PD-AAC-889

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P 5/21

CLASSIFICATION

PROJECT EVALUATION SUMMARY (PES) — PART I

Report Symbol U-447

1. PROJECT TITLE	2. PROJECT NUMBER	3. MISSION/AID/W OFFICE
Artificial Propagation of Milkfish	931-0526	DS/AGR 931-0526 (3) PD-AAC-889-A

4. EVALUATION NUMBER (Enter the number maintained by the reporting unit e.g., Country or AID/W Administrative Code, Fiscal Year, Serial No. beginning with No. 1 each FY) 89p

☐ REGULAR EVALUATION ☒ SPECIAL EVALUATION

5. KEY PROJECT IMPLEMENTATION DATES			6. ESTIMATED PROJECT FUNDING	7. PERIOD COVERED BY EVALUATION
A. First PRO-AG or Equivalent	B. Final Obligation Expected	C. Final Input Delivery	A. Total $ 1,224,000	From (month/yr.) 7/77
FY 75	FY 78	FY 80	B. U.S. $ 1,224,000	To (month/yr.) 9/78
				Date of Evaluation Review 9/22/78

8. ACTION DECISIONS APPROVED BY MISSION OR AID/W OFFICE DIRECTOR

A. List decisions and/or unresolved issues; cite those items needing further study. (NOTE: Mission decisions which anticipate AID/W or regional office action should specify type of document, e.g., airgram, SPAR, PIO, which will present detailed request.)	B. NAME OF OFFICER RESPONSIBLE FOR ACTION	C. DATE ACTION TO BE COMPLETED
1. Team recommended that project be funded for its 5th year with some modification of goals, and a small increase in budget. New work plan completed.	-	9/28/78
2. RAC, after review of team report recommended further reduction in scope of work and reduction of budget. Revised scope of work completed.	Neal DS/AGR/F	11/15/78
Revised PIO/T submitted	Neal DS/AGR	11/24/78
3. Amended contract for modified scope of work completed.	CM/COD	12/10/78

9. INVENTORY OF DOCUMENTS TO BE REVISED PER ABOVE DECISIONS

- ☐ Project Paper
- ☐ Financial Plan
- ☐ Logical Framework
- ☐ Project Agreement
- ☐ Implementation Plan e.g., CPI Network
- ☐ PIO/T
- ☐ PIO/C
- ☐ PIO/P
- ☐ Other (Specify) _____
- ☐ Other (Specify) _____

10. ALTERNATIVE DECISIONS ON FUTURE OF PROJECT

- A. ☐ Continue Project Without Change
- B. ☐ Change Project Design and/or
  ☒ Change Implementation Plan
- C. ☐ Discontinue Project

11. PROJECT OFFICER AND HOST COUNTRY OR OTHER RANKING PARTICIPANTS AS APPROPRIATE (Names and Titles)

Richard A. Neal DS/PO/RES

Richard A. Neal DS/AGR/F DS/AGR, Dean Peterson

SEEN BY P.G, DS/PO DS/AGR, J. WILSON

12. Mission/AID/W Office Director/Approver

Signature

Typed Name DAA/DS/FN, Tony Babb

Date 5/9/79

Background: Because of successful work of this nature completed by the Oceanic Institute with a similar marine species, the gray mullet, the initial research plan was broad, including research peripheral to the central problem of spawning inducement but important to milkfish culture in the long run. As research progressed it became obvious this species would be more difficult to spawn in captivity, and the research approach was modified to include rearing of greater numbers of captive experimental animals. The research has been narrowed in scope in response to past reviews. On a year-to-year basis planned research has been accomplished, and while the results of this successful experimentation have identified additional problems not anticipated earlier, steady progress toward accomplishment of the underlying objective has been made.

Following a review in March, 1977 (about midway in five-year project) RAC recognized that progress toward the objective of spawning milkfish in captivity was slow and that it would be impossible to demonstrate a working procedure by the fifth year of the project as had been planned. RAC asked that a special review be held during the summer of 1978 to evaluate progress and to make new plans for the fifth year of the project.

An annual report for 1977 (Attachment A) and a special report of research activities during the period January, 1978 - August, 1978 (Attachment B) are presented.

Evaluation Procedure:

A review team composed of the following people reviewed research activities at the Oceanic Institute September 19-22, 1978:

Dr. C.P. Idyll (team leader) National Marine Fisheries Service, Washington, D.C.

Dr. John Hunter, National Marine Fisheries SErvice, La Jolla, California

Dr. D.R. Mitchell, AID, ASIA Bureau

Dr. M. Rechcigl, AID, Program Office

Dr. S. Wishik, RAC Member

The team conducted a thorough review of research progress and scientific competence of the contractor. They reviewed plans and discussed a series of specific problems. A number

of options were considered by the review team including termination of the project, modification of the 5th year plan and/or budget and continuation of the project without change in plans. A revised set of research plans for the 5th year were developed in consultation with the review team (Attachment C).

Other Project Evaluations:

A review conducted in May, 1976 (16 months into the project) led to the conclusion that work was in accordance with terms of the contract, that it was generally on schedule, and that it was deserving of continued support.

A second review in March, 1977 raised several specific issues requiring modification of research plans but recommended continuation of the research. RAC acted upon this team's findings recommending extension of the funding and modification of the scope of work.

Progress Toward General Objectives:

See "Summary of Findings" in attached review team report (Attachment D).

Progress Toward Specific Objectives:

See "Accomplishments" in attached review team report (Attachment D).

Overall Evaluation:

Contract work is given an overall rating of "satisfactory." There have been a number of disappointments, logistical problems and in the early stages of the project poor planning on the part of the contractor and by AID; nevertheless, the contractor has generally been responsive to the terms of the original contract and to modification of the research plan suggested by the project manager and the three review teams. Each review team has been supportive of the work done previous to the review, but has suggested changes. The contractor might complain that AID has asked for excessive modifications of the

original contract. In retrospect it can be seen that the original contract was too broad and that objectives were too optimistic.

Research to date has been conducted in a satisfactory manner even though results have not produced the breakthroughs anticipated earlier.

Attachment A

XD-AAC-889-A1

THE OCEANIC INSTITUTE
ANNUAL REPORT
1977

THE OCEANIC INSTITUTE

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Honolulu, Hawaii

Dr. Athelstan Spilhaus, Soecial Consultant
National Sea Grant Program, NOAA
Washington, D.C.

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President Emeritus, The Oceanic Institute
Woods Hole, Massachusetts

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Kamehameha Schools/B.P. Bishop Estate
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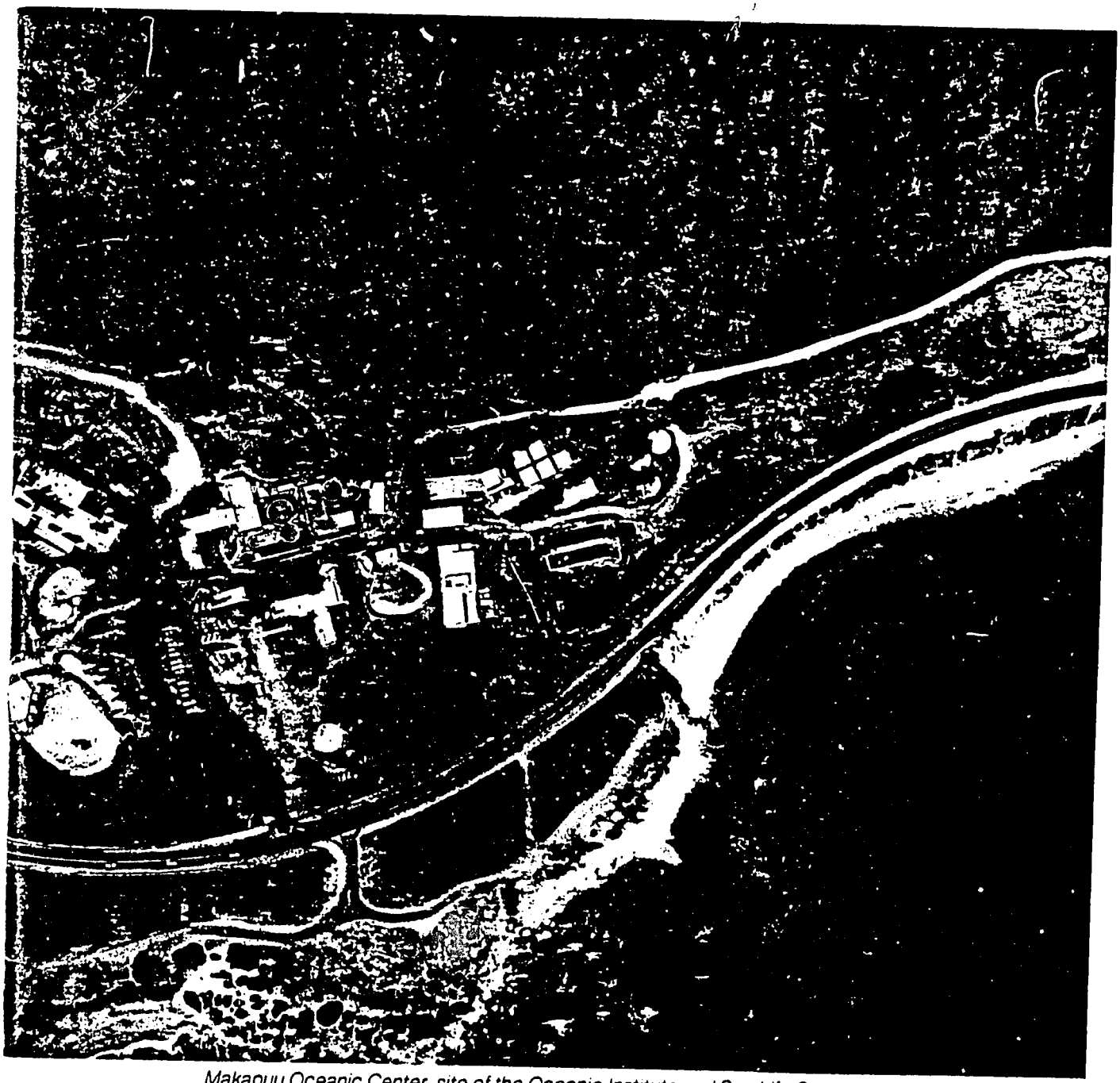

Makapuu Oceanic Center, site of the Oceanic Institute and Sea Life Park
(photo by Tryck, Nyman & Hayes, Inc.)

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THE CHAIRMAN'S REPORT

Robert H. Rath, Sr.

We succeeded, through the combined efforts of our staff, trustees, financial contributors, and state and federal agencies in concluding a rewarding year. Financially, we operated in the black and scientifically we have embarked on several exciting new projects which will be explained in some detail in this annual report. The Institute continues to gain stature and recognition both nationally and internationally and is gaining increased support locally as one of the keystones to future success by the State in aquacultural areas.

During the past year, one of the Institute's prime emphases was to improve communications and exchange information with the local business and community leaders, as well as to continue working with key members of both national and state legislatures and agencies. We hosted more than 280 individuals at the Institute during the year at our monthly luncheons and other social occasions which have greatly broadened interest in the Oceanic Institute.

From the funding point of view, this has been a year of policy change. Many local foundations altered their donation policies from the support of operating overhead to specific project grants. The local foundations contributed to the building of four large holding ponds which will support our finfish hatchery program. In addition to the gifts themselves, these donations enable the Institute to use such awards as matching funds for federal and private grants. We are looking toward the overhead allowances on grants, the resumption of rentals from Sea Life Park, and contributions from friends to carry our operating costs from here on out.

During the past year, Dr. H. Burr Steinbach resigned as President to become President Emeritus from his residence at Woods Hole, Massachusetts. Dr. Steinbach contributed considerably toward the Institute's reestablishment of its national and international reputation. Richard W. Power moved from Administrative Vice President to President and Chief Executive Officer upon Dr. Steinbach's departure and injected new activity and considerable energy to all phases of operation and financing. Dr. Colin Nash, on the other hand, returned to Oceanic on a full-time basis after having been assigned on a half-time basis to ICLARM.

We welcome and need the total community support that we received during 1977 to assure the Institute's continued successes in its varied fields.

THE PRESIDENT'S MESSAGE

Richard W. Power

We are looking ahead optimistically to 1978. We are continuing to stress the practical side of our research and development projects, particularly in the field of aquaculture. We are, for example, exploring the underwater environment for fish farming with cages and other facilities, and developing a new program on the feasibility of marine shrimp farming in Hawaii. These projects are in addition to the many research aspects of our long-term aquaculture program which is involved in responding to the demands of world hunger.

We were disappointed during the year when the funds appropriated by the State legislature for completion of the hatchery were not released. The Institute has held a challenge grant of $150,000 from the Henry L. and Grace Doherty Foundation for over twelve months to complete the hatchery and laboratory facility. It was hoped that the conditions would be met with the State funds and final construction could begin. However, we are hopeful that support by the State will be renewed this year and we can be in production next year.

The Chairman of the Board of Trustees, in his report, referred to the changes in operational procedures enforced by internal policy changes of the private foundations. These have directly influenced the management of the Institute and it is necessary, more than ever before, to maintain a continuous projection for the Institute's interests and income for at least two years ahead. In the world of federal grants and contracts, with its notorious uncertainty and bureaucratic slowness, those are difficult projections to make, and are often beyond our control. The Institute has a need for its independence, and is a more useful organization in that capacity than if it is enveloped in any safe state or federal organization. However, in order to remove it in part from an annual dependence on government agencies, we are looking at some close associations with certain universities and international organizations which, we hope, will lead to some basic long-term funding. We are, in particular, seeking to move into the capacity of a technical education center, offering facilities for technical training and research. This education role will, we feel, provide the Institute with a strong base for its future. We hope that some of these long-term plans bear fruit in the coming months as we now move ahead into the challenging and exciting days of 1978.

THE DIRECTOR'S REVIEW

Colin E. Nash, Ph.D.

National Resources, Environment, Energy — such everyday words of life and language that it is almost impossible to imagine a time without them. Within the last ten years each has risen from obscurity in the dictionary to the heights of international and national authority, complete with bureaucracy and budget. Concern for them is now so important a part of modern society that one wonders why each was not a priority decades ago.

In spite of a seemingly overnight emergence of these major social issues, in reality not a great deal in general attitude has changed. New watchwords may have appeared, but beneath the facade of reorganization and awareness, man is maintaining a concern which he developed through the eons of his existence on earth. Historically, man has always been conscious of the possibility of earthly imbalances. Socially he has had a rationale for specific progress, but with the advantage of evolving the process through leisurely and benign paths.

The problem at present is that man and his society are advancing rapidly up an exponential curve, and there is little room for error. To put this in perspective, compare the slow historical evolution of the stone and bronze ages with the headlong rush of the petrochemical and nuclear ages of modern times. Such comparisions cannot be lightly dismissed. At their respective periods in man's evolution, each was or still is a critical factor influencing man's entire future.

In many ways, man has become a victim of his own ingenuity and technical achievement. For example, the rapidity of modern communication and television can promote a minor national issue into an international problem overnight. In earlier times, man was much more in control of his destiny and at one with his surroundings — and not because of compulsion. In spite of the low odds against individual survival, man's social instincts for doing the right thing in order to increase his chances were probably far higher than those of today.

Time, of course, is truth's great foe. With time the real truth often becomes lost or unalterably embroidered. Villains become folk heroes, and benign cultures are dismissed as unimportant, unprogressive eras in the civilization of man. Probably most of the faculties of man to exist as a benign part of the earth's ecosystem have been lost within the last hundred years. The Indians of North and South America were probably the last societies which utilized an inherent philosophy, evolved through centuries, for living with nature. Their instinct for self-preservation was management — not the management of the plants and animals on which they depended for their existence, but management of themselves within the boundaries of their own environment. Their societies, in fact, may have been the height of man's integration within the earth's ecosystem.

Modern society seems to be unable to manage itself and almost always impulsively overreacts to management issues. The last two decades, for example, illustrated spontaneous attempts to deal with a wide variety of social problems, but with poor and even retrogressive results. Although government by nongovernment is widely accepted as an effective form of managing society, the last two decades have been landmarks for shotgun legislation in vain attempts by man to manage his existence within his world.

And it is going to go on. Throughout 1977, and unquestionably tabled for 1978, the social issues were subjected to much international and national discussion for the purpose of enacting protective laws. The country and the states struggled with energy plans, conservation and environment laws, coastal zone regulations, and all with only half an eye turned toward their impact on the real economies of life.

Such decisions by government on social issues have an immediate effect on the existence and future of the Oceanic Institute. New departments of science and technology are created, others get

folded into bigger bureaucracies, and occasionally some are wiped out. As a consequence, research and development contract funds are to be found in a maze of shifting government corridors, but more and more specific to political priorities. The Institute staff, therefore, has to keep itself continuously informed of the trends and funding programs being developed in the capitol buildings in order to guarantee an existence for the Institute.

However, the Institute still values its independence and its ability to direct its own priorities for project development. More and more the help for such activities is provided by the many private foundations which have supported the Institute for many years. Although private foundations have been redirecting their own efforts to the support of the new watchwords, such as energy and resource management, they are probably more responsible than any other group of organizations for the backing of real innovation. This is greatly to their credit.

Although topical social issues are undeniably important, the Institute is continuing to maintain its objectives to secure scientific and technical knowledge for a wiser use of the oceans and to share that knowledge with the peoples of the world.

The year 1977 has had both its peaks of high hope and its troughs of discouragement. Obviously many projects take years to promote and mature and consequently a great deal of the Institute's work depends on long-range planning and project development — often with nothing to show for it at the end. For example, nowhere was the Institute more hopeful than in the area of finfish hatchery construction. In 1975 the Institute was awarded a challenge grant by the Henry L. and Grace Doherty Foundation for the completion of its unfinished laboratory. In 1976 several State senators worked hard to appropriate funds to comply with the challenge grant and to produce a hatchery and aquaculture educational facility that would be an asset to marine research and development in Hawaii. After 18 months the progress has been thwarted at every turn, and at the end of 1977 the construction had yet to start. Nothing could be more frustrating, particularly at a time when the State authorities are developing an aquaculture master plan which is going to be dependent on pilot-scale facilities, and large resources of federal funds are becoming available that are earmarked for research and development consortia that have both technical talent and large-scale facilities to work on the real problems of aquaculture as a food-production system.

A peak in the year was the signing of a two-year contract with the U.S. Agency for International Development to continue work on the spawning and production of the milkfish. This fish, which is so vital to the protein requirements of many countries in Southeast Asia, must be reared in captivity to guarantee future supplies of fingerlings for the fish farms of those countries. The contract was probably the largest the Institute has signed in its short history.

The Institute is gearing to move into commercial aquaculture in addition to its humanitarian activities with a major program in marine shrimp production. Because of the climate in Hawaii, marine shrimp could be grown successfully in ponds similar to existing practices in Panama and Costa Rica. The Institute has excavated ponds on site for maintenance of the shrimp broodstock that are being imported into the country.

The Institute produced two books during the year in addition to all its other regular publications. Under the editorship of Joe Hanson and Hal Goodwin, "Shrimp and Prawn Farming in the Western Hemisphere" was published by Dowden, Hutchinson & Ross, Inc., of Pennsylvania; and the Director edited a staff publication on mullet breeding and larval rearing techniques for the International Center for Living Aquatic Resources Management. The publication will be produced as a manual for distribution by ICLARM, particularly in the developing countries.

Work on the salmon project in Chile was delayed for 12 months awaiting the restructuring of the fisheries sectors in the country and the appointment of an Undersecretary for Fisheries. This was completed late in 1977 and the Director made one further visit to Chile to initiate the project once more. Hopefully there will be positive progress in 1978.

For 15 months the staff has been involved in the planning of an environmental assessment program for the proposed new superport in Palau. Again this is just one of many similar programs that may break at any time. Alternatively, it could be wiped out overnight by a political decision beyond the Institute's control. It is therefore a continuous process to develop the innumerable leads in the hopes that a small percentage will succeed and provide the year's activities.

Staff and Affiliates

People, of course, make places, and it was with some disappointment that the staff learned of the intention of the President, Dr. H. Burr Steinbach, to retire yet again from an active scientific life and make an early return to his home at Woods Hole. During his tenure of office at the Institute, Burr was responsible for its scientific and technical strength, and he has already been greatly missed on his daily rounds to talk with the staff in the laboratories and offices. Fortunately, he accepted an honorary title of President Emeritus, and he is still therefore with us in spirit if not in daily presence. Certainly we wish both he and Susie a happy return to Woods Hole and a fond hope that they both can visit Hawaii often. Burr, of course, continues to be active on the east coast on behalf of the Institute.

Dick Power took over the role of President following his year with us as Vice President, Administration. In his short tenure, he has been responsible for opening the eyes of the local community to the work of the Institute and its potential role within the State's marine affairs. He has conducted a series of monthly business lunches and other social programs that have had great benefit to the Institute and opened avenues of contractual research with local industry. He has also developed an extensive program for people to become associates of the Institute.

