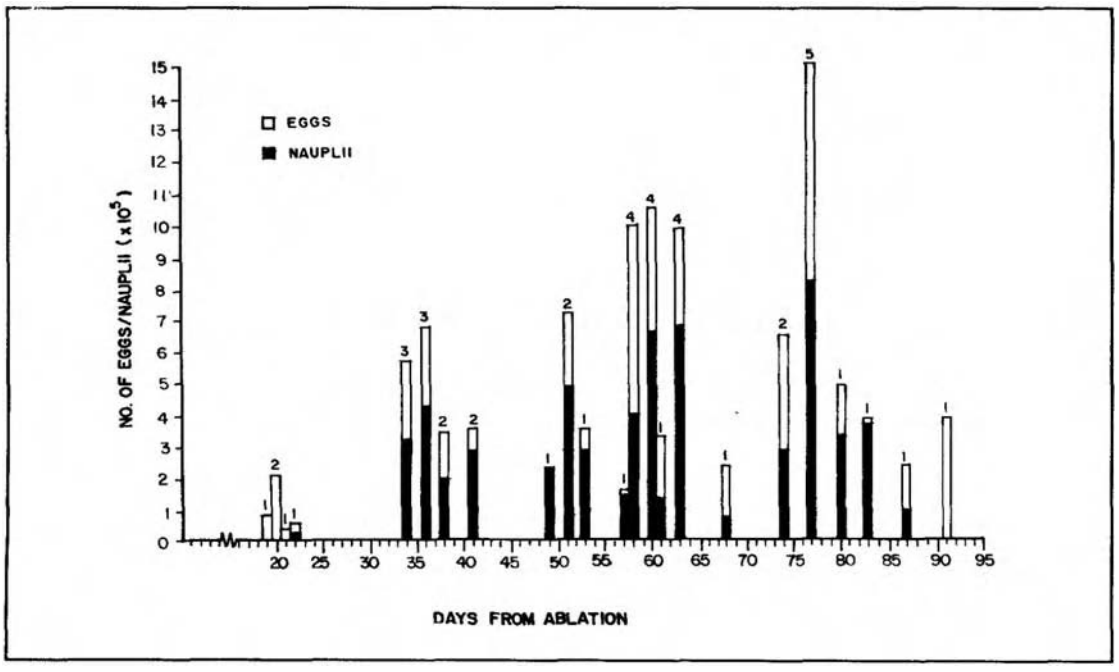
Penaeus monodon juveniles, mean weight 0.38 g, were fed twelve practical diets with 30, 20 or 16% Peruvian fish meal, 15 or 35% defatted soybean meal (DSM), 10% *Leucaena leucocephala,* leaf meal (LM), and 15% shrimp meal with and without vitamins and/or minerals. The diets contained 42-48% crude protein and 11-13% crude fat. The animals were stocked at 10 per fiberglass tank, and reared in 40 liters aerated seawater in a flowthrough system for 8 weeks. There were eleven replicates in a completely randomized design. Growth and survival were not affected by the level of DSM but significantly decreased ($P<0.05$) in prawn fed diets with LM. Feed conversion ratios of prawn were better for complete diets than those where vitamins only were added. Poor feed conversion ratios and specific growth rates were obtained when no vitamins and minerals or only minerals were added to the diets.



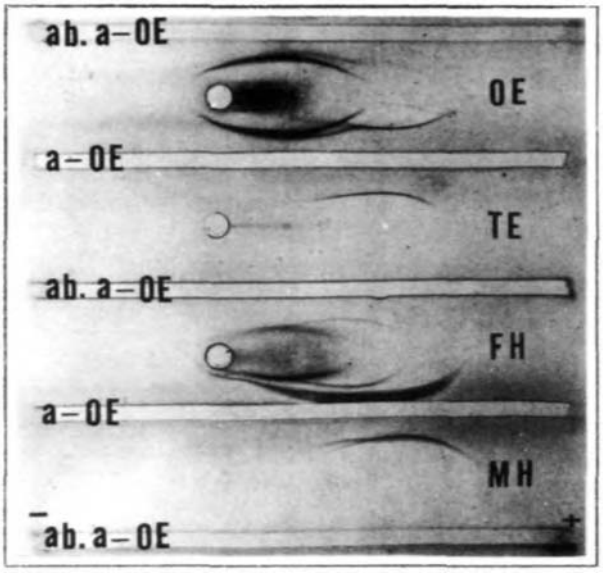
Number of eggs, nauplii and hatch rates of first and repeat spawns in ablated Penaeus monodon. Numbers in parentheses refer to number of spawns (After Primavera& Caballero, Philipp. Sci. 26: 12. 1989)

- **Primavera JH, Caballero RV. 1989. Effect of tagging on maturation and survival of ablated *Penaeus monodon* in painted and unpainted tanks. *Philipp. Sci.* 26:5-20.**

A study was conducted to determine the effect of tags and tank color on reproductive performance and survival of pond-reared *Penaeus monodon* after eyestalk ablation. In 1986, the best over-all reproductive performance was from eyestalk-tagged females in black tanks. Because of



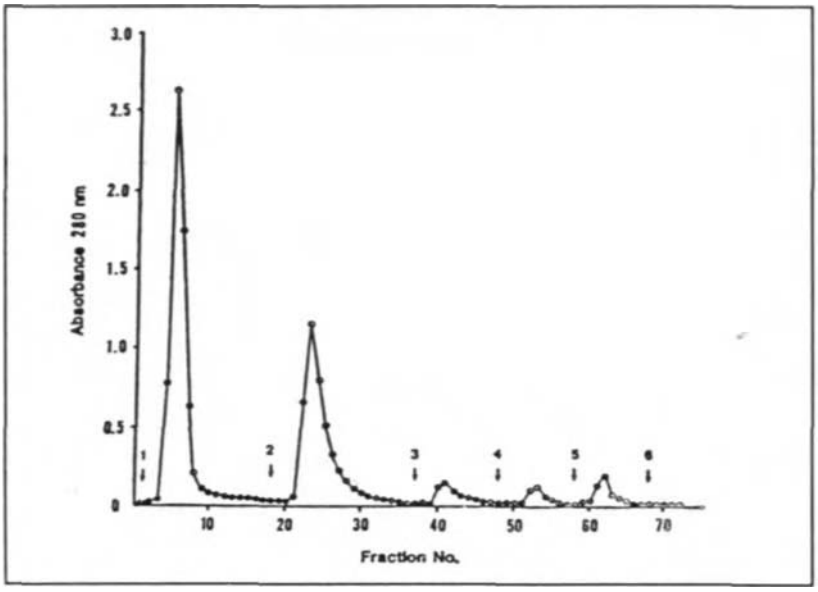
Daily egg and nauplii production from ablated Penaeus monodon (n=31) in a 12 m³ tank. Numbers on bars refer to number of spawns (After Primavera & Caballero, Philipp. Sci. 26: 13. 1989)



Immunoelectrophoretic pattern of ovarian extract (OE), testis extract (TE) and hemolymph from female (FH) and male (MH) P. monodon with antiserum against ovarian extract (a-OE) and Us absorbed antiserum with male hemolymph (ab.a-OE) (After Quinitio et al., Invert. Reprod. Dev. 17:223. 1990)

varying water flowthrough rates and loss of carapace tags, statistical analysis was not possible. The 1987 study show that tagging and tank color had no effect on maturation but survival rates of tagged females were significantly lower (54.2-62.9%) than those of untagged females (83.5%) in black and unpainted tanks. The 1986 results also showed lower survival rates of eyestalk-tagged females (31.2-59.0%) compared to untagged ones (90.0%).

Monitoring of rematurations (by means of eyestalk tags) showed that among ablated females, 64% spawned once, 35% a second time, and 12% a third time with a maximum of seven spawns from a single female. Uniform spawn sizes characterized first and repeat spawns but hatch rates decreased with each consecutive spawn.



Elution pattern on hydroxylapatite column of P. monodon ovarian extract. Numbers above arrows indicate the stepwise elution with potassium phosphate buffer: 0.1 M (1). 0.2 M (2), 0.3 M (3), 0.4 M (4), 1.2 M (5). and 2.0 M (6) (After Quinitio et al., Invert Reprod. Dev. 17:223. 1990)

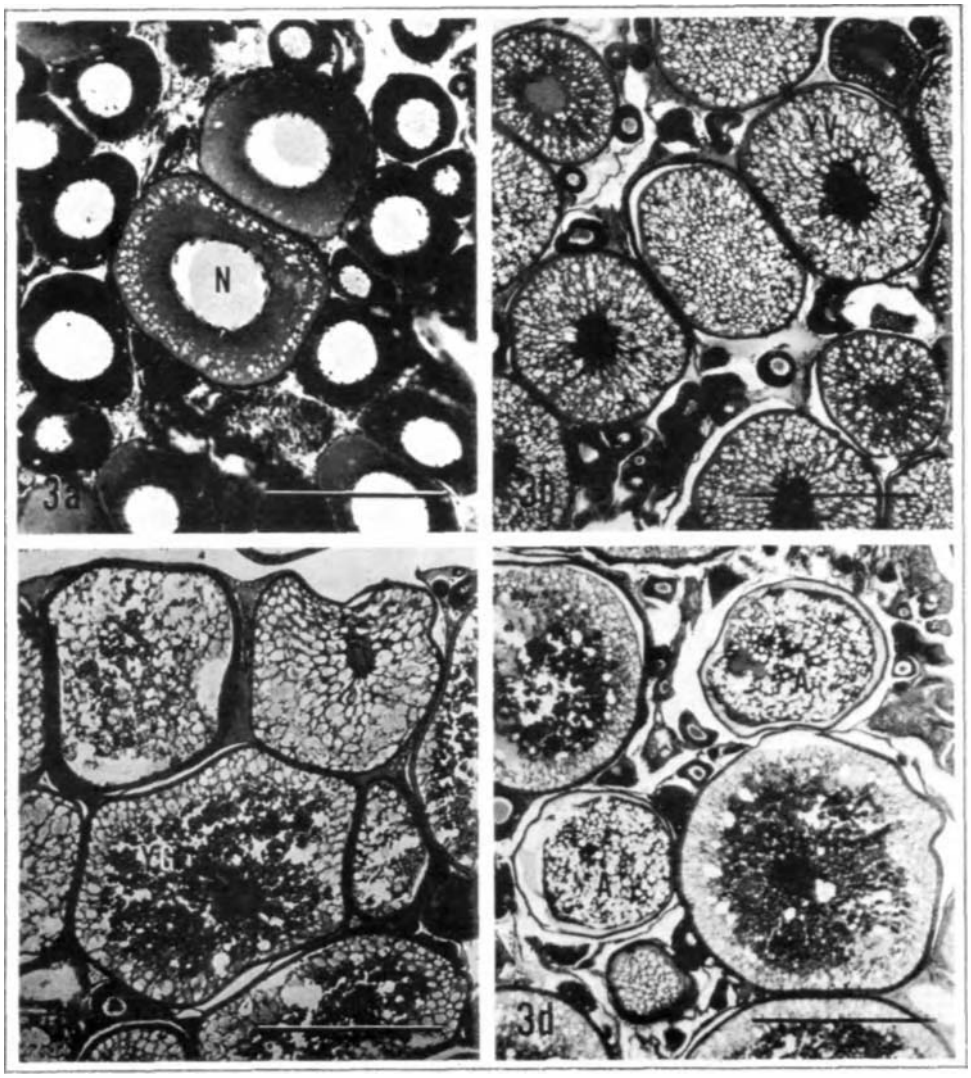
• Quinitio ET, Hara A, Yamauchi K, Fuji A. 1990. Isolation and characterization of vitellin from the ovary of *Penaeus monodon*. *Invert. Reprod. Dev.* 17:221-227.

