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ANNUAL REPORT SUMMARY SHEET

RESEARCH IN ARTIFICIAL PROPAGATION OF MILKFISH AID/ta-C 1189
Project Title and Contract Number

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1/13/75 - 1/12/80 Contract Period (as amended) From - To	1/13/78 - 1/12/79 Reporting Period From - To
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A. SUMMARY

The program objective is to breed the milkfish, Chanos chanos Forskal, in captivity and to raise the fry from egg to fingerling size. Carrying out any research and development which may be necessary to attain the goal is within the scope of this work.

During this year, holding capacity has been greatly expanded to accommodate increased numbers of captive milkfish stock which resulted from intensive fishing activities at sea and in local fishponds. A total of 31 adults, 175 subadults, and 650 juveniles were added to the resident population of fish on site. A substantial number of subadults (360) and juveniles (226) were also recruited in the Ahuipuaa pond complex on the island of Hawaii, where resident stocks of milkfish adults have been matured in captivity.

During the past year, additional sources of mature adults from Nomilu pond on Kauai, Isle and Pelican Lagoons on Christmas Island, and Rangiroa Atoll in French Polynesia were explored. Induced-spawning trials have also been conducted in these locations.

The induced-breeding technique using carp pituitary homogenate (CPH) and human chorionic gonadotropin (HCG) as triggering agents is being used to reinitiate the final oocyte maturation of the milkfish. The responsiveness of the breeder to the hormone treatments for producing fertilizable eggs depends on the physical conditions of the recipient and the maturity stage of ovarian oocytes. The injection of a priming dose, 10 to 20 mg CPH and 1,000 to 2,000 IU HCG per kg body weight, followed by double-dose injections of the hormones, stimulates oocyte hydration when the recipient fish, with eggs at yolk globule stage larger than 0.7 mm in mean diameter (preferably 0.73 mm), is hypophysated.

An individual female fish with yolk-laden eggs smaller than 0.7 mm is considered to be in the maturation phase of ovarian development. At this stage the number of injections of hormones required to advance development may unnecessarily stress the fish and cause death. The response of such a fish to the hormone treatment was found to be negligible.

Mature milkfish adults were found in the various environments with salinities ranging from 5 to 196‰. Osmotic acclimation and periodic egg-sampling of the captive mature individual from either brackish or hypersaline waters produce physiological stresses which cause atresia of the oocytes. This characteristic is evident in the female at the late phase of maturation, but not observed in those at the early phase of ovarian development. Injection of about 10 mg CPH and 1,000 IU HCG per kg body weight, given within 26 hours after the egg-sampling stress, has been found to be an effective method of preventing the oocytes from atresia.

Studies on fry transport were considered essential to establish the most efficient means of distributing the stocks to the farmer. It has been established that 10- to 14-day-old fry can be safely transported in 18‰ brackish water in sealed plastic bags filled with air, at a density of up to 500 fry per liter for as long as 7 days, and at a density of 1,000 fry per liter for 4 days.

Work on health care has been diversified. The use of automated chemical analysis of blood and mucus has been investigated as a means of monitoring health conditions of broodstock. The levels of Cl, K, and LDH can be used as indications of change in the physical condition, resulting from oxygen deprivation or other stresses. Commercially available test kits for these determinations are presently being evaluated as a means of providing the aquaculturist with an easy way to assess the health of milkfish.

Routine autopsies of fish which died in our program have revealed in vivo blood clots which appear to be related to mortality. A protamine sulfate test is being adapted for milkfish sera to detect pathologic conditions (e.g., infection, traumatic tissue injury, shock, malignancy, etc.). This and a new test from the medical field, the Thrombo-Wellcotest, have produced promising results and will be applied to the mucus sampling work.

Simple tests on mullet serum to identify sex are in progress. This approach will be applied to the milkfish sera and skin mucus for the purpose of carrying out sex identification of broodstock.

B. PROJECT OBJECTIVES

The main objective of the project is to develop effective and controlled means of producing seedstock of the milkfish, Chanos chanos Forskal, upon which 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. Collect mature fish in season (at sea).

   b. Collect and grow out immature fish (from brackishwater ponds).

   c. Collect migratory fish (from known runs).

   d. Develop broodstock husbandry methods.

   e. Develop holding, handling, and sampling methods for large fish.

f. Identify broodstock individuality.

g. Establish year-round breeding through environmental control.

2. Establish Conditions for Spawning

a. Determine natural spawning conditions (through location of spawning sites).

b. Simulate spawning conditions in laboratory (by environmental control).

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

3. Induce Spawning by Hormone Injection

a. Define the optimum induced-spawning procedure (for salmon gonadotropin, specifying correct time for treatment, dosage, dose rate, response, etc.).

b. 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, and

(4) Testing reactions to other cheaper hormones.

4. Improve Survival of Larvae in Laboratory

a. Nursery I (days 0-21) development
Define Nursery I rearing procedure with recommended facilities, food and food density, rearing density, water quality, and external environmental conditions, etc.

b. Produce high survival (%) from available eggs.

5. Increase Hardiness of Larvae to Juvenile Stage

a. Nursery II (days 21-50) development
Define Nursery II rearing procedure with recommended facilities, food and food density, rearing density, water quality, and environmental conditions, etc.

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

c. Establish economics of operations; low cost of juveniles.

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.

C. ACCOMPLISHMENTS TO DATE

1. Broodstock Collection and Husbandry

a. Wild Fish

The fishing effort for milkfish was intensified in Pearl Harbor and in Kaneohe Bay during the breeding season of 1978. Additional adult milkfish were captured from private fishponds on Oahu. The purpose of intensive fishing activities is to increase the size of milkfish stock in captivity. Access to fish in these waters requires several permits. Fishing within Pearl Harbor is severely restricted by the Navy, and all fishing, particularly in the breeding season, requires permits from the State of Hawaii Division of Fish and Game. Fullest cooperation was received from both military and State authorities for Institute staff to collect milkfish in these waters.

A total of 31 adults, 175 subadults, and 650 juveniles were successfully transported to the Institute. The mature adults were used for induced breeding and the others were added to the milkfish stock on the OI campus for various purposes.

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. 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 abrasians 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 four days' acclimation period during which the salinity is adjusted to the natural conditions prior to the distribution of fish into the permanent holding tanks or the experimental environment.

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 12 fish under the experimental conditions 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 freshwater 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 are available for milkfish growth and maturation trials.

(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.

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

b. Pond Fish

The captive milkfish adults in the Hopeaia and Manoku ponds of the Lahuipuaa pond complex located on the Kona coast of Hawaii were again found to be sexually mature. The ponds cover 3.5 acres in area. Biologically and geologically they are unique, lying exclusively in recent lava flows and harboring both marine and brackishwater biota. They are irregular inland ponds with no open sea connection, but are connected to the subsurface seawater table and have tidal rhythm. Flushing rates are moderate with a high level of nutrients entering the pond system with high freshwater flow. High primary productivity of the ponds with a high standing crop are evident. Extensive growths of Ruppia maritima provide significant surface area for epiphytic diatom growth. The algal species include Navicula, Nitschia, Gyrosigma, Ulva, Rhizosolenia, Chlamydomonas, Chlorella, and others. Extreme porosity of the bottom virtually eliminates any possibility of fertilization. The salinity ranges between 5 and 8 ‰ and the temperature between 25 and 29° C in the summer season.