On the scientific and technical staff, there have been several landmark events during the year. At the end of the year, Dr. Robert A. Shleser joined the staff as the Assistant Director. Bob has been involved in a broad range of aquaculture activities from the specialties of crustacean culture to the politics of preparing aquaculture legislation for the U.S. Congress. His career has been distinguished and visible, and his association with the Institute is most welcome.

Dr. Albert C. Smith joined the Institute on a full-time basis from the University of Hawaii Medical School in July. His unusual qualifications of M.D. and Ph.D. in marine pathology provide the Institute with a unique talent, and already his research in pathology using his medical knowledge has produced some spectacular results.

In December, Paul Bienfang was awarded his Ph.D. in oceanography from the University of Hawaii. Paul was one of the few people in the country who had a research grant from the National Science Foundation without having that degree — an unusual achievement in itself. However, it was through his research and with the help of a fellowship by the Institute from the Jessie Smith Noyes Foundation that he was awarded his doctorate just before the end of the year.

Joe A. Hanson left the Institute in midyear on completion of his work with the National Sea Grant Program. During his years with the Institute, Joe was responsible for some of its most futuristic projects. He edited two volumes on mariculture and produced innumerable publications on the Institute's work. He left the Institute and took a position with Jet Propulsion Laboratory in Pasadena, California. Also with the termination of the same contract, Hal Goodwin ended his consulting service arrangement with the Institute. It was a great pleasure to have Hal's association with the staff, and we appreciate his work on a number of projects which have been published under the Institute's name.

Other Institute staff who left during the year were Craig Pelton, Debbie Goodwin, and three of the administrative staff. To them all we send our appreciation and good wishes.

The Institute continued its arrangement with Hawaiian Electric Company's Environmental Department under Dr. John McCain to conduct experimental work. It was a pleasure to have on site Dr. David Ziemann who was working for HECO. The greenhouse project being conducted by the University of Hawaii Department of Botany also continued to function under the direction of Drs. Sanford and Barbara Siegel.

The brine shrimp project under the State Comprehensive Education and Training Program of the Department of Labor and Industrial Relations came to an end during the year. The team, led by Terry Astro, obtained land on Kauai and relocated there to continue its work as a private enterprise.

Dr. Michael C. Mix, Associate Professor of Biology in the Department of General Science, Oregon State University, worked in the summer on marine pathology projects in association with Dr. Smith, as did Dr. Franklin Ramos of the University of Hawaii, Hilo, and David Coleman of the University of Hawaii, Manoa.

Staff Representation

In April, Dr. Ching-Ming Kuo, Craig Paulsen, and Bill Madden spent several weeks in the Philippines working at the Southeast Asian Fisheries Development Center near Iloilo. They went to work with the SEAFDEC personnel on joint research to try to spawn the milkfish. The program at the Institute is supported by the U.S. Agency for International Development, while work at SEAFDEC is supported by the International Development and Research Center of Canada. Following the visit, the staff went on individual visits to Taiwan, Hong Kong, and Japan.

The Director presented a paper on hatchery economics at the annual meeting of the World Mariculture Society in Costa Rica in January, and then another paper on fish larvae survival at the meeting of the International Commission for Exploration of the Seas in Brest, France, in May. A third paper on "Ocean Ranching and Fisheries Management" at a second ICES meeting in Reykjavik, Iceland, was not presented in person because of other commitments.

In September, Dr. Ching-Ming Kuo chaired a session at the international symposium on the reproductive physiology of fish, sponsored by the National Institute of Agronomic Research, in Paimpont, France. He also presented a paper on the use and function of prostaglandins and gonadotropins for the induced breeding of fish.

Fellowships and Awards

In 1975, the Jessie Smith Noyes Foundation of New York awarded the Institute a major grant for it to conduct educational and training activities in marine-related fields at a variety of academic levels. This grant established the first such programs at the Institute and it made possible many national and international awards to postgraduates, graduates, and high school students to conduct joint or independent research at the Institute, or to receive specific training and instruction in aquaculture techniques. In its short history, the education and training program which was regulated by the President and Director of the Institute, made a total of seven international awards, two national scholarship awards, two senior fellowship awards, four Institute staff fellowships, and gave an annual prize for the best marine-related project at the Science and Engineering Fair of the State of Hawaii.

Of the four members of staff receiving fellowships, Paul Bienfang was the first to complete his program and obtain his doctorate in December. Wade Watanabe continues his research for his doctorate at the University of Hawaii, and Craig Paulsen pursues a degree in business administration. Dr. Franklin Ramos and David Coleman were both given scholarships to undertake specific research in association with the Institute's marine pathology program, and Toby Morris from Punahou School was given a summer scholarship to study the potential for using water storage tanks as aquaculture production units. He conducted his work on a farm in Waimanalo.

Receiving can also be as pleasant as giving, and it was pleasing for Al Smith to be awarded a prize for meritorious research work by the American Foundation for Oceanography.

Buildings and Grounds

Thanks mainly to the gift of paint by Mr. Jim Wada and the labor of Steve Ribakoff and his grounds staff, almost all the buildings were painted during the year. Many of the buildings have weathered kindly during the ten years of their existence but were in need of some further protection. The Institute's lease requires that the design and color of the buildings be in keeping with the conservation priority of the area. The buildings were therefore painted in colors which made them aesthetically fitting to the landscape, and the results have been excellent.

As many projects planned for the future had overlapping requirements for building space and outdoor facilities, the master plan for the site was reformed. A picture of the Institute and its facilities, including those now proposed for 1978, is shown in Fig. 1. The drawing was made by Conrad J. Oliver of Makai Ocean Engineering.

Although the building of the hatchery had not been started in 1977 as it was first hoped, four new ponds were excavated between the hatchery and the road. The funding was kindly provided by the Atherton Family Foundation, Inc., the Samuel N. and Mary Castle Foundation, the McInerny Foundation, the G.N. Wilcox Trust, and Charles M. and Anna C. Cooke, Ltd. The new ponds will be an asset for holding broodstock shrimp and finfish, which are so neccesary to the aquaculture projects. The ponds have dirt bottoms, and will provide a good substrate for the feeding behavior of the two groups of animals.

A few other minor projects were undertaken during the year. The larval feeding system operating in the covered tanks beside the Brittingham Laboratory was upgraded. Ten new units were built giving the production unit a capacity of 6,000 gallons. Some indoor changes were made to the round buildings and to the Brittingham Laboratory, improving the laboratory and office space.

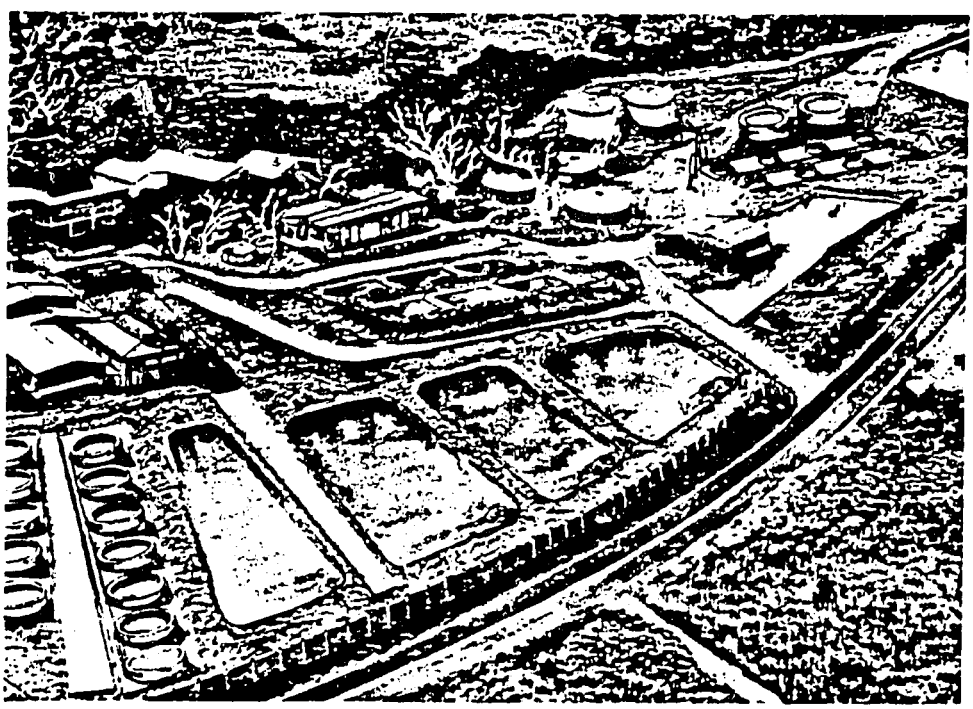

Fig. 1 The Oceanic Institute hatchery and aquaculture facilities.

STATUS OF MAJOR PROJECTS

Larval Food Production

C. Paulsen, D. Goodwin, K. McGovern

In support of expanding larval-rearing experiments, the production of larval food organisms was scaled up and consolidated (Fig. 2). Large-scale phytoplankton cultures are grown in twelve 2,000-liter tanks. More than 4,000 liters of dense (3×10^6 cells/ml) *Chlorella* sp. and *Dunaliella tertiolecta* culture can be harvested daily. This phytoplankton is used to maintain the water quality in larval-rearing tanks as well as to culture the rotifer, *Brachionus plicatilus*. Rotifers are now cultured in two 16,000-liter tanks which can supply some 2×10^7 rotifers/day for larval rearing. As described, this system can sustain 70,000 liters of larval-rearing volume on a continual basis. Techniques learned in developing this system will be applied in the planned pilot-scale hatchery.

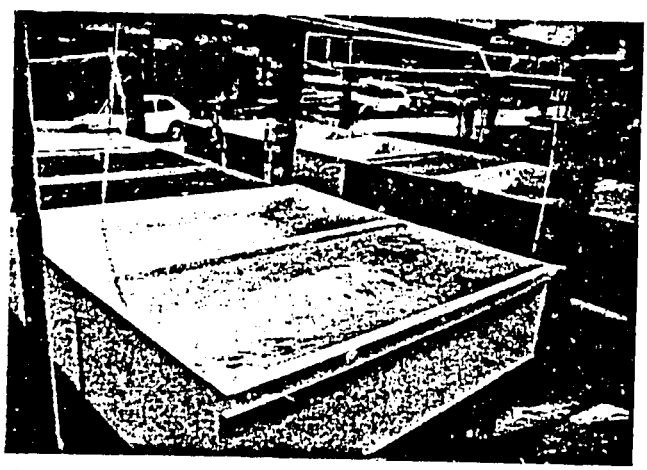

Fig. 2 Large-scale phytoplankton culture facility.

Artemia Culture

C. Paulsen

Brine shrimp nauplii (*Artemia salina* L.) used to feed fish and crustacean larvae are becoming scarce and expensive. This shortage threatens aquaculture development, so a program has been initiated that is intended to conserve available brine shrimp cysts and, ultimately, provide a supply of cysts. Cyst demand can be substantially reduced by feeding smaller numbers of older nauplii to larger fish larvae rather than using only small, newly-hatched nauplii.

To provide larger nauplii on demand, a large-scale, intensive *Artemia* culture system is being developed. Two 1,000-liter prototype systems have been constructed and are being tested (Fig. 3). They are presently capable of culturing 2,000 nauplii/liter using a diet of dried algae (*Spirulina* sp.). The tanks are rectangular, semistatic raceways that use an array of airlift pumps to aerate and circulate the water. Nauplii can be harvested from any of the airlifts as required. Once a culture has been started, the only labor involved is the preparation and filling of a food reservoir twice a week.

It is hoped that this system can also be used to maintain breeding *Artemia* as a source of cysts.

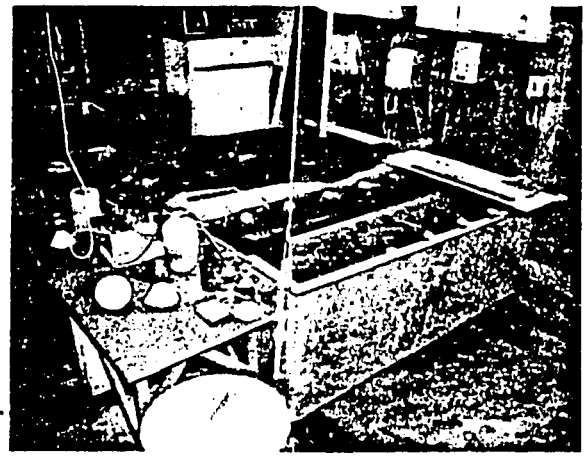

Fig. 3 Artemia culture tanks depicting semi-static raceways.

Invertebrate Separator
C. Paulsen, C. Pelton

Refinements have been made on the separator system used to harvest invertebrate animals from ponds for use as larval food. This system has submersed lights and pumps to attract and collect invertebrates which are then sorted according to size. Experiments using different light wavelengths at the same intensity show that these invertebrates are differentially attracted to colored lights. It was determined that only an isopod (*Paradynamena)* is attracted toward a red light of 635 nm wavelength. Phototropism becomes progressively more positive as the wavelength decreases, peaking at a green color of about 565 nm.

A beam of light focused on the pump intake was also found to attract more organisms than a spot-type light source of the same intensity and wavelength. Combining these findings in the separator system has increased its efficiency as a source of food for larval fish.

Another prototype invertebrate separator has been developed using solar-generated electricity to power the system (Fig. 4). It does not require any additional utilities and consequently can be used to gather food for larval fish in remote pond areas.

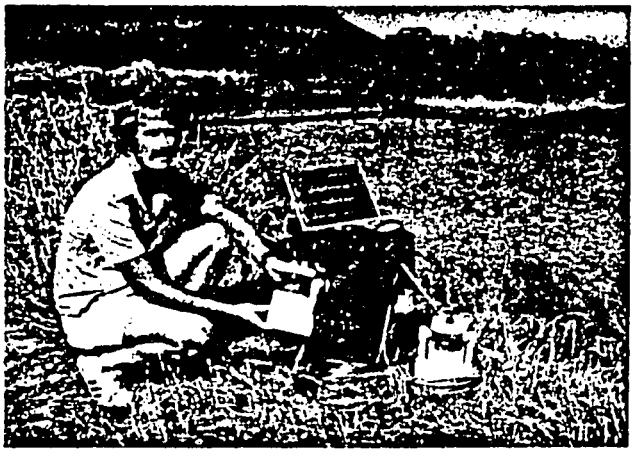

Fig. 4 Solar-powered invertebrate separator prototype.

Larval Fish Production
W. Madden, C. Tamaru

Over 10,000 postlarval mullet were produced during 1976-1977 season. Most of these fish were stocked in four fishponds on Oahu and Hawaii, these being Haena Pond, Keaau, Hawaii island, and three freshwater ponds at Haleiwa, Kualoa, and Waimanalo on Oahu. The remainder are on hand at O.I. to be used for genetic selection of future broodstock.

The most significant success of this past year's rearing was the increased survival rates of the fry. This was accomplished through the identification of some of the factors contributing to the mass mortality of the larvae by gas bladder disease between the second and third weeks after hatching. Specifically, better management of the systems permitted increased survival rates which exceeded 30% (Fig. 5). This was effected by controlling the food densities and by increasing aeration which reduced or eliminated a supersaturated gas condition of the water, resulting from high phytoplankton productivity.

As a result of high larval fish densities in the rearing tanks, feeding of artificial foods after day 30 was possible as a supplement to live *Artemia* nauplii. This was accomplished by the larvae exhibiting intensive and aggressive feeding behavior which was apparently stimulated by the higher numbers of fish. Once accustomed to prepared feeds, the cost and effort required to produce brine shrimp should be substantially reduced.

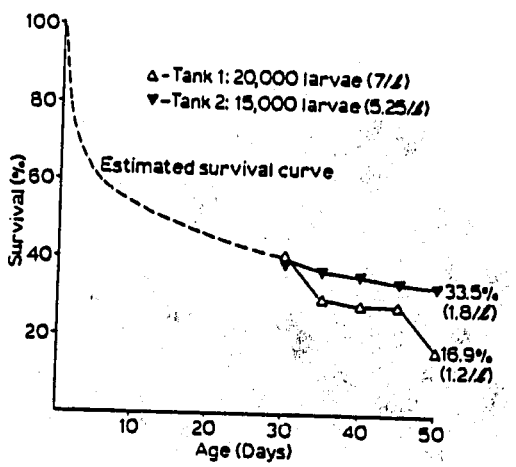

Fig. 5 Survival of grey mullet, 1977 season.

Fish Collection and Fieldwork
W. Madden, C. Tamaru, M. Suiso

Fieldwork on the induced spawning of milkfish at the Lahuipuaa Pond complex on Hawaii was a principal part of this year's work. Three separate trips were made during the summer to capture adult milkfish for injection trials of gonadotropins.

As in previous years, over 60 fish were seined and examined for gonad development. Ripe males and females were placed in pools and injected with crude pituitary extracts from carp and salmon. Induced spawning by hormone injection occurred on June 22 when a female, which had received two injections of crude salmon pituitary mixed with chorionic gonadotropin, began to release eggs in the holding pool. The fish was quickly caught and the eggs expressed into a shallow pan (Fig. 6). Several males were then caught and stripped of their milt in order to fertilize the eggs. Once this was done, the eggs were allowed to stand for 10 minutes and then placed in a clean container and washed with

seawater several times. After washing, the eggs were transferred (Fig. 7) to a large 1,000-liter tank with aeration to incubate. While many of the eggs 1.2 mm in diameter sank and appeared to be infertile, a number remained in the water column undergoing initial cleavage and development. Unfortunately, the eggs did not develop beyond 3 hours after spawning. The probable cause for this was the timing of the stripping relative to the readiness of the eggs. It was determined that the fish had ovulated earlier and had only "dribbled" eggs instead of releasing them enmasse. Thus it is probable that the ovulated eggs were past the critical period when successful development could occur, and that they were "old eggs". Continued efforts to induce these fish to spawn are expected to be successful if sufficient broodstock can be obtained.

Results of the collection this season showed that there were fewer mature fish than in the previous two years. While no reasons for this are known, it is possible that sampling-induced stress contributed to the low levels of gonad production. For this reason and to ensure a continued supply of these fish for future work, nearly 100 subadult and adult fish which had not matured were captured from other ponds and placed in the two best ponds at Lahuipuaa. It is expected that these will mature and be available to continue the injection trials next year.

Fig. 6 Milkfish eggs collected in shallow pan.

Fig. 7 Transfer of eggs by dipnet to incubation tank.

Ornamental Fish Culture
W. Madden, C. Tamaru

A substantial potential exists for ornamental marine fish culture in Hawaii if certain technical constraints can be removed. The most significant technical problem involves obtaining eggs of the species of interest—through induced spawning or otherwise—and rearing the larvae which hatch from these eggs. A research program which would remove these constraints and lead to an industry for culturing ornamental marine fish in Hawaii is desirable and would be an extension of existing culture practices.

An initial project aimed at developing techniques for ornamental marine fish culture was carried out at the Oceanic Institute and the Hawaii Institute of Marine Biology as a coooperative program during 1977. Funding for the work was provided by the office of the Marine Affairs Coordinator for the State of Hawaii.

The project concentrated on five species: *Centropyge potteri* (Potter's angelfish), *Dascyllus albisella* (aio'ilo'i), *Chaetodon miliaris* (lemon butterflyfish), *Abudefduf abdominalis* (maomao), and *Forcipiger flavissimus* (long-nosed butterflyfish). Additional work was done with the triggerfish, *Balistes fuscus.*

The species initially chosen have two distinct spawning modes which we wished to examine. The butterflyfish exhibit pelagic spawning where the eggs are broadcast into the water, fertilized, and allowed to drift while incubating. Laboratory spawning for this group was expected to be unlikely unless induced by injection of pituitary and chorionic gonadotropins. The damselfish, on the other hand, are demersal spawners, laying adhesive eggs in a nest which is guarded by the male parent during incubation. Demersal egg masses of the damselfish, *Abudefduf abdominalis* (the maomao), were collected and the larvae successfully raised. A small number of juveniles were produced as a result and further trials are warranted to develop reliable rearing methods.

Spawning of *Forcipiger flavissimus,* the long-nosed butterflyfish (Fig. 8) was an important success of the program. These desirable aquarium fish were induced to spawn in captivity by a sequence of two injections of Antuitrin S, a gonadotropin, at 2.5 I.U./g. The effect of the gonadotropin is to precipitate the hydration or uptake of water by the eggs and to induce ovulation. When the eggs have hydrated fully, spawning can take place, and this usually occurs within 6-12 hours after the second injection. From earlier work it was determined that injections of hypophysial hormones were effective in inducing ovulation and some egg release when the mean diameter of the eggs was greater than 0.36 mm.

