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**TERMINAL REVIEW OF THE PROGRAM
'RESEARCH IN ARTIFICIAL PROPAGATION OF MILKFISH'
CONDUCTED AT THE OCEANIC INSTITUTE**

The terminal review of this program was conducted at the Oceanic Institute, Waimanola, Hawaii, April 28-30, 1980. The program was established by contract AID/TA-G-1189.

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PROJECT HISTORY

Funding for the first three years of this five year research project was \$528,000. The research plan approved was a broad one, encompassing activities related to the ecology, rearing, spawning, pathology and larval culture of milkfish.

Original plans for the 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. However, it was soon recognized 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 concluded that work was in accordance with terms of the contract and that it was generally on schedule.

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

AID's Research Advisory Committee approved funding for the final two years of the project in July 1977, at a level of \$738,000 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.

A third review was conducted September 19-22, 1978. It concluded that although the research effort had not achieved its full objectives, significant progress had been made. The review recommended that the program be funded for the fifth year, but urged that the goals should focus more strongly on the development of techniques to promote maturation and artificial spawning of milkfish and development of the larvae through the early states. The review urged that more attention be put on the collection of additional brood fish, and that formal and close links be established with the SEAFDEC laboratory in the Philippines.

This terminal review examined the results and conduct of the project under the following headings:

Quality of planning by the contractor.

Qualifications and use of staff.

Adherence to the scope of work.

Adherence to work schedule.

Quality of reports and scientific publications.

Coordination with other institutions.

Effectiveness of administration of the project.

Value of the research contribution.

Quality of planning by the contractor

The contractor understood the importance and purpose of this project, which was to develop reliable supplies of milkfish fry to fish farmers in Southeast Asia and elsewhere.

Planning by the contractor to achieve the purpose of the project was basically satisfactory. However, planning fell short in several areas, and this may have contributed to the failure to reach the stated goals fully. Too much reliance was placed on the success of the mullet program so that the investigators were led improperly to believe that the mullet findings were readily transferable to the milkfish. It turned out that the two species were different in a number of respects that affected the success of the project: milkfish are easily stressed, reproductive physiology is different and more complicated. The success of the mullet program was therefore a liability in the sense that it biased the planning and approaches required to fulfill the objectives of the milkfish studies.

The initial plans developed for the program proved to be too broad and to encompass too many components for available funding and personnel. This was recognized by the second review panel in 1977. At that time, suggestions were made to shift from the broad plans to a more restricted program. The team reiterated that the major program thrust was to develop methods for induced spawning of the milkfish, with racial identification having a much lower priority. The review suggested that other research elements "concerned with application of research findings should not commence until successful and reproducible methods of controlling spawning have been developed. These same shortcomings in planning were also identified by the 1978 review panel. At that time the pathology research was suggested to be dropped.

Qualification and use of staff

Some change in project personnel occurred during the 5-year period of the project but well-qualified senior scientific personnel worked on the project continuously. The principal investigator throughout the project was a scientist recognized internationally as a leader in the field of fish reproduction, and endocrinology. Second level technical staff were well-qualified biologists who gained important experience over the course of the research project, and who contributed substantially to the project. During the project the Oceanic Institute has been the leading research organization internationally on milkfish reproduction.

Adequate numbers of personnel were assigned to the research throughout the contract period. Because of the seasonal nature of parts of the research, staff members were shifted back and forth among several projects to utilize personnel and funding efficiently. Some logistical problems encountered could possibly have been overcome if additional manpower had been available, but these problems occurred because of unforeseen circumstances and not because of a shortage of personnel.

Adherence to the scope of work

The original scope of work was set out as follows:

The single objective of the project was to develop effective and controlled means of producing seedstock of the milkfish, Chanos chanos, upon which mature fish production enterprises are based, and to develop subsequently effective distribution systems for the fry.