Maintaining the milkfish population in this pond complex is crucial in order to ensure mature adult availability for induced-breeding use and to establish environmental conditions for gonadal development.

A total of 77 subadults were stocked in the Lahuipuaa pond complex in 1977. They were examined in July 1978. The growth of these 3-year-old fish was found to be satisfactory; they are expected to reach maturity and to be available for induced breeding in the 1979 season. In addition to these previously stocked subadults, continuous recruitment of juveniles and subadults was emphasized; a total of 360 subadults (2 years old) and 226 juveniles were added to the resident population in 1978.

An additional source of milkfish adults suitable for induced breeding was explored in the Nomilu pond on the island of Kauai. This is a larger (26 acres), highly productive pond with a population of about 40 mature adults. Salinity ranges from 38 to 42 ‰. The pond has a satisfactory water-control mechanism for nutrient accumulation and a low flush rate. There is a large diversity of algal species: Thalassiosira, Navicula, Chlorella, Gyrosigma, Ulva, Rhizosolenia, Nitschia, Coscinodiscus, and others.

Preliminary surveys of the broodstock availability on Christmas Island were conducted in August of 1976 and in February of 1978. The milkfish population has proven to be abundant and accessible to induced-breeding trials. The mature adults were captured mostly from Isle Lagoon and Pelican Lagoon, which are both hypersaline; salinity is 76 ‰ in Pelican Lagoon and 102 to 106 ‰ in Isle

Lagoon. Measurements of fork length, body weight, and gonad weight were taken, and gonadal development was also examined (Appendix I). The size of these mature adults ranged between 0.58 and 1.56 kg in body weight and between 31.4 and 46.2 cm in fork length, although they were 4 years of age.

The size at which a milkfish reaches sexual maturity is dependent upon the environmental conditions; age is also a determining factor for this species. A mature milkfish in brackishwater ponds in Hawaii, and in the open sea in Hawaii and the Philippines, is usually about one meter long and weighs in excess of 14 pounds. In comparison, milkfish in the confined lagoons of Christmas Island where salinity ranges from 76 to 102 ‰ reach maturity at an average size of 0.37 m in fork length and 1.5 pounds in body weight.

c. Fry Transport

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 growth studies.

The transport experiments of milkfish fry up to the density of 100 fry per liter were conducted in 1977. The results are shown in the report of 1977. The experiments continued this year when new recruits of milkfish fry became available from the wild. Densities from 50 to 1,000 fry per liter were examined. The results indicated that 10- to 14-day-old fry can be safely transported at densities up to 500 fry per liter for periods as long as seven days and at a density of 1,000 fry per liter for four days (Table 1). Salinity was 18 ‰, with an air-to-water ratio of 5:1.

Table 1. Results of fry transport experiments for a 7-day period

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

*In the preliminary test, 100% mortality was noted at the end of the 7-day experimental 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.

d. Environmental Regulation of Captive Stock

The approach of developing conditions which mimic the natural environments where milkfish were known to achieve sexual maturity was taken to establish mature captive stock. The environmental conditions in which milkfish with mature eggs are found were assumed to be 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, a two-pronged approach to spawning milkfish in captivity was emphasized: establishing stocks in environments which are known to be suitable, and building manageable environments which mimic the conditions where the milkfish normally mature to spawning condition.

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 12 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.

The gonad development of these fish was examined after 10 and 20 weeks and no development of eggs had occurred at those times. The health condition of the adult fish in this confinement was shown to be excellent, and the study is still in progress.

Hopeaia and Manoku ponds, in which the resident population matured in the summer season, were used for maintaining the broodstock in the natural pond environment. These ponds presently have 50 resident adults and 77 of the 427 subadults that should reach maturity in the breeding season of 1979.

Two 1/8-acre dirt ponds on the OI campus mimic the conditions of these natural ponds for establishment of mature broodstock. The experiment is presently in progress. One pond maintains salinity at 8 ‰ and the other at 32 ‰. The most common algal species found in the ponds where the milkfish matured were introduced and have become established.

e. Nutrition

Nutrition plays an important role in the maturation process. However, until sufficient stocks of milkfish and manageable facilities are established, it is premature to start on a nutrition program.

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

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 nutritional requirements of the milkfish. Previous works have 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. However, a basal diet of high protein was formulated and this consists 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 diets as supplements to natural nutrition. A more practical food for these herbivorous captive fish is a modified form 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.

2. Induced Breeding by Hormone Injection

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

a. The Reproductive Cycle

Individual statistical data have been accumulated to define the reproductive cycle of milkfish in 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; 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.

b. 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 duration of the fieldwork activities was determined by charter flight availability between Hawaii and Christmas Island and accessibility of mature milkfish adults.

There were many logistical problems, e.g., gear shipment from Hawaii to the field site, limited availability of transportation, and a petroleum shortage on the island when the fieldwork was undertaken. The field activities during the first week were, therefore, directed only to facility setup at the base located at Artemia Corner on the island (Fig. 1). No seawater system was available upon arrival, so a temporary seawater pumping system was installed with available materials. The distance between the seawater intake and the holding tanks was about 150 yards. Due to the oceanographic and physical conditions at the site, the system became a troublesome part of the field operation requiring frequent maintenance and attendance.

Despite the various difficulties, significant biological information was obtained. For example, sexually mature milkfish adults are readily obtainable from Pelican and Isle Lagoons, where salinities are 76 ‰ and 106 ‰, respectively. The milkfish in these highly saline lagoons are smaller with mature adults ranging between 34 and 40 cm in forklength. They have adapted to these high-salinity conditions and, hence, differ from the breeders found elsewhere, where salinity is 35 ‰ in the Philippines and 7 ‰ in Hopeaia pond on the island of Hawaii.

Two problems were solved before induced-breeding experiments could be pursued: (1) acclimation conditions to mature breeders in captivity following capture and long-distance transport, and (2) optimal salinity for reproduction and embryonic development.