Eggs from each spawning were successfully fertilized and embryonic development, never before observed for this species, was recorded photographically (Fig. 9). As soon as cleavage in the egg was seen, the eggs were transferred to an incubator tank and vigorously aerated. Development was followed hourly and photomicrographs were taken. Development was rapid, with the blastula and gastrulation occurring by the tenth hour. Hatching was completed by 36 hours after fertilization, and the newly hatched larvae were free swimming. Upon hatching, the larva is approximately 1.7 mm long (Fig. 10) and retains a yolk sac with an oil droplet. This food reserve is used up gradually until the third or fourth day, when the eyes become pigmented and the larva is ready to feed.

No larvae survived more than six days (Fig. 11) after hatching; the most likely cause of larval mortality was the unavailability of enough food particles small enough to be consumed by the larvae.

Based on the results of this preliminary project and on assessments of the state of marine fish culture technology, continued efforts are expected to ensure satisfactory control over the culture of these fish.

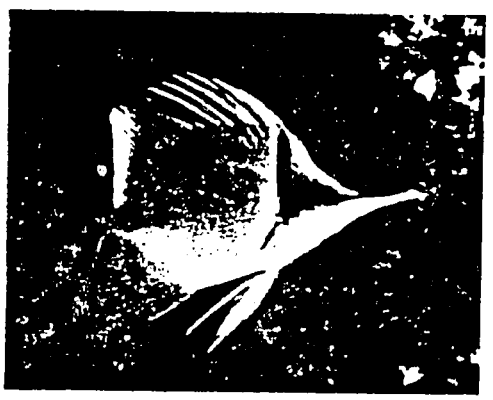

Fig. 8 Adult long-nosed butterflyfish, Forcipiger flavissimus.

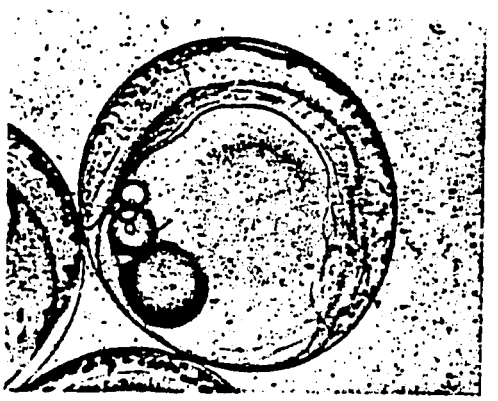

Fig. 9 Butterflyfish embryo in developing egg.

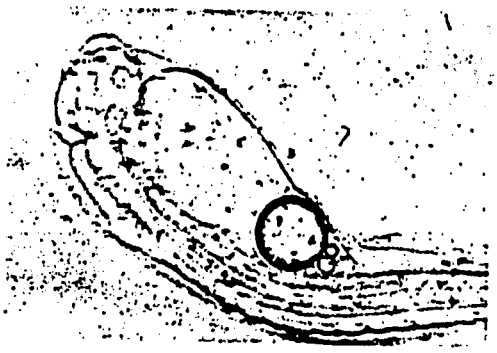

Fig. 10 Newly hatched butterflyfish larva.

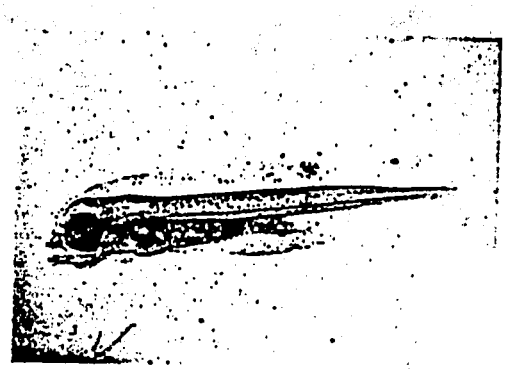

Fig. 11 Six-day-old butterflyfish larva.

Reproductive Physiology

C-M. Kuo and W. Watanabe

Mass propagation of marine fishes has been the ultimate goal of the aquaculture program at the Oceanic Institute. The program has emphasized the control of teleostean reproduction and production of quality seedstock of cultivable marine fish. These objectives have been approached by intensive research in the reproductive physiology, behavior, and ecology of the grey mullet.

The reproductive physiology program has directed its work toward better understanding of fish physiology and the related problems by investigating (1) the processes and mechanisms involved in the gonadal maturation and ovulation, (2) influences of exogenous hormones on eggs, (3) substitution of gonadotropic hormones for induced spawning under controlled conditions, (4) possible use of prostaglandins as an ovulatory agent, (5) circadian rhythm of oocyte responses to exogenous gonadotropins and prostaglandins, (6) osmotic regulation of oocytes during the preovulatory hydration period, (7) physiological causes of larval mortality, (8) preferential physical conditions for embryonic development, and (9) preparation of piscine pituitary gonadotropin. These research activities have aimed to improve continuously the induced-breeding and larval-rearing methods (Figs. 12 and 13).

Fig. 12 Examination of the sex and maturity of adult milkfish.

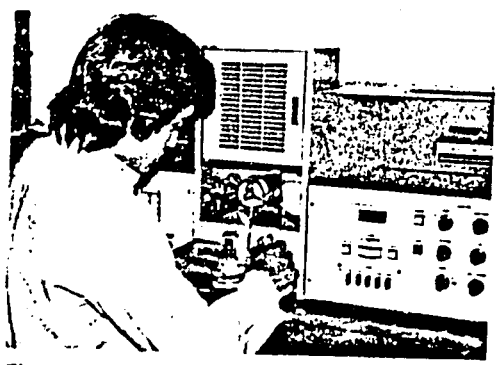

Fig. 13 Analysis of egg quality.

Establishment of a reliable method for inducing spawning and better understanding of physical preferences and nutritional requirements of the grey mullet larvae have led to the readiness for the pilot hatchery. Improvements in the controlled-breeding and larval-rearing techniques have been emphasized in the past years and will continue to be the main goal toward the success of aquaculture programs. The search for the effective triggering agents for gonadal maturation and ovulation, and the most efficient methodology for the artificial propagation of marine fishes, is of continuing interest.

The gonad maturation of the grey mullet has been induced by manipulation of temperature and photoperiod regimes. To date, clomiphene citrate was found to be effective in inducing maturation. The spawnings of these mature females can be induced not only by piscine and mammalian gonadotropins, but prostaglandins are also effective. The presence of prostaglandins in the mature eggs of the grey mullet was further confirmed. The physiological roles of these compounds in fish reproduction are now under investigation.

Circadian rhythm of oocyte responses to gonadotropins and prostaglandins was examined. Effectiveness of the hormone treatment is definitely determined by the endogenous rhythm of oocyte sensitivity to the hormones present. The results were presented to the Symposium of Reproductive Physiology of Fish, held in France, in September 1977. Understanding of this physiological phenomenon will certainly provide basic information needed to develop the most effective and economical induced-spawning method.

The program of artificial propagation of milkfish, supported by the U.S. Agency for International Development, has been continued for the third year. Efforts were continued to investigate the annual cycle of gonadal development, to control the gonadal maturation in captivity, and to induce spawning. The study of the reproductive cycle is completed. The response of the milkfish breeders to hormone treatments was widely variant. Prevention of the mature eggs from atresia, caused by the osmotic and handling stresses, is vital to the success of artificial induction of spawning. This problem has been solved, and the spawnings have been induced by the injections of piscine pituitary homogenate combined with human chorionic gonadotropin.

The limitation on the milkfish breeders available in Hawaii has hampered the progress of the program in the past. The field operation of the program is now in the process of transferring to Christmas Island, where accessible mature milkfish breeders are abundant. The size at sexual maturity of milkfish adults is much smaller and they are mature in the saline lagoons. The problem of the stress causing egg atresia will be solved and the success of the artificial propagation of milkfish is then foreseen.

Marine Pathology

A. Smith and L. Broadgate

Continuing routine autopsies of milkfish from Hawaiian waters has confirmed the impression of a high incidence of a pathological stomach condition, gastritis. In addition, autopsies of milkfish as well as other species continue to strengthen the hypothesis of clotting within the live animals occurring frequently in many disease conditions, and perhaps being a major unsuspected cause of death. Clotting is not a well-studied process in fishes but from the standpoint of fish health, it may be a major factor in whether or not they are able to survive any number and variety of crises (stress, infection, trauma, etc.). Preliminary efforts to develop simple methods to identify the formation and possible propagation of clots in living fishes have been tested and show considerable promise. Diet, to some extent, may be able to control or at least reduce the tendency for clot formation in certain fishes.

The studies in stress have led to the development of a trio of practical tests for detection of early stress. These are (1) the occult hemoglobin test, which can identify nonspecific stress by the presence of trace amounts of hemoglobin in fish skin mucus (and continuing studies may lead to methods of

mass-screening swimming fish for this indicator of stress); (2) the ketone body test, which can identify specific stress due to starvation; and (3) the C-reactive protein (CRP) test, which can detect the specific stress associated with disease. Positive results from any of these tests can alert the fish farmer to a need for corrective action well before irreversible health problems set in.

Identification of sex by external signs is a difficult problem in the milkfish. Unlike some other species, e.g., goldfish and tilapia, there are no apparent external anatomical differences between male and female milkfish. In humans, simple scrapings of surface membranes such as from the mouth cavity reveal sex differences by characteristic patterns of nuclear genetic material in the cells as seen microscopically. This approach, known as exfoliative cytology, was tested in a double blind study of milkfish, goldfish, and tilapia. In none of these species did the results show definite differences between the sexes. However, the cell preparations were excellent and exfoliative cytology has not been used extensively in fishes. Therefore, other possible research using this method is under consideration. For external identification of sex in milkfish, a hormone test using skin mucus will be evaluated next.

The field of immunology has become a major one in recent years, and allergy may play a bigger role in the marine environment than has been previously realized. Basic research and applications have gone hand in hand as rarely seen before. Work on the common Hawaiian sea cucumber, *Holothuria cinerascens*, has revealed the first allergy-type reactions reported for an invertebrate. These animals also, like humans, appear to be able to be desensitized, but much more rapidly. It is hypothesized that this capability for rapid desensitization is an adaptation to minimize tissue reactions from the many symbiotic animals that they frequently harbor. Other studies in the immunology-allergy area have led to: (1) A hypothesis linking in evolution certain pharyngeal structures (Polian vesicles) in the sea cucumber to organs of defense against disease (e.g., lymph nodes) in the higher vertebrates, including man. (2) The identification of naturally occurring substances from invertebrates that behave like antibodies in producing precipitation reactions with foreign proteins, but appear to differ in the type of proteins with which they react. These invertebrate reacting substances are being "packaged" to place them in a convenient form for large-scale pathology and biological studies. It is hoped that these substances will be able to discriminate between normal and cancerous cells, and generally resolve differences not otherwise possible or done as well by antibodies. Their use has already been applied to discriminate fine biochemical genetic differences between fish of the same species. (3) The identification of allergy-like reactions in fishes, with mucus substances from some likely to disrupt the red cells of others in the same tank (Fig. 14). In the figure are shown mixtures of red cells and skin mucus that were prepared on microscope slides: "A" reveals a powerful clumping and destruction of the red cells (central dark mass with radiating, branched strands) from a milkfish, *Chanos chanos*, after mixing with mucus from a snapper, *Lutianus fulvus*. The reaction was rapid, occurring within a few seconds. "B" shows two negative reactions of red cells with mucus from other individuals of these same species. Here, the red cells simply remain dispersed, the crystallin patterns being an effect of drying. Reactions between red cells and mucus occurring in the live fish could conceivably result in anemia and weakness. Such debilitated fish might also be the first to develop disease which, once

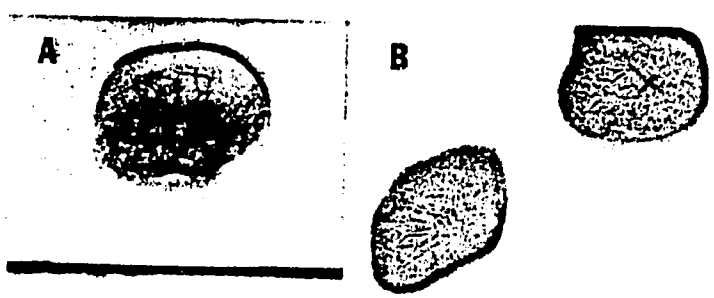

Fig. 14 Reactions of fish red blood cells with skin mucus.

established, may then spread to others in the tank. This phenomenon probably has an immunologic (allergic) basis; its significance to aquaculture and a possible simple preventive are described in a recently published paper.

Proteins of the eye lens core, because of their extreme inertness and other advantages, have found much practical use as genetic markers. Since those in the middle of the lens core are deposited earliest in development and the proteins in the outer layers deposited later, it seemed possible that the former proteins might also be phylogenetically older. Studies to test this hypothesis were carried out on yellowfin tuna and skipjack tuna, the latter being a more primitive species. In both tunas, electrophoretic patterns of the proteins from deeper lens core layers were simpler and more alike (= protein pattern of a common ancestor?); the patterns from progressively more superficial layers showed increasing species specificity.

A number of studies were conducted during the summer with two visiting scientists, Dr. Michael C. Mix of Oregon State University, Corvallis, and Dr. Franklin Ramos of Hilo College, Hawaii. Dr. Mix is a specialist in mollusks, so these animals were used extensively in the research. The possibility that bivalves have different blood types was explored, and no evidence for this was found. An attempt was made to develop a method for collecting and concentrating the low numbers of hemocytes (blood cells) of mollusks by adapting a filtration technique from the medical field. Some progress was made with this technology, but it will require further research to become operational. The last project with Dr. Mix involved developing an optimal extracting fluid for bivalve adductor muscle proteins. This effort was successful, and the extracting fluid was used to produce high-quality electrophoretic patterns of adductor muscle proteins from Hawaiian and northwestern mainland bivalve species. With the development of this extracting fluid and its use in conjuction with electrophoresis, a great range of biological studies (genetic, taxonomic, physiological, environmental) on bivalve mollusks can now more effectively be carried out.

Dr. Ramos is a psychologist/psychobiologist, so the research emphasis was on projects within these areas. A classical experiment in psychology was the demonstration that hyperphagia (overeating) could be experimentally produced in laboratory mammals by administration of gold thioglucose. This substance is selectively concentrated in the appetite-suppression center of the hypothalamus of the brain and destroys this site. Animals so treated have been known to increase their body weight as much as 300%. With a view toward producing increased weight at an accelerated rate in fishes, gold thioglucose was administered to tilapia. The result was hypophagia, or a decrease in eating and, consequently, weight loss, contrasting with the findings in mammals. Toward the end of this experiment, a publication by Canadian workers appeared which reported similar results in goldfish as were found in tilapia. Why and how gold thioglucose produces hypophagia in fishes is currently under investigation. A second experiment was to produce a biochemical or immunological image of the brain. Various studies have indicated that chemical boundaries do not always follow obvious anatomical boundaries; special techniques are, therefore, required to visualize the biochemical architecture of the brain. In this experiment, brain slices were applied to a plastic membrane impregnated with whole brain antiserum. In this way, a biochemical map, or "immunogram", of the brain structures developed on the basis of sites of concentration of reacting antigens. Such information could lead to methods of modifying various behavioral traits, e.g., aggression, eating, reproduction, etc., and nonsurgically destroying areas of brain that are diseased, as with a tumor. The final project with Dr. Ramos was to develop the CRP test mentioned earlier as an indicator of stress specifically associated with the onset of a disease process. The test was adapted from a commercial kit used in human medicine. It was evaluated in tilapia with induced areas of inflammation and necrosis, which are the hallmarks of disease and primarily responsible for the elevated levels of CRP.

Dr. Ramos was supported in his research by a Jessie Smith Noyes Fellowship.

David Coleman, a Sea Grant intern and candidate for a Master's degree from the University of Hawaii, carried out directed research in pathology during the winter months. He was also supported by a Jessie Smith Noyes Fellowship.

Marine Engineering
G. Rothwell, R. Koningsberger, S. Ribakoff, M. Hites

During 1977 planning work was completed for a finfish hatchery to be built on the Institute campus. Documents prepared included a lengthy Project Definition Statement. Environmental Impact Assessment, Construction Plans and Specifications. All required permits have been obtained. This work was done under a contract with the State of Hawaii Division of Fish and Game, the responsible agency for coordinating the State's contribution to the project. The hatchery complex, utilizing and expanding a partly completed building on the Institute campus, will house research facilities, a classroom, and a general-purpose hatchery capable of producing at least a million 50-day fry of any of several species of brackishwater and marine fish of interest to aquaculture. The construction of the hatchery is expected to begin in 1978, with funding from the State and private sources.

In conjunction with the hatchery development, the Institute has completed four new one-eighth-acre (0.05 hectare) experimental ponds, equipped with fresh and saltwater supply, plus discharge gates designed to control water level and circulation, recover and hold finfish, or harvest marine shrimp. With all accessories, these ponds cost $40,000, provided through the generosity of several private Hawaiian foundations. These ponds will be a very important addition to the Institute's facilities in support of both finfish and marine shrimp breeding and growout programs (Fig. 15).

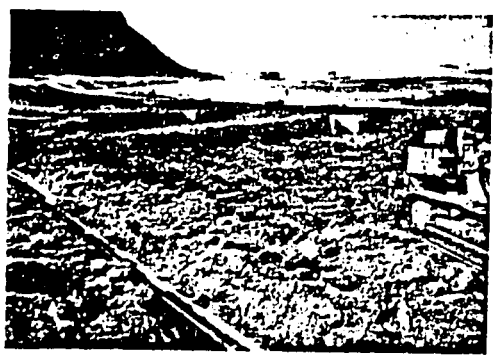

Fig. 15 Saltwater/brackishwater unlined ponds (each is 0.05 Ha.) can be filled and drained independently, and can hold shrimp or finfish juveniles or adults.

The second year of the Sea Cage Project has been successfully completed under the sponsorship of the National Sea Grant Program and the Charles E. Culpeper Foundation of New York. The goal of this project is to develop a technology for cage culture of marine fish in areas of rough water, and to derive criteria for the design of commercial marine aquaculture systems. During this year, the investigation was moved into the sea with an intermediate-sized array of floating, flexible net pen enclosures, each with a volume of 30 m^3 (Fig. 16), and a rigid metal and plastic bottom-located cage

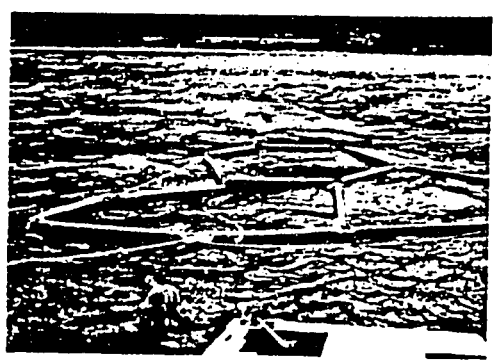

Fig. 16 Flexible float arrangement allows this net pen array to experience large seas without damage.

of 60 m^3 volume (Figs. 17 and 18). These enclosures were stocked with carangid fish (three species of *papio*) and feeding and harvesting methods were investigated. At the same time biofouling and durability observations were made on the arrays. Results were very encouraging. The enclosures withstood periods of rough water very well, with only minor damage to the floating array during a storm, in which no fish were lost. Feeding and harvesting methods for the bottom-located cage were worked

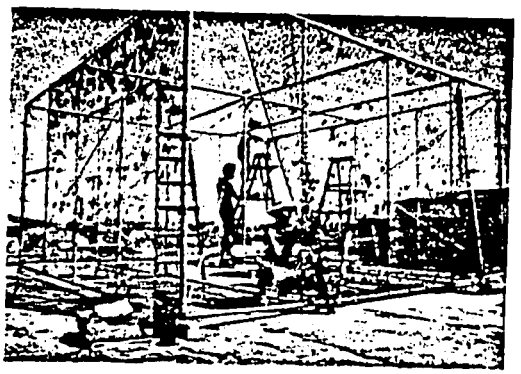

Fig. 17 Rigid bottom-located cage being assembled. White tubes are floodable buoyancy tanks for delivering cage to its ocean site.

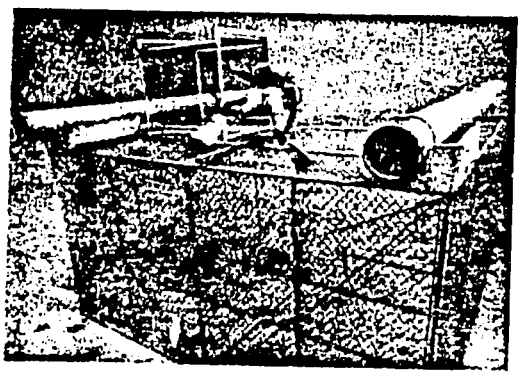

Fig. 18 Rigid cage on site in 30 ft. of water. Small cage on top is harvesting chamber.

out. Feeding by a buoyed hose from the surface proved fully feasible and efficient (Fig. 19), while use of feeding response of the fish to attract them into a harvesting trap proved workable. Trials are now underway with 100 *papio* in a 12,000-liter tank on shore to determine the optimum shape and dimensions of the feeding chamber for attraction, capture and sorting of fish at the stage where they are ready for market. Funding for the coming year has been approved. During 1978 several lines of investigation will be pursued, including deployment and stocking of larger sea cages and experiments with improved feeding and harvesting gear.