Female-specific protein (FSP, vitellogenin) in *Penaeus monodon* hemolymph and its related ovarian protein (vitellin, lipovitellin) were identified and characterized using electrophoretical and immunological procedures. The purification of vitellin from mature ovaries was carried out using hydroxylapatite and Sepharose 6B columns. Results indicated that there are two proteins specifically existing in the hemolymph of the mature female which are immunologically identical to ovarian protein. These are absent in the male. The isolated vitellin has a molecular weight of approximately 540 kDa and is composed of 4 major (polypeptide) subunits, 74, 83, 104 and 168 kDa and 1 minor (polypeptide) subunit. 90 kDa. The purified protein stained positively with periodic acid-Schiff and Sudan black B and thus is a glycolipoprotein.

Results of double immunodiffusion demonstrate the cross-reactivity of *P. monodon* vitellin antiserum with the ovarian extract from mature females of *Penaeus indicus, Penaeus merguiensis* and *Penaeus semisulcatus,* but not with *Pandalus kessleri,* indicating that there is no antigenic difference at species level in Penaeidae.

• Quinitio GF, Takemura A, Goto A. 1989. Ovarian development and changes in the serum vitellogenin levels in the river sculpin, *Cottus hangiongensis,* during an annual reproductive cycle. Bull. *Fac. Fish. Hokkaido Univ.* 40: 246-253.

Annual changes in the ovarian development and serum vitellogenin concentration were investigated in the



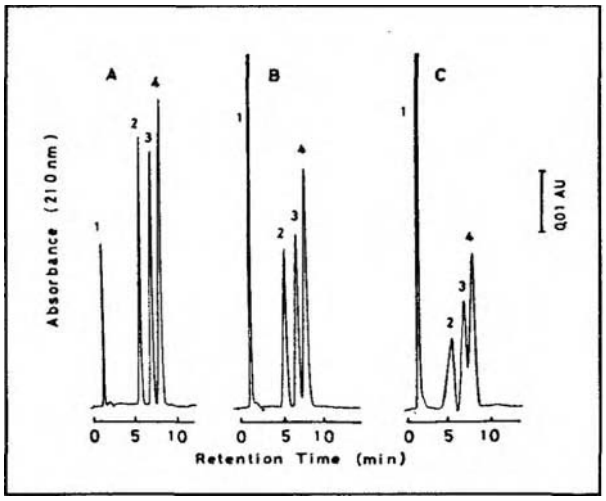
Cross-sections of ovary qf Cottus hangiongensis: fish collected in July had oocytes mostly at the peri-nucleolar stage and few at the yolk vesicle stage (3a)(N, nucleus; scale bar. 200 μm); fish collected in October with oocytes mostly at the yolk vesicle stage(3b) (scale bar, 500 μm); fish collected in February had abundant oocytes at the primary to tertiary yolk stages (3c) (scale bar,200 μm); and fish collected in May with tertiary yolk stage and atretic oocytes (3d) (scale bar, 200 μm) (After Quinitio et al., Bull Fac. Fish. Hokkaido Univ. 40: 250. 1989)

river sculpin, *Cottus hangiongensis,* sampled monthly from a river in southern Hokkaido, Japan. Ovarian development started advancing from summer and continued during the winter months until March with a maximum mean gonadosomatic index (GSI) of 15.99%. Hepatosomatic index (HSI) was also highest in March with a range of 5.13-5.95%. Spawning season usually occurred from April to May. Annual changes in serum vitellogenin level correlated very well with the patterns of GSI and HSI, as well as histological changes of the ovary. However, high serum vitellogenin was maintained in March and April.

• **Rokushika S, Kihara K, Subosa PF, Leng WX. 1990. Ion chromatography of nitrite, bromide and nitrate ions in brine samples using a chloride-form anion exchange resin column. *J. Chromatogr.* 514: 335-361.**

In aquaculture research, maintenance of water quality is of great importance. Recent improvements in fishpond management and fish culture systems such as intensive feeding and fertilization have created complex problems. Deterioration of water quality is primarily the resulting effect of these improvements. For example, high-protein feeds and nitrogen fertilizers applied in fishponds produce considerable amounts of nitrite in the water. Moreover, their synergistic effects cannot be ignored. Hence the routine determination of nitrite in fishpond water is required.

Since its introduction by Small et al., ion chromatography has been



Effect of chloride ion concentration in the sample solution on the peak shape of anions. Chloride ion concentrations: (A) 5; (B) 20; (C) 50 µg/mL Peaks: 1=Cl; 2=NO_2; 3=Br; 4=NO_3 (After Rokushika et al., J. Chromatogr. 514:357.1990)

widely used for the determination of ions in water. However, the presence of very high concentrations of chloride is the main obstacle in the analysis of seawater samples, affecting the separation and detection not only of nitrite but also of other anions.

Koh and Shinbori applied ion chromatography to the analysis of seawater using a 125-cm long column and a conductimetric detector. This technique provided a simple and sensitive analytical method for brine samples. However, nitrite could not be determined owing to the presence of a large chloride peak.

Attempts to determine nitrite in seawater also led to innovations in the methodology. Lee and Field employed a post-column cerium flourescence detection system to determine nitrite and nitrate in drinking water and seawater. The use of a pretreatment column in the silver form for removal of chloride has been reported.

Various detectors have been applied in ion chromatography in addition to the conductimetric detector. The UV detector has been shown to be useful detector for several kinds of inorganic anions. Selective detection of specified inorganic ions can be achieved by tuning the wavelength of the UV detector in ion chromatography and also in ion-exclusion chromatography. The elimination of the chloride matrix interference in the sub-ppm determination of nitrite in seawater was achieved by a heart-cutting and recycling method using a dual detection system consisting of conductimetric and UV detectors. This method, however, requires valve switching during analysis and needs a long analysis time.

This paper describes a simple and rapid method for UV-absorbing anions, such as nitrite, nitrate and bromide, in brine samples using achloride-formanion-exchange resin column combined with a UV detector.

- **Santiago CB. 1989. Nutrition and feeds of Nile tilapia broodstock and fry. Fortes RD, Darvin LC, De Guzman DL, eds. Fish and Crustacean Feeds and Nutrition: Proceedings of the Seminar-Workshop on Fish and Crustacean Feeds and Nutrition; 25-26 February 1985; University of the Philippines in the Visayas, Iloilo City. Los Baños, Philippines: PCAMRD; 40-49.**

Studies on some aspects of tilapia nutrition and feed formulation conducted at BRS are reviewed in this paper.

The effect of varying dietary crude protein on growth and spawning frequency of tilapia broodstock was determined under laboratory conditions. The weight of male fish increased as dietary crude protein increased; however, weight gain of male fish did not follow a definite trend due to the asynchronous nature of spawning of the tilapia. Results suggest that when diets contain high-quality protein, and feeding is at satiation level, the influence of increasing dietary protein on spawning frequency and growth of the females is not considerable.

In another study, tilapia broodstock in cages and tanks had the best growth and highest fry production when fed a 40% CP diet.

Ipil-ipil leaf meal as a sole or major source of dietary-protein caused weight loss among tilapia breeders, drastic reduction in fry production, and eventually cessation of reproduction. The growth of breeders decreased significantly with the incorporation of more than 40% ipil-ipil in the diet.

For Nile tilapia fry, growth was enhanced by availability of high phyto-

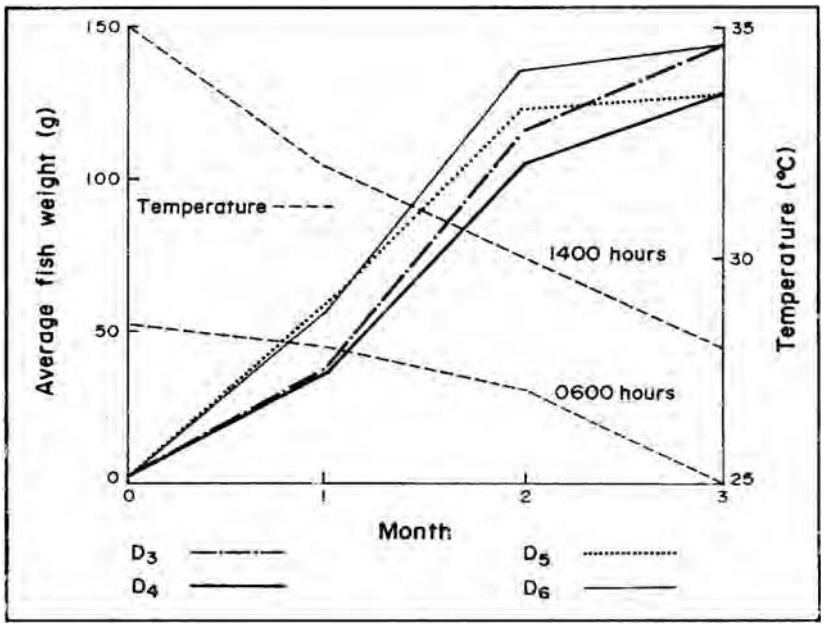
plankton densities in the rearing medium throughout the culture period. Feeding the fry with unialgal culture of *Navicula* and *Chroococcus* resulted in highest weight gains and survival rates. *Chlorella* gave poor survival and growth. Rice bran or *Moina* as feed for the fry was nutritionally inadequate. Their nutritional value improved when both were combined as feed.

- **Sumagaysay NS, Chiu-Chern YN, Estilo VJ, Sastrillo MAS. 1990. Increasing milkfish (*Chanos chanos*) yields in brackishwater ponds through increased stocking rates and supplementary feeding. *Asian Fish. Sci.* 3:251-256.**

Brackishwater milkfish culture in the Philippines is normally practiced at fish stocking rates of 2,000-3,000 ha^{-1} with fertilizers as the sole nutrient input. Supplementary feeding is not common. We stocked two 1-ha ponds with 6,000 fish and another two 1-ha ponds with 9,000 fish with an average weight of 2 g. The fish at each stocking rate were given diets with two different energy levels (2,950 and 3,265 kcal kg^{-1}) at 3% body weight, on the second and third month of culture. An average of 0.69 and 1.04 t were produced at 6,000 and 9,000 ha^{-1}, respectively. Low temperature and dissolved oxygen levels appeared to limit the growth of milkfish masking the effect of dietary energy. The results suggest that supplementary feeding can have a marked effect on milkfish yield when stocking rates are 6,000 ha^{-1} or above.

- **Tabbada RA, Florendo PE, Santiago AE. 1989/1990. Uptake and some physiological effects of mercury on water hyacinth, *Eichhornia crassipes* (Mart.) Solms. *BIOTROPIA*(3):83-91.**

The uptake and growth effects of mercury (Hg) on *Eichhornia crassipes* under field conditions and with lakewater as culture medium were investigated. Absorption of the heavy metal increased with higher levels of Hg in the culture solution. Roots accumulated much more of the heavy metal than the leaves. The addition of 1 and 2 ppm of Hg into the culture medium was not toxic but did significantly reduce fresh matter production, root elongation, ramet formation and total chlorophyll content of mature leaf blades during a four-week culture period. The results strongly suggest a beneficial role of the plant, long considered as a noxious weed, as a bioaccumulator of Hg in polluted lakes.