During the period of April 27 through May 16, a total of 71 milkfish adults from nine fishing trips were subjected to various holding systems. Three types of holding systems were tested:

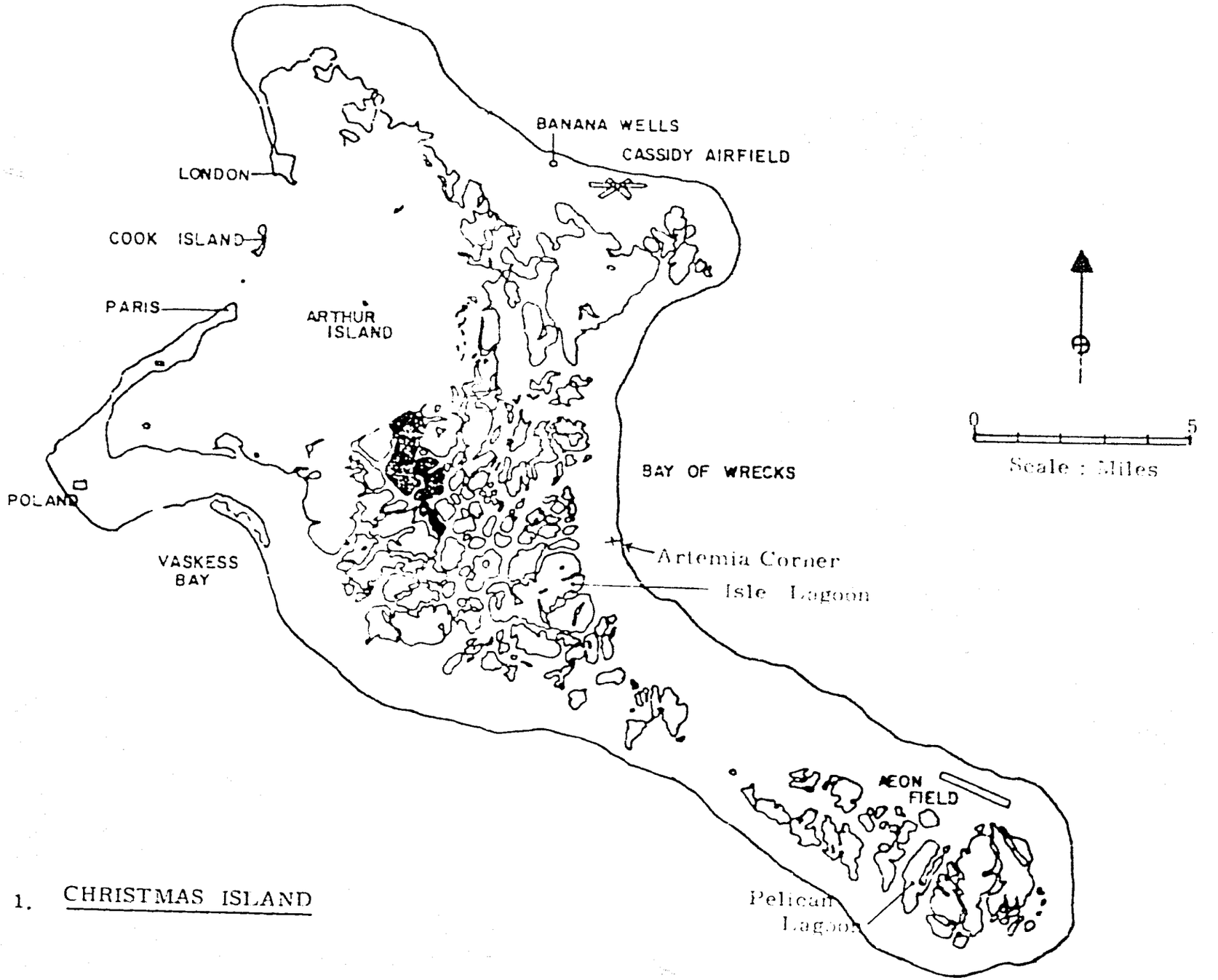


Fig. 1. CHRISTMAS ISLAND

1. Open System: Initial salinity was adjusted to that of the lagoon where the fish were captured. Salinity was subsequently reduced 20‰ per day by a continuous flow of seawater (35 ‰) into the tanks.

2. Closed System: The salinity in the system was adjusted to that of the capture site and maintained at that level throughout the experimental period.

3. Semi-open System: Two holding tanks were set up. One of them was used for holding the broodstock, and the other was used as a reservoir. The initial salinity in both tanks was adjusted to that of the fishing site. A slight water exchange was achieved by pumping reservoir water into the holding tank. Seawater was simultaneously pumped into the reservoir to maintain reservoir volume. Hence, a slow but progressive salinity drop (1 ‰ every 3 hours) was obtained in the holding tank.

The results of the acclimation experiments are summarized in Table 2. The proper acclimation conditions for the broodstock were therefore established. The best results were attained with the semi-open system.

To induce breeding of milkfish, carp pituitary homogenate (CPH, purchased from Spirit Lake, Iowa) combined with human chorionic gonadotropin (HCG, Ayerst) was used. The mature females were injected twice a day at 9 a.m. and 6 p.m., the times of the day when eggs are more sensitive to hormone stimulation. Varying doses of hormones, ranging from 12.5 to 50 mg of CPH, and 1,250 to 20,000 IU of HCG, were tested. The results are summarized in Table 3. Fish response to the hormone treatment in general depended upon the condition of the fish during the acclimation period. Most fish were found to respond to the hormone injections of CPH and HCG in the dose range examined. Most of the ovarian eggs (95%) were found to be undergoing the hydration process. They were at an advanced stage of hydration or even at the fertilizable state (Fig. 2). Several attempts at artificial fertilization were made, but no development was observed.

The best result was obtained from one injected female that released tremendous numbers of hydrated eggs into the holding tank 13 hours after the injection of 25 mg CPH and 2,500 IU HCG. The salinity at that time was 72 ‰. However, natural fertilization of these eggs was not possible because no males were present at that time.

Fieldwork on induced breeding was again conducted in the Lahuipuaa pond complex on the island of Hawaii. Additional mature adults from Nomilu pond on the island of Kauai became available to the program this year.

Over 80 milkfish adults were available from these ponds in addition to 15 adults captured from Kaneohe Ranch pond for induced breeding work during the months of June through August.

Table 2. Percent mortality of milkfish adults in captivity, Christmas Island, 1978

System	Initial Salinity (‰)	4 hr	8 hr	12 hr	18 hr	24 hr	30 hr	36 hr	42 hr	48 hr	60 hr	66 hr	Maturity
Open	35	10	50	100									Immature
	65	0	33.3	33.3	33.3	33.3	100						Mature
	65	0	37.5	100									Immature
	76	0	0	0	33.3	33.3	33.3	33.3	66.7	66.7	66.7	83.7	Mature
Closed	76	0	0	0	50.0	100							Mature
	76	0	0	66.7	100								Mature
Semi-open	76	0	0	0	0	33.3	50.0						Mature
	76	0	0	0	0	66.7	100						Immature
	85	0	50.0	50.0	50.0	83.7							Mature
	76	0	0	0	0								Mature

Note: Arrows indicate the time of hormone injections.

Table 3. Summary of induced breeding of milkfish at Christmas Island, 1978.