Fig. 19 Caged carangids take readily to a diet of chopped herring delivered from the ocean surface through a base.

The Institute has for several years conducted an ongoing mullet-breeding and larval-rearing program, which has led to the accumulation of much practical experience in animal husbandry and hatchery techniques at the laboratory level. Last spring, the engineering group took on the task of codifying and organizing this experience, under a contract with the International Center for Living Aquatic Resource Management. All available data were compiled through interviews with staff members, by observation and measurement of current methods, and by consulting past records. The resulting materials were carefully analyzed, engineering quantities were extracted, and hatchery design criteria were formulated. The result was a draft of a four-part book comprising (1) History of Mullet Culture, (2) Mullet Biology and Physiology, (3) Manual on Hatchery Techniques, and (4) Hatchery Design, Sizing, and Siting.

Environmental Consulting
G. Rothwell, R. Koningsberger

During this year, final plans and specifications were approved by the State Division of Harbors for the small boat launching facility at Mala, Maui. This project involved site studies, a concept plan, an Environmental Impact Statement, and construction plans and specifications for a $350,000 facility, conceived as an environmentally sound way to relieve the use pressures on the existing Lahaina boat harbor nearby.

Early in 1977, the engineering group prepared and submitted to the Department of the Interior an unsolicited proposal to perform environmental consulting services in Palau, Western Carolines District, Trust Territory of the Pacific, in connection with the possible development of a major seaport there (Fig. 20). Palau has an almost undisturbed natural aquatic biota of great richness, which makes the notion of a major port development there highly controversial. During the year, substantial changes have occurred in the political situation respecting Palau, both in Washington and in the negotiations now underway to end the U.N. Trust. Guy Rothwell went to Palau in September, and while there had an opportunity to visit the more promising sites for such a development. As a result of that trip, a revised proposal was submitted to the Palau Legislature, which is under consideration at this time. If awarded, the contract will call for intensive surveys of at least two harbor sites by a team of experts, many of whom will be recruited from other institutions to augment the Institute staff, and preparation of preliminary environmental evaluations. The work will extend over 12 to 15 months.

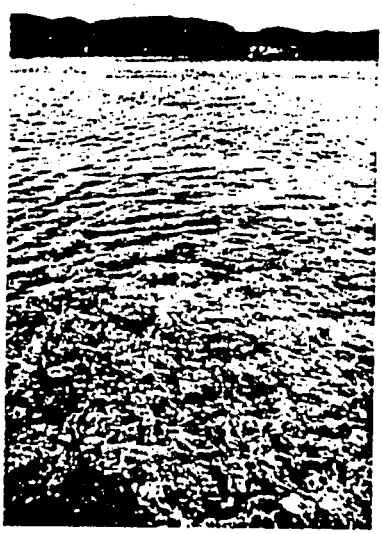

Fig. 20 View across Malakal Harbor, Palau. Even in the harbor, the water is clear and marine life abounds.

MARS Development and Evaluation
P. Bienfang, G. Rothwell, W. Johnson

As a complement to laboratory research on the sinking rates of phytoplankton, Oceanic Institute personnel have developed an instrument called MARS (Multichannel Assembly for Radioplankton Sinking) which enables researchers to measure the sinking rates of natural assemblages of phytoplankton in the open ocean. This instrument detects "labeled" phytoplankton cells which have metabolically absorbed radioactive carbon and is able to register biomass changes with the high sensitivity necessary for studies in the open ocean environment where phytoplankton concentrations are naturally very low.

A grant from the National Science Foundation supports the research program concentrating on development and evaluation of various methodological aspects of the MARS technique. These investigations include laboratory studies with a variety of labeled phytoplankton cultures as well as experiments at sea with MARS capabilities being tested for shipboard use (Fig. 21). It was necessary to develop highly efficient gimbal components in order to dampen ship motion and vibrations since the MARS technique involves the layered introduction of a phytoplankton sample to the surface of a stable seawater column. Cells sink at their own rate to the bottom of the chamber where beta particles emitted by the cells are detected by a Geiger-Müller type assembly and counted by a microcomputer. An important aspect of the development of this technique is a theoretical framework which allows us to deal with the data accurately and in a way that will be meaningful to other investigators in this field. Further research objectives include continued instrument refinements and an evaluation of the MARS experimental environment and its influence on the sinking behavior of test populations.

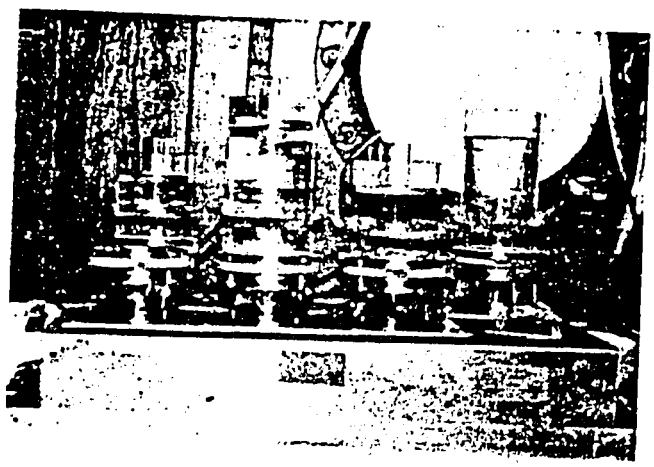

Fig. 21 MARS assembly and gimbal aboard ship.

Thermal Pollution Research
P. Bienfang, W. Johnson

Institute personnel have completed a two-year study, funded by the Hawaiian Electric Company, to determine the effects of thermal pollution on the suspended plants (phytoplankton) in the coastal waters. The photosynthetic activity of phytoplankton provides the primary source of food for higher organisms and any circumstances which influence the phytoplankton may in turn affect the entire marine ecosystem.

The fieldwork was conducted near Waianae at the Kahe Power Plant where seawater is drawn in to cool the condensers and then discharged off the coast. After a residence time of four minutes in the generating facility, an average temperature increase of about 5° C occurs. Changes in biomass and production rates of the phytoplankton were measured during various climatic conditions in the

incoming waters and again after traveling through the power plant. Laboratory experiments were conducted under controlled conditions where the effects of abrupt temperature increases could be investigated without the added variables of pressure or mechanical stress which occur when the microscopic plants are drawn through the generating facility. Field and laboratory data show that exposure to this type of thermal pollution has a rather small effect on phytoplankton biomass. The rate at which phytoplankton may grow and thus produce food does decrease, however, by about 30% immediately after thermal elevation, indicating that the phytoplankton are stressed or shocked but not killed by passage through the power plant. Time sequence experiments suggest that the phytoplankton are able to recover from this stress and original (preentrainment) productivity levels may be attained by seven to eight hours after thermal shock.

Research on the Nutritional Control of Phytoplankton Buoyancy
P. Bienfang, W. Johnson

Work has been completed on a project, funded by the National Science Foundation, to study the biological factors involved with the sinking rates of phytoplankton. These microscopic cells are slightly more dense than the seawater in which they exist so they tend to sink slowly through the ocean. The resulting vertical distribution of phytoplankton is an important factor in the function of marine food webs which are supported by the photosynthetic activity of phytoplankton.

Extensive laboratory investigations were conducted with several different species of phytoplankton which were grown using continuous culture techniques (Fig. 22) with a wide range of controlled nutrient inputs and growth rates. Using a modification of techniques found in the literature, phytoplankton samples were taken from the large growth chambers and poured into specially designed cuvettes which are used in a fluorometer (Fig. 23). This instrument is able to detect the presence of fluorescent material (chlorophyll) and as the cells sink to the bottom, darkened region of

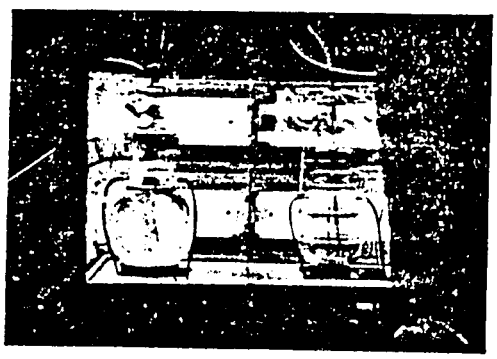

Fig. 22 Continuous culture apparatus.

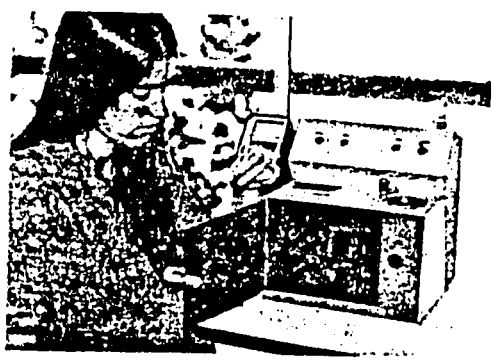

Fig. 23 Fluorometer and cuvette used in sinking rate determinations.

the cuvette, the fluorometer registers a gradual loss of fluorescence which is then used to calculate the mean sinking rate of the phytoplankton population. These experiments yielded results indicating that sinking rates are approximately 10 times slower (.03 - .5 m/day) than had been estimated by other researchers who made measurements only on cells grown in batch culture. We found that the uniform characteristics of phytoplankton populations with a constant growth rate and our revised method of interpreting fluorometric data give a more accurate representation of phytoplankton buoyancy response.

Generally sinking rates do not vary greatly at different growth rates or as a result of varied nutrient inputs. The most pronounced changes in sinking rates occur on a daily cycle with the highest sinking rates at the end of the dark period and the lowest ones at the end of the light period. Further experiments with cells which had been removed from the continuous culture regime and had no fresh nutrient inputs for as long as two weeks also showed a diel buoyancy response, indicating that the physiological control of sinking rates is related to a basal metabolic function.

Gas Chromatography
W. Johnson, P. Bienfang

The analytical capabilities of the Oceanic Institute are being used to assist the Naval Ocean Systems Center in the study of short- and long-term effects of petroleum products on marine communities. Their research involves the introduction of diesel fuel #2 and/or crude oil to a flow-through tank simulating natural conditions and containing semitropical assemblages of marine organisms. The effects of this hydrocarbon stress will be monitored for various time periods by various techniques. One of these is the quantitative analysis for oil in tissue, sediment, and water samples by gas chromatography. The Oceanic Institute possesses a Hewlett Packard 5710A gas chromatograph and integrator suitable for the required analysis and the staff will conduct hydrocarbon concentration determinations on extracted samples provided by the Naval Ocean Systems Center according to their specifications.

Kaunakakai Water Quality
P. Bienfang, W. Johnson

Oceanic Institute staff participated in the Kaunakakai Baseline Environmental Study conducted by Hawaii Planning Design Research for the U.S. Army Corps of Engineers. We provided baseline water quality information which was needed to describe the site of the projected Kaunakakai Deep Draft Harbor and to assess the possible effects of this project on the marine environment. Physical oceanography and fish and coral inventories of the area were investigated by other consultants.

The water quality study included pH, nitrate, total phosphorous, dissolved solids, and chlorophyll determinations as well as bacteriology sampling at several stations in the area of the proposed project. Within the existing Kaunakakai wharf area proper, water quality conforms to the criteria set for class B waters on the basis of requirements set by chapter 37-A of the Department of Health water quality standards. By the same standards, waters adjacent to the wharf area conform to criteria established for class A or AA coastal waters.

Anaehoomalu Ponds Study
P. Bienfang, W. Johnson, W. Madden

A comprehensive survey of anchialine ponds at Anaehoomalu on the island of Hawaii was conducted last summer for Boise Cascade. These ponds occur in shallow depressions and fissures along the flat lava coastline and their slightly saline waters communicate and mix underground. Twelve ponds in the area are to be filled for construction of a resort complex and 50 other ponds were studied in order to establish predevelopment conditions and provide a framework for assessing any direct or indirect impacts on this unique environment. During the field survey a photograph was taken of each pond at high and low tide (Fig. 24) and size and depth data were recorded while surrounding vegetation and aquatic organisms were identified and enumerated. Water quality parameters including temperature, salinity, nitrate, phosphate, ammonium, and dissolved oxygen concentrations were determined for each pond at high and low tide. The water chemistry measurements show relatively high concentrations of nutrients which are typical of groundwater nutrient levels and the pond biota is found to be characterized by low species diversity. Daily flushing of the ponds' water volume is caused by tidal forces affecting the groundwater sources and may serve to control diurnal temperature and salinity ranges within the ponds while maintaining extreme water clarity. The data collected in this study will be used as a baseline for further investigations of the ponds at Anaehoomalu.

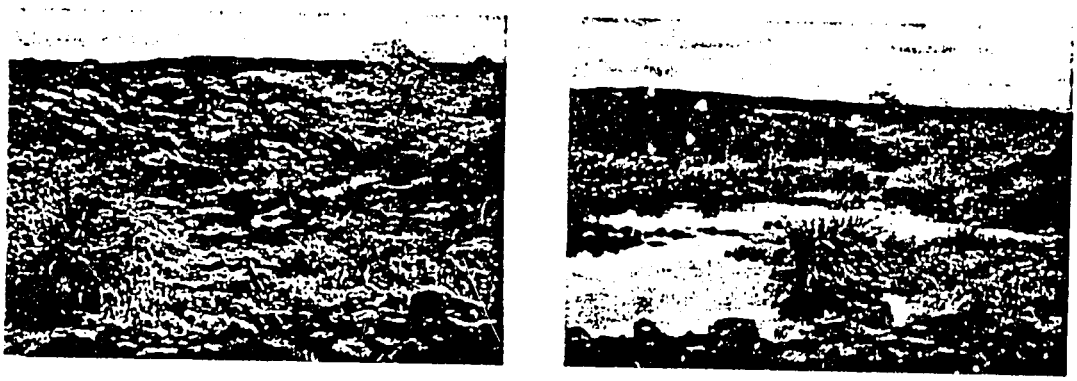

Fig. 24 Low- and high-tide views of one of Anaehoomalu ponds.

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Rothwell, G.N. Environmental aspects of site selection. Prepared for the Special Committee on Palau Port Authority, Palau Legislature, September.

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Smith, A.C. Formation of specific binding activity in fish lens protein. Submitted to Ophthalmic Research.

The Oceanic Institute Marine Science Award — 1977

Joel Yuen of McKinley High School was the winner of the Institute's 1977 trophy in the Zoology Division at the 20th Hawaiian Science and Engineering Fair. The title of his project was "The Utilization of *Zooxanthellae* Photosynthate in the Development of the Coral *P. damicornis*."

FINANCIAL SUMMARY — 1977

The following statement of revenues and expenditures is made on an accrual basis and shows an excess of revenues over expenditures of $41,941. Included in the statement is the final sum of $80,000 of the operating fees from Sea Life, Inc., which were prepaid in 1972 for six years as part of the reorganization agreement. As these advance payments were used to settle the accounts at that time, there has been no cash available from this source for the last six years. On an accrual basis, however, it was necessary to include them in revenues in the years for which they were prepaid. 1977 was the last year of this financial adjustment.

Revenues and Expenditures (Accrual Basis)

(Unaudited)

	1977	1976
REVENUES EARNED		
Research grants and contracts	$640,576	$589,573
Unrestricted gifts and contributions	46,595	176,746
Operating fees (sublease to Sea Life, Inc.)	128,283	121,347
Interest and miscellaneous	19,770	24,393
Total	$835,224	$912,059
EXPENDITURES		
Administration	$119,565	$143,367
Scientific/technical direct expenses	474,773	486,879
General (including indirect expenses and support of scientific work, landscaping and improvements)	198,945	174,004
Total	$793,283	$804,250
Excess Revenues over Expenditures	$ 41,941	$107,809

Contributions Received — 1977

INDIVIDUALS

Mr. & Mrs. Page Anderson
William E. Aull
Lawrence S. Berger
Paul Bienfang (in kind)
Mr. & Mrs. Edward Brennan
Howard Butcher III
Mr. & Mrs. Samuel A. Cooke
Mr. & Mrs. Herbert C. Cornuelle
Mr. & Mrs. Jim Deane
Tilghman Boyd Evans
Dean A. Eyre, Jr.
Marshall Field
George E. Freitas
Mr. & Mrs. Howard H. Hamamoto
General Hunter Harris, Jr.
Mr. & Mrs. Herbert D. Hart
Mr. & Mrs. Thomas P. Huber
Mr. & Mrs. Andrew T.F. Ing
Mr. & Mrs. Davis G. Juell
Malcolm MacNaughton
W.C. Morris (in kind)
Robert C. Oshiro
Mr. & Mrs. A.J. Ostheimer
John Peacock
Charles J. Pietsch, Jr.
David T. Pietsch
Mr. & Mrs. R.W. Power
Mr. & Mrs. R.H. Rath
Nancy Roberts (in kind)
Willis H. Sanburn
F.A. Schaefer III
Mr. & Mrs. C. Frederick Schutte
Mr. & Mrs. William W. Sellew
Charles C. Spalding
Dr. & Mrs. H. Burr Steinbach
George W. Sumner, Jr.
Nadine V. Taylor
Henry A. Walker, Jr.
Mun Charn Wong

Total Individual Contributions $11,682

TRUSTS AND FOUNDATIONS

Amfac Foundation	$ 100
Alexander S. Atherton Trust (in kind)	763
Cyjo Foundation, Inc.	350
Hawaii Newspaper Agency Charities	2,000
Chinn Ho Foundation	2,000
F.M. Kirby Foundation, Inc.	1,000
Boyd and Roberta MacNaughton Foundation	95
Arthur Murray Foundation, Inc.	250
Union Oil Company of California Foundation	1,000
C.S. Wo Foundation	225
	$ 7,783

RESTRICTED

Atherton Family Foundation	$ 17,000
Bank of Hawaii	1,725
Samuel N. and Mary Castle Foundation	10,500
Castle & Cooke, Inc.	15,000
Charles M. and Anna C. Cooke, Limited	3,000
Mr. & Mrs. C.M. Cooke	100
Charles E. Culpeper Foundation, Inc.	30,120
Gordon H. Damon	1,000
The Henry L. and Grace Doherty Charitable Foundation, Inc.	50,000
The Charles Engelhard Foundation	5,000
Frear Eleemosynary Trust	1,000
Mr. & Mrs. Bruce P. Harden	500
Hawaii Newspaper Agency Charities	2,225
McInerny Foundation	10,276
Bernice and Conrad von Hamm Foundation	400
Elsie H. Wilcox Foundation	430
G.N. Wilcox Trust	5,000
	$153,276

BUSINESSES

American Savings	$ 500
Anonymous (in kind)	300
E.E. Black, Ltd.	500
C. Brewer and Company, Ltd.	500
Castle & Cooke, Inc.	9,000
Diamond Head Sprinkler Supply	100
Dillingham Corporation	1,000
First Federal Savings & Loan Association of Hawaii	100
First Hawaiian Bank	3,000
Foodland Super Market, Ltd.	1,000
HC&D, Ltd.	200
Hawaiian Electric Company, Inc.	1,000
Hawaiian Trust Company, Limited	500
Hilton Hawaiian Village	50
Holsum (Hawaii) Baking, Inc.	250
Honolulu Advertiser, Inc.	1,000
International Savings & Loan Association, Ltd.	500
J B G Corporation	500
Johnson & Higgins of Hawaii, Inc.	100
Molokai Ranch Ltd.	1,000
Niwa Painting, Inc. (in kind)	1,845
Olsen Tool & Supply Co., Inc. (in kind)	5
Pacific Resources, Inc.	300
QM Productions	100
Sea Life Incorporated	100
Sears, Roebuck and Company	250
Edward D. Sultan Co., Ltd.	500
Tongg Publishing Co., Ltd.	100
	$ 24,300
Total Contributions	$197,041

SCIENTIFIC AND SUPPORT STAFF

Director Colin E. Nash, Ph.D.

Assistant Director Robert A. Shleser, Ph.D.

Senior Staff

Paul K. Bienfang, Ph.D.
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Ching-Ming Kuo, Ph.D.
Fish Physiology
Guy N. Rothwell, B.A.
Engineering
Albert C. Smith, Ph.D., M.D.
Pathology

Technical Staff

Linda Broadgate, B.A., Technician
David Coleman, B.A., Technician
Wendy Johnson, B.S., Technician
Rosine M. Koningsberger, M.S., Data Analyst/Programmer (part time)
William D. Madden, B.S., Research Assistant
Kathleen McGovern, B.A., Technician
Craig L. Paulsen, M.S., Research Assistant
Stephen B. Ribakoff, M.S., Engineer
Shirley Robinson, M.L.S., Librarian (part time)
Dorothea Rosinsky, Publications
Clyde Tamaru, B.S., Technician
Wade O. Watanabe, M.S., Research Assistant

ADMINISTRATIVE STAFF

R.W. Power, B.A., President
Colin E. Nash, Ph.D., Vice President
Nadine V. Taylor, CPA, Controller and Assistant Treasurer
Nancy A. Roberts, B.A., Paymaster and Assistant Secretary
Helen Carroll, B.S., Accounting Clerk (part time)
Shirley Gregory, A.A., Accounting Clerk
Stephen B. Ribakoff, M.S., Maintenance Supervisor
Edward Porter, B.A., Maintenance
Philip D. Parker, Maintenance

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January 1978

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Cable address:
OCEANINST

Attachment B

XD-AAC-889-B

THE OCEANIC INSTITUTE

An Interim Report

on

RESEARCH IN ARTIFICIAL PROPAGATION OF MILKFISH

and

PROPOSED WORK

to

the Research Advisory Committee of

U.S. AID

Principal Investigator:

Ching-Ming Kuo, Ph.D.