Growth of milkfish in earthen ponds at two stocking densities and two levels of supplementary dietary energy. Solid lines, weight offish in the four ponds D_3-D_6 broken lines, water temperature. D_3 - 6000 fish ha^{-1}, lower energy; D_4 - 6000, higher energy; D_5 - 9000, low; D_6 - 9000, high (After Sumagaysay et al., Asian Fish. Sci. 3:255. 1990)

- **Toledo JD, Kurokura H. 1990. Cryopreservation of the euryhaline rotifer *Brachionus plicatilis* embryos. *Aquaculture* 91:385-394.**

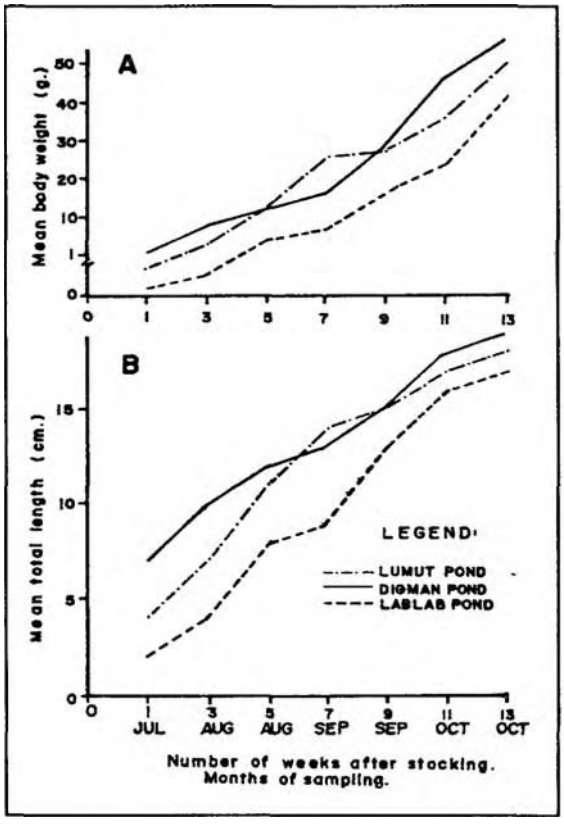
A method for the separation and cryopreservation of *Brachionus plicatilis* embryos is described. Juveniles with uniform development were collected from a cultured stock by passing them through a series of nets. Collected juveniles were cultured and the embryos separated by vigorous vortex mixing as soon as the majority had laid their first eggs Separated embryos at stage I (cleavage stage), stage II (invagination stage), stage III (symmetrical embryo stage), or stage IV ("eyed" stage) were frozen to -196°C using various concentrations of DMSO and a two-step freezing procedure. No stage I embryos survived freezing and the highest post-thaw survival was obtained with stage III embryos. A DMSO concentration of 10% of the freez-

ing medium resulted in high post-thaw survival while concentrations higher than 10% appeared to be harmful to embryos. Prolonged incubation in 10% DMSO for up to 30 min before freezing increased post-thaw survival.

Incorporating the above results, stage III embryos from a single batch culture were incubated in 10% DMSO for 30 min and frozen to -196°C. Post-thaw survival rates of 63%, 62%, 53%, and 55% were obtained after 3,7, 15, and 30 days of storage in liquid nitrogen, respectively. Survivors fed actively on marine *Chlorella* and started to lay eggs 2-3 days after thawing.

- **Triño AT, Bolivar EC. 1990. Growth performance of *Penaeus monodon* in *lablab, lumut,* and *digman* ponds under various farm practices. *J. Aqua. Trop.* 5:123-129.**

The influence of *lablab, lumut* (filamentous algae), and *digman* (*Najas graminea*) on the growth of prawns as associates of natural food biomass in brackishwater ponds was investigated. Ponds, each grown with the above macrophyte species and with no supplementary feed used, were selected as sampling areas. Sampling was conducted on a biweekly basis. Results showed that although the specific growth rate of prawns reared in the *lablab* pond was significantly higher than that of prawns reared in the lumut and *digman* ponds ($P<0.01$), absolute values in growth showed otherwise. Prawns reared in the *digman* pond attained highest growth which was significant ($P<0.01$). The *digman* pond was able to support a much higher density and produce a higher growth, coupled with a substantial survival. The *digman* pond yielded 606 kg of prawns, while the *lablab* pond yielded 569.5 kg and the *lumut* pond 305 kg. At value of $10 per kg, the rearing of prawns in the *digman* pond would be more profitable than the other treatments.



Mean length and weight of Penaeus monodon in brackishwater ponds under different treatments (After Trino & Bolivar, J. Aqua. Trop. 5:125. 1990)

- **Villaluz AC. 1990. Milkfish fry collection and handling. Tanaka H, Uwate KR, Juario JV, Lee CS, eds. Proceedings of the Regional Workshop on Milkfish Culture Development in the South Pacific; 21-25 November 1988; Tarawa, Kiribati. Suva. Fiji: South Pacific Aquaculture Development Project/ FAO; 77-87.**

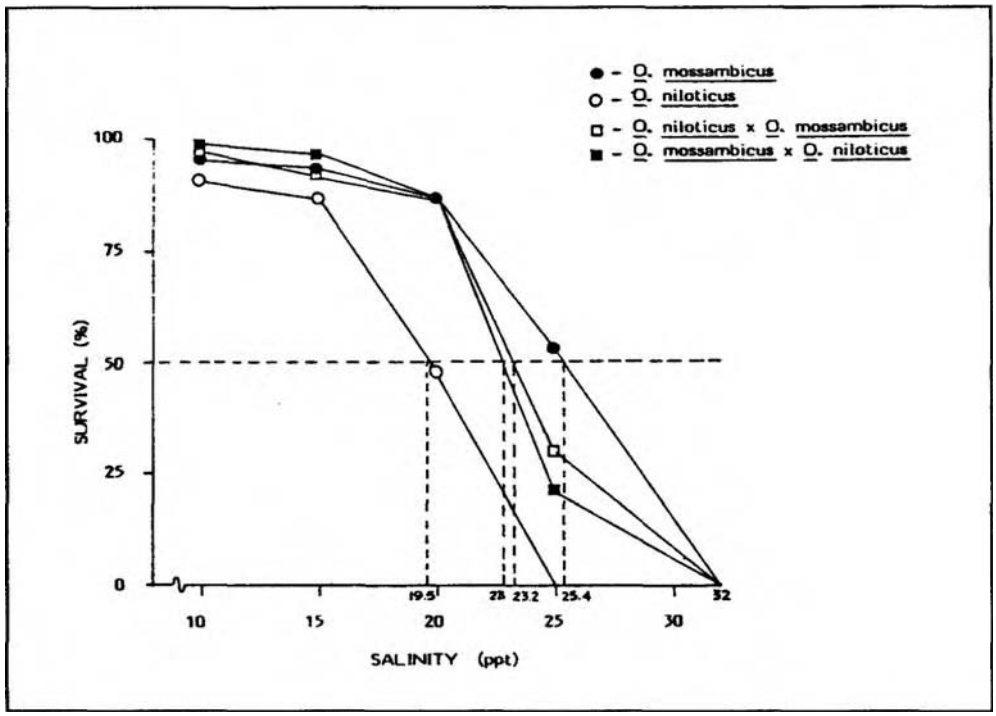
Milkfish fry can be collected in almost all the coastal waters of the tropical Indo-Pacific region. The fry season occurs at different times of the year in various sections of the species' geographical range. The season is longer near the equator and become progressively shorter at higher latitudes. In regions affected by monsoon or trade winds, the peak fry season typically coincides with one or both of the biannual wind shifts. These seasonal peaks are more or less predictable, but fry abundance may vary from year to year.

This paper summarizes the methods and practices of collection, storage, transport and acclimation of milkfish fry in various countries.

Villegas CT. 1990. Evaluation of the salinity tolerance of *Oreochromis mossambicus, O. niloticus* and their F_1 hybrids. *Aquaculture* 85:281-292.

The salinity tolerance of freshwater-spawned and reared *Oreochromis mossambicus, O. niloticus* and their F_1 hybrids of various ages was studied. Several tests were conducted using three indices as practical measures of salinity tolerance: (1) mean survival time (MST); (2) median survival time (ST_{50}); and (3) median lethal salinity-96 hours (MLS-96).

Interspecific and age-specific differences ($P<0.01$) in salinity tolerance were observed in these species and their F_1 hybrids on the basis of MST and ST_{50} indices, with salinity tolerance generally increasing with age of brood. No significant age-specific differences ($P>0.05$) in salinity tolerance were observed in all four groups on the basis of MLS-96 index. At the same salinity *O. niloticus* fry at ages from 15 to 90 days post-hatch exhibited significantly lower ($P<0.05$) salinity tolerance than *O. mossambicus* and F_1 hybrids. Changes in salinity tolerance were determined to be more closely related to body size than age.



Survived patterns for 15- to 90-day old freshwater-spawned and reared O. mossambicus, O. niloticus, and their F_1 hybrids 96 h following direct transfer to various salinities. MLS-96 for each species is indicated by the value beneath each dotted vertical line (After Villegas, Aquaculture 85:286.1990)

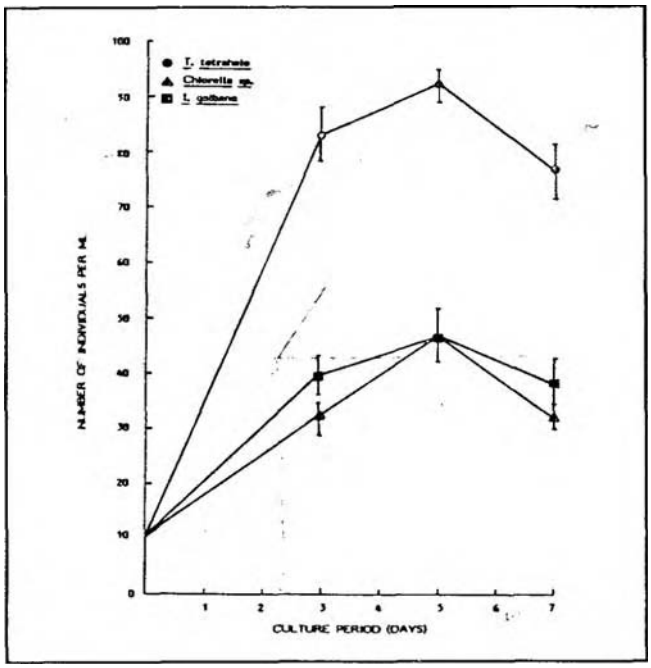
- **Villegas CT. 1990. Growth and survival of *Oreochromis niloticus, O. mossambicus* and their F_1 hybrids at various salinities. Hirano R, Hanyu I, eds. Proceedings of the 2nd Asian Fisheries Forum; 17-22 April 1989; Tokyo, Japan. Manila, Philippines: Asian Fisheries Society; 507-510.**

Growth and survival of *Oreochromis niloticus, O. mossambicus,* and their F_1 hybrids were studied at various salinities (0-32 ppt) in net cages inside 12-tonne concrete tanks after acclimation. Growth, measured as weight gain, and survival were assessed after three months of culture. Results showed that although *O. niloticus, O. mossambicus,* and their F_1 hybrids can be acclimated and grown at varying salinities, optimum salinity ranges for good growth varied greatly. The salinity ranges for good growth of *O. mossambicus* and F_1 hybrids were wider than *O. niloticus. O. mossambicus* had the highest growth rates at 15 and 32 ppt. The optimum salinity range for growth of F_1 hybrids was 15-32 ppt, while for *O. niloticus* it was 0-10 ppt. Salinity up to 20 ppt had no significant effect ($P>0.05$) on survival of all test strains. However, at 25-32 ppt survival of *O. niloticus* was significantly lower ($P<0.05$) than that of *O. mossambicus* and the F_1 hybrids.

- **Villegas CT. 1990. The effect of feeding water fleas (*Moina macrocopa* Straus) and rotifers (*Brachionus plicatilis*) on the growth and survival of milkfish (*Chanos chanos*) fry. *Israeli J. Aquacult. -Bamidgeh* 42:10-17.**

The effects of feeding water fleas, *Moina macrocopa* Straus, and rotifers, *Brachionus plicatilis,* to milkfish, *Chanos chanos* Forsskal, fry at different feeding rates and frequencies were investigated. Fry (4.66 mg average weight and 13.45 mm standard length) were stocked in 20 1 round plexiglass tanks at l00 per tank in a completely randomized design with four replicates per treatment.