Capture Date and Location	No. of Fish	Initial Salinity (‰)	Injection Dose CPH (mg) + HCG (IU)	Hour after Capture	Water System	Results
May 1 Pelican Lagoon*	2	65	50 + 5,000	30	Closed	Adaptability of mature milkfish in captivity was the primary concern. In acclimation period, the fish swam most of the time near the surface. The breeders survived for 30 hours after capture even though the fish conditions were unsatisfactory. They died right after the handling for hormone injection. It was probably due to the stress from a rapid salinity change.
May 3 Pelican Lagoon	6	76	25 + 2,500	5	Closed	First injection was given in a short period of acclimation. The stress from salinity change was reduced, but three fish died in 13 hours. No further injections were given because of the condition of fish observed.
May 5 Pelican Lagoon	4	76	50 + 5,000 50 + 5,000	43 52	Open	Four females were injected 43 hours after capture. Two of them died before second injection and other two received the second hormone injection. Only one fish survived until 57 hours. Artificial fertilization was attempted when the fish began to show erratic motions. Oocyte hydration nearly completed. No fertilization was observed.

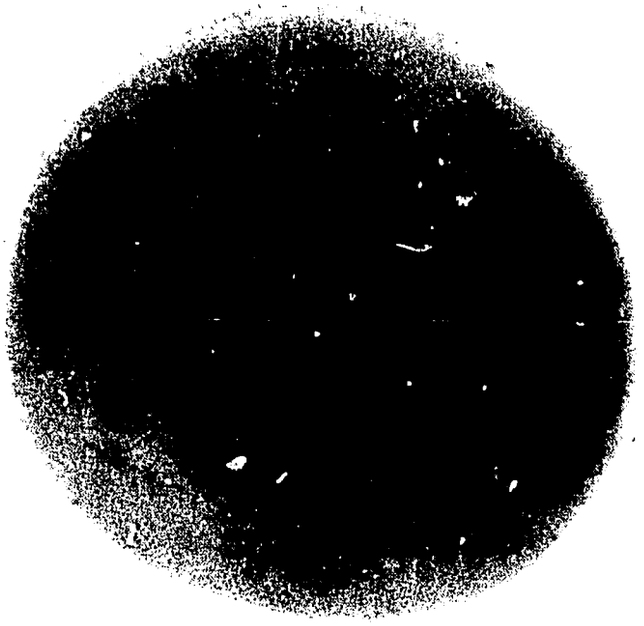
Table 3 (cont.)

Capture Date and Location	No. of Fish	Initial Salinity (‰)	Injection Dose CPH (mg) + HCG (IU)	Hour after Capture	Water System	Results
May 8 Pelican Lagoon	4	76	50 + 5,000 or 37.5+3,750	7	Semi-open	First injections were given to four females at 7 hours after capture. Two of them died before the second injection at 9 a.m. of the following day. Only one female survived until 27 hr. The fish was stripped. About 95% of eggs hydrated. Artificial fertilization failed.
			50 + 5,000	22		
May 12 Pelican Lagoon	3	85	12.5 +1,250 or 37.5+3,750	6	Open	Two injected females died before the second injection. The remaining female died right after the handling for the injection. The fish did not behave normally from the very beginning of stocking, probably due to high initial salinity.
			50 + 5,000	22		
May 15 Pelican Lagoon	3	76	25 + 2,500	3	Semi-open	One of the injected females released many eggs in the tank in 13 hrs. Another female showed near completion of oocyte hydration. The other showed progressive hydration. No fertilization was observed because no males were present in the tank at the time.
May 29 Isle Lagoon*	2	100	50 + 5,000	1	Open	Ten milkfish captured by throw-net from Isle Lagoon. Two mature females with hydrating oocytes. Both died within 3 hours of capture. They were stripped but no fertilization was observed.

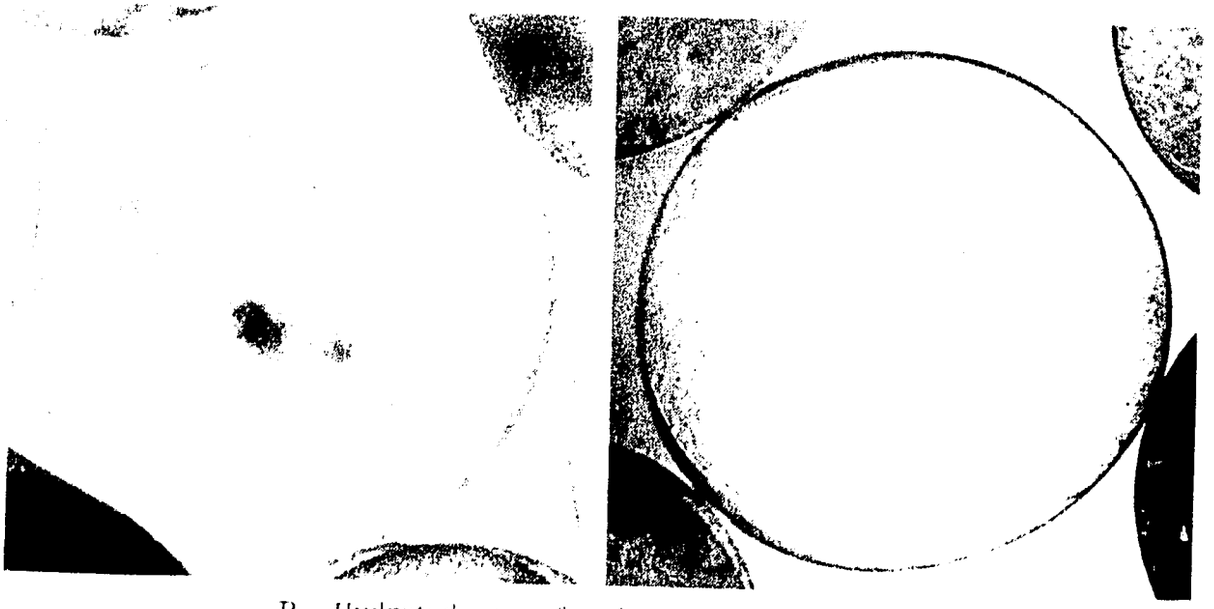
Table 3 (cont.)

Capture Date and Location	No. of Fish	Initial Salinity (‰)	Injection Dose CPH (mg) + HCG (IU)	Hour after Capture	Water System	Results
June 3 Isle Lagoon	1	100	50 +10,000	1	Closed	Three adults captured by throw-net. Two running males died within 12 hours of capture. Injected female died at 17 hours after capture. No hydration was noted.
			50 +10,000	13		
June 4 Isle Lagoon	2	95	50+20,000 or 50+10,000	1	Open	Two mature females were injected. One of them showed hydrating oocytes and started to dribble the eggs 2 hours after the injection, although the eggs were not fully clear. Dribbling continued until noon, June 5. The fish then showed erratic behavior and was stripped. Majority of the stripped eggs were still at the early stage of hydration process. No fertilization was observed. The other fish died 2 hours earlier. She showed a response to the hormone treatments, but not much progress was observed.
			50+20,000 or 50+10,000	12		

*The salinity in Pelican Lagoon is 76 ‰; in Isle Lagoon it is 102 to 106 ‰.



A. Mature eggs (tertiary yolk globule stage) before hormone injection.



B. Hydrated eggs after hormone injection.

Figure 2. Morphological changes of egg after hormone injection(s) of carp pituitary homogenate combined with human chorionic gonadotropin. The hydrated eggs are at the fertilizable stage.