The scope of work is summarized as follows:

1. Establish broodstock in captivity
 - a. Collection of mature fish in season (at sea)
 - b. Collection and growing-out of immature fish (from brackishwater ponds)
 - c. Collection of migratory fish (from known runs)
 - d. Development of broodstock husbandry
 - e. Holding, Handling, and sampling large fish
 - f. Identification of broodstock individuality
 - g. Year-round breeding through environmental control.
2. Establish conditions for spawning
 - a. Determination of natural spawning conditions (through location of spawning sites)
 - b. Simulation of spawning conditions in laboratory (by environmental control)

- c. Attempt spawning without hormone treatment (by behavioral responses).

3. Induce spawning by hormone injection

- a. To define the optimum induced spawning procedure (for salmon gonadotropin, specifying correct time for treatment, dosage, dose rate, response, etc.)
- b. To determine cost effectiveness of readily available hormones.

Experiments will include:

- 1) Determination of natural reproductive physiology (for both sexes) from immaturity
- 2) Determination of responses to hormone treatment
- 3) Testing reactions to salmon pituitary gonadotropin
- 4) Testing reactions to other cheaper hormones.

4. Improve survival of larvae in laboratory

- a. Nursery I (day 0 - day 21*) development
Definition of Nursery I rearing procedure with recommended facilities, food and food density, rearing density, water quality, and external environmental conditions, etc.
- b. Production high survival (%) from available eggs.

5. Increase hardiness of larvae to juvenile stage

- a. Nursery II (day 21 - day 50*) development
Definition of Nursery II rearing procedure with recommended facilities, food and food density, rearing density, water quality, and environmental conditions.

Produce juveniles larger and healthier than those caught and distributed by the existing farming operators and define expected products.

- b. Economics of operations; low cost of juveniles.

*An estimated stage differentiation.

6. Improve handling and husbandry of juveniles

- a. Improve collection of juveniles from Nursery II facilities
- b. Develop safe transportation methods
 - 1) Develop safe procedures for mass collection and transportation of nursery stock.
 - 2) Recommend optimum economic transfer method for fry distribution, from unknown operational costs and survival factors.
 - 3) Restrict receiving facilities on farms

This scope of work was amended by a review team from AID in May, 1976. The team recommended that a request by the Institute to add funds for pathology research be approved, and that proposed research, design and construction of low cost pens and enclosures for production of fish be removed from the scope of work.

In July, 1977, AID's Research Advisory Committee reduced the scope of work so that additional effort could be directed toward key problems of spawning and nutrition.

A brief discussion is given below on each of the points listed in the scope of work.

1. Establishment of broodstock in captivity

Not enough brood animals were captured or maintained to carry out fully the major parts of the program. The insufficient numbers of brood fish that were available for the spawning induction experiments may have resulted from a misjudgement as to the ease with which fish could be captured off Hawaii, and to insufficient effort to capture and keep enough fish. There may have been too great a reliance on the fish kept in the ponds on the island of Hawaii. The numbers of brood animals there (77 in July and August 1979) were thought to be enough for the induction experiments. Yet in the experiments of that year only 6 fish were apparently in proper reproductive condition, and in fact none was successfully induced. This disappointing result may be because the time of maturity of these fish was earlier than expected or that the conditions in the ponds (e.g., only 6 ‰ salinity were not conducive to spawning). Yet, the fact is that not enough ripe fish were available.

A tantalizing aspect of this project is that when it ended it seemed to be on the verge of success. The results in the Philippines and on Tahiti encouraged the expectation that with more fish the successful induced spawning could be repeated; and with still more fish, modifications to the techniques could have been tried which would have led to successful fulfillment of the basic objective of the project: the development of effective means of producing young milkfish to supply to commercial farmers.

In the Philippines, where the work in 1979 might well have resulted in several more successful induced spawnings if more fish had been available, a typhoon destroyed the traps that were being used to supply brood stock. Thus, bad luck has to be accepted as one factor contributing to this deficiency in the program. Nonetheless, the Institute staff may have failed to pay sufficient attention to the necessity to collect enough experimental animals for the core activity of the program.