September 1978

MAKAPUU POINT
WAIMANALO, HAWAII 96795
(808) 259-7951

35

INTRODUCTION

This is an interim report to the Research Advisory Committee of U.S. AID. It is written in the context of a period of 8 months of work on a program whose scope and objectives were modified in May 1977. The results presented describe progress on a program scheduled for completion in January 1980.

The seemingly simple objective of using hormones to induce spawning in milkfish is not so simple when you look at the steps involved. A description of the steps involved in obtaining broodstock and spawning these animals provides a perspective for the accomplishments to date:

- finding the fish
- catching the fish
- handling the fish
- transporting the fish
- holding the fish
- spawning the fish
- fertilizing the eggs
- hatching the eggs
- holding the larvae
- feeding the larvae
- transporting the juveniles (fry)

Developing methods and carrying out each of these activities revealed a whole new set of unsolved problems. The solution of these problems in a practical and repeatable fashion is the minimum requirement for the development of transferable technology that will realistically contribute to the production of fish in any context.

GOALS AND OBJECTIVES

A. Goals

The ultimate goal of this project is to develop methods and systems that will result in the capability to breed milkfish. This will result in the capacity to supply stocking seed for culture ponds in Southeast Asia and other areas where milkfish farming is important to food production. To accomplish this goal, we have identified a list of activities or problems to be solved. These activities are the objectives of the current program; the scope of activities range from finding broodstock to eventually solving the food requirements for larvae. For the purpose of this report, the activities of the program are grouped into seven distinct categories which, when complete, should fulfill the goal of this program. These are the specific objectives:

B. Objectives

1. Establish broodstock in captivity
2. Establish conditions for spawning
3. Induce spawning by hormone injection
4. Establish conditions for larval rearing in the laboratory
5. Develop practical methods for large-scale fry production
6. Determine the economics of hatchery operation
7. Conduct health care and pathology research

I. ESTABLISH BROODSTOCK IN CAPTIVITY

A. Scope

The scope of these activities include:

1. Collect mature-sized fish from wild populations.

2. Collect immature fish and establish populations for spawning in future years.

3. Develop appropriate holding facilities for these stocks.

B. Results to Date

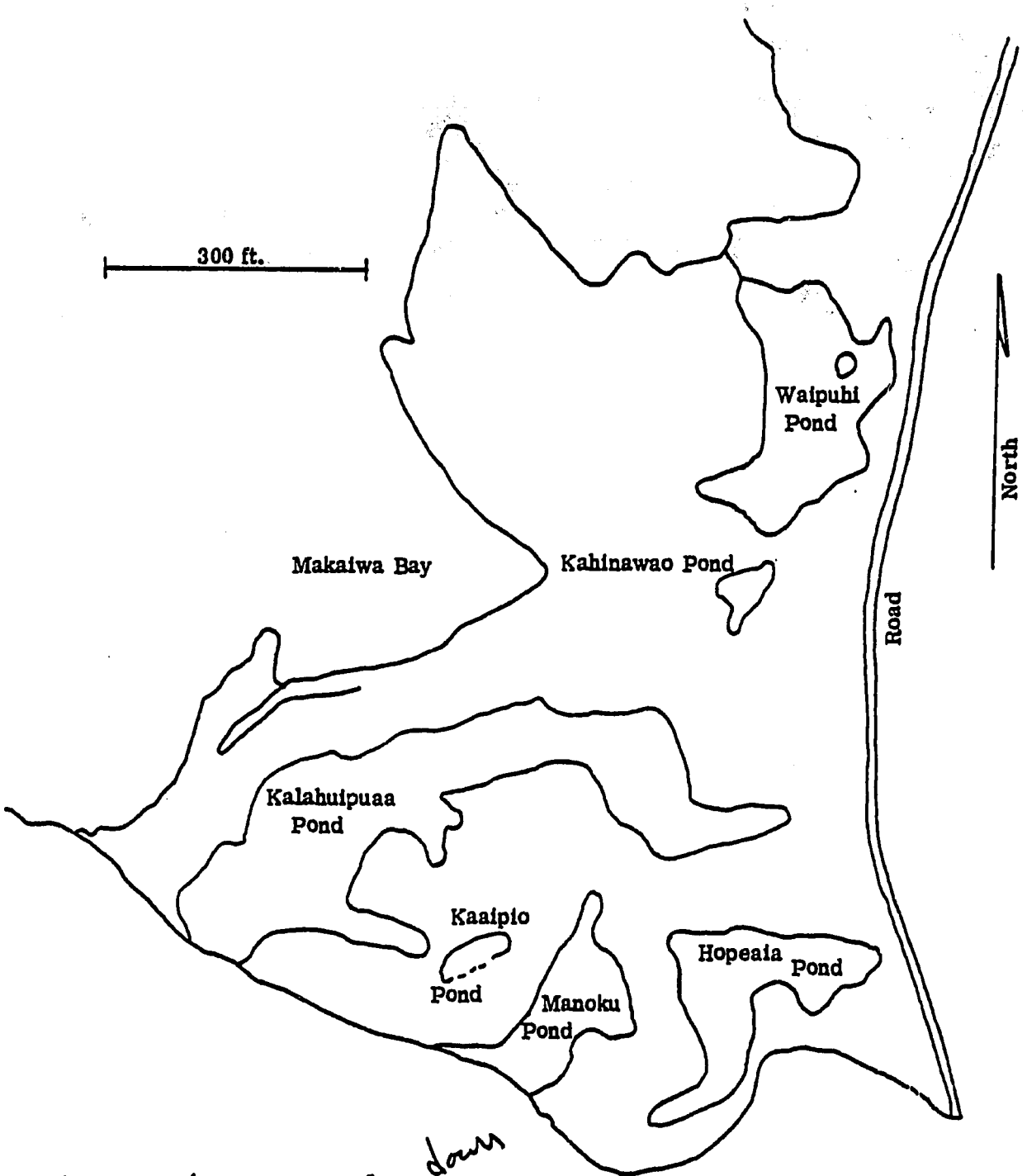
Holding facilities are essential to being able to hold stocks. We have taken the dual approach of developing artificial holding facilities at the Oceanic Institute as well as management of natural pond areas where milkfish are known to reach sexual maturity. These were then stocked with fish. The use of natural ponds involved obtaining permission from owners and managers of the land. Specifically, three ponds are now being used; these are: Hopeaia and Manoku ponds on Hawaii, and Nomilu pond on Kauai. In addition, we have designed and built three experimental environments at the Oceanic Institute. These include four 1/8-acre ponds, one 0.88-acre pond, and a 400 sq. ft. controlled-environment tank.

1. Fishing Program

To provide stocks for these ponds and tank, the Oceanic Institute has, in cooperation with Alu Like (who have provided the manpower for the program), developed a fishing team to capture wild stocks in Pearl Harbor, Kaneohe Bay, and other ocean locations. This team, consisting of five persons, has been successful in locating several wild populations and has developed capture methods for milkfish juveniles and subadults.

a. Juvenile Fish. The methods of capturing juveniles are relatively straightforward and survivals in excess of 70% are routinely achieved. To date, we have captured 805 immature fish with sizes ranging from 1.3 cm to 40 cm.

b. Mature Fish. The size at which a milkfish reaches sexual maturity is dependent upon the environmental conditions; age is also a determining factor for this species. For example, in Hawaii and the Philippines, a mature milkfish is usually about 1 meter long and weighs in excess of 14 pounds, in comparison with Christmas Island where mature fish are found in salinities ranging



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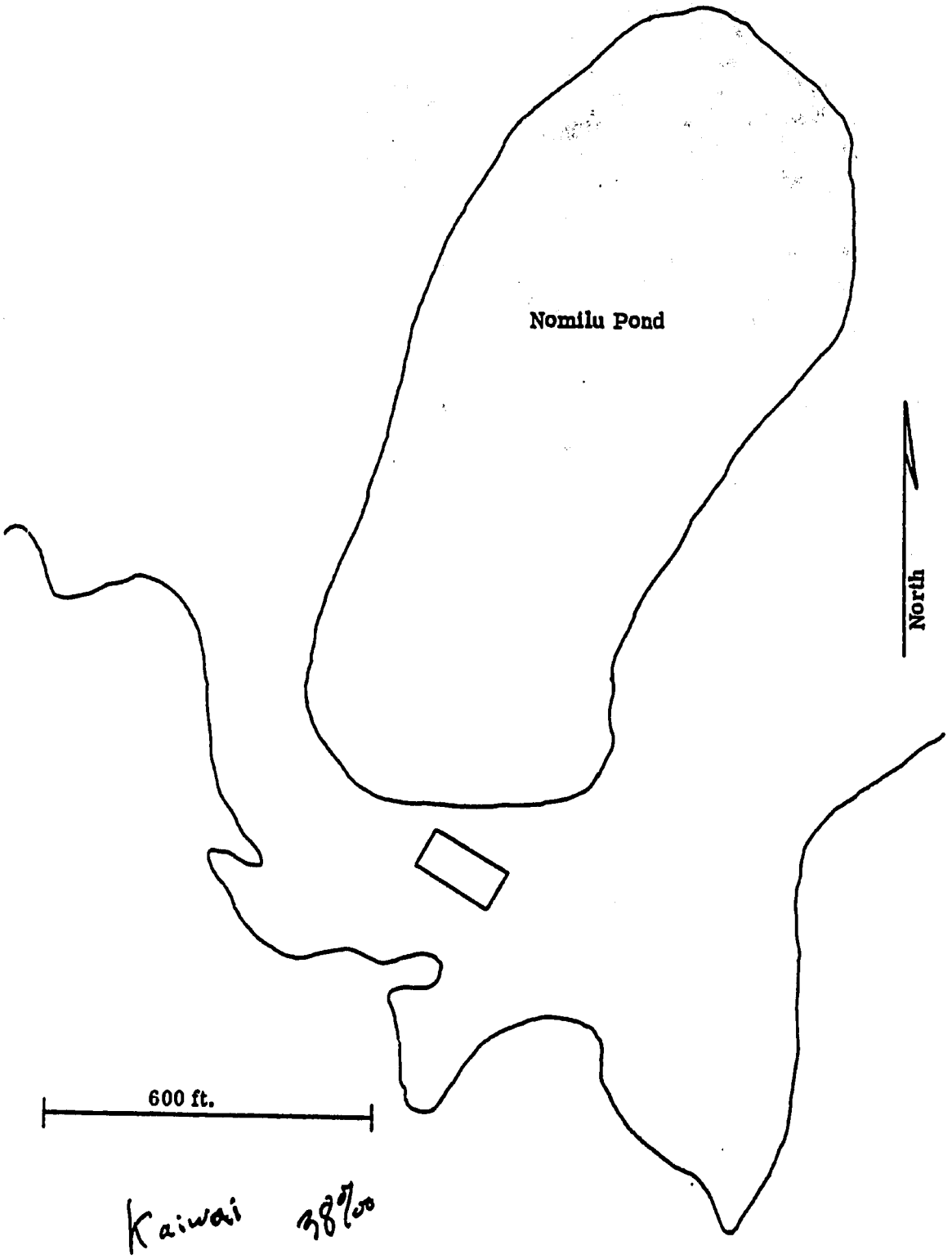

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from 76 ppm to 102 ppm and reach maturity with lenghs of 0.38 m and weighing 2.2 pounds.

Capturing mature fish in ponds at Christmas Island and pond locations in Hawaii has been relatively successful with about 90% survival. By comparison with Christmas Island, the number of mature fish in ponds in Hawaii is relatively small. Transportation from neighbor islands to Oahu is logistically difficult and expensive. Thus we have used fish on Kauai and Hawaii for pond studies and developed artificial environments on Oahu where the Institute is located. The following is a summary of the livestock in captivity.

The subadults stocked last year in the Lahuipuaa pond complex on the island of Hawaii were examined in July. The growth of these fish was found to be satisfactory and they can be used for induced breeding in the 1979 season.

In addition to these 77 stocked subadults, recruitment of subadults to this pond complex is continuing. A total of 360 subadults and 115 juveniles have been stocked in Hopeaia and Manoku ponds this year.

The fishing effort for milkfish is continuing and intensifying in Pearl Harbor and Kaneohe Bay during the breeding season of 1978. This is intended to increase the size of milkfish stock in captivity. They will be used for growth and maturation studies in the coming year(s).

A total of 150 subadults and 650 juveniles were captured this year. As of August 1978, milkfish inventory are:

(1) On the Oceanic Institute campus:

a. Rubber-lined ponds: 19 adults, 74 subadults, 650 juveniles

b. Environmental tank: 10 adults

c. New dirt pond #2: 0 adults, 11 subadults

d. New dirt pond #3: 13 adults, 47 subadults

e. Beer vats: 2 adults, 29 subadults

(2) Off campus:

a. National Marine Fisheries Service, Kewalo: 6 adults

b. Kualoa pond: 15 adults

c. Kaneohe Ranch pond: 30 subadults

(3) Neighbor islands:

a. Lahuipuaa pond complex, Hawaii: 30 adults, 433 subadults, 400 juveniles

b. Nomilu pond, Kauai: 30 adults

(4) Totals:

a. 1977: 32 adults,* 74 subadults*

b. 1978: 125 adults, 624 subadults

Fry were again caught by a fine seine net at Hawaii Kai, Oahu, during several field trips in the months of July and August. All of the fry exhibited the elongation and transparency characteristics of new recruits to the estuarine population. The length of the fry at the time of collection was 1.3 - 1.6 cm (1.5 cm average). They were used for transport experiments and then for the growth studies.

2. Holding Facilities Development Program

a. New Facilities

To accommodate the increasing milkfish stock at the Institute, new holding facilities have been added. They are:

(1) A large indoor tank. It is a rectangular concrete block tank measuring 5.2 m x 4.3 m x 1.2 m deep with a volume of 29.6 m^3. Two collecting channels, 4.3 m x 0.6 m deep, were incorporated into the bottom of the tank to facilitate the catching and handling of the fish. The photoperiod, salinity, and temperature are controlled. Its purpose is to test diet and environmental control for stimulating gonad maturation. This system will hold 20 or more large milkfish. At present there are 10 fish in this system.

(2) Four 1/8-acre by 4 ft. deep outdoor dirt ponds have been completed and brought to productive condition. They have both fresh and saltwater supplies and discharge gates for trapping fish by lowering the pond level. They are general-purpose ponds which were constructed with private funds, and they are available for milkfish growth and maturation trials.

*Adults are fish older than 4 years, and subadults are those 2 to 3 years old.

(3) A new 0.88-acre by 6 ft. deep dirt pond was constructed. The pond construction and catchment gate are similar to the ponds mentioned above. This pond is exclusively for the milkfish program and will be used to study maturation and carry out nutrition studies.

b. Existing Holding Facilities

(1) Six butyl rubber-lined ponds, 20 ft. x 30 ft. x 3 ft. (L x W x D) have been used to stock mullet and subadult milkfish. These ponds will be used for nutrition studies prior to the construction of the new pond.

(2) A dirt pond in the neighborhood of 0.88 acre has been used to maintain broodstock. This pond will be converted to an oxygenated pond when the water system is completed.

These new facilities increase the holding capacity from 7,200 cu. ft. to 250,000 cu. ft. and remove a critical limitation we have suffered in the past.

3. Handling and Transport Program

Upon capture, the procedures for handling and transport have been established. The fish were handled by either dipnet or water-filled plastic bags. To minimize the handling stress and damage, the latter is preferred. An 800-liter circular tank with a firm cover is used for transporting the milkfish. By reducing the salinity and providing strong aeration, the best results are achieved.

Newly captured fish almost always have some injury. We have found that holding fish in a 16,000-liter tank and treating with Furacin greatly decreases the incidence of infection and mortality. A salinity of 18‰ is maintained throughout the treatment period. The fish are treated with Furacin or Furan 2 (+)(-), once a day for 10 days or more until all visible lesions or abrasions are healed. The length of treatment is dependent upon the amount of handling damage and the recovery time required for the particular fish. When the fish has recovered, this procedure is followed by another 4 days' acclimation period during which the salinity is adjusted to the natural condition prior to the distribution of fish into the permanent holding tanks or the experimental environment to which the fish will be transferred.

II. ESTABLISH CONDITIONS FOR SPAWNING

A. Scope

Under this section of the work, we have taken the approach of developing conditions which mimic the environments where milkfish were known to achieve sexual maturity under natural conditions. We have assumed that the environmental conditions in which milkfish with mature eggs are found are suitable for gonadal maturation. Fish with mature ovaries have been found in several locations: Kauai, Hawaii, Oahu, and Christmas Island. On the basis of these observations, we have decided to emphasize a two-pronged approach to spawning milkfish in captivity: establishing stocks in environments which are known to be suitable; and building environments that can be managed to mimic the conditions where the milkfish matured to spawning condition.

B. Results

1. Environmental Regulation of Captive Stock

Intensive fishing efforts are being directed at obtaining additional milkfish adults for induced-spawning trials and to increase broodstock. In captivity, resident milkfish have been subjected to a variety of modified environments in order to evaluate the conditions required for gonadal maturation in captivity.

One of the photoperiod laboratories at the Oceanic Institute has been converted to a controlled holding facility. A total of 10 adult milkfish were stocked and the environmental conditions have been maintained at a constant photoperiod of 18L/6D, a temperature of 25° C, and light intensity averaging 7.5 footcandles. Naviculoid diatoms and enteromorpha have been provided as natural food sources in this concrete tank. In addition, the diet is supplemented with catfish chow and protein supplement in the form of processed Spirulina. 3'x6' tank

The gonad development of these fish was examined after 10 weeks (on July 11). No development of eggs had occurred at that time. Since the time was short between stocking and sampling, the study is still in progress and the fish will be sampled again in 10 weeks.

2. Ponds

We are using Hopeaia and Manoku ponds where previous work with hormones resulted in release of fully hydrated eggs as a location for captive stocks. These ponds have 50 resident adults and 77 subadults that should reach maturity next breeding season.

We have made arrangements to use two ponds on Oahu as sources of mature milkfish for future years. We have stocked one of these ponds in the past and feel confident that this environment will continue to produce mature females. Since mature females tend to leave brackishwater environments and head for the sea as the time of spawning approaches, fish which come to the gates between the pond and the sea can be assumed to be mature. Intensifying our efforts to retrieve fish from the gates is expected to be a productive approach in years to come. This behavior may be a bioassay for maturation in captivity.

As described in the previous section, we have built and stocked a number of ponds at the Oceanic Institute. The 1/8-acre ponds can be controlled for salinity and food. 7‰

With the expansion of fish-holding facilities at the Institute and on Oahu, we expect to be able to provide strong logistics support to the spawning program in the next year.

3. Nutrition

We have recognized that nutrition plays an important role in its contribution to the maturation process and the composition of the eggs. However, until we were in a position to have sufficient stocks of immature and maturing and mature fish and manageable facilities, it was premature to start on a nutrition program.

In starting on a nutrition program, we have assumed that in locations where milkfish farming would be supported by a hatchery program the broodstock would probably be held in ponds. Thus we have attempted to extrapolate from pond observations and consider pond management as part of the nutrition program. This year, only a few preliminary steps were taken in the area of nutrition. We anticipate that in 1979 we will be in a position to carry out an experimental program in this area.

Since milkfish were shown to sexually mature in natural ponds by grazing on indigenous foods, we have taken a preliminary approach to nutrition by transplanting species of algae that milkfish are observed to be using as feeds to OI ponds and maintaining outdoor environments which mimic the ponds where sexual maturation has been demonstrated. Providing supplemental feed by transplanting the species of algae and aquatic plants observed to be natural food for maturing animals may be productive. This work is now in progress.

Since we have captured significant numbers of subadult fish, it may be possible to evaluate various feeds and supplements for juvenile and subadult fish if it is the desire of the committee.

Without adequate nutrition, even the most precisely controlled environmental conditions would not likely produce mature eggs. No information is available on the qualitative and quantitative requirements of the milkfish diet. Previous work by Halver and others has established that even when extensive studies of essential amino acids, lipids, carbohydrates, and vitamins have been completed, diets suitable for reproduction will not necessarily result. Since these kinds of studies require many years and thousands of dollars, we have taken the advice of the 1977 RAC committee and formulated a basal diet consisting of:

Wheat middlings 20% Soybean meal 20% Tunafish meal 60%	Premix--97 parts
Propylene glycol Visorbin (Vitamin B complex) Vitamin premix	3 parts

By feeding this diet to captive populations at the Institute, it will be possible to compare the diets as a supplement to natural nutrition. The more practical food used for these herbivorous captive fish are modifications of the pelletized food used for catfish. During shortages of this catfish chow supply, the diet is then substituted by the above-mentioned prepared feed being developed at the Institute.