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 often 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, however, 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.

Osmotic acclimation and periodic sampling and handling of the captive mature female produces physiological stresses which cause atresia or resorption of the oocytes. This happens in both resident captive fish and in fish caught at sea when the ovarian development is examined by the cannulation method. This characteristic is not evident in the males, as milt can be very easily expressed from a ripe male by pressing the abdomen.

The mature females were injected with 50 mg carp pituitary homogenate combined with 5,000 IU human chorionic gonadotropin within 26 hours of egg-sampling stress to prevent the oocytes from atresia. The experimental adults were acclimated in 7‰ brackish water for a few hours after their capture from the pond, where the salinity is 7‰. The salinity was gradually adjusted to 35‰ in 30 hours.

Of the 31 readily identifiable females available in the 1978 season from a total of 95 fish, 12 failed to advance vitellogenesis beyond the yolk vesicle stage. Nine females underwent vitellogenic development into the yolk globule stage, but only 6 advanced and were suitable for induced breeding by hormone injections. The other 4 females developed to the yolk globule stage, but the size of the oocytes was around 0.62 mm in diameter.

All the males from the Lahuipuaa pond complex and Nomilu pond were sexually mature.

Six females with eggs larger than 0.8 mm were injected with 25 to 100 mg of carp pituitary homogenate (CPH) and 2,500 to 10,000 IU of human chorionic gonadotropin (HCG) as a primary dose, and the various doses of 100 to 200 mg of CPH and 10,000 to 40,000 IU HCG followed (Table 4). In fish 780806, a low dose of the hormones was given primarily to prevent the egg condition of atresia while efforts to capture mature males were continued.

Another three mature females with egg diameters between 0.6 and 0.65 mm were subjected to a series of hormone injections. Two of them received injections

of 50 mg CPH and 5,000 IU HCG daily for three days to examine the oocyte response to the hormone treatment. The response was not significant. Another female received a total of seven injections of high-dose CPH (ranging from 100 to 200 mg each) and HCG (ranging from 5,000 to 20,000 IU each). The schedule of two injections daily, 9 a.m. and 6 p.m., was followed. At the end of four days' injection period, the fish conditions were excellent, but no response of the yolk-laden eggs to the hormone treatments was evident. These results supported the previous finding that the hormone injections are effective to reinitiate the final maturation of oocytes if the fish with oocytes larger than 0.7 mm (average) are hypophysated.

Three females with a mean egg diameter of 0.32 mm were trapped from Hopeaia pond on June 28. They were still far from sexual maturity and were released back to the pond. Two of them were recovered at the end of August. One of them was full of atretic eggs which indicated onset of egg reabsorption, while the other showed significant progress in ovarian development. Egg diameters had 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-breeding trials. This indicated 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.

The first trip to Nomilu pond was made primarily for determining (1) the feasibility of capturing milkfish adults from this large pond, (2) gonadal development of resident adults, and (3) the possibility of establishing a fieldwork site.

A total of five mature adults were captured; one was a running male, while the others were mature females. Of the females captured, two of them (780703 and 780704), with eggs around 0.5 mm in diameter, were injected with 100 mg CPH and 5,000 IU HCG, although the field facilities were not set up at that time. The fish responded positively to the hormone treatments, and one of the treated females released eggs in a temporary holding area. Unfortunately, no spawned-out eggs were recovered due to an inadequate holding system setup.

The results of the induced-breeding trials conducted at Rangiroa Atoll, French Polynesia, in the month of December was satisfactory. All the females injected with the hormones responded significantly and hydration of nearly all the eggs advanced to the completion of final maturation in 24 hours. Two of them showed premature spawnings, even though the eggs advanced to the stage of being nearly fertilizable, mainly due to poor water conditions caused by the lack of a water-pumping system. With an improvement of the water system, an acceptable water quality was maintained throughout the experiments by a continuous water exchange. As a result, two females (781209 and 781210) were stripped and the fertilizations of both spawnings were obtained (Table 4).

Table 4. Summary of induced breeding of milkfish in 1978.

Fish No. and Location	Date	Time	Initial mean egg diameter (mm)	Body wt. (kg)	Dose CPH (mg) + HCG (IU)	Observation
780701	7/19	9:00 a.m.	0.77	8.5	100 + 10,000	The fish condition was excellent throughout
Oceanic	7/20	9:00 a.m.			100 + 10,000	the experimental period. The progress of
Institute,		6:00 p.m.			200 + 20,000	oocyte hydration was satisfactory. Continu-
Oahu	7/21	9:00 a.m.			200 + 20,000	ous deposition of calcium material observed.
		6:00 p.m.			200 + 20,000	Body swelling and cloaca extruded.
	7/22	9:00 a.m.			200 + 35,000	
		6:00 p.m.			200 + 40,000	
	7/23	9:00 a.m.			200 + 40,000	Eggs examined microscopically. All the eggs hydrated; at least 16% of eggs were at fertil- izable condition.
		7:15 p.m.				Fish stripped. Fertilization failed due to difficulty of obtaining semen from males.
780702	7/19	9:00 a.m.	0.73	7.5	100 + 10,000	The fish was not in healthy condition. The
Oceanic	7/20	9:00 a.m.			100 + 10,000	oocyte hydration progressed.
Institute,		6:00 p.m.			200 + 20,000	
Oahu	7/21	9:00 a.m.			200 + 20,000	
		6:00 p.m.				Injection terminated because of the physical damage of fish and appearance of atretic eggs.
780703	7/26	5:00 p.m.	0.81	7.0	100 + 5,000	
Nomilu	7/27	8:00 a.m.				The fish spawned in a temporary holding area.
pond,						No eggs recovered due to improper setup.
Kauai						Spawning confirmed by examination of ovaries.

Table 4 (cont.)

Fish No. and Location	Date	Time	Initial mean egg diameter (mm)	Body wt. (kg)	Dose CPH (mg) + HCG (IU)	Observation
7S0704 Nomilu pond, Kauai	7/26 7/27	5:00 p.m. 8:00 a.m.	0.81	6.7	100 + 5,000 100 + 10,000	The fish died after second injection, due to shallow and muddy condition in the temporary holding section on the pond.
7S0805 Nomilu pond, Kauai	8/9 8/10	6:00 p.m. 9:00 a.m. 3:00 p.m.	0.81	4.5	25 + 2,500 25 + 2,500	Injections of low-dose hormone to prevent the eggs from atresia until the mature males were captured. The fish released the eggs in the holding tank. Fertilization was not obtained because no male was present.
7S0806 Nomilu pond, Kauai	8/9 8/10 8/11	6:00 p.m. 6:00 p.m. 6:00 p.m.	0.79	4.8	25 + 2,500 25 + 2,500 25 + 2,500	Daily injection of low-dose hormones was given to maintain the egg condition. Experiment terminated after 3 days' injections due to the difficulty of capturing males from Nomilu pond.
7S1107 Rangiroa Atoll, French Polynesia	11/30 12/1	4:00 p.m. 9:00 a.m.	0.82	5.5	50 + 5,000 100 + 10,000	Egg hydration well progressed. The fish maintained under improper condition due to lack of water exchange (failure of water-pumping system).