Results indicated beneficial effects of feeding *M. macrocopa* to



Population growth of Brachionus plicatilis fed three selected algal diets. Plotted points, means (n=4); vertical bars, standard deviation (After Villegas et at, Aqua. Fish. Mgt. 21:216. 1990)

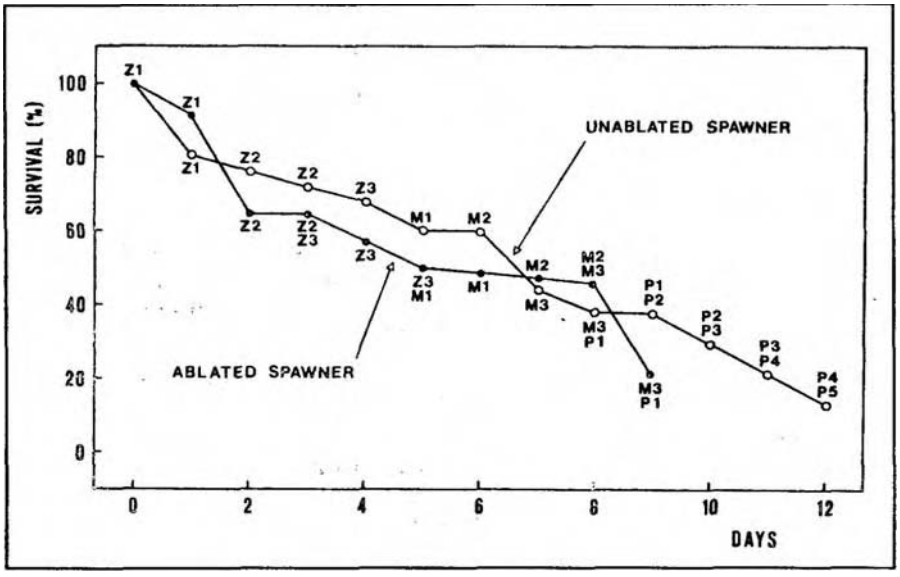
milkfish fry. Fry fed *M. macrocopa* reached a mean individual final weight, daily gain in weight and yields which were significantly higher (P<0.05) than fry fed *B. plicatilis.* Feeding the fry with *M. macrocopa* at the rate of 60 individuals per ml resulted in fish that were 3 to 4 times bigger than fry fed *B. plicatilis.* Feeding *M. macrocopa* to fry did not significantly increase survival rates.

• **Villegas CT, Millamena OM, Escritor F. 1990. Food value of *Brachionus plicatilis* fed three selected algal species as live food for milkfish, *Chanos chanos* Forsskal, fry production. *Aquacult. Fish. Mgt.* 21:213-219.**

The effects of three selected algal species, *Tetraselmis tetrahele, Isochrysis galbana* and marine *Chlorella* sp. on the population growth of *Brachionus plicatilis* was evaluated after 3, 5 and 7 days of culture. The rotifers fed on *T. tetrahele* showed superior growth with mean peak density of 92.5 individuals per ml to those fed on /. *galbana* (48.2 individuals per ml) and *Chlorella* sp. (47.2 individuals per ml) in 5 days.

In another experiment, milkfish, *Chanos chanos* Forsskal, fry with initial mean body weight and standard length of 6.26 ± 1.07 mg and 13.04 ± 1.40 mm, respectively, were fed *B. plicatilis* reared on the three algal species for 30 days. Results showed that growth, measured in terms of gain in weight and length, was significantly different (P<0.05) among treatments. Fastest growth was obtained in fry fed *B. plicatilis* cultured on *T. tetrahele* with mean gains in weight and length of 61.48 ± 8.40 mg and 9.37 ± 1.68 mm, respectively, followed by fry fed *B. plicatilis* reared on *J. galbana* (43.56±8.35 mg and 6.25±0.48 mm). Milkfish fry fed *B. plicatilis* cultured on *Chlorella* sp. gave the poorest growth with mean gains in weight and length of 11.05 ± 1.82 mg and 2.33±0.29 mm. respectively. Percentage survival ranged from 93.1 to 95.3 percent and was not significantly different between treatments (P>0.05).

The differences in population growth of *B. plicatilis* and consequently growth of milkfish fry was almost certainly attributed to the differences observed in the biological values of their algal diet,



Comparison of larval mortality from unablated and ablated spawners across stages (zoea 1 to postlarva 5). Larvae from ablated female were observed only until day 9 (Z, zoea: M, mysis; P, postlarva) (After Vogt et al., Aquaculture - A Biotechnology in Progress. 567. 1989)

and in particular their protein and lipid contents. *T. tetrahele* and J. *galbana* improved the food value of *B. plicatilis* as live food for the milkfish fry. Their high dietary value was related to the higher algal protein and fat levels and contents of n-3 HUFA particularly 20:5n-3 and 22:6n-3 compared to *B. plicatilis* cultured on *Chlorella* sp. The results of the present study point to T. *tetrahele* as the most suitable food for the mass culture of the rotifer, *B. plicatilis.*

- **Vogt G. Quinitio ET, Pascual FP. 1989. Interaction of the midgut gland and the ovary in vitellogenesis and consequences for the breeding success: a comparison of unablated and ablated spawners of *Penaeus monodon.* De Pauw N, Jaspers E, Ackefors H, Wilkins N, eds. Aquaculture - A Biotechnology in Progress; Bredene, Belgium: European Aquaculture Society; 581-592.**

The midgut glands and ovaries of unablated and ablated females of *Penaeus monodon* were examined before and after spawning by light and electron microscopy to elucidate the role of the midgut gland during vitellogenesis. In addition, the larvae of both were divided into fed and starved groups and the mortalities were recorded up to stage postlarva 5 in order to compare the quality of the offspring from unablated and ablated spawners. Although a limited number of spawners was used in this preliminary study, a close interaction of the midgut gland and the ovary during vitellogenesis is evident. The influence of eyestalk ablation on the breeding success is discussed and hypothetically correlated to endocrinology. In late vitellogenesis, the resorptive cells of the midgut gland of an unablated female exhibited specific ultrastructural characteristics such as whirls of rough endoplasmic reticulum, conspicuous Golgi bodies and highly active smooth endoplasmic reticulum. The ablated female showed these features only in moderate development. After spawning, the "normal" structures of the midgut gland cells were restored to a large extent. In the ovary of the unablated female before spawning, mature oocytes were dominant. After spawning, the ovary of the unablated female contained only immature oocytes. In contrast, all stages of maturation could be found in the ablated female 2 h after spawning. The hatching rate was much lower in the ablated female. Up to stage postlarva 5, however, fed larvae from the ablated and unablated spawners had the same mortality rates. Starved zoea 1 from both types of spawners could not reach the next moulting stage without feeding. This indicates the urgent necessity of good feed at the very beginning of larval development. When the larvae were fed until moulting to mysis and then starved during mysis stage, the offspring of the ablated spawner died earlier than that of the unablated female. Only a very few starved larvae reached the postlarval stage. In any case, starvation led to an extension of the various larval stages and substages.

- **Wilson JM, Meier AH, Emata AC, Wahba MT. 1989. Ovine growth hormone and prolactin stimulate increases of immunoreactive somatomedin-C in plasma of gulf killifish, *Fundulus grandis. Sci. Res. J.* 1:1-7.**

Injections of ovine growth hormone (oGH) and ovine prolactin (oPRL) stimulated 2- to 4-fold increases in plasma immunoreactive somatomedin-C (ism-C) in gulf killifish, *Fundulus grandis.* However, Opel was more effective. The ism-Ac responses to both hormones were equivalent to those reported in mammals and recently in a teleost, the gilthead seabream. Somatomedins may mediate the activities of growth hormone and prolactin in teleosts.

Research seminars

Date	Title	Speaker
8 Feb.	Japanese scallop culture and benthos community in scallop beds	S. Nakao*
15 Feb.	Studies on the energy metabolism of milkfish during starvation and growth	K. Schroeder**
22 Feb.	Role of aquaculture in the degradation and rehabilitation of the coastal environment	J.H. Primavera
8 Mar.	Agar yield and gel strength of three species of *Gracilaria* from Panay and Guimaras Islands	M.T.R. de Castro
22 Mar.	Alternate sources of protein for fishmeal in *Lates calcarifer* diets	V.R. Alava
29 Mar.	Variations in plasma somatomedin-C and thyroid hormone responses in the gulf killifish, *Fundulus grandis*	A.C. Emata
24 Apr.	Marine Ranching Project in Japan	J. Tsukidate*
10 May	Ion chromatography of inorganic anions in brine samples	P.F. Subosa
31 May	Development of a milkfish practical diet	F.P. Pascual
7 June	Effect of light quality on *Penaeus monodon* maturation and survival	J.H. Primavera
28 June	Occurrence of *Vibrio* infection in groupers, *Epinephelus* spp.	C.L. Pitogo
12 July	Growth and survival of *Penaeus monodon* juveniles fed diet without vitamin supplement in a modified extensive culture system	A.T. Trino
19 July	Effect of dehulling on digestibility of various leguminous seeds as protein sources for tiger shrimp juveniles	P.S. Eusebio
2 Aug.	Artificial diet for larval rabbitfish, *Siganus guttatus* Bloch	M.M. Parazo
9 Aug.	Modular method of rearing milkfish with artificial feeds	N.S. Sumagaysay
16 Aug.	Effect of supplemental feeding on the growth, survival, and production of milkfish with *lablab* as natural food base	N.B. Solis

Research seminars

Date	Title	Speaker
23 Aug.	Culture of *Artemia, Moina,* and *Euplotes* with microbes	I.J. Dogma, Jr.
30 Aug.	Freshwater cladoceran *Moina macrocopa* as an alternative live food for sea bass fry	A.C. Fermin
6 Sept.	Spawning response latency and egg production capacity of LHRHa-injected mature female sea bass	L.Ma.B. Garcia
20 Sept.	Preliminary study on extensive pond culture of *Penaeus monodon* with various natural food	LB. Tuburan
26 Sept.	Research needs of the shrimp farming industry	P.F.S. Cruz*
27 Sept.	Essential fatty acid requirements of milkfish	I.G. Borlongan
4 Oct.	Physico-biological assessment of five candidate sites on Panay for a pilot seafarming and searanching center	R.M. Cheong
	Social and economic considerations in the site selection of a pilot seafarming and searanching center	R.F. Agbayani
18 Oct.	Mollusc research and development at SEAFDEC/AQD	W.G. Gallardo
25 Oct.	Seaweed culture in Panay - farming practices	A.Q.H. Ponce
	Seaweed culture in Panay - production, economics, and marketing	G.P.B. Samonte
7 Nov.	Metabolism of L-cysteine in mammalian cells	R.M. Coloso
8 Nov.	*Aeromonas hydrophila* in the epizootic ulcerative syndrome (EUS) of snakehead (*Ophicephalus striatus*) and catfish (*Clarias batrachus*): quantitative estimation in natural infection and experimental induction of dermonecrotic lesion	G.D. Lio-Po
5 Dec.	Studies on the reproductive physiology of milkfish (*Chanos chanos* Forsskal)	C.L. Marte
6 Dec.	Acute toxicity of mercury to *Oreochromis niloticus* fingerlings	M.L. Cuvin-Aralar

*Guest. **Visiting scientist.

TRAINING

In pursuit of the mandate to develop human resources for aquaculture development in the region, AQD continued to offer national and international training programs on various aspects of aquaculture.

Conducted during the year were the following:

Training courses

Fish Health Management

In attendance were 17 participants from 4 countries: Malaysia (2), Thailand (3), Philippines (11), and Sri Lanka (1). Six participants received SEAFDEC fellowship grants. The training course was conducted from 21 Feb. to 27 Mar. at Tigbauan Main Station.