III. INDUCE SPAWNING BY HORMONE INJECTION

A. Scope

This part of the program focuses on producing sufficient seedstock in captivity by developing a reliable and defined spawning procedure. A consideration of cost of hormones is part of this approach.

The present state of the art indicates that unless adult milkfish are subjected to environmental conditions which result in the stimulation of gonad development, induction of spawning by hormone injections will not be possible. We have shown that adult milkfish of both sexes can reach sexual maturity in confined brackish water and hypersaline ponds and other coastal environments. At the time of spawning, the fish migrate to sea where the natural conditions trigger spawning and provide an environment that favors larval survival. In this part of the program we are attempting the artificial induction of milkfish spawning by injections of exogenous hormones, as a means of bypassing the unknown environmental requirements, for the last step.

B. Results

Understanding of the reproductive cycle and spawning conditions are essential to control the breeding in captivity.

1. The Reproductive Cycle

Individual statistical data have been accumulated to define the reproductive cycle of milkfish in the Hawaiian waters. The gonadosomatic index (GSI) and the monthly variation of oocyte composition are used to postulate the cycle.

In Hawaii, the breeding season of milkfish occurs in the months of June through August, and September is the postspawning period. The data from the milkfish population in Hawaii suggests a synchronous spawning behavior, namely all the mature oocytes are released at one time and the adults spawn once a year. The details of the reproductive cycle are shown in Appendix A.

2. Work on Induced Spawning

To exploit an alternative milkfish population prior to the beginning of the breeding season in Hawaii, fieldwork on induced spawning was conducted on Christmas Island from April 20 through June 7, 1978. The report on the fieldwork on Christmas Island is attached (Appendix B).

To conduct fieldwork on induced spawning in the neighbor islands (Hawaii and Kauai), five trips were made in this breeding season. The trips were:

a. June 26 - July 1, 1978: Hopeaia pond, Hawaii

b. July 17 - July 22, 1978: Manoku pond, Hawaii

c. July 25 - July 29, 1978: Nomilu pond, Kauai

d. August 7 - August 16, 1978: Nomilu pond, Kauai

e. August 28 - September 1, 1978: Hopeaia pond, Hawaii

The details of each trip are described as follows:

a. June 26 - July 1, Hopeaia pond, Hawaii

Over 100 milkfish adults were trapped in Hopeaia pond and examined. Most of them are sexually immature and they were presumably stocked in the previous year. Only five maturing or mature females were available; two of them were at the stage of yolk globule, with a mean egg diameter of 0.62 mm, and the other three females were still far from sexual maturation. The mean egg diameters were around 0.32 mm.

All the males from the original broodstock were again sexually mature.

Previous results indicated that the oocytes should be 0.7 mm in diameter or above to offer the greatest chance for successful induced breeding. A priming dose of homogenate is injected on capture. This has been shown to be most successful when it is followed by a minimal number of injections of gonadotropin in the ensuing period. It should be noted that although an individual female fish may be in the maturation phase on capture, the number of low-level daily injections of hormone required to advance development may unnecessarily stress the fish and possibly kill it. Thus when the oocyte diameter is smaller than 0.7 mm it is necessary to wait for female fish to undergo further vitellogenic development before injecting the hormone.

For this reason, only two females were subjected to three injections, 50 mg CPH and 5,000 IU HCG daily for three days, to examine the oocyte response to the hormone treatments. The response was not significant and they, along with two other females, were released back to the Hopeaia pond.

b. July 17 - July 22, Manoku pond, Hawaii

Only one mature female, with eggs of 0.55 - 0.6 mm, was captured. Running males were obtainable from the pond. Because of the maturation state of the female, no attempt was made for induced breeding trials.

c. July 25 - July 29, Nomilu pond, Kauai

This trip was made primarily to evaluate the potentiality of milkfish populations in Nomilu pond for induced breeding use. The pond, about 26 acres with a population of about 30 milkfish adults, presently is not in use. The purpose of this trip was to examine (1) the feasibility of capturing milkfish adults, (2) gonadal development of milkfish adults, and (3) the possibility of establishing a fieldwork site.

Because of the size and depth of the pond, a method of trapping is used. A seine net, 300 x 18 ft. (L x D) with a pulley system was used to intersect the 1/6 section of the pond; fish are then trapped in the pocket. A total of five adults were trapped: four mature females and one running male. Only two females with eggs larger than 0.8 mm were injected with 100 mg CPH and 5,000 IU HCG as a primary dose. This was followed by 100 mg CPH and 20,000 IU HCG each on a daily basis. Since the Kauai work was a new location for the team, almost no support facilities were available at this location.

A temporary holding facility was set up by enclosing a small section of the pond. It was inadequate to maintain the milkfish adults, as it was too shallow and muddy.

After two injections, one of the females released eggs. The second female died of handling stress and infection. Since these first results seemed to offer promise, we decided to establish a working facility on Kauai to work on this captive population. A second trip to the Nomilu pond was planned.

d. August 7 - August 16, Nomilu pond, Kauai

A temporary holding tank with necessary equipment was set up on site. The fishing operation, as in previous trips, was conducted to capture milkfish adults.

The milkfish were captured two times from a total of five fishing trials. In the first catch, only four mature females were caught; the egg diameters were 0.6 and 0.8 mm (two fish each). No males were captured at this time. Two of the captured females, with eggs of 0.8 mm in diameter, were injected with 25 mg CPH and 2,500 IU HCG. One of the treated females released the eggs in the holding tank 22 hours after injection. Unfortunately, since no males were captured,

fertilization was impossible. The remaining two females proved to be too immature to be injected (egg diameter of 0.6 mm). No injections were given to these females and they were then released.

Further efforts were made to obtain ripe males to continue the induced-breeding trials. The fish soon learned to avoid the net and in this deep pond, our equipment was not adequate for further fishing. All the captured females were then released back to the pond after four days in captivity.

A second attempt to catch milkfish adults was initiated. Only one ripe male was caught. Continued fishing failed to capture any female for induced-breeding trials; therefore, the fieldwork at Nomilu pond, Kauai, was terminated.

Several comments may provide important perspective. The fish that spawned produced a full complement of fully hydrated eggs. There is every reason to believe that if a mature male or cryogenically preserved sperm were available, fertilization might have been accomplished.

This almost result emphasizes the need for more organization and preplanning. We feel that the captive populations now established will largely overcome these problems. Cryopreservation of sperm may be useful for backup support to the programs.

e. <u>August 28 - September 1, Hopeaia pond, Hawaii</u>

The gonad development of the resident broodstock in Hopeaia pond was reexamined. Most of the males were still at the running condition. Although the number of original broodstock identified in 1975 was numerous, losses occurred mainly through poaching, with smaller losses from handling and an early shipment of 30 adults to OI offset by recruitment from subadults and juveniles in the subsequent years.

Two females, not fully mature (0.32 mm in egg diameter) at the end of June, were recovered on this trip. One of them was full of atretic eggs, while the other showed significant progress in the ovarian development (vitellogenesis). The egg diameter increased from 0.32 mm to 0.62 mm in two months. These eggs were full of yolk materials, yet they were smaller than the optimal egg size proposed for induced spawning trial; 0.7 mm or above was reported to be an adequate size range.

The experimental adults were acclimated in 7‰ brackish water for a few hours after their capture from the pond where the salinity is 7‰, and the salinity was gradually adjusted to 35‰ in 30 hours. A total of <u>seven injections</u> of carp pituitary homogenate (ranging from 100 mg to 200 mg each) and HCG (ranging from 5,000 IU to 20,000 IU each) were given. The schedule of two injections daily, 9 a.m. and 6 p.m., was followed. 7

At the end of four days' injection period, the fish conditions were excellent. However, no response of the yolk-laden eggs to the hormone treatments was accomplished. From these observations we can conclude that progress of ovarian eggs from the early phase of yolk globule stage to the late phase of yolk globule stage is possible, even when sampling stress is introduced. This requires approximately two months. However, yolk-laden eggs smaller than 0.7 mm in diameter are inadequate for hormone-induced spawning.

f. At OI

A total of 15 milkfish adults were obtained from Kaneohe Ranch pond owned by Mr. Ueymura. Two females were found to be mature (egg diameters of 0.83 - 0.87) and ready for induced-breeding trials. Another four adults were identified as males, although they were not fully ripe.

Both females were injected with carp pituitary homogenate combined with human chorionic gonadotropin twice a day. The times of injections were 3 hours after sunrise and the interface between light and darkness. At these times, the female is more receptive to the hormone treatments (obtained from the work on the mullet, refer to Appendix C).

Injections in one of the females were terminated after four treatments because of the physical damage and appearance of atretic eggs. Induced-breeding trials on the remaining female were continued for two more days. The progress of oocyte hydration was satisfactory and at least 16% of total oocytes were completely clear and ready for fertilization as determined by microscopic examination 12 hours prior to stripping. The stripping was made when the fish showed continuous dribbling. Fertilization of these eggs was impossible because no semen was obtainable from the males (possibly more justification for a sperm cryopreservation activity).

In summary, limited numbers of milkfish adults were available for breeding trials. The mature adults were often collected from either brackish water (7 ~ 12‰) or hypersaline water (76 or 102‰ at Christmas Island).

Acclimation of these fish to the natural breeding condition, presumably 35‰, imposes osmotic stress to the breeders being handled. The salinity adjustment is usually completed in 24-30 hours. The milkfish available in the Philippines may overcome these problems. We feel that the successful demonstration of hormone-induced spawning of fully hydrated eggs, even without fertilization, provides a good foundation for next year's work.

3. Future Work

The major failing of this year's activities was the inability to capture fully developed females and males at the same time. This is especially true in the fieldwork in the neighbor islands.

Establishing broodstock in captivity is the key to successful artificial propagation of milkfish. Although wild fish can be captured and fertilization of eggs completed following killing and stripping, the long-term benefits will be best obtained through management of the broodstock in captive situations.

We feel that now the establishment of realistic-sized facilities and captive populations of presexed fish should enhance the probability of succeeding in achieving fertilization and carrying out the further studies.

Stocking of juvenile fish in ponds is priority.

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IV. ESTABLISH CONDITIONS FOR LARVAL REARING IN THE LABORATORY

A. Scope

Larval culture involves fertilization, hatching, and then rearing larvae to the juvenile stage. In general, it is essential to maintain optimal environmental conditions and to provide foods suitable for larval growth and survival. The following subobjectives have been defined:

1. To develop larval-rearing techniques with descriptions of physical and food requirements of milkfish larvae, and

2. To improve larval survival by finding the optimal conditions of larval life.

B. Results

Although we have not achieved a fertilization this year, four successful spawnings which produced a full complement of hydrated eggs suggest that we are one step away from fertilization.

1. Food and Physical Systems

Considerable experience in rearing larvae of the grey mullet (*Mugil cephalus*) has resulted in development of facilities and food-production capacity for all stages envisioned as required for larval culture. Foods envisioned to be necessary are phytoplankton and rotifers in culture and *Artemia* nauplii. A review of our facilities should be given at this point.

2. Present Hatchery

The present experimental hatchery at the Oceanic Institute consists of tanks for the growth of algae, phytoplankton, rotifers, and brine shrimp. The hatching facilities allow holding of 10 mullet with four indoor culture tanks with a capacity to rear 15,000 mullet larvae to stocking size. The other six outdoor culture tanks are also available.

3. New Facilities

The above hatchery was adequate to carry out research, development, and demonstration of methods of spawning and larval culture for mullet. However, this facility is clearly inadequate for production of levels of fingerlings that would allow economic evaluation. Recognizing this failing and realizing the necessity of

having a production hatchery as well as facilities that support holding broodstock for large milkfish, considerable expansion of facilities was required. This has been accomplished during the past year. The new hatchery will make the required economic evaluation possible.

4. Hatchery

A production-size hatchery facility was designed which would make use of an existing unfinished building. In August 1978, the governor of Hawaii signed the order releasing the funds for finishing the building; $300,000 of State money coupled with $150,000 of funds from the Doherty Foundation are committed to the program. This provides matching funds of $450,000 to this program.

5. Larval Rearing

Efforts have been made to develop techniques for larval rearing by using wild milkfish larvae before the larvae production is realized in captivity. Collection of newly recruited fry from the wild continued to conduct experiments on larval survival and growth. Various feeds including Spirulina supplement were examined.

The larvae were caught near Hawaii Kai in brackish water and were estimated to range in age from 10-14 days. Total length at this stage averaged 15 mm (range 13-16 mm) and average weight was 13 mg. Fifty larvae were placed in each of six 80 l aquaria with a water exchange rate of 3/day. Other parameters measured in this experiment were salinigy (32‰), temperature (25-26° C) and dissolved oxygen levels (7.0-7.5 mg/l). Aquaria were cleaned daily and rations, mortalities, and other observations were noted. The larvae in each tank were fed one of six different rations daily so that food would not be a limiting factor.

Treatment and results are described as follows:

Treatment	Daily Ration	Total Length (av.) (range)	Weight (av.) (range)	Survival Rate (%)
Chlorella salina	10^4 cells/ml	-- --	-- --	0
Brachionus plicatilus	5 rot./ml	27 mm (18-39 mm)	0.25 g (0.12-0.37 g)	10
C. salina + B. plicatilus	10^4 cells/ml 5 rot./ml	23 mm (18-32 mm)	0.13 g (0.04-0.28 g)	54

Treatment	Daily Ration	Total Length (av.) (range)	Weight (av.) (range)	Survival Rate (%)
Purina catfish chow	0.5-1.0 g	31 mm (25-37 mm)	0.20 g (0.10-0.29 g)	12
Spirulina maxima (spray-dried)	0.5-1.0 g	34 mm (28-45 mm)	0.33 g (0.15-0.73 g)	34
S. maxima + chow	0.5-1.0 g	30 mm (17-40 mm)	0.26 g 0.04-0.58 g)	66

There was a rapid decline in the numbers of larvae fed only the alga Chlorella salina and that treatment was terminated on day 12 when there were no remaining survivors. The other treatments were continued for the full 30-day period. By day 30 the survivors from all of the treatments were noticeably smaller than those caught at the same time but which were stocked in an outdoor pond. These fish were fed supplementally with a 1:1 mixture of spray-dried Spirulina maxima and finely-ground Purina catfish chow.

These preliminary studies demonstrated that Spirulina maxima and S. maxima mixed evenly with finely-ground Purina catfish chow can be successfully used to feed milkfish larvae of 10 or more days. These diets provide good growth and survival and are easily handled and stored at room temperature. It would appear, however, that these rations might be used most effectively as a supplemental feed to natural biogrowth in outdoor ponds rather than as a sole source of nutrition. Further feeding trials are necessary to ascertain whether S. maxima and Purina catfish chow are nutritionally complete for milkfish larvae at this stage.

In preparation for larval fish-rearing efforts with milkfish, the food production system was scaled up to meet future demands. Emphasis has been placed upon improving and expanding phytoplankton and rotifer culture techniques. The ubiquitous algae, Chlorella spp. and Tetraselmis spp., have been selected for their high productivity and ease of culture in large volumes. Brachionus plicatilus was cultured using Chlorella achieving densities of 150 rotifers/ml in 10 days. Tetraselmis spp. was demonstrated to be acceptable to three-week-old milkfish larvae.

A new alga species, Nannochloris spp., is now being mass-cultured for use in milkfish larval rearing. This alga appears to be a superior base for our food chain for two reasons: it can be intensively cultured under a wider temperature range than the Chlorella and Dunaliella previously used, and it has a smaller percentage of cell wall than does the Chlorella, making it easier to digest.

6. Fry Transport

The transport experiments of milkfish fry up to the density of 100 fry per liter were conducted in 1977. The results are shown in Appendix D. The The experiments continued this year when new recruits of milkfish fry became available from the wild. The densities from 50 to 1,000 fry per liter were examined. The results indicated that up to 1,000 10- to 14-day-old fry per liter can be safely transported for periods as long as four days. Salinity was 18‰ in a ratio of 5:1 (air to water).

Results of fry transport experiments for a 7-day period are as follows:

Density	Initial D.O. (ppm)	Mortality (%)	Final D.O. (ppm)	Temperature (° C)
50 fry/l	7.5	2	5.1	24
100 fry/l	7.5	3	4.9	24
500 fry/l	7.5	1.2	2.5	24
1,000 fry/l	7.5	1.1	4*	24

*In the preliminary test, 100% mortality was noted at the end of the 7-day experiment period. The test was repeated and terminated with only 1.1% mortality observed at the end of four days. The loss of the first trial was due to bag leakage.

V. DEVELOP PRACTICAL METHODS FOR LARGE-SCALE FRY PRODUCTION

A. Scope

To develop practical rearing procedures for large-scale production of milkfish seeds through understanding of ecological and nutritional requirements of postlarvae.

The scope of this work starts with the recognition that methods that are developed at a well-equipped research institution are dependent on pumps, filters, and expensive equipment. These methods must be adapted to a set of simple procedures that can be scaled to production-level activities.

B. Results

The completion of this objective will follow from an understanding of the requirements for culture of the milkfish larvae. We have not succeeded in obtaining fertilized eggs this year.

We have made arrangements to interact with the workers at SEAFDEC where the spawning and fertilization of milkfish has been accomplished. Although large numbers of fish have not been produced, we anticipate that based on our past experience with mullet culture, it may be possible to plan for large-scale culture.

However, until specific larval foods, stocking density, flow rates, etc., can be determined, it will not be possible to go any further with this study area.

VI. DETERMINE THE ECONOMICS OF HATCHERY OPERATION

A. Scope

Operating a larval-rearing program of significant size is essential to determine the economics of such an activity. In the absence of a fertilization this year, we cannot carry out this objective at this time.

B. Approach

We will work with the SEAFDEC team to review their program and develop a preliminary cost analysis for the economics. We are working with James M. Montgomery Consulting Engineers who have considerable experience in construction in Southeast Asia to develop preliminary estimates of costs of operation from existing data.

VII. REPORT ON MILKFISH PATHOLOGY PROGRAM

Basic to the spawning and rearing efforts of milkfish in captivity is maintaining them in a healthy condition. This requires in the aquaculture program a pathology/health component. Because of the youthfulness of pathology in the aquaculture field, a program in this area should be broad and diverse. This is the case with the program here at the Oceanic Institute, which has made the following contributions:

I. To milkfish health directly

A. Provided first description of a pathologic condition, gastritis, in the milkfish. The gastritis may be an etiologic factor in producing the well-known nervousness of the milkfish, or the nervousness may be the cause of the gastritis. Paper in press: Smith, A.C. Pathology and Biochemical Genetic Variation in the Milkfish, Chanos chanos. J. Fish Biol.

B. Developed a trio of simple noninvasive tests for identifying early nonspecific stress, stress related to starvation, and stress related to the early onset of disease. Papers published: (1) Smith, A.C., and Ramos, F., 1976. Occult Haemoglobin in Fish Skin Mucus as an Indicator of Early Stress. J. Fish Biol., 9:537-541. (2) Ramos, F., and Smith, A.C., 1977. Ketone Bodies in Fish Skin Mucus as an Indicator of Starvation: A Preliminary Report. J. Fish Biol., 12:105-108. (3) Ramos, F., and Smith, A.C., 1978. The C-Reactive Protein (CRP) Test for the Detection of Early Disease in Fishes. Aquaculture, 14:261-266.

C. Provided first description of allergy-like incompatibilities between milkfish and products (skin mucus) of other fishes. Article published. Smith, A.C., 1977. Reactions of Fish Red Blood Cells with Mucus and Sera from Other Fish(es). Calif. Fish Game, 63:52-57.

D. Determined in milkfish by automated chemistry analysis the baseline serum and skin mucus values for 24 chemical tests before and after the application of stress sufficient to compromise health.* The purpose was to identify (1) the key tests providing health information, particularly with mucus because of its ready availability, and then (2) the simple inexpensive commercially available kit tests for individually carrying them out.

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*Supported by the Rockefeller Foundation and the Engelhard Foundation.

E. Developed from autopsy examinations of milkfish and other fish species a concept of in vivo clotting from multiple etiologies (infection, trauma, etc.) possibly playing an unsuspected major role in causing mortalities.

F. Contributed practical information to field personnel on handling shock-like conditions and other health problems in milkfish related to capture and subsequent containment.

II. To general milkfish biology

A. Developed a simple inexpensive method for identifying intraspecific molecular variation in milkfish. Application of this technique may help to understand the basis for variations in breeding cycles among milkfish of different geographic areas by linking the variations to different subpopulations. Submitted m.s.: Smith, A.C. Reactions of Fish Nuclear Lens Proteins with Sea Cucumber Coelomic Fluid.