The lack of sufficient numbers of brood fish was a matter of concern to the AID review team in late 1978, and one of its principal recommendations was to increase the effort to catch and maintain more fish. Results of the work suggest that not enough effort was applied to this activity in the last year of the project.

2. Establish conditions for spawning

Simulation of spawning conditions in the laboratory by environmental control was attempted by holding 12 fish in a concrete tank at a constant photoperiod (18L/6D) at ambient temperatures (20° to 27° C). After 15 months of holding, these fish exhibited no gonad development.

It appears that this experiment could have been designed with more skill. Rather than a single photoperiod/temperature regime, one or more variables could have been tested. However, given the numbers of experimental fish available, and the laboratory facilities of appropriate kind, it may be that OI adhered as closely as feasible to this part of the scope of work.

Subadults were placed in the pond at Lanuipuaa on the island of Hawaii to provide brood stock for the induction experiments and to determine under which environmental conditions maturity takes place. This work started in 1977, and in 1979, 77 adults were found to be sexually maturing in July and August. However, while the males were fully ripe, few of the females were at the proper stage of ovarian development to be used for induction experiments, the oocytes being smaller than 0.6 mm diameter.

Milkfish were established in 1/8 acre ponds on the Institute grounds. One pond was maintained at a salinity of 32 ppt and another at 15-20 ppt. None of the fish in the latter pond showed signs of gonad maturation, while the majority of the fish in the former pond showed some signs of maturing: five were running males, two were vitellogenic females showing significant progress in gonad maturation, but were not fully mature; five showed no signs of maturation. If it transpires that fish maturing in ponds can be induced to spawn, these results will be useful. Thus, after suitable experimental ponds had been built (the need for which was recognized after the program began) this aspect of the scope of work showed progress.

It seems certain that nutrition plays an important role in the maturation process. Late in the program, OI launched research on nutrition in an attempt to determine nutritional needs of milkfish as a guide to preparation of appropriate diets. With the collaboration of faculty and graduate students of the Department of Animal Science at the University of Hawaii, studies were launched on the biochemical analysis of the gut contents, digestibility, dietary composition passage and protein requirements. Such work is judged to be a useful contribution to the fulfillment of the scope of work.

3. Induced spawning by hormone injection

This part of the work can be regarded as the central activity of the milkfish project. The project was conceived by the Institute and accepted for funding by AID to a large measure by the success of induced breeding of mullet by OI staff. It was believed that techniques used for the mullet should be transferable to the milkfish. Because of this background, it can fairly be said that other aspects of the program were ancillary or supportive of the development of procedures to produce viable milkfish fry by induced spawning with hormones.

The Institute staff kept this phase of the work foremost in their minds, and they therefore did adhere to this phase of the scope of work. OI biologists made good progress in identifying the stage of ovarian development at which the oocytes are responsive to exogenous hormone treatment. It is clear, however, that more experience and skill must be accumulated on this point, since some fish apparently ready for hormone injection failed to mature while others seemingly in the same stage did so.

The kind of hormone and the size and frequency of dose have been determined so that at least in some cases (a total of four) successful spawning was induced. Clearly, considerable refinement of this technique is necessary, but it appears that enough is known that success can be achieved in cases where the gonadal development of the fish is appropriate.

As discussed above, it seems possible that if more fish at the proper stage of development had been available, the project would have ended with induction procedures having been demonstrated on a reproducible basis, and the foundation having been laid for designing a production system to supply milkfish fry on a commercial basis. Further work seems likely to achieve this, and the system will probably be built to an important degree on the results of the Oceanic Institute research.

Among the several differences between the mullet and the milkfish that made transferability of the mullet research difficult was that it proved impossible during these experiments to accomplish milkfish spawning in tanks. The alternative was to strip eggs from ripe females and fertilize them with sperm from a stripped male. In some cases ripe females were on hand following successful induction, but no ripe male was available. Late in the project experiments were conducted which suggested that milkfish sperm can be stored for considerable periods and still be effective. This has still to be confirmed, and more work requires to be done in this area. Nonetheless, this aspect of the research will be valuable in assisting future work.