Table 4 (cont.)

Fish No. and Location	Date	Time	Initial mean egg diameter (mm)	Body wt. (kg)	Dose CPH (mg) + HCG (IU)	Observation
781107 (cont.)	12/1	6:00 p.m.				The fish released eggs in the tank. A great majority of eggs advanced to nearly fertilizable state. The premature spawning caused by improper water condition. The fish was stripped, but fertilization failed.
781208 Rangiroa Atoll, French Polynesia	12/1 12/2	7:00 p.m. 8:00 a.m.	0.80	4.5	50 + 5,000	The fish spawned in tank. Progress of egg hydration better advanced than 781107. No stripping and fertilization were attempted. Water condition was as bad as above.
781209 Rangiroa Atoll, French Polynesia	12/6	9:00 a.m.	0.73	4.1	100 + 10,000	Water-pumping system improved. The proper holding condition obtained by continous water exchange.
		6:30 p.m.			100 + 10,000	The fish responded to the hormone injection. Egg hydration progressed.
	12/7	7:00 a.m.				The fish started to dribble the eggs at 6:30 a.m. Eggs stripped and fertilized. Embryonic development followed.
781210 Rangiroa Atoll, French Polynesia	12/7	10:00 a.m. 5:30 p.m.	0.74	6.5	100 + 10,000 100 + 15,000	Proper water condition in the holding tank was maintained by sufficient water exchange. Fish in an excellent condition. Egg hydration progressed.

Table 4 (cont.)

Fish No. and Location	Date	Time	Initial mean egg diameter (mm)	Body wt. (kg)	Dose CPH (mg) + HCG (IU)	Observation
7S1210 (cont.)	12/7	10:00p.m.				Dribbling of eggs began. The eggs stripped and fertilized. Embryonic development followed.
7S0711-12 Hopeaia pond, Hawaii	6/28	6:00 p.m.	0.6 - 0.65	4.8 - 5.0	50 + 5,000	Two fish were injected daily to examine oocyte response to hormone treatments. The experiment was intended to reconfirm the previous statement that the egg smaller than 0.7 mm is unsuitable for induced-breeding use.
	6/29	6:00 p.m.			50 + 5,000	
	6/30	6:00 p.m.			50 + 5,000	
	7/1	9:00 a.m.				No progress in oocyte hydration was observed.
7S0S13 Hopeaia pond, Hawaii	S/29	6:00 p.m.	0.62	4.5	50 + 5,000	The purpose of the experiments was the same as in 780711-12. A series of high-dose injections were given twice a day at 9:00 a.m. and 6:00 p.m.
	S/30	9:00 a.m.			100 + 10,000	
		6:00 p.m.			100 + 10,000	
	S/31	9:00 a.m.			100 + 10,000	
		6:00 p.m.			200 + 20,000	
	9/1	9:00 a.m.			200 + 20,000	
		6:00 p.m.			200 + 20,000	
	9/2	9:00 a.m.				No response of the yolk-laden eggs to the hormone treatments was noted.

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 on the neighbor islands in Hawaii. Establishing broodstock in captivity is therefore 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 under captive conditions.

3. Larval Rearing

Efforts have been made to develop techniques for larval rearing by using wild milkfish larvae before larvae production is realized in captivity. Collection of newly recruited fry from the wild continued in order 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 to 14 days. Total length at this stage averaged 15 mm (range 13 to 16 mm) and average weight was 13 mg. Fifty larvae were placed in each of six 80-liter aquaria with a water exchange rate of 3 times per day. Other parameters measured in this experiment were salinity (32‰), temperature (25-26° C), and dissolved oxygen levels (7.0 to 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
Purina Catfish Chow	0.5-1.0 g	31 mm (25-37 mm)	0.20 g (0.10-0.29 g)	12

Treatment	Daily Ration	Total Length (av.) (range)	Weight (av.) (range)	Survival Rate (%)
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 plicatilis was cultured using Chlorella, achieving densities of 150 rotifers/ml in 10 days. Tetraselmis spp. was demonstrated to be acceptable to 3-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.

4. Health Care of Captive Fish

The project objectives are (1) to continue to develop methods for practical health monitoring and treatment, and (2) to pursue all research toward practical application for the hatchery and farmer.

a. Sex Identification

The need for a test that would simply and practically identify the sex of milkfish was given emphasis in recent months. Preliminary studies indicated that antibody-like chemicals in invertebrate body fluids could be treated to combine with only male or female fish red blood cells. These fluids could then be used to indicate the sex of a fish. Specifically, the studies involved red cells from 5 female and 3 male mullet. Repeated test trials with the red cell samples gave results that were 100% reproducible. A followup study utilized the skin mucus rather than the red cells from the same mullet. This approach looks promising, and future work will involve further technique development which we hope will result in a sex identification test for milkfish.

b. Studies in Clotting in vivo

Other studies focused on new methods of obtaining serum and of detecting in vivo blood coagulation in fishes. The motivation for these studies came from autopsies of approximately 20 fish of several species (primarily milkfish, skipjack, yellowfin, and mullet) which provided evidence of in vivo clotting. This appeared in the form of "old," organized clots in the aorta and recent antemortem clots that occluded the main outflow tract from the heart. It is known, primarily from human medicine, that such clots are incompatible with life and form as a result of a wide variety of pathologic conditions, e.g., infection, traumatic tissue injury, shock, malignancy, etc. It is therefore important to be able to determine if in vivo clotting is occurring in living fish in order to take appropriate measures. Standard clotting tests from human medicine were evaluated. The protamine sulfate test was applied to the sera of 10 milkfish, with and without clots. This and a newer test from the medical field, the Thrombo-Wellcotest, have produced promising results. Skin mucus will also be evaluated in the near future.

c. Immunopathology

Milkfish serum was used to investigate allergy in marine animals. Specifically, the common Hawaiian sea cucumber, Holothuria cinerascens, was found to have a natural allergy to this serum, which offered the opportunity to study the little-known subject of allergy in a marine animal. Ten milkfish provided pooled sera for these experiments. Results demonstrated reactions in the sea cucumber resembling allergy, e.g., in the form of sensitized muscle.

Further, a loss of precipitin in response to the fish serum, as well as other changes, indicated a marked capacity for desensitization. Finally, responses of pharyngeal structures led to the proposal that these structures and the system to which they belong may be precursors to the vertebrate lymphoreticular system, and models of human immunopathology (e.g., bronchial asthma) can now be proposed as a result of this work. This and other recent research suggest that allergy may play a greater role in the health of marine animals than has previously been suspected.

d. Studies in Oncofetal Protein in Milkfish

An oncofetal protein, carcinoembryonic antigen (CEA), which is best known from developing mammals and some of their pathological conditions (particularly certain types of cancer), was detected in a sample of pooled sera from six milkfish. The level of CEA was 1.5 ng/ml, which is within the range normal for humans. This is the first known report of the presence of an oncofetal protein in fish.

e. Health Study by Automated Blood and Mucus Test

Automated chemical analysis of blood and skin mucus, with a view toward identifying test kits for farmer-level use, was investigated. The studies were carried out on six milkfish before and after oxygen deprivation and the application of other stresses to undermine their health. Three chemical components, viz., Cl, LDH, and K, showed significant differences (95% confidence) between treatment groups. Commercially available test kits for such chemicals are presently being evaluated so as to provide the aquaculturist with an easy means to assess the health of milkfish.