B. Developed working models of a simple slide test for identifying the sex of milkfish by blood and skin mucus reactions.

III. To other scientific fields

A. Invertebrate pathology

Demonstrated with the aid of milkfish serum the first known allergy-like reactions in a marine invertebrate. Three papers published: (1) Smith, A.C., 1977. Immunologic Reactions of the Sea Cucumber, Holothuria cinerascens, to Serum from the Milkfish, Chanos chanos. J. Invert. Path., 29:326-331. (2) Smith, A.C., 1978. A Proposed Phylogenetic Relationship between Sea Cucumber Polian Vesicles and the Vertebrate Lymphoreticular System. J. Invert. Path., 31:353-357. (3) Smith, A.C., 1978. Hypersensitivity and Desensitization in the Sea Cucumber, Holothuria cinerascens. Dev. Comp. Immunol., 2: 355-360.

B. Human medicine

1. Developed with the aid of milkfish serum a model of human immunopathologic conditions, viz., bronchial asthma and autoimmune disease. This work is in an advanced stage of writeup.

2. Determined that the milkfish could provide a model of human a- or hypocatalasemia. Published article: Smith, A.C., 1976. Catalase in Fish Red Blood Cells. Comp. Biochem. Physiol., 54B:331-332.

C. Comparative medicine

Described the presence of oncofetal protein (carcinoembryonic antigen, or CEA) in the milkfish. This type of protein is well known in mammals in association with certain types of pathology, particularly malignancy, but had not previously been described in fish. Paper in press: Smith, A.C. Oncofetal Proteins in Marine Animals. Comp. Biochem. Physiol.

VIII. FACILITIES AND STAFF SUMMARY

During the last year, the Oceanic Institute has made a major commitment to marine finfish aquaculture. This is best evidenced in a summary of the facilities directly involved and the milkfish program and the general support laboratories that are involved in the program.

A. Finfish Hatchery

The marine finfish hatchery has been discussed previously. This 13,400 sq. ft. building will have 4,500 sq. ft. committed to larval culture with complete capability for phytoplankton, algae, rotifer, and *Artemia* culture. The building will also have complete capability for water quality analysis.

B. Ponds

The Institute has a variety of experimental ponds available to the program. These range from 20' x 30' rubber-lined ponds for holding juvenile stocks and carrying out feeding experiments to 1/8-acre ponds for conducting experiments with adult and subadult milkfish. The ponds have a full range of water quality and salinity control. Three ponds are equipped with auxiliary aeration systems (these ponds and support systems were constructed with private funds).

C. Photoperiod Control Laboratory

The photoperiod control laboratory contains a 16' x 14' concrete tank. The lab is equipped to control temperature, salinity, and all aspects of water quality and photoperiod. Smaller tanks are also available in similar environments if the program requires these facilities.

D. Support Programs

Water Quality. A fully equipped water quality lab is kept operational at the Institute. Under the direction of Dr. Paul Bienfang and Ms. Wendy Johnson, this laboratory monitors water quality in all programs including microbiological determinations.

Pathology. The pathology program under the direction of Dr. Al Smith provides facilities to autopsy dead animals and diagnose disease situations.

Feed Lab. The Oceanic Institute maintains a basic capability to blend ingredients for the formulation of experimental diets. As the kinds of feeds required are better known, we have been promised support from the Waldron Company which operates a large feed mill on Oahu.

Engineering Services. The Institute has an ongoing engineering program headed by Guy Rothwell. He and Steve Ribakoff are responsible for the design of facilities, tanks, pond water systems. In conjunction with Robert Shleser, they carry out work on economics.

Data Processing. Rosie Rothwell-Koningsberger is responsible for data processing.

Administrative Staff. Richard Power is in charge of the administrative program. Bookkeeping, secretarial services, and drafting are provided to the program.

Library. The library maintains active subscriptions to the major journals that report results in aquaculture, physiology, fish nutrition, and endocrinology.

University of Hawaii. A close working relationship has been developed with the University of Hawaii. William Furtick, Dean of Tropical Agriculture, is one of the trustees of the Institute. John Craven, Marine Affairs Coordinator of the State of Hawaii and Dean of Marine Programs, is also a trustee.

Attachment C

XD-AAC-889-C

September 28, 1978

Dr. M. Rechcigl, Jr.
Chief, Research & Methodology Division DS/PO
Agency for International Development
U.S. Department of State
Washington, D.C. 20523

RECEIVED
OCT - 5 1978

Dear Dr. Rechcigl:

In accordance with your request for a restatement of the project scope, including a timetable and revised budget, we have prepared the enclosed documents. The primary emphasis of the program is hormone-induced spawning of milkfish. The activities identified fall into seven project areas that focus directly on the primary objective. For simplicity, we have used the numerical approach with a single number, e.g., 1, identifying the major activity. The next set of numbers, e.g., 1.1, identifies the subset of specific actions, and the next digit, e.g., 1.1.1, identifies the next level of refinement.

The program timetable diagram accompanying the program description shows the timing of the activities and their relationship to one another. Also enclosed is a chart describing the relative levels of activities. This is a companion to the program timetable and indicates the amount of emphasis which will be given to each activity for the duration of the program.

Finally, enclosed is a revised budget which was prepared in accordance with the guidelines outlined at the summary meeting on September 23. The $25,000 budget for the joint SEAFDEC program was identified separately.

If there are any questions that we can answer, please call Richard Power, Jimmy Kuo, or me at any time.

Sincerely,

Signed

Robert Shleser
Director

RSdr

Enclosures
cc: Richard Neal

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PROGRAM DESCRIPTIONS

1. Fishing Program - collection of immature and mature fish

A fishing program will be intensified to augment the milkfish stocks available to the experimental programs. This effort will emphasize cooperative efforts with private individuals as well as Institute resources. Alu Like training personnel will be involved in the fishing program.

1.1 Adult Fish

Recruitment of sexually mature milkfish will be given priority in this fishing program. The immediate goal is 50 additional fish for our broodstock by February 1.

1.1.1 Collection from local fishponds

Fishing efforts will initially focus on several privately-owned fishponds where milkfish populations have been identified. These fish will be caught by our staff and transferred to various test environments.

1.1.2 Collection from wild

Mature milkfish will be taken opportunistically from offshore areas where milkfish populations have been located in previous fishing programs.

1.2 Subadult Fish

Subadult milkfish from one to four years of age will be recruited for the Institute's milkfish stocks.

1.2.1 Collection from fishponds

Subadult milkfish captured during the pond fishing phase of this program will be added to the Institute's available milkfish populations.

1.2.2 Collection from wild

Subadult milkfish caught in the offshore fishing effort will also be added to the Institute's milkfish populations. These fish have been identified in Pearl Harbor and Kaneohe Bay.

1.3 Juvenile Fish

Juvenile milkfish of 10 or more days will be captured following the natural spawning season. These fish will be used for environmental and dietary growout studies.

1.3.1 Collection from wild

Juvenile milkfish will be captured in identified brackishwater locations when they migrate inshore. This period coincides with the natural spawning season of the milkfish.

2. Broodstock Gonad Maturation in Captivity

The progress of milkfish maturation in captivity will be monitored. This will take place in established conditions at the Oceanic Institute as well as at field sites.

2.1 Stimulation of gonad maturation through environmental control

Experimental environments will be established at the Institute to induce natural maturation processes in milkfish. A segment of the Institute's broodstock population will be maintained in each of these environments.

2.1.1 Manipulation of photoperiod and temperature regimes

A milkfish holding facility will have a regulated photoperiod of 18L:6D (to induce gonad maturation).

2.1.2 Establishment of natural pond conditions

Other holding ponds at the Institute will be manipulated to simulate salinity and food conditions found in natural settings where milkfish have been previously shown to mature.

2.2 Test various environments as locations for maturation of broodstock

Experimental pond conditions established at the Institute will be used to evaluate these manipulations on the maturation of milkfish.

2.2.1 Mature broodstock in natural ponds

The procedure established for spawning the milkfish will be tried on the adult milkfish held in natural ponds to determine its applicability. Hopeaia and Manoku ponds have been shown to produce mature fish. This will be contrasted with environments simulated after this pond (2.2.2).

2.2.2 The milkfish spawning procedure will also be tested on the broodstock fish held in the several manipulated pond environments recently constructed at the Institute. These will be compared with natural environments (2.2.1).

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2.2.3 Captive mature broodstock at SEAFDEC

SEAFDEC broodstock constitute yet another milkfish race held in different conditions which necessitate the further testing of the spawning procedure under these conditions. Results will be compared with 2.2.1 and 2.2.2.

2.2.4 Mature broodstock from wild in Philippines

Wild-caught milkfish in the Philippines represent yet another test group which must be treated to assess the milkfish spawning methods. After the available test groups have been evaluated, an optimal spawning procedure, and its possible variations, may be adequately described.

2.3 Stimulation of gonad maturation through dietary control

Supplemental and staple feeds will be varied to assess dietary effects on gonad maturation. Feed compositions will be based upon past experience and current available nutritional information on similar fish. Subpopulations of fish in test environments at the Oceanic Institute will be given supplemental feed.

2.4 Establishment of broodstock in natural ponds with supplemental feed

Adult milkfish held in natural ponds will be fed artificial feedstuffs to supplement the natural biogrowth found in these ponds. Supplemental feeding will be done on a regular basis preceding and during the maturation of gonads in milkfish held in a subpopulation.

3. Induced Spawning

Attempts will be made to spawn captive milkfish using techniques that utilize hormone injections.

3.1 Stimulation of spawning by environmental manipulation by behavioral response

Spawning of milkfish will be tried by managing environmental parameters or behavioral responses. Salinity, temperature, and photoperiodicity will be manipulated on the basis of the best information available.

3.2 Induced spawning by hormone injections

Hormone injection treatments will be used to induce spawning in captive milkfish. Various selected hormones and hormone combinations will be used on fish described (2.2.1, 2.2.2, 2.2.3, 2.2.4).

3.2.1 Define the optimal breeding procedure

When successful spawning of milkfish has been demonstrated, the optimal hormone/environmental combination will be determined. The hormone treatment schedule will also be investigated.

4. Establish Optimal Egg Incubation System and Procedure

A facility and method for the incubation of fertilized milkfish eggs will be developed using milkfish rearing literature and past experience in hatchery operations for other finfish.

5. Establish Larval Rearing Procedure/System

An experimental-scale rearing program will be developed for milkfish larvae. Experiments will be conducted to determine larval rearing techniques and other information necessary to scale up to a more practical level.

5.1 Larval food culture

Species of phytoplankton and zooplankton that have been found useful in larval rearing programs will be cultured for use with the milkfish larvae. These will include the phytoplankters Chlorella, Dunaliella, Nannochloris and the zooplankters Brachionus, Artemia, and possibly others.

5.2 Optimal environmental conditions for larval survival

Variations of environmental parameters in rearing larval milkfish will be evaluated to determine those conditions that ensure maximum larval survival. Multiple trials using different environmental parameter combinations will be tested for each possible spawning.

5.3 Test food preference of larvae

Experiments will be conducted using different food organisms at varying densities to assess the food preferences of the larvae against survival. This series of experiments may also include the use of different stages of growth found in these food organisms.

5.4 Test the relationship of food density for larval survival

Once suitable food organisms have been identified for rearing larval milkfish, experiments will be done to determine optimal combinations and densities of these organisms. These tests may extend to the use of artificial food particles.

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5.5 Evaluate stocking density to maximize larval survival

Experiments designed to establish optimal stocking densities of larvae will be performed coincidentally with the larval food experiments. Stocking densities will be tested in various rearing environments that include water exchange rates and photoperiod control.

5.6 Define optimal larval rearing procedure/conditions

The series of experiments designed to establish optimal individual rearing parameters will be consolidated to form a set of guidelines which can be used for the rearing of milkfish larvae at an experimental level.

6. Establish Fry Production Facilities

The results of all the rearing experiments performed on a laboratory scale will be used to develop specifications for prototype facilities and techniques to raise milkfish fry. This stage of the rearing period begins at day 10-14 when the larvae metamorphose and become able to accept artificial food particles.

6.1 Test temperature and salinity requirements for fry survival

Milkfish at this stage will be subjected to a variety of feeds, temperatures, and salinities in order to ascertain an optimal set of conditions for survival.

6.2 Test food density for fry survival

Rearing trials will be performed utilizing different densities of food particles of varying sizes to establish an optimal particle density and size for rearing milkfish fry.

6.3 Test various stocking densities to maximize fry production

Fry survival will be measured in experiments using different stocking densities. Water exchange rates will constitute another variable in this series of experiments.

6.4 Define food requirements of fry

Small-scale experiments will be conducted to determine the specific food requirements of milkfish fry at different stages in their growth. Available literature on rearing milkfish as well as comparison to similar fishes will serve as a starting point.

6.5 Define optimal fry production/conditions

The culmination of all the fry rearing experiments will enable the description of a model fry production facility and techniques. After testing, this guide could then be scaled up to a practical level.

6.6 Evaluate economics of fry production operation

Determination of optimal procedures for rearing milkfish fry will ultimately result in a production-oriented approach. The testing of this system will allow for extrapolations to commercial-scale operations with a concomitant economic assessment of such an operation.

7. Biomedical Program

The Oceanic Institute's Pathology Laboratory will continue studies designed to aid in the husbandry and propagation of milkfish. Results of these experiments will be implemented as improved care and treatment techniques.

7.1 Sex identification

Mucus and blood serum from milkfish specimens are being tested to develop a simple and reliable method for sex identification. Such a method will allow rapid sexing of milkfish with a minimum of handling stress. This work is a continuation of program begun in 1978.

7.2 Identification of stress symptoms

Additional experiments will establish techniques for the identification of stress symptoms in milkfish under field conditions. Medical techniques of mucus and serum testing (SMA-3) will be used to compare captive fish with wild-caught fish.

7.3 Anesthetic studies

Experiments will be conducted using different chemical compounds and techniques to determine safer procedures for using anesthesia on milkfish. Results and observations from these experiments will be implemented whenever possible during the course of this program to minimize stress to experimental animals.

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PROGRAM SUMMARY

1. Fishing Program - collection of mature and immature fish

    1.1 Adult Fish

        1.1.1 Collection from local fishponds

        1.1.2 Collection from wild

    1.2 Subadult Fish

        1.2.1 Collection from fishponds

        1.2.2 Collection from wild

    1.3 Juvenile Fish

        1.3.1 Collection from wild

2. Broodstock Gonad Maturation in Captivity

    2.1 Stimulation of gonad maturation through environmental control

        2.1.1 Manipulation of photoperiod and temperature regimes

        2.1.2 Establishment of natural pond conditions

    2.2 Test various environments as locations which favor maturation of broodstock

        2.2.1 Mature broodstock in natural ponds in Hawaii (Hopeaia, Manoku and Nomilu ponds)

        2.2.2 Mature broodstock in environmentally controlled ponds at OI

        2.2.3 Captive mature broodstock at SEAFDEC

        2.2.4 Mature broodstock from wild in Philippines

    2.3 Stimulation of gonad - maturation through dietary control

    2.4 Establishment of broodstock in natural ponds with supplemental feed

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3. Induced Spawning

3.1 Stimulation of spawning by environmental control or behavioral responses

3.2 Induced spawning by hormone injections

3.2.1 Define the optimal breeding procedure

4. Establish Optimal Egg Incubation System and Procedures

5. Establish Larval Rearing System and Procedures

5.1 Larval food culture (zoo- and phytoplankton)

5.2 Optimal environmental conditions for larval survival (temperature, salinity, water quality, etc.)

5.3 Test larval food preferences

5.4 Test relationship of food density for larval survival

5.5 Evaluate stocking density to maximize larval survival

5.6 Define optimal larval rearing procedure/conditions

6. Establish Fry (Juvenile) Production/Facilities

6.1 Test temperature and salinity conditions for fry survival

6.2 Test food density for fry survival

6.3 Test stocking density to maximize fry production

6.4 Refine food requirements of fry

6.5 Define optimal fry production/conditions

6.6 Evaluate economics of fry production operation

7. Biomedical Program

7.1 Sex identification

7.2 Identification of stress symptoms

7.3 Anesthetic studies

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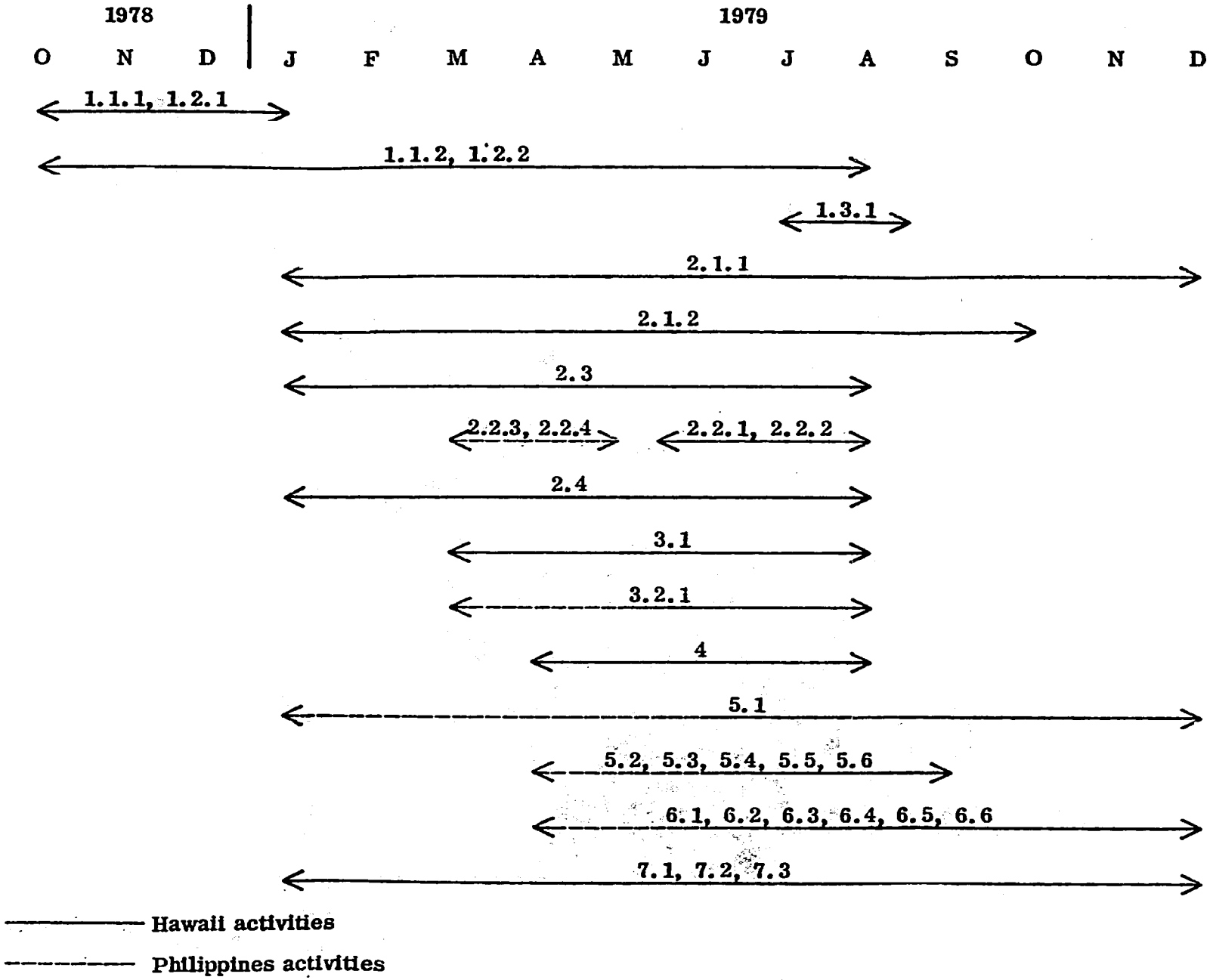