4. Improve survival of larvae in the laboratory

Very few larvae were available for testing their survival in the laboratory. The only case where this was possible was after the spawning in the Philippines in 1979. These larvae were raised with a satisfactory survival rate. They began to feed from the third day and reached the fry state (12-15 mm) in 15 days. They were described as hardy and very resistant to environmental fluctuation. They were "easy to raise" on a rotifer diet.

Within the severe constraints of the lack of experimental animals, this part of the work was performed satisfactorily.

Parts 5 and 6 of the scope of work ("Increase hardiness of larvae to juvenile stage" and "Improve handling and husbandry of juveniles") could not be accomplished for lack of experimental animals.

In summary, the Institute adhered satisfactorily to the scope of work. The only significant reservation to this was one of emphasis: that more attention should have been paid to the procuring of adequate supplies of experimental animals. Unfortunately, this deficiency made it either more difficult or in some cases impossible to fulfill the other objectives of the program.

Adherence to work schedule:

The contractor was unable to adhere to the original work schedule because the overall research problem proved to be more complex than had originally been anticipated. AID staff and the AID Research Advisory Committee recognized this difficulty and revised the work schedule twice to permit more effective use of available resources.

The problems resulting in failure to adhere to this work schedule were characteristic of those encountered in pioneering research with a poorly understood species. The slow progress toward research objectives during the first 2½ years of the project is not indicative of unsatisfactory performance but resulted from the multifaceted approach used rather than a narrow focus on the most critical research topics. Following redefinition and narrowing of the scope of work and the work schedule for the fourth year, and again for the fifth year of the project, the contractor was still unable to adhere to the work schedule. Technical problems listed below contributed to this

The principal obstacle to completion of planned work on inducing maturation and spawning was a lack of adequate numbers of maturing fish. This is discussed above, but it is worthwhile listing some factors contributing to the shortage of fish: (1) difficulty in capturing wild fish in Hawaiian waters, (2) difficulty in handling and holding the large, easily traumatized fish, (3) theft of fish from holding ponds, and (4) failure of fish to mature in captivity. Failure to mature in captivity may have been partly due to the young age of fish being held, plus the fact that large numbers were not introduced into holding ponds until the third year of the project.

A second obstacle to completion of the work was that during the early years of the project research activities were varied and numerous, and this prevented sufficient time being spent on the most critical problem areas. Thirdly, poor communication or misunderstandings with SEAFDEC personnel during the first visit of Institute personnel to the Philippines was an obstacle to cooperation between the groups. This was not resolved until the final year of the project.

Logistical problems of several types hampered research progress. Field activities on the Christmas Islands (as well as those on the Island of Hawaii) ran into difficulties from lack of vehicles, fuel and tanks, or from shortages of ripe males.

Controlled and replicated experiments on hormone dosage was not possible because no more than a few maturing females were ever available at the same time.

Quality of reports and scientific publications

Technical and fiscal reports were submitted to AID on schedule and in an acceptable form. The annual reports were candid and useful to the reviewers. Areas of difficulty and barriers to success were as readily identified as the accomplishments. These reports afford the opportunity to review progress of the program chronologically so that progress as well as recurring problems can be noted. Changes to program elements can also be identified.

The annual reports are also of benefit to the research team. In addition to the chronological listing of events, accomplishments and problems, the reports provide a review mechanism for the status of the program.

Beginning with the third annual report the Oceanic Institute listed scientific publications, "...either directly concerned with or related to the current milkfish propagation project at the Institute". Lists were also included in the fourth and fifth annual reports. A total of 34 titles were provided, but some were duplicates because their first listing had been when the paper was submitted or when publication was pending, and a later listing was made when publication occurred.

Three papers (published in refereed journals) are judged to be major publications directly related to the central problem of developing a method of induced spawning for milkfish; five papers (some also published in refereed journals) were related to associated problems (such as detecting stress and monitoring the health of milkfish); seven papers were related to associated or peripheral problems. Some manuscripts listed were only loosely related to the project.