The five-week course covered topics on the occurrence and spread of diseases in aquaculture operations, microbial diseases, non-infectious diseases, and disease prevention and control.

Participants from different countries come to AQD to train in various aspects of aquaculture.

Aquaculture Management

This was attended by 13 participants from 4 countries: Malaysia (2), Thailand (2), Philippines (8), and Sri Lanka (1). Nine participants received SEAFDEC fellowship grants. The training course was conducted from 18 Apr. to 23 May at Tigbauan Main Station.

The five-week course aimed to develop the skills of project managers in the areas of aquaculture planning and implementation, monitoring, and evaluation. The course was designed for middle to top level government planners, executives, bankers, aquaculturists, and decision makers. It covered technical topics in seed production of finfishes and crustaceans, grow-out techniques, nutrition, and fish diseases. The management module covered topics in production, marketing, financing, business, and government policies.

Hatchery/Nursery of Marine Finfishes

The training course was conducted from 13 June to 1 Aug. at Tigbauan Main Station. Fourteen participants from 3 countries attended the course: Malaysia (2), Thailand (2), and Philippines (10). SEAFDEC provided fellowships to 9 participants from Member Countries, one was sponsored by the Seafarming Research Development Center (Philippines), and 4 were funded by private institutions.

The seven-week course aimed to equip the participants with the basic technical skills in the operation and management of marine finfish hatcheries.

Shrimp Hatchery/Nursery Operation and Management

This seven-week course conducted from 29 Aug. to 18 Oct. had 10 participants from 4 countries: Malaysia (3),

Thailand (2), Philippines (2), and Vietnam (3). SEAFDEC provided fellowships to 7 participants from Member Countries, while 3 participants from Vietnam were funded by the Food and Agriculture Organization (FAO) through the Network of Aquaculture Centres in Asia (NACA).

The course aimed to develop skills in operating a small-scale shrimp hatchery including broodstock and nursery with emphasis on *Penaeus monodon.* Participants conducted larval rearing runs in small-scale tanks, eyestalk ablation, broodstock sampling, transport of spawners, monitoring of embryonic stages after spawning, natural food production, feeding and water management, and harvesting and packing.

Fish Nutrition

This course was conducted from 3 Oct. to 13 Nov. with 12 participants from 4 countries: Malaysia (2), Thailand (3), Philippines (6), and Vietnam (1). Ten participants received SEAFDEC fellowship grants; 1, sponsored by FAO; 1, funded by a private institution.

The five-week course covered topics on basic nutrition and feeding theories in determining requirement of cultivable species, formulation and preparation of artificial feeds based on feeding habits/behavior and digestive physiology, evaluation of feeds with respect to conversion rates, digestibility, and efficiency.

Fish Health Management training course in session.

Milkfish Hatchery

The Regional Agricultural and Fisheries Council (RAFC)-Region VI through the Department of Agriculture (DA) asked AQD to conduct a training course in *Milkfish Hatchery* in response to requests from shrimp hatchery operators who are also milkfish aquaculturists. Two sessions were conducted: 30 July- 28 Aug. with 9 participants and 27 Aug. to 25 Sept. with 8 participants. These were shrimp hatchery operators/technicians (13) and DA extension workers (4). Participants of the 2 sessions came from Western Visayas (Negros, Capiz, Aklan, and Iloilo Provinces) - Region VI. IDRC provided funding assistance for the conduct of the training.

The one-month course was designed primarily to update shrimp hatchery operators/technicians and DA extension workers on new technologies of milkfish hatchery. It covered the basic concepts of milkfish larval rearing and production of natural food organisms required by larvae and fry.

Practicum Training

Forty-four graduating students from Philippine fisheries schools and universities availed themselves of the program.

Internship Training

Seventeen trainees underwent training in phycology, pathology, microtechnique, physiology, larval rearing, and chemical analyses.

Summer Youth Training Program

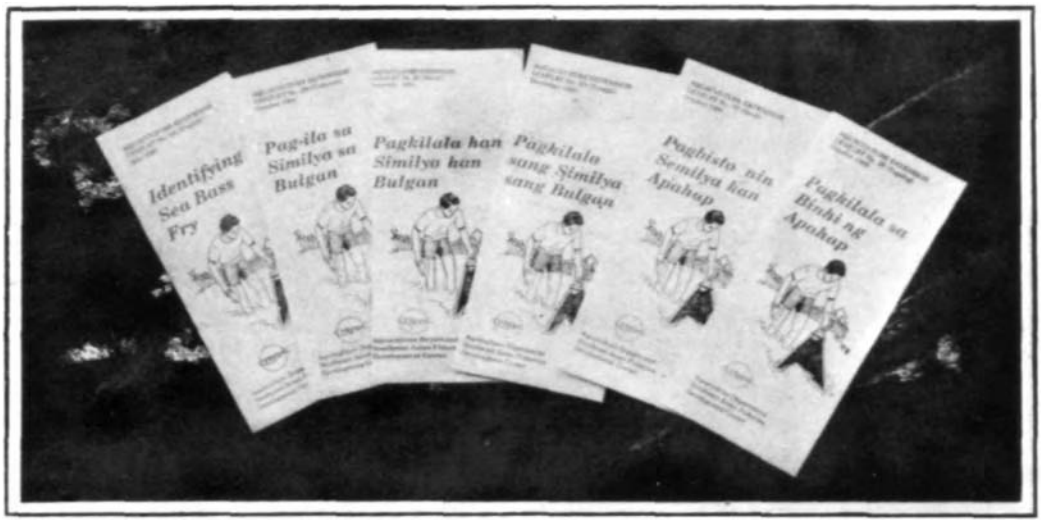
A total of 51 students and out-of-school youths from the vicinity of AQD research stations participated in the program from 1 Apr. to 1 June.

Other training programs

INFORMATION

The year witnessed peak production of manuals, pamphlets, leaflets, and other extension materials. Acquisition and processing of information materials continued through the Library and Documentation Services.

Publications



AQD gives out free, highly informative how-to leaflets on fry identification, spawner or broodstock collection and handling, etc. in the language the small fishfarmers understand.

Publications issued by AQD during the year were the following:

Proceedings

Culture and Use of Algae in Southeast Asia. Proceedings of the Symposium on Culture and Utilization of Algae in Southeast Asia. Tigbauan, Iloilo, Philippines, 8-11 December 1981

Aquaculture extension manuals

- *Diseases of Penaeid Shrimps in the Philippines,* AEM No. 16
- *Milkfish Hatchery Operations,* AEM No. 17
- *Sea Bass Hatchery Operations,* AEM No. 18

Monograph

- *Biology and Culture of Siganids*

Poster

- *Anatomy of Adult Penaeid Prawns/ Shrimps* , No. 3.

Newsletters

- *SEAFDEC Asian Aquaculture* (quarterly; Vol. XII, 1990)
  - No. 1, Mar.
  - No. 2, June
  - No. 3, Sept.
- *Aqua Farm News* (bimonthly; Vol. VIII, 1990)
  - No. 1, Jan.-Feb.
  - No. 2, Mar.-Apr.
  - No. 3, May-June
  - No. 4, July-Aug.
  - No. 5, Sept.-Oct.

Aquaculture extension leaflets

- *Identifying Siganid Fry* AEL No. 1A (English)

No. 1B (Cebuano)
No. 1C (Waray)
No. 1D (Ilonggo)
No. 1E (Bikol)
No. 1F (Tagalog)
No. 1G (Ilokano)

Identifying Sea Bass Fry
No. 2A (English)
No. 2B (Cebuano)
No. 2C (Waray)
No. 2D (Ilonggo)
No. 2E (Bikol)
No. 2F (Tagalog)
No. 2G (Ilokano)

Internal newssheet

- *Aqua Dep't News* (fortnightly; Vol. V, 1990)
  Twenty-four issues

Other publications

- *Mud Crab Abstracts*
- *AQD Annual Report 1988*
- *AQD Annual Report 1989*
- *AQD Information Brochure*
- *AQD Aquaculture Training Program* (brochure)
- **Press releases.** - Thirteen were submitted to different media outlets.
- **Job requests.** - A total of 125 were accommodated covering the following: printing, binding, reproduction, photo and video coverage, film processing, graphics, public address system, movie, slide and overhead projectors, stage props and design, and exhibits.

Library/Documentation Services

Accessioned during the period were 474 monographic volumes, 171 pamphlets, and 166 SEAFDEC publications. Checklisted were 1708journal issues. Received as Gifts and Exchanges were 1301 journal issues, 357 monographs, 216 reprints, 8 theses, 12 pamphlets, and 41 brochures and posters.

The *SEAFDEC/AQD Library has one of the largest collection of aquaculture literature in Asia.*

The present collection stands at 9700 monographic volumes, 4897 pamphlets, 2114 SEAFDEC publications, and 3471 journal volumes.

A total of 11 891 readers (students, researchers, faculty members, practitioners, trainees) were recorded for 1939 hours of library services, making an average of 6 readers/h. Six thousand one hundred four materials were borrowed, averaging 25 materials/day for 238 days of library service.

The Library catalogued and classified 538 volumes of books and pamphlets. One hundred fifty-six queries from 55 countries were received on literatures of specific topics, titles of material, and queries requiring specific answers. The queries came mostly from researchers and students.

SEAFIS project

A second partial list containing 266 bibliographical citations of Philippine fisheries literature was sent to SEAFDEC Secretariat in January, bringing a total of 1320 records sent for the SEAFIS Project. Total number of records in the SEAFIS data base as of June was 3556.

The Library continued to publish the monthly Library Acquisition List and to send out photocopied table of contents of new journals through its *Library Awareness Bulletin.*

Technology generated from research gets widely disseminated through participations in fairs and exhibits. DA Secretary Senen Bacani (left in top photo) visits AQD booth in the Tacloban Agri-Food Fair.

Extension

Extension activities were carried out in coordination with the host country's Department of Agriculture which sponsors fairs and exhibits. AQD participated with a booth alongside exhibits of agricultural products and technology generated by the government and the private sector from all over the Philippines.

AQD participated in five fairs and exhibits: (1) *Tigbauan Fair on* the occasion of the Tigbauan Town Fiesta, 19-21 Jan.; (2) *Agri-Food Fair* in Tacloban City, 21-25 Mar.; (3) *Agri-Food '90* (International Exhibition) at the Folk Arts Theater, Manila, 22-26 May; (4) *Agri-Food Naga '90,* 28 Apr. -2 May where AQD also gave a seminar on aquaculture; and (5) *Book Fair and Exhibits,* Philcite, CCP Complex, Metro Manila, 21-29 July.

The AQD booth displayed and sold publications and video tapes and featured continuous video showing of research activities. The booth also served as an "Aquaculture Clinic" where AQD researchers gave advice on aquaculture problems presented by the visiting public.

Visitors

AQD received 3650 visitors comprising students and teachers (94%); government workers, fish farmers, and the general public (4%); foreign visitors and dignitaries (1%). Visitors were oriented on AQD's research and development efforts.

ADMINISTRATION

Pursuant to the revised *Plan of Operation* and *Program of Work of AQD* as approved by the SEAFDEC Council at its 22nd Meeting in Singapore in November 1989, the new organizational structure of AQD was adopted. The new structure comprises the Office of the Department Chief and 4 divisions - Research, Training and Information, Administration, and Finance.

Personnel

As of 31 December, the permanent staff of AQD totalled 375, with 166 in Research, 37 in Training and Information, 126 in Administration, 25 in Finance, and 21 in the Office of the Chief.