TIMETABLE OF PROGRAM

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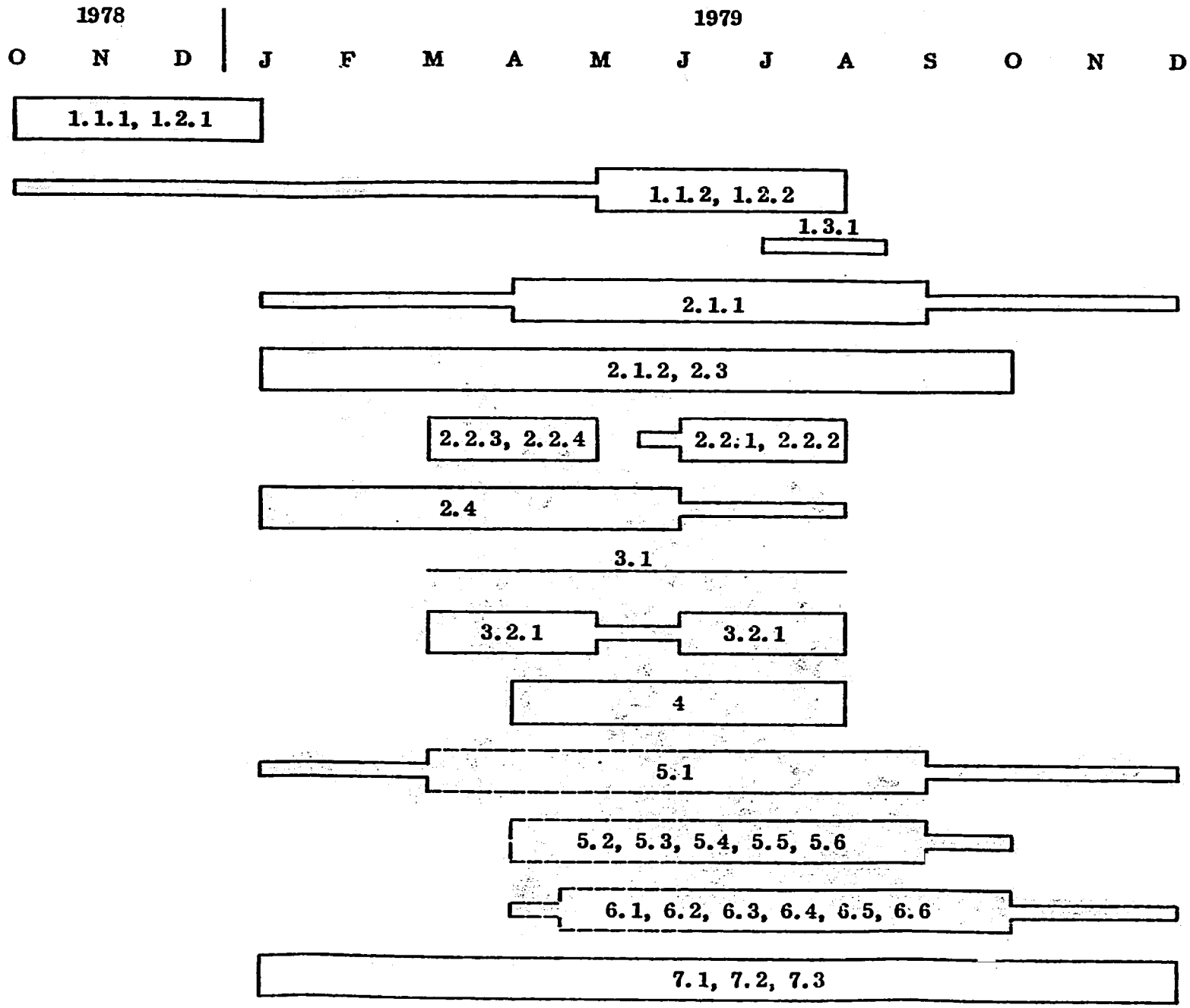
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5.2, 5.3, 5.4, 5.5, 5.6
6.1, 6.2, 6.3, 6.4, 6.5, 6.6
7.1, 7.2, 7.3

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FISCAL 1979 (JANUARY 13, 1979 TO JANUARY 12, 1980)

	Salaries and Overhead	Capital Items	Supplies	Travel Local & U.S.	Travel Overseas	Publications	Consultants	OI-SEAFDEC Program	Total
1. Broodstock management (12 mm)	17,698	1,000	5,000						23,698
2. Broodstock husbandry (12 mm)	28,318		3,000				4,000		35,318
3. Spawn condition, Induced spawning, Field breeding (38.1 mm)	117,230	2,000	3,800	2,600	4,500	1,700		25,000*	156,830
4. Larval rearing, Larval survival (35.1 mm)	71,524		5,000						76,524
5. Pathology (18 mm)	57,288	1,000	2,600			1,000			61,888
6. Coordination (4 mm)	20,766								20,766
7. Fishing (6 mm)	12,176		1,000						13,176
Totals	325,000	4,000	20,400	2,600	4,500	2,700	4,000	25,000	388,200

*For cooperative program between CI (USA) and SEAFDEC (Philippines) to conduct milkfish program in Philippines (March through May) and in Hawaii (June through August), recommended by U.S. AID RAC review team and subject to approval of U.S. AID RAC committee.

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Attachment D

XD-AAC-889-D

REVIEW OF THE PROGRAM
"RESEARCH IN ARTIFICIAL PROPAGATION OF MILKFISH"
CONDUCTED AT THE OCEANIC INSTITUTE

This review was held at the Oceanic Institute, Waimanola, Hawaii, and atfield facilities near Kona, on the island of Hawaii, September 19-22, 1978. The program under review was that established by contract AID-TA-O-1189. The original proposal provided for five years of work, and at the time of the review the program was in its fourth year of operation. A previous review conducted July 14-15, 1977, had recommended that before the fifth year of funding was approved that "the project be reviewed for adequate progress and brought back to RAC within a year". The present report results from that review.

The review team consisted of:

John Hunter
C.P. Idyll
Donald R. Mitchell
Miloslav Rechcigl
Samuel Wishik

Staff contacted included:

Richard Power
Robert Shleser
Ching-Ming Kuo
Albert C. Smith
Wade Watanabe
Craig Paulson
Guy Rothwell

SUMMARY OF FINDINGS

The team concluded that while the research effort had fallen short of achieving its full objectives, significant progress had been made, and that there is a reasonable chance that valuable results can be obtained from the fifth year of activity. The team therefore recommends that the project be approved for this final year, although with some tightening of goals to focus even more strongly on the development of techniques to promote maturation and artificial spawning of the milkfish and development of the larvae through the critical early stages. In pursuit of these objectives, the team proposes some shifts in emphasis in the work, and consequently some modification of the budget.

The availability of substantial number of ripe fish is critical to the success of this project, and the team therefore spent considerable time in evaluation of the number of fish likely to be available at various locations. There seem to be a reasonable chance that adequate numbers will be ready for hormone induction in Hawaii when the spawning

occurs in June to August of 1979, provided that efforts be started immediately to capture more fish in Pearl Harbor and other local areas. In additional supply of mature fish suitable for induction is available in the Philippines and the team strongly endorses the proposal by the Institute to accept the invitation of the Director of the Southeast Asia Fisheries Development Center (SEAFDEC) to collaborate with them in conducting spawning and larval rearing trials in that country. In order to increase the chance of success of this cooperative effort, the team proposes that AID provide funds for a small sub-contract through the Oceanic Institute to SEAFDEC to enable the latter organization to provide the necessary equipment and facilities and to help train staff at SEAFDEC in procedures.

PROJECT HISTORY

Initial funding for the first three years of this first year research project began in January 1975. Funding for this 3-year period was $528,000. The research plan approved was a broad one, encompassing a variety of activities related to the ecology, rearing, spawning, pathology and larval culture of milkfish.

Original plas for this project called for four years of research followed by a year of demonstration, and refinement of successful spawning and larval culture methods. Initial expectations of both AID and the Oceanic Institute were that methods developed earlier by the Institute for mullet could be modified for use on milkfish with relative ease. However, a number of unforeseen difficulties arose, leading to recognition that the maturation and spawning of milkfish in captivity was much more difficult to accomplish than had been anticipated. A review conducted in May, 1976 (16 months into the project) led to the conclusion that work was in accordance with terms of the contract, that it was generally on schedule, and was deserving of continued support.

A second review in March, 1977 raised several specific issues requiring modification of research plans, but the review team recommended continuation of the research.

RAC considered funding for the final two years of the project in July 1977. Funding of $738,000 for the final two years was approved with the proviso that a review team reconsider the fifth year's activities in mid-1978 and that RAC reserved the right to modify or withdraw the funds for the fifth year on the basis of the review team's findings. In addition the scope of work was reduced so that additional effort could be directed toward key problems of spawning and nutrition.

ACCOMPLISHMENTS

Methods of capturing, handling, and transporting milkfish have been improved, and a procedure for determination of the stage of gonad maturity has been developed. Holding facilities at the Institute have

been improved to enable brood stock rearing and maintenance for experimental purposes. Additions include four 1/8 acre ponds, one 0.88 acre pond and 400 ft^2 controlled environment tank. Major increases in the numbers of broodstock being held in captivity have been accomplished. At present 125 adults, 624 subadults, and 650 juveniles are being held.

Experimentation with control of light, temperature, salinity, and diet with groups of 10 adult fish has begun in the new controlled environment facility. Inability to produce fry from eggs has prevented experimental comparisons of feeds except with wild fry. A milkfish diet has been formulated based upn the best available information on nutritional requirements. During the last year a series of induced spawning experiments have been completed in Hawaii and at Christmas Island. Fish in late stages of maturation (27 on Christmas Island and 15 in Hawaii) were injected in an effort to induce spawning. Partial responses to the injections led to modification of the dosage level and procedures. Even though only some of the fish hydrated and only two actually released eggs, the researchers feel confident that revised procedures will be adequate for future induction of spawning without major modification.

Preliminary experimentation with larval culture procedures has been conducted with wild-caught, 10-day old fry. Culture procedures and equipment for live foods needed by the fry have been used successfully at the Oceanic Institute. Procedures for packaging and shipping fry have been established based upon experimentation.

Work in the pathology section has led to a better understanding of the same mechanisms of mortality of captive fish, and simple non-invasive testing procedure are being developed which will aid in early detection of stress and trauma.

Reports from the Oceanic Institute and other communications have been of high quality, timely and informative. Expenditures have been reasonable and fiscal reporting and financial record keeping procedures are in order.

PROBLEMS

In spite of the accomplishments noted above it is apparent that several of the original objectives of the project will not be accomplished during the coming year. Progress has not been entirely satisfactory because of a variety of problems encountered, especially within the last year. Present research plans are realistic and are in line with budget and professional capabilities.

Major problems encountered are as follows:

1. The capture of wild adults at sea and transport of fish to shore facilities has been extremely difficult. It has thus not been

possible in Hawaiian waters to capture the numbers of adult fish needed for experimental spawning inducement. Therefore, increased emphasis has had to be placed on maturing juveniles and sub-adults (a process requiring several years.)

2. The Oceanic Institute did not have adequate holding facilities on campus until recently.

3. Dr. Colin Nash, the principal investigator, left the Institute in June, 1978.

4. General procedures which had been successfully developed at the Oceanic Institute for inducing maturation and spawning of mullet do not work for milkfish, even though the life histories of the two fish are similar. Milkfish are much more fragile and vulnerable to injury and they require different conditions for maturation, and different techniques may be needed for induction for spawning.

5. Communication and cooperation with other research groups has not been fully effective.

6. Initial research plans were unrealistically broad and complex.

7. During the early years of the project manpower was diverted to a number of peripheral activities and consequently insufficient time was devoted to the core problems.

ESTIMATE OF THE NUMBERS OF FISH WHICH WILL BE AVAILABLE FOR INDUCED SPAWNING IN THE COMING YEAR

It is estimated that about 230 adult milkfish will be available for induced spawning in Hawaii next year, and about 200-450 in the Philippines. Some of the fish are at hand in ponds, and plans have been made to catch the rest. Some of those in captivity are pre-adult, but are expected to mature before the coming spawning season.

The fish are located in at least five different places. In Hawaii, present holding facilities are three different islands, and additional holding ponds may also be used. The remainder of fish are those at SEAFDEC in the Philippines. The supplies of fish from the two countries have to be considered separately, since each group would be used for separate series of experiments, without transfer of specimens. Among the separate facilities in Hawaii, careful planning, coordination and timely transfer of fish will be necessary to exploit the fish available.

The figures below are believed to be conservative:

Number of mature adults likely to be available;

In Hawaii - about 230

In Philippines - about 200-450

Number of mature females, assuming about a 1:1 sex ratio:

In Hawaii - about 100

In Philippines - about 100-225

Number of females ready for induction of spawning:

In Hawaii - about 45

In Philippines - about 60

Number of running males available:

In Hawaii - about 80

In Philippines - about 80

If in fact the number of fish do not exceed these conservative levels, the stocks available will be at the marginal minimal levels for spawning induction. But there is a reasonable expectation of success even at these levels, and if induction procedures (form, timing and dosage of hormones) are successful, spawning should be achieved in some of the cases. But with these numbers it will not be possible to accumulate enough data on favorable and unfavorable variables to define fully the method for replication by others.

A strong reason exists, therefore, to expend every reasonable effort beginning immediately to catch more wild adult fish in Hawaiian waters, and to place them in ponds or tanks under conditions favorable for reproductive maturation. Full documentation of experience and exposures in captivity should be maintained at least for groups of fish and if possible for identified individuals.

The Institute has suggested that staff should travel to Tahiti to assist scientists there with milkfish work. The team suggests that this not be done, since this will dilute the essential effort to complete the objectives of this project.

EVALUATION OF METHODOLOGY FOR INDUCTION OF SPAWNING AND LARVAL REARING

The methodology being used by the Oceanic Institute for induction of spawning in sexually mature milkfish is typical of techniques used successfully on other marine fishes. The method is a trial and error testing of a combination of fish pituitary extract and common mammalian gonadotropins, usually injected over an interval of 1-2 days. The variables include 1) choice of mammalian hormones (HCG or PMS are frequent choices) 2) dosage of hormones, 3) timing of the injections, and 4) the reproductive state of the female. Since the method depends little on theory, and is essentially trial and error, it requires a substantial number of sexually mature females to insure success. Further, the large size of mature milkfish increases the difficulty of handling and achieving natural spawning and fertilization. Handling often results in death or atresia of the gonads, making spawning impossible. The group at the

Oceanic Institute appears to have solved the handling problem by experience and specially developed techniques and equipment. But the small swimming pools available may be inadequate for normal spawning and fertilization because the fish may not go through their normal spawning behavior in such environments. If so, stripping and artificial fertilization may have to be employed. The Institute scientists should consider this as an alternative and make the necessary preparations. The review team recognizes that this is a less desirable technique because the number of viable eggs are often fewer than in natural spawning, but it would be a reasonable backup procedure.

Thus, if an adequate number of mature females can be obtained, the techniques to be used offer a reasonably good chance of being successful. These techniques have worked on at least five species of marine fishes, and the results of last year's work at the Institute suggest that female milkfish are responsive to HCG and fish pituitary extract.

The methods proposed for larval rearing are similar to those used successfully for a wide variety of fish and invertebrate larvae throughout the world. Food managment is one of the most critical factors. It is therefore essential that the present research plan be expanded and that an experimental design be established that includes rearing larvae under additional feeding conditions beyond those now proposed. Although filtered "small" Brachionus (rotifers) may be the solution to the small prey requirement of first feeding milkfish larvae, other foods should be included. Some possible foods treatments include Gymnodinium + Brachionus wild or culture copepod nauplii (50-100mm diameter) + Brachionus etc. As many containers as possible should be used in the design since variability among containers can be great, and unknown contagious effects within containers often occur.

MANAGEMENT

The team concluded that management and logistical problems had been responsible to a significant degree for the relatively slow progress of the project to date. We suggest that the Institute should make special efforts to overcome these management problems, to increase the probability that the project objectives can be met. It is recommended:

1. That measures be taken within the next few months to assure an adequate supply of mature fish for spawning in 1979. Although the Kona pond facilities on the island of Hawaii have a number of fish which are expected to become mature, it is possible that this number would not be sufficient for the individual studies. While it is believed that there will be at least the minimal number of fish available in Hawaii, it would be prudent to establish a link with SEAFDEC so that the fish available there can be used to supplement local supplies.

2. A close cooperation with SEAFDEC would not only increase the number of fish available to the project but it would also make available excellent modern marine laboratory facilities suitable for the experiment, and thus further reduce some of the logistical problems.

Furthermore, it would provide an LDC setting for the project and thus bring it closer to the ultimate objectives of the project. The SEAFDEC management is very much interested in this milkfish research, which they hope will lead to an increase of the available animal protein. The team emphasizes that collaboration between the Oceanic Institute and SEAFDEC would have to be based on a well-defined partnership. The roles of scientists of both institutions should be clearly set forth in a memorandum of agreement. To increase the effectiveness of the collaboration between the Oceanic Institute and SEAFDEC, the team recommends that a small amount of money (perhaps $25,000) be added to the Institute budget to support a subcontract to SEAFDEC. This would be for the purpose of training Philippine technical staff and to provide for additional facilities and equipment that might be necessary to perform the Oceanic Institute's activities.

The Oceanic Institute should take steps immediately to establish a working relationship with SEAFDEC, including a visit by senior personnel. Later, Dr. Kuo and other Institute staff should prepare for coordination of the proposed work by visiting the SEAFDEC facilities well in advance of the spawning season, to set up equipment and apparatus and to establish the relative roles of the two staffs.

NEXT YEAR' S WORK

In the remaining time available the objectives of the project should be focused on:

a. Induction of spawning

b. Rearing of the larvae in the critical days of their life (up to 10 mm in size)

Activities which might increase the effectiveness of the project include:

a. Carefully recorded data on observed life experience of groups of fish. Special events pertaining to individual non-surviving fish should be recorded, if possible, for correlation with ante mortem test results and post mortem findings.

b. Newly developed laboratory methods, such as mucus testing for early adverse reaction to trauma, should be correlated with response to attempted induction of spawning. When reasonably validated this should be incorporated into decisions on selection of experimental individuals and timing of injections.

c. If spawning follows injection of more than one female it would be desirable to plan for separate treatment and rearing of respective eggs, larvae and young fry for correlation of outcome with data on the experimental animals, injection dosage and other relevant factors.

d. Capturing more adult fish in Hawaiian waters should be organized far in advance of the spawning season. Fish should be relocated among holding facilities according to a plan for preinduction testin, examination and preparation.

e. Techniques for assessing the nutritional status of individual fish should be chosen and used in classification of candidates for induction.

There is a need for an overall sequential research plan with systematic recording of observations in form available for different purposes. Different aspects of the project, including endocrinological, pathological and husbandry components, need to be coordinated and integrated. The overall project design should spell out in detail the individual responsibilities of staff members, in a specific time frame. Work which does not relate directly to the narrowed objectives should be dropped from this project. This might include part of the pathology research.

Feeding and nutritional work should be carried out in close cooperation with a recognized fish nutritionist, and the team is pleased that the Institute is making arrangements to use the advice of Dr. John Halver. No major capital investment should be undertaken during the remaining phase of the project.

STAFF

The team reiterates the judgment of the previous reviewers of this project that the staff is competent to carry out the research. We were, furthermore, impressed with the dedication and energy of the staff. With the loss of Dr. Nas, the original principal investigator, the project had obviously been handicapped by adding a burden of logistics and other tasks to the technical responsibilities of Dr. Kuo, and the team is concerned that this has affected his efforts. It is expected that Dr. Shleser will fill this gap, and the team suggests that while Dr. Kuo retain the scientific responsibility for the maturation/spawning research, Dr. Shleser can give the necessary overall technical and logistics overview.

BUDGET

The funds budgeted for the fifth year of this project amount to $363,000. The Oceanic Institute has requested additional funds for:

1.	Pond renovation	$55,000
2.	Improvement of water system	100,000
3.	Pond Management	20,000

The team recommends against these extra items since they do not seem to be essential for the completion of this project. However, in order to increase the effectiveness of the cooperative work in the Philippines, the team suggests that about $25,000 be provided to the Oceanic Institute for the purpose of sub-contracting with SEAFDEC for training, special facilities and equipment.

The Institute may have to reprogram funds available in the budget in order to fulfill the objectives of the project. For example, travel to Tahiti

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should be dropped, while other travel may require additional funds. It may be necessary to shift project support for some parts of the pathology program to other sources of support. More support may have to be given to the fishing operations.

THE NEXT STEPS

While the goal of the present project is to induce spawning in adult milkfish and to rear the larvae through the first critical stages, the ultimate objective of the project is to develop methods for the hatchery sufficient to supply commercial producers.

To accomplish this long term objective, additional support will for specific research activities be necessary. Tasks will include:

1. Improve the reliability of spawn production and the viability of eggs.

2. Develop and implement methods to control sexual maturity such that fish in spawning condition are available on a year round basis. This work should involve descriptive studies of maturation and growth in large commercial ponds as well as experimental manipulation of variables in smaller experimental containers.

3. Evaluate growth, nutrition and stock density, with the objective or reducing the time from recruitment of juveniles to formation of sexually mature brood stock.

4. Adapt small experimental scale larvae culture and larvae food culture to large scale hatchery systems.

5. Develop a demonstration hatching and brood stock maintenance system in an LDC.

Seedstock production is seen as a separate problem from that being studied in the research under way at the Oceanic Institute. It does not necessarily have to be undertaken by the same researchers, although the Oceanic Institute may be well qualified to conduct the follow-on work. The pilot hatchery now under construction at the Institute for grey mullet would likely be suitable for milkfish. Their skills in handling brood stock might enable them to develop hatchery techniques earlier than other institutions. A Title XII arrangement with an LDC institution may be an alternative; funding through SEAFDEC may be another.

Techniques to mature and spawn milkfish in captivity seem likely to be developed soon. Plans must be developed for the follow-on project and provisions made for necessary funding in a timely manner.

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