Coordination with other institutions

Coordination with other institutions doing research on milkfish or interested in the activity was extensive. This included contact with a number of foreign laboratories and fisheries departments, and with United States institutions.

Since the major thrust of the work was to develop methods of producing large numbers of milkfish fry for farmers in Southeast Asia, communication with institutions in the developing countries was especially desirable. In the reviews by AID the Oceanic Institute was encouraged, and indeed prodded, to pay special attention to such relationships.

The Institute realized early that it would be valuable to work closely with the Southeast Asia Fisheries Development Center (SEAFDEC), and the OI Director visited the Aquaculture Department of that institution in the first year of the project. An international symposium on milkfish was sponsored by SEAFDEC in May 1976. Scientists from the Institute attended this, and agreed to assume responsibility for coordination of data on reproductive physiology, health and stress of the fish, and racial identification. Unfortunately, this promise of collaboration among research institutions did not come to much, and the machinery for exchanging information has been little used.

In 1977 arrangements were made for staffs of the Institute and of SEAFDEC to take part in induced spawning trials at the other's facilities. Three OI scientists visited the Philippines for this purpose. In this instance they were disappointed to be restricted to the role of spectators, and this experience reduced the enthusiasm for collaboration.

The next year on the advice and urging of the AID review team, the President and the OI chief scientists visited Iloilo, the site of the SEAFDEC aquaculture facility and concluded a formal agreement for collaboration. This has resulted in fruitful exchanges. In 1979, four OI staff took part in attempts at induced spawning experiments in the Philippines. The team was able to assist the SEAFDEC scientists to improve their techniques in induction and in larval food reproduction systems. In return, four SEAFDEC biologists took part in induced spawning experiments in Hawaii.

The collaboration with SEAFDEC will be further strengthened since Dr. Kuo, the Institute's former chief scientist on the milkfish project, is now stationed at the SEAFDEC facility, supported by the International Center for Living Aquatic Resources Management (ICLARM).

Collaboration has also been carried out with French Polynesia and Taiwan. At the request of officials in Tahiti, induced spawning trials were carried out at Rangiroa Atoll on two occasions. Taiwan was visited several times by OI scientists, especially the Fishery Centers in Tungkung and Tainan where milkfish spawning induction trials are proceeding. Information and advice were provided. Further, commercial milkfish farmers were given information on these techniques, and reports have been received that some of these persons have succeeded in spawning some fish and in raising small numbers of larvae.

Effectiveness of administration of the project

Administration of the project by the Oceanic Institute has been good. The three reviews of the project conducted by AID praised the Director and his staff. The first review stated that the "leadership provided by Colin Nash appears to be most effective", and "the staff is extremely competent professionally." The second review echoed this, saying that the team was "impressed by the calibre of the scientific staff and its director, Dr. Nash....It is a tribute to this director that the staff works well together as an interdisciplinary unit....(and that he has) the ability to keep the staff together in the face of moderate salaries and inadequate fringe benefits...(this) speaks well for the respect and confidence that this staff has in the director."

The fact that the milkfish project did not accomplish its major objectives is due in major part to the fact that the problem is bigger than had been conceived by the Oceanic Institute and AID, but some deficiencies of management may have contributed to the partial failure. For example, the third review noted that "management and logistical problems had been responsible to a significant degree for the relatively slow progress," and the other reviews pointed out some deficiencies in the planning and conduct of the project, which may reflect on the management. A major deficiency seen was that the work plan was too ambitious. This is more easily recognized in hindsight, and any criticism here must be shared with AID, which approved the research plans. Yet review teams pointed out that the Institute was spending effort on a number of activities which were only peripheral to the main objective of the project. Such efforts may have prevented better progress in other areas.