AQD also availed itself of the services of foreign personnel and local consultants: S. Fukumoto, Deputy Chief; Dr. T. Kume, Dr. J. Tsukidate, Mr. A Nagai, JICA Experts; K. Schroeder. Exchange Scientist from the University of Hohenheim, Germany; Prof. N. Rossel, Consultant (Mollusc Project); Dr. G.Trono, Jr., Consultant (Seaweeds Project); R. Gotera. Editorial Consultant; Atty. R. Encarnacion and Atty. H. Teodosio, Legal Counsels/Consultants; Atty. E. Rondain, Legal and Personnel Consultant; and Dr. R. Certeza and Dr. E. Tubungan, Retainer Physicians; Dr. T. Hilomen, Dr. M. Espinosa, Jr., and Dr. P. Calingo, Dentists.

Four fixed-term employees were given permanent appointments; 6, hired on a fixed-term basis; 38, reappointed for fixed terms. Two retired upon reaching the age of 60; 9, resigned; 1, deceased.

Deputy Chief Satoru Fukumoto is one of the expatriates boosting the foreign personnel component.

Policy on study leave

The policy on study leave was revised, particularly on the return service: for study leave with pay, return service is 2 years for every year of study; and for study leave without pay, one-half year.

Accountability of study leaders

In reference to Section 16.4 of the Administrative Regulations of SEAFDEC, study leaders are held accountable for the budget of their research studies. Clearance can only be issued to study leaders upon submission of either a copy of scientific publication, an acceptable manuscript, or a terminal report with raw data from the research study.

Staff development and activities

Human resource development was a continuing management concern. Personnel who benefited from the staff development program are as follows:

Degree program

R. Coloso completed his Ph.D. in Nutritional Biochemistry from Cornell University, U.S.A. through a Fulbright-Hays scholarship grant.

A. Emata completed his Ph.D. in Physiology from Louisiana State University, U.S.A. through a Fulbright-Hays scholarship grant.

C. Marte completed her Ph.D. in Fish Reproductive Physiology from the National University of Singapore through IDRC fellowship grant.

J. Toledo completed an M.Sc. degree (Fisheries) from Hiroshima University through Monbusho scholarship grant of the Government of Japan.

T. Bagarinao is pursuing Ph.D. program in Marine Biology at the University of California-La Jolla under Fulbright-Hays scholarship grant.

S. Baldia is pursuing Ph.D. degree at the United Graduate School of Ehime University through Monbusho scholarship grant of the Government of Japan.

Z. Basiao is pursuing Ph.D. in Fish Genetics at Dalhousie University, Halifax, Canada through an IDRC fellowship grant.

R. Fernandez is pursuing Ph.D. in Fish Diseases (Fish Virology) at Hokkaido University through Monbusho scholarship grant of the Government of Japan.

A. Gallego is pursuing Ph.D. in Microbiology at the Universidad de Alcala de Henares, Spain through a university grant.

E.C. Lacierda is pursuing Ph.D. in Tropical Fish Health at the Faculty of Fisheries and Marine Science, Universiti Pertanian Malaysia through an IDRC fellowship grant.

G. Lio-Po is pursuing Ph.D. in Biological Sciences at Simon Fraser University, Canada through an IDRC fellowship grant.

V. Alava is pursuing Ph.D. program in Nutritional Chemistry at Kagoshima University through Monbusho scholarship grant of the Government of Japan.

J. **Almendras** is pursuing Ph.D. degree program at Rennes University, France through fellowship grant from the Government of France.

J. Primavera is pursuing Ph.D. degree program in Marine Biology at UP Marine Science Institute, Quezon City, through fellowship grant from the Philippine Council for Aquatic and Marine Research and Development.

E. Quinitio is pursuing Ph.D. degree program in Fisheries at Hokkaido University under the Japan Society for the Promotion of Science (JSPS) Ronpaku Program.

D. Chavez and **R. Gapasin** are pursuing M.Sc. degree programs in Marine Ecology at Vrije Universiteit, Brussels, Belgium through fellowship grant from the *Artemia* Reference Center of Belgium.

G. Miñoso is pursuing M.Sc. degree in Environmental Sanitation at the State University of Ghent, Belgium through fellowship grant from the *Artemia* Reference Center of Belgium.

Non-degree program

E. Lacierda, C. Pitogo, and **F. Palisoc** - Biostatistics Course, University Sains Malaysia, Penang, Malaysia through IDRC grant, 20 Apr.-19 May

E. Rodriguez and **J. Sarroza** - 10th Integrated Fish Farming Course, Asian - Pacific Regional Research and Training Center, Jiangsu Province, People's Republic of China through FAO/UNDP grant,

19 Apr.-18 Aug.

M.C. Baticados - 3-month advanced research study in the diagnosis and control of fish diseases, University of Arizona, Tucson, U.S.A. under the Senior Scholar Fulbright Program

Z. Basiao - Workshop on Design of Biological Experiments. Dalhousie University, Halifax, Canada through IDRC grant, 6-8 June

R. Eguia - Training Course in Aquaculture Genetics, Bamfield Research Station, British Columbia. Canada through IDRC grant, 13-31 Aug.

F. Ayson - Training on RIA of Thyroid Hormones, National University of Singapore, through IDRC grant, 10 Oct.-10 Dec.

E. Aralar and **R. Travina** - Fish Health Management Training Course, SEAFDEC/AQD, Tigbauan, Iloilo, 21 Feb. - 27 Mar.

R. Alger, N. Ebron, and **E. Natividad** - Management Accounting Seminar, Iloilo City, 2-4 Mar.

M. Castaños - Course in Scriptwriting, Philippine Human Resources Development Center (PHRDC), Metro Manila, 5-16 Mar.

Thirty SEAFDEC/AQD supervisors attended the Basic Supervisory Course conducted by the University of the Philippines in the Visayas/School of Development Management, SEAFDEC/AQD, Tigbauan, Iloilo, 22-25 Mar.

R. Lacierda - Course in Curriculum Design and Lesson Planning, PHRDC, Metro Manila, 26-28 Mar.

A. Arisola - Seminar-Workshop on Improved Librarianship and Library Services for the 1990's, Iloilo City, 18-20 Apr.

C. Animas - PageMaker Seminar, DynalinkServices, Inc., Iloilo City, 7-11 May

R. Tumaliuan - DBase Computer Programming Training Course, Dynalink Services, Inc., Iloilo City, 7 May- 6 June

J. Lagoc, L. Plondaya, J. Requintina, M. Surtida - PageMaker Seminar, Dynalink Services, Inc., Iloilo City, 28 May-1 June

K. Corre - Basic Trainors' Course, University of Life, Pasig, Metro Manila, 18-29 June

Samonte - DA-BFAR Training Program on Design and Analysis of Socioeconomic Research, San Pablo City, Laguna, 5-23 Nov.

Bombeo - DA-BFAR Training Program on Research Design, Analysis, and Technical Writing, UP Los Baños, Laguna, 12-29 Nov.

Attendance in international workshops and conferences

F.J. Lacanilao - SEAFDEC Technical Working Group Meeting, Bangkok, Thailand, 16-19 Jan.; Asian Fisheries Society Council Meeting, Singapore, 17-21 Apr.; World Aquaculture Society 13th Meeting, Halifax, Canada, 10-16 June; 13th Meeting of the SEAFDEC Program Committee. Bangkok, Thailand, 14-17 Aug.; and 23rd Meeting of the SEAFDEC Council, Bangkok, Thailand, 11-14 Dec.

Z. Basiao - World Aquaculture Society Meeting, Halifax, Canada, 10-16 June

G. Po - Bacterial Diseases of Fish Conference, Scotland, 26-29 June

M.L. Aralar - Symposium on Inland Aquatic Environmental Stress Monitoring, SEAMEO-BIOTROP, Bogor, Indonesia, 25-27 July

F. Palisoc, Jr. - Regional Workshop on the Relationship of Epizootic Ulcerative Disease Syndrome to the Environment, Bangkok, Thailand, 13-25 Aug.

S. Fukumoto - 13th Meeting of the SEAFDEC Program Committee, 14-17 Aug.; 23rd Meeting of the SEAFDEC Council, 11-14 Dec.

C. Santiago, F. Pascual, M. Parazo, and **N. Sumagaysay** - 4th Fish Nutrition Workshop, Vijayawada, India. 3-7 Sept.

A. Santiago - 4th International Congress on Conservation and Management of Lake, Hangshou, China, 5-9 Sept.

C. Pitogo - Workshop on Fish Disease and Fish Health Management, Pusan, Korea, 8-15 Oct.

G. Lio-Po, E. Lacierda, and **C. Pitogo**

- 5th Asian Fish Health Network Meeting, Malang, Indonesia, 19-23 Nov.

M.C.L. Baticados, G. Lio-Po, E. Lacierda, C. Pitogo, and **F. Palisoc Jr.** - Symposium on Diseases in Asian Aquaculture, Bali, Indonesia. 26-29 Nov.

Attendance in national seminars, workshops, and conferences

J. Primavera - Seminar-Workshop on the Management of Nearshore Fisheries, Cebu City, 23-25 Jan.; Seminar-Workshop on Marine Ecosystems and the Future, Iloilo City, 20 Mar.; 1st National Symposium in Marine Science, Bolinao, Pangasinan, 16-18 May; 1st Regional Media Seminar/Workshop on Marine Ecosystems, Iloilo City. 21-22 July

F.J. Lacanilao - Seminar-Workshop on Marine Ecosystems and the Future. Iloilo City. 20 Mar.; 1st International Symposium in Marine Science, Bolinao, Pangasinan, 16-18 May; 1st Regional Media Seminar/Workshop on Marine Ecosystems, Iloilo City, 21 -22 July

J. Lagoc - Seminar-Workshop on Marine Ecosystems and the Future. Iloilo City. 20 Mar.; 1st Regional Media Seminar/Workshop on Marine Ecosystems, Iloilo City, 21-22 July

I. Borlongan and **V. Peñaflorida** - 6th Chemistry Congress. Cebu City. 24-26 May

M. Ortega - Annual ISISPHIL General Assembly, U.P. Diliman, Quezon City, 28-29 June

R. Agbayani - 2nd DA-BFAR National Symposium, UP Diliman, Quezon City, 23-24 July

D. Tupas and **L. Torres** - Secretary's Seminar, Bacolod City, 2-3 Aug.

C. Villegas and **R. Romana** - 2nd Training Workshop on Quantitative Genetics on Farmed Tilapias, Central Luzon State University, Nueva Ecija. 20-25 Aug.

A. Ponce and **M.T. de Castro** - Regional Workshop on Seaweed Culture and Utilization, Cebu City, 27 Aug.-1 Sept.

L. Cababasay - Seminar on Effective Computer User Documentation, National Computer Center, Manila, 2-4 Sept.

S.R. Tillo - Orientation Seminar on Technological Breakthroughs in Electro-Mechanical Products, Iloilo City. 7 Sept.

F. Jarder - Seminar/Demonstration on Infratec Auto Analyzer. Metro Manila. 8 Oct.

A. Santiago - 19th Annual Convention of Chemical Society of the Philippines (Los Baños Chapter), Los Baños, Laguna, 24-27 Oct.

G. Quinitio and **J. Toledo** - 1st Philippine-French Technical Workshop on Advances in Finfish and Mariculture, Los Baños, Laguna, 24-28 Oct.

R. Agbayani, G. Samonte, and **S. Siar** - Contributions of Social Anthropology in Fisheries Development, ICLARM, Manila, 23 Oct.

R. Agbayani, G. Samonte. S. Siar, and **R. Tumaliuan** - Seminar/Workshop on Fisheries Economics, ICLARM, Manila, 5-10 Nov.

R. Alger and **R. Agbayani** - Seminar/Workshop on Financial Management in Research (sponsored by SEARCA). UP Los Baños, Laguna, 26-29 Nov.