The system used was the Sequential Multiple Analyzer Computer (SMAC), manufactured by Technicon, Inc., which can perform the following 24 tests: albumin, alkaline phosphatase, BUN, Ca, CO_2, Cl, cholesterol, CPK, creatinine, glucose, A/G, creatinine/BUN, Fe, LDH, K, SGOT, SGPT, Na, total bilirubin, total protein, triglycerides, uric acid, globulin, and balance = Na - (Cl + CO_2).

In addition, the pathology program at the Institute has made the following contributions:

(1) First description of a pathologic condition, gastritis, in the milkfish. 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.

(2) A trio of simple noninvasive tests for identifying early nonspecific stress, stress related to starvation, and stress related to the early onset of disease.

(3) First description of allergy-like incompatibilities between milkfish and skin mucus of other fishes.

(4) A concept of in vivo clotting from multiple etiologies (infection, trauma, etc.) possibly playing an unsuspected major role in causing mortality.

(5) Practical information to field personnel on handling shock-like conditions and other health problems in milkfish related to capture and subsequent containment.

(6) With the aid of milkfish serum, a model of human immunopathologic conditions, viz., bronchial asthma and autoimmune disease.

D. DISSEMINATION OF RESULTS

This annual report ends the fourth year.

The extensive international interest in the milkfish at the present time has had some relevant repercussions which are beneficial to the Agency and to the participants. Following the international Bangos Symposium in the Philippines in May 1976, when representatives of international development agencies and research centers agreed to coordinate their research efforts on the milkfish, there has been a continuous interchange of personnel and of research data.

The Oceanic Institute program has obviously been intimately involved with most aspects of the artificial propagation of milkfish, but the Institute has agreed to act as coordinator for data on reproductive physiology, health and stress, and sex determination. Other centers accepted similar or other components. The project at the Institute has continued to send information to these associated centers, particularly the Southeast Asian Development Center for Aquaculture in the Philippines, the Fisheries Research Institute at Tainan and Tungkang in Taiwan, the United Nations Development Programme Research Center at Japara in Indonesia, the United Nations Development Programme Baitfish Project in the Gilbert Islands, the Territorial Government of French Polynesia in Tahiti, and the Hawaii Institute of Marine Biology.

Personal overseas working visits of seven weeks were made by a team of Dr. Ching-ming Kuo, Wade O. Watanabe, and Clyde Tamaru to conduct fieldwork on induced breeding on Christmas Island. The scientific activities and achievements from this fieldwork have resulted in the Gilbert Government Authority's decision to undertake protective measures for milkfish resources on the Island.

Richard W. Power, President of the Oceanic Institute, and Dr. Kuo visited the Aquaculture Department, Southeast Asian Fisheries Development Center in

the Philippines, under recommendation by the U.S. AID RAC Review Team, to establish a joint cooperation between the two institutions in 1979 for continued work on the artificial propagation of milkfish. Dr. Kuo then traveled to the Primary Production Department, Singapore, to view its intensive culture and cage culture systems of milkfish. He then extended his trip to the milkfish propagation program in Taiwan. These visits gave the Agency program wide dissemination throughout the Asian countries.

Dr. Kuo, upon request by the Territorial Government of French Polynesia in Tahiti, visited the Rangiroa Atoll to evaluate the potential for the development of a milkfish program and to demonstrate artificial propagation methods. All expenses were paid by the Territorial Government of French Polynesia. Success in induced breeding and in artificial fertilization were achieved. The fieldwork on Christmas Island and Rangiroa Atoll, Tahiti, also extended the program of the Agency, disseminating its progress and potential to the governmental authorities of those South Pacific countries.

Again a great deal of travel was conducted between Oahu and the pond sites on Hawaii and Kauai by most members of the research team.

Finally, there has been an increase in inquiries from private pond operators and research institutions interested in the development of brackishwater fish farming in Hawaii, New Guinea, Poland, and Brazil using milkfish and mullet.

The following articles published during the year are either directly concerned with or related to the current milkfish propagation project at the Institute:

Kuo, C-M. and C.E. Nash. Annual reproductive cycle of milkfish, Chanos chanos Forskal, in the Hawaiian waters. Aquaculture. In press.

Kuo, C-M. and W.O. Watanabe (1978) Circadian responses of teleostean oocytes to gonadotropins and prostaglandins determined by cyclic AMP concentration. Ann. Biol. anim. Bioch. Biophys. 18(4):949-956.

Nash, C.E. (1978) Institute gains international reputation for aquaculture research, development. The Commercial Fish Farmer 4(3):27-33.

Nash, C.E. (1978) Milkfish at Christmas: How a lonely Pacific island could play a major role in fish farming development. Fish Farming International 5(2):8-13.

Ramos, F. and A.C. Smith (1978) Ketone bodies in fish skin mucus as an indicator of starvation: a preliminary report. J. Fish Biol. 12:105-108.

Ramos, F. and A.C. Smith (1978) The C-reactive protein (CRP) test for the detection of early disease in fishes. Aquaculture 14:261-266.

Smith, A.C. (1978) A proposed phylogenetic relationship between sea cucumber Polian vesicles and the vertebrate lymphoreticular system. J. Invert. Path. 31:353-357.

Smith, A.C. (1978) Hypersensitivity and desensitization in the sea cucumber, Holothuria cinerascens. Dev. Comp. Immunol. 2:355-360.

Smith, A.C. (1978) Oncofetal proteins in marine animals. Comp. Biochem. Physiol. 61B:499-500.

Smith, A.C. (1978) Pathology and biochemical genetic variation in the milkfish, Chanos chanos. J. Fish Biol. 13:173-177.

Smith, A.C. Reactions of fish nuclear lens proteins with sea cucumber coelomic fluid. Dev. Comp. Immunol. In press.

Smith, A.C. Immunopathology in an invertebrate, the sea cucumber, Holothuria cinerascens. Submitted to American Journal of Clinical Pathology.

Smith, A.C. and M.C. Mix (1978) The effects of sodium chloride concentration on electrophoretic patterns of adductor muscle proteins from bivalve molluscs. Comp. Biochem. Physiol. 61B:169-171.