The lack of fully effective contact and collaboration with other groups working in this field is discussed above, where it is recognized that cooperation is a two-way street. The review team is aware of efforts by staff of the Oceanic Institute to work cooperatively with other groups, only to have these offers turned aside. Yet collaboration with LDC countries was stressed from the beginning of the project, and the Institute did not push this aspect very hard until after the third review, in which the Institute staff was urged to do so in specific terms.

The Oceanic Institute was responsive to most of AID's suggestions for changes in the project and its conduct.

As a result of reviews and Research Advisory Committee evaluations, new directions were called for in 1977 and again in 1978. The Institute was responsive to most of the suggestions made in 1977, which narrowed the research area slightly. Notable exceptions were that interaction with other institutions was not improved substantially and that nutritional work suggested by the first review team was not carried out satisfactorily.

It should be kept in mind the second review called for a specific operating plan for years four and five, with emphasis on induced spawning. The Institute was responsive, since it organized and followed a specific research plan of the type requested. The second review noted the need for better holding facilities for milkfish, and drew attention to the unsatisfactory nature of the system of using old fish rearing ponds owned by others. Following the review the Institute improved its facilities, but the changes were made too late to be of significant value. In response to questions raised by the second review team and the Research Advisory Committee regarding the value of racial studies, this work was discontinued.

The third review team concluded that although the research had fallen short of its full objectives there was a reasonable chance of obtaining valuable results during the fifth year. The Institute was encouraged to tighten its focus still more, to improve cooperation with SEAFDEC, and to take additional steps to insure that adequate numbers of maturing adults would be available during the final spawning season. The Institute made a serious effort to be responsive: research focus was changed, and cooperation with SEAFDEC was improved. But another of the review teams requests was not complied with fully since sufficient number of adults were not obtained for the induced spawning research. This was for a variety of reasons, some of which were beyond the control of the Institute.

It appears that management by AID was generally satisfactory, but some reservations are necessary. The original research plan and scope of work was too ambitious for the amount of money and time available. As indicated above, it is easier to see this after the fact. As is also mentioned, the responsibility for this error in judgement must be shared by AID. As the project proceeded both sides recognized that the range of activities had to be narrowed, and the Institute readily acceded to AID suggestions that this be done.

Value of the research contribution

Program goals set for this study were to develop techniques to breed the milkfish (Chanos chanos) in captivity and to raise the resultant fry. The techniques developed and information gained would be transferred to other research and user groups through publications, communications, workshops, and cooperative working arrangements. Although research efforts have fallen short of these goals, significant progress was made in increasing our understanding of various components of the life history of the milkfish. These advances will increase the chance of developing commercial culture technique in the future.

While some of the research findings are substantial, in many cases they are incomplete, presenting only a partial solution to the major barrier to milkfish culture, the shortage of fry. In other cases, findings identified a need for further research, including areas where information is sparse or is totally lacking. Nevertheless, the research accomplishments integrated with findings from complementary studies carried out by other groups in (Taiwan, French Polynesia and the Philippines) are forming the basic building blocks necessary for milkfish culture. One point of concern is whether the technology for culturing the Hawaiian milkfish is fully adaptable to other races of this species. Hawaii is at the edge of the milkfish range, and oceanic conditions there are not typical of the regions where milkfish farming takes place. Several differences have already been found, such as growth and maturation. Whether these differences will manifest themselves in culture technology is not known.

In summary, substantial progress was made by the Oceanic Institute in solving the problem of developing procedures for producing large quantities of milkfish fry in hatcheries. The Institute failed to fulfill its objectives completely, partly because the difficulties of accomplishing the task proved to be considerably greater than had been expected. Further, the project staff encountered a number of mechanical, logistical and other difficulties that could not have been anticipated. What the project did accomplish was to increase knowledge of the biology and behavior of the milkfish; to demonstrate that spawning could be induced and artificial fertilization be accomplished; and to show that fry could be raised in the laboratory. Enough progress was made to suggest strongly that milkfish could be induced to spawn and that vigorous fry could be produced. While many questions remain to be answered, the research performed by the Oceanic Institute will be of significant value in future work on this problem.