Like other academic institutions, AQD equates human resource development with institutional growth

Cooperation with non-member governments and other organizations

Government of Belgium

• Staff development

Two fellowship grants for M.Sc. degree program in Marine Ecology at Vrije Universiteit, Brussels

Fellowship grant for an M.Sc. degree program in Environmental Sanitation at the State University of Ghent

Government of France

• Staff development

Fellowship grant for a Ph.D. degree program at Rennes University

International Development Research Centre (IDRC) of Canada

• Research projects

(1) Growth, feed utilization, and body composition of young red tilapia given diets with varying protein and energy levels

(2) Genetic evaluation and selective breeding of *Oreochromis niloticus* for broodstock development

(a) Development of a high-yield red tilapia strain through introgressive hybridization

(b) Development of genetic evaluation and selection criteria for tilapia broodstock: IV. Effect of restrictive and non-restrictive feeding on growth of *Oreochromis niloticus* strains

(c) Performance evaluation of two *Oreochromis niloticus* strain in two lake environments

(d) Practical methodology for genetic strain evaluation of tilapia in small- to medium-sized experimental facilities

(3) Seafarming in the Philippines (1989-1990)

(a) Evaluation of credit needs and its availability in five selected fishing villages in Panay

(b) Factors affecting the acceptability of Territorial Use Rights in Fisheries (TURFs) among fisherfolks in five fishing villages in Panay Island

(c) Social and economic considerations in the implementation of an integrated seafarming program

(d) An assessment of the marine communities of five candidate sites in Panay for a pilot seafarming project

(4) Fish Microbiology (1989-1992; Also in collaboration with Simon Fraser University, Burnaby, B.C., Canada)

(a) Investigations on the vertical and horizontal transfer mechanisms of the luminescent bacterium, *Vibrio harveyi*

(b) Histopathology of epizootic ulcerative syndrome in some freshwater fishes of Laguna de Bay, Philippines

(c) Viral and bacterial etiology of the epizootic ulcerative syndrome (EUS)

• Staff development

Four Ph.D. grants: 1, National University of Singapore; 1, Dalhousie University, Halifax, Canada; 1, Simon Fraser University, British Columbia, Canada; 1 .Universiti Pertanian Malaysia

Attendance of AQD research staff to workshops/symposia: *Biostatistics Course* at Universiti Sains Malaysia, 20 Apr.-19 May (3 researchers); *Workshop on Design of Biological Experiments* at Dalhousie University, Halifax, Canada, 6-8 June (1); training course in *Aquaculture Genetics* at Bamfield Research Station, British Columbia, Canada, 13-31 Aug. (1); *2nd Training Workshop on Quantitative Genetics of Farmed Tilapias,* Central Luzon State University, Nueva Ecija, 20-25 Aug. (2); *4thNutrition Workshop,* Vijayawada, India, 3-7 Sept. (4); training on *RIA of Thyroid Hormones* at the National University of Singapore, 10 Oct.-10 Dec. (1); *5th Asian Fish Health Network Meeting,* Malang, Indonesia, 19-23 Nov. (3); *Symposium on Diseases in Asian Aquaculture,* Bali, Indonesia, 26-29 Nov. (5).

• **Training**

Financial assistance for the conduct of *Milkfish Hatchery Training Program* . Two sessions were conducted (30 July-28 Aug.; 27 Aug.-25 Sept.) with a total of 17 participants.

International Foundation for Science (IFS)

• **Research**

(1) Effect of temperature and ration size on growth and energy utilization of sea bass (1988-1991)

(a) Food consumption of sea bass (*Lates calcarifer*) in captivity: II. Group feeding

(2) Quality assessment of shrimp feeds and feed ingredients: thiobarbituric acid value, aflatoxin level and urease activity, and the biological effects on tiger shrimp

(3) Prevention of *Aeromonas hydrophila* infection among *Chanos chanos* (Forsskal) by vaccination

• **Staff development**

Attendance of a researcher in the *Bacterial Diseases of Fish Conference,* Scotland, 26-29 June

Network of Aquaculture Centres in Asia

• **Training**

Training of 3 Vietnamese fishery officials in *Shrimp Hatchery/Nursery Operations and Management* at Tigbauan, 29 Aug.- 18 Oct.

University of Hohenheim, Germany

• **Research**

(1) Energy metabolism of milkfish *Chanos chanos* during starvation and growth (1990-1991)

(a) Determination of the maintenance rate of milkfish of varying body size at different rearing temperatures

(b) Measurement of the critical oxygen tension of milkfish at different rearing temperature and for different body mass groups

(c) Studies on the oxygen consumption of milkfish with different body mass and at different rearing temperatures

(2) *In vitro* determination of protein digestibility in milkfish

International Center for Living Aquati Resources Management (ICLARM)

• **Staff development**

Attendance of AQD research staff to a one-day lecture on "Contributions of Social Anthropology in Fisheries Development," ICLARM, Manila, 23 Oct. (3 researchers); *Seminar/Workshop on Fisheries Economics,* ICLARM, Manila, 5-10 Nov. (4)

U.P. in the Visayas

• **Research**

(1) Larval rearing of white shrimps (*Penaeus indicus/P. merguiensis*): water management techniques

(2) Algal food preference and effect on growth of *Placuna placenta* larvae and spat

Department of Science and Technology

• **Research**

Luminescent bacteria and their bacteriophages from coastal waters

Bureau of Agricultural Research - Department of Agriculture

• **Staff development**

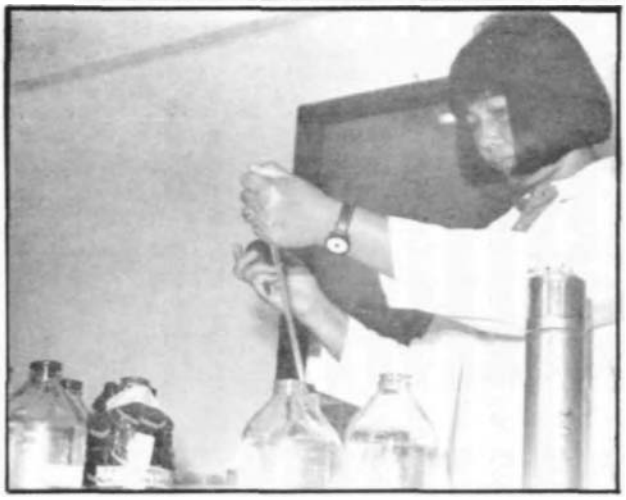
Attendance of AQD research staff to a DA-BFAR training program on "Research Design, Analysis, and Technical Writing," Los Baños, Laguna, 12-29 Nov. (1 researcher); DA-BFAR training program on "Design and Analysis of Socioeconomic Research," San Pablo City, Laguna, 5-23 Nov. (1)

Philippine Council for Aquatic and Marine Research and Development

• Staff development

Attendance of AQD research staff to the *1st Philippine-French Technical Workshop on Advances in Finfish and Mariculture,* Los Baños, Laguna, 24-27 Oct. (1 researcher); scholarship grant award for a Ph.D. program in Marine Biology at U.P. Marine Science Institute, Quezon City, beginning 1 Nov. (1)

The Centralized Analytical Laboratory accommodates feed samples for analysis from the private sector aside from AQD's own research studies.



The capability to mass produce natural food organisms in an artificial environment matches AQD's strides in hatchery technology.

Facilities

Service laboratories

Larval Food Laboratory. *A* total of 9124 1 of different phytoplankton starters and 8 sets of different enriched media stock solutions were serviced to research studies and training courses conducted at AQD. Starter cultures of various phytoplankton and zooplankton in liquid media (463 l) and enriched media stock solutions (17 sets) were prepared for the private sector at minimal costs.

Artemia nauplii (450 million), enriched *Artemia* (200 million), and adult biomass (130 kg) were used in different research studies at AQD. Seventeen *Artemia* cyst samples were also analyzed for hatching efficiency, hatching percentage, and naupliar size analysis.

Centralized Analytical Laboratory. Samples analyzed during the period consisted of feed (799), soil (151), and water (3195). All the feed samples came from AQD's ongoing studies while 57 samples of soil and 95 of water were analyzed for the private sector. Feeds were analyzed for moisture content, crude protein, crude fat, crude fiber, ash, calcium, and phosphorus. Water quality analyses included pH, nitrite-nitrogen, ammonia-nitrogen, phosphate, total alkalinity, dissolved oxygen, total hardness, and turbidity. For soil, the analyses involved pH, organic matter, available phosphorus, potential acidity, lime requirement, available sulfate, and available iron.

Fish Health Laboratory. A total of 321 cases submitted for disease diagnosis consisted of 265 (82.6%) shrimp,

16 (4.9%) finfish, and 40 (12.6%) rearing water samples. The bulk of the cases came from the private sector (90%) while the rest came from AQD studies (10%).

Microtechnique Service Laboratory. A total of 4727 samples consisting of liver, gills, skin, kidney, muscles, hepatopancreas, and gonads from different species of fish and crustaceans were processed. Samples of seaweeds, *Placuna placenta* and *P. sella* gonads, and larval stages of *Penaeus monodon* were also processed histologically. A total of 8497 typical and serial section slides were released to the respective study leaders for analysis.

Closure of Leganes Brackishwater Station

With the redirection of R & D efforts to seafarming, the Leganes Brackishwater Station was closed in May. The experimental ponds and buildings were turned over to the Municipality of Leganes, in accordance with the Memorandum of Lease Agreement.

Infrastructure development

Completed in Tigbauan Main Station were the (1) renovation of the Wet Lab Building; (2) construction of the access way for disabled persons at the Administration, Research, and Library Buildings; (3) installation of 2 new deepwell pump units (20 hp); (4) installation of 2 FEX Philippine Long Distance Telephone lines; (5) renovation of the Feed Laboratory Building; (6) repair of the Barangay Crustacean Hatchery; (7) repair of 3 units of 75 KVA distribution transformer; (8) renovation of 250 KVA generator house; (9) repair and repainting of the Executive and Guest Houses. Repair of the station house at the Igang Marine Substation was undertaken. Repairs were also started on other facilities destroyed by the November typhoon (*Ruping*).

Two major infrastructure projects funded by the government of Japan were undertaken: the pilot feedmill plant which was completed in December and the one-module seawater supply system, expected to be completed in early 1991.

Funding

Contributions, grants, and other receipts received by AQD for the year are as follows:

• Contributions	
Philippine Gov't	$2,390,438
Gov't of Japan	421,139
• Grants	
IDRC	89,601
IFS	7,748
University of Hohenheim	5,000
Cultor Limited	2,066
• Other Receipts	129,725
Total	**$3,045,717**

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Santiago AE. 1990. Limnological behavior of Laguna de Bay: Review and evaluation of ecological status. *19th Annual Convention of the Chemical Society of the Philippines;* 16 Oct. 1990; Los Baños, Laguna.

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Senior staff

Management		
Lacanilao, Flor	Ph.D. (Fish Physiology) University of California at Berkeley 1971	Chief
Fukumoto, Satoru	B.S. (Fisheries) Kagoshima College of Fisheries 1949	Deputy Chief
Marte, Clarissa	Ph.D. (Zoology) National University of Singapore 1990	Head, Research Division
Villegas, Cesar	Ph.D. (Plant Breeding) Iowa State University 1970	Head, Training and Information Division
Cuevas, Rufil	B.S. (Agricultural Economics) University of the Philippines at Los Baños, 1970	Head, Administrative Division
Alger, Rene	B.S. (Accounting/Economics) University of Iloilo 1964/1965	Head, Finance Division

Research		
Agbayani, Renato	M.B.A.(Business Management) University of the Philippines, Diliman 1972	Aquaculture economics
Alava, Veronica*	M.S. Fisheries (Aquaculture) University of the Philippines in the Visayas, 1979	Aquaculture nutrition
Almendras, Jesus Manolo	M.S. (Marine Biology) University of the Philippines, Diliman 1982	Fish physiology
Aralar, Ma. Lourdes	M.S. (Biology)/M.S. (Zoology) University of the Philippines, Diliman/ Glasgow University, 1984/1985	Lake ecology
Avila, Enrique	Ph.D. (Biology) University of Heidelberg, 1987	Fish biology
Ayson, Felix	M.S. (Marine Biology) University of the Philippines, Diliman 1987	Fish breeding
Bagarinao, Teodora*	M.S. (Marine Biology) University of California-San Diego 1982	Larval ecology
Baldia, Susana*	M.S. (Biology) University of the Philippines, Diliman 1984	Larval food culture

Basiao, Zubaida	M.S. (Zoology) University of the Philippines, Diliman 1976	Aquaculture genetics
Baticados, Ma. Cecilia	M.S. (Biology)/M. Aquaculture University of the Philippines, Diliman/ University of the Philippines in the Visayas, 1980/1983	Fish health
Bautista, Myrna	M.S. (Food Science) University of the Philippines, Diliman 1980	Aquaculture nutrition
Bombeo, Ruby	M.S. Fisheries (Aquaculture) University of the Philippines in the Visayas, 1983	Larval food culture
Borlongan, Ilda	M.S. (Chemistry) University of the Philippines, Diliman 1982	Aquaculture nutrition
Buensuceso, Robmar	M. Aquaculture University of the Philippines in the Visayas, 1989	Mollusc culture
Castillo, Antonio Jr.	M.S. (Fisheries) Miyazaki University 1988	Fish hatchery
Catacutan, Mae	M.S. Fisheries (Marine Animal Nutrition) Kagoshima University 1982	Aquaculture nutrition
Cheong, Ronald**	M.S. Fisheries Louisiana State University 1986	Seafarming
Coloso, Relicardo	Ph.D. (Nutritional Sciences) Cornell University 1990	Aquaculture nutrition
De Castro, Ma. Teresa	M.S. (Environmental Engineering) University of the Philippines, Diliman 1982	Seaweed culture
De la Pena, Milagros	M.S. (Marine Biology) University of the Philippines, Diliman 1983	Larval food culture
Duray, Marietta	M.S. (Biology) University of San Carlos 1977	Fish hatchery
Emata, Arnil	Ph.D. (Physiology) Louisiana State University 1990	Fish physiology
Estenor, Demetrio	M.S. (Marine Ecology) Vrije Universiteit 1989	Larval food culture

Estepa, Fe Dolores	M.S. Fisheries (Aquaculture) University of the Philippines in the Visayas, 1982	Crustacean hatchery
Eusebio, Perla**	M.S. (Animal Science) University of the Philippines at Los Banos, 1978	Aquaculture nutrition
Fermin, Armando	M.S. (Aquaculture) Central Luzon State University 1985	Fish hatchery
Fermin, Josefa	M.S. (Zoology) University of the Philippines, Diliman 1982	Fish breeding
Fernandez, Roselyn*	M.S. Fisheries (Aquaculture) University of the Philippines in the Visayas, 1983	Fish health
Gallardo, Wenresti**	M. Aquaculture University of the Philippines in the Visayas, 1989	Mollusc culture
Gallego, Amalia*	M.S. Fisheries (Aquaculture) University of the Philippines in the Visayas, 1986	Fish hatchery
Garcia, Luis Ma.	M.S. (Zoology) University of Alberta 1984	Fish physiology
Golez, Nelson	M. Agriculture (Agricultural Chemistry) Kyoto University 1988	Soil chemistry
Gonzal, Angelito	B.S. (Chemical Engineering) Adamson University, 1979	Fish breeding
Javellana, Gilda	M.S. (Zoology) University of the Philippines, Diliman 1985	Crustacean hatchery
Hurtado-Ponce, Anicia**	D. Agriculture (Phycology) Kyoto University 1988	Seaweed culture
Lacanilao, Flor	Ph.D. (Fish Physiology) University of California at Berkeley 1971	Fish physiology
Lacierda, Erlinda	M.S. (Marine Biology) University of the Philippines, Diliman 1981	Fish health
Ladja, Jocelyn	M. Aquaculture University of the Philippines in the Visayas, 1987	Mollusc culture

Lio-Po, Gilda	M. Public Health (Microbiology) University of the Philippines, Diliman 1973	Fish health
Marte, Clarissa	Ph.D. (Zoology) National University of Singapore 1990	Fish physiology
Millamena, Oseni	M. Engineering (Environmental Eng'g) Asian Institute of Technology 1968	Aquaculture nutrition
Palisoc, Fermin Jr.	M.S. (Zoology) University of the Philippines, Diliman 1982	Fish health
Parazo, Monina	M.S. Fisheries (Nutritional Chemistry) Kagoshima University 1987	Aquaculture nutrition
Peñaflorida, Veronica	M. Agriculture (Animal Science) West Visayas State College 1979	Aquaculture nutrition
Pitogo, Celia	M.S. (Marine Biology) University of the Philippines, Diliman 1984	Fish health
Primavera, Jurgenne	M.A (Zoology) Indiana University 1969	Crustacean breeding
Quinitio, Emilia	M.S. Fisheries (Aquaculture) University of the Philippines in the Visayas 1980	Crustacean breeding
Quinitio, Gerald	D. Fisheries Science Hokkaido University 1989	Fish breeding
Rodriguez, Eduard	M.S. Fisheries Science Nagasaki University 1987	Pond culture
Romana, Ma. Rowena	M.S. (Genetics) University of Wales 1985	Aquaculture genetics
Samonte, Giselle**	M. Management University of the Philippines at Los Baños, 1988	Aquaculture economics
Santiago, Alejandro	M.A. (Zoology) Indiana University, 1978	Lake ecology
Santiago, Corazon	Ph.D. (Fish Nutrition) Auburn University 1985	Aquaculture nutrition

Solis, Noel	M.S. (Biology)/M. Aquaculture University of San Carlos/ University of the Philippines in the Visayas, 1976/1983	Ecology and pond culture
Subosa, Precilla	B.S. (Chemical Engineering) Mapua Institute of Technology 1971	Aquaculture nutrition
Sumagaysay, Neila**	M.S. Fisheries (Aquaculture) University of the Philippines in the Visayas, 1988	Pond culture
Tamse, Catherine*	M.S. Fisheries (Aquaculture) University of the Philippines in the Visayas, 1979	Fish health
Toledo, Joebert	M.S. (Fisheries) Hiroshima University 1990	Fish breeding
Toledo, Nieves	M.S. Fisheries Kagoshima University 1988	Crustacean breeding
Trino, Avelino	M.S. Fisheries (Aquaculture) University of the Philippines in the Visayas, 1986	Pond culture
Tuburan, Isidra	M.S. Fisheries (Aquaculture) University of the Philippines in the Visayas, 1980	Pond culture
Ver, Leo Michael*	M.S. (Marine Biology) University of the Philippines, Diliman 1981	Shellfish culture
Villegas, Cesar	Ph.D. Agriculture (Plant Breeding) Iowa State University 1970	Aquaculture genetics

Training and Information

Amar, Edgar	M. Aquaculture University of the Philippines in the Visayas, 1987	Training Officer
Corre, Kaylin	M.S. Fisheries (Aquaculture) University of the Philippines in the Visayas, 1983	Training Officer
Lacierda, Rodrigo	M. Aquaculture University of the Philippines in the Visayas, 1984	Training Officer
Lagoc, Julia	A.B. (English) University of the Philippines, Diliman 1959	Information Associate

Natividad, Teresita	M. Library Science University of the Philippines, Diliman 1987	Information Associate
Ortega, Marubeth	M. Library Science University of the Philippines, Diliman 1986	Librarian
Saliente, Jessica	M. Aquaculture University of the Philippines in the Visayas, 1989	Training Officer
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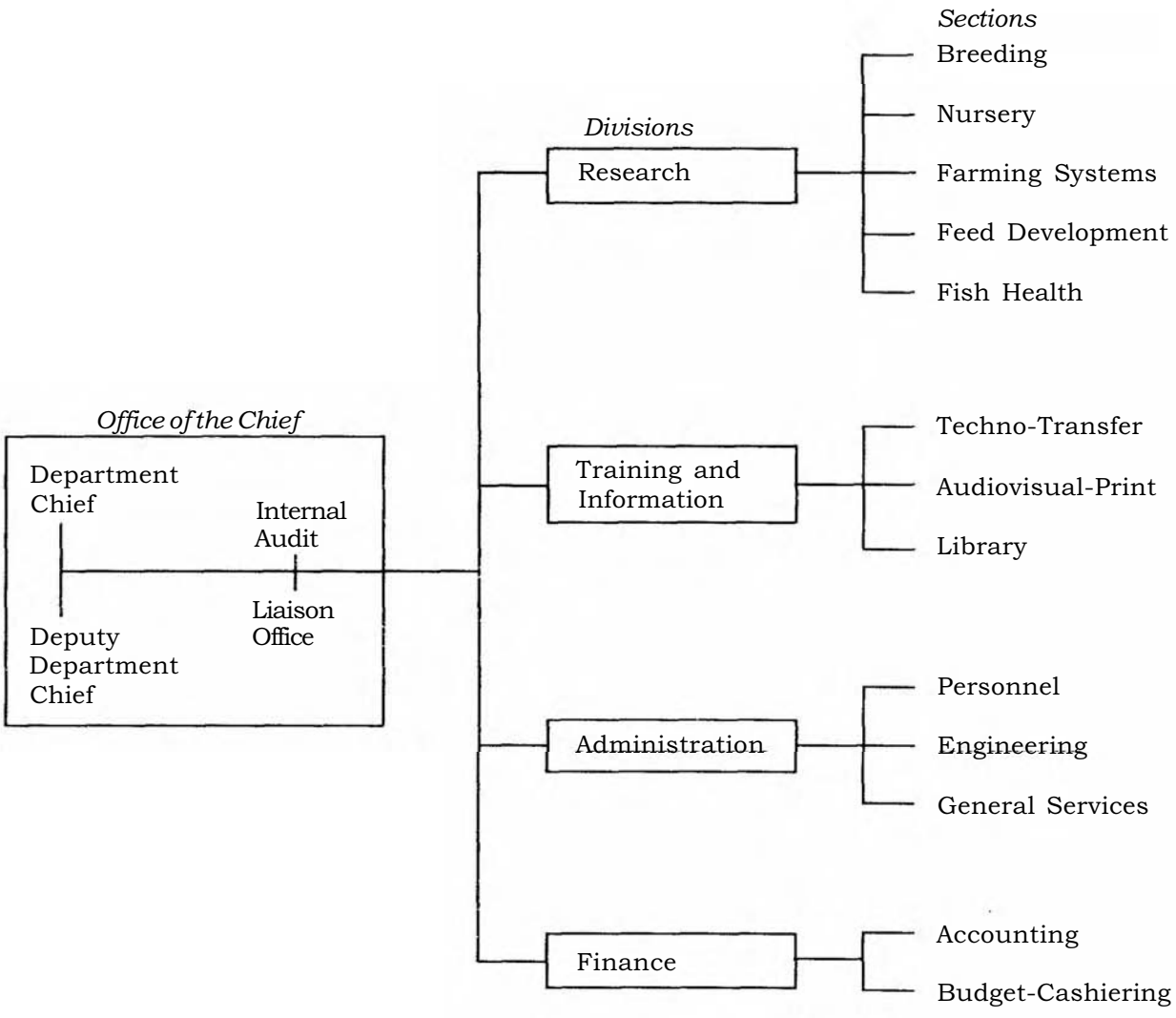
*On leave. **Contractual.

AQD organizational chart



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Aquaculture Department
Southeast Asian Fisheries Development Center
June 1991