E. WORK PLAN FOR THE COMING YEAR

The work plan for the year of 1979 is revised according to the recommendation of the U.S. AID RAC review team. The complete project work plan is as follows:

Objective 1. Fishing Program - collection of mature and immature fish

- Task 1.1 Adult Fish
  - 1.1.1 Collection from local fishponds
  - 1.1.2 Collection from wild
- Task 1.2 Subadult Fish
  - 1.2.1 Collection from fishponds
  - 1.2.2 Collection from wild
- Task 1.3 Juvenile Fish
  - 1.3.1 Collection from wild

Objective 2. Broodstock Gonad Maturation in Captivity

Task 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

Task 2.2 Testing of 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

Task 2.3 Stimulation of gonad maturation through dietary control

Task 2.4 Establishment of broodstock in natural ponds with supplemental feed

Objective 3. Induced Spawning

Task 3.1 Stimulation of spawning by environmental control or behavioral responses

Task 3.2 Induced spawning by hormone injections
- 3.2.1 Define the optimal breeding procedure

Objective 4. Establish Optimal Egg Incubation System and Procedures

Objective 5. Establish Larval Rearing System and Procedures

Task 5.1 Larval food culture (zoo- and phytoplankton)

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

Task 5.3 Test larval food preferences

Task 5.4 Test relationship of food density for larval survival

Task 5.5 Evaluate stocking density to maximize larval survival

Objective 6. Biomedical Program

Task 6.1 Sex identification

Task 6.2 Identification of stress symptoms

The description, timetable, and level of activities of the project are given in Appendix II.

In 1977, the research team worked with the Aquaculture Department of SEAFDEC on the program of artificial propagation of milkfish. For a number of reasons, the research team confined its activities this year to the Hawaiian Islands and Christmas Island.

Upon recommendation of the U.S. AID RAC review team, a joint cooperation between the Institute and the SEAFDEC Aquaculture Center has been established. The details of the work plan on artificial propagation of milkfish have also been developed for the year 1979. The objective of this cooperation is to undertake a joint effort to develop methods and systems resulting in breeding milkfish in captivity in order to be able to supply seedstock for ponds in Southeast Asia and other areas where milkfish farming is important for food production.

Mature milkfish adults are captured in the Philippines by the method of trapping, or Otoshi-ami, from the spawning runs at sea. The system of collection and transport of the adult milkfish have been established and the breeders will be available for induced-breeding use. Close cooperation with SEAFDEC would not only increase the number of fish available to the project, but it would also make available excellent marine laboratory facilities suitable for the experiments, and thus further reduce some of the logistical problems which are often encountered in the fieldwork. Furthermore, it would provide an LDC setting for the project and thus bring it closer to the ultimate objectives of the project. It is firmly believed that the results of the artificial propagation of this species will be productive in the coming year.

APPENDIX I

Measurements and GSI of Milkfish, Chanos chanos, from Christmas Island, 1978

Location, Date & Salinity	Total length (cm)	Fork length (cm)	Body weight (gm)	Sex	Gonad weight (gm)	GSI	Maturity* Stage
Pelican	42.0	35.4	866.4	M	0.21	0.024	I
Lagoon,	39.0	31.4	580.6	M	17.0	2.928	IV
2/18/78	44.8	38.0	784.7	M	22.4	2.855	IV
76 ‰	46.2	37.7	970.7	M	3.4	0.350	I
	46.6	37.8	870.9	M	22.4	2.572	IV
	45.0	37.9	929.9	M	18.4	1.979	IV
	45.8	37.9	898.1	M	4.7	0.523	I
	41.6	33.8	689.5	M	19.5	2.828	IV
	45.7	36.8	748.4	M	23.1	3.087	IV
	42.4	34.4	730.3	M	22.6	3.095	IV
	45.4	37.5	1165.7	F	18.2	1.560	II
	43.8	35.3	707.6	F	25.8	3.646	III
	46.2	37.3	916.3	F	25.3	2.761	III
	49.3	39.6	1138.5	F	15.8	1.388	II
	46.0	37.3	966.2	F	10.7	1.107	II
	43.1	36.9	843.7	F	10.5	1.245	II
	45.9	38.1	1016.1	F	76.4	7.519	III
	48.2	39.4	1043.3	F	19.2	1.840	II
	44.8	36.2	780.2	F	49.0	6.280	III
	50.7	40.4	1093.2	F	37.4	3.421	III
	50.4	41.3	1283.7	F	10.4	0.810	I
	44.5	36.5	975.2	F	45.8	4.696	III
	44.3	37.5	866.4	F	53.5	6.175	III
	43.2	35.4	793.8	F	2.9	0.365	I
	44.0	36.4	880.0	F	24.6	2.795	III
	44.2	36.1	762.0	F	12.2	1.601	II
	43.2	36.1	957.1	F	6.9	0.721	I
	44.2	36.1	762.0	F	12.2	1.601	II
	43.2	36.1	957.1	F	6.9	0.721	I
	47.0	38.0	1156.1	F	15.1	1.306	II
	43.2	36.4	798.3	F	31.5	3.946	III

Additional 9 fish were examined, but no measurements were taken. They include 2 immature males, 4 maturing males, 1 immature female (stage I), and 2 mature females (stage II).

Location, Date & Salinity	Total length (cm)	Fork length (cm)	Body weight (gm)	Sex	Gonad weight (gm)	GSI	Maturity* Stage
Te Bati,	85.8	69.2	6705.6	M	7.8	0.116	I
2/19/78	68.1	62.7	3704.6	M	6.5	0.175	I
56‰	67.2	54.2	2773.9	M	1.6	0.058	I
	89.2	71.9	6660.2	F	54.2	0.814	I
	81.6	65.4	5874.8	F	18.8	0.320	I
	63.7	51.3	2310.9	F	2.6	0.113	I
	63.8	51.1	2088.4	F	1.8	0.086	I
	60.5	47.4	1602.6	F	1.2	0.075	I
	46.0	36.9	726.4	F	0.4	0.056	I
	66.6	52.4	2510.6	F	4.0	0.159	I
	38.3	31.4	544.8	**	0.2	0.037	
	40.3	33.2	590.2	**	0.2	0.034	
	46.2	36.8	799.0	**	0.5	0.063	
	47.8	38.5	808.1	**	0.5	0.062	
	47.5	37.2	794.5	**	0.2	0.025	
	45.3	36.8	790.0	**	0.2	0.025	
	44.6	35.8	735.5	**	0.2	0.027	
**Sex undecided							
I4	56.3	45.0	1634.4	**	0.8	0.049	
2/21/78	57.4	45.9	1679.8	**	0.7	0.042	
60‰	53.7	43.3	1652.6	**	0.8	0.048	
	62.6	51.4	2138.3	**	1.3	0.061	
	55.8	46.8	1943.1	**	0.3	0.015	
	59.0	48.2	1861.4	**	0.8	0.043	
	51.2	42.0	1389.2	**	0.6	0.043	
	54.5	44.9	1507.3	**	0.3	0.020	
	54.3	43.6	1507.3	**	0.4	0.027	
	56.4	45.7	1793.3	**	0.8	0.045	
	58.4	47.1	1843.2	**	1.1	0.060	
	63.2	51.1	2428.9	**	0.6	0.025	
	56.2	45.4	1725.2	**	0.6	0.035	
	54.1	43.8	1652.6	**	0.6	0.036	
	56.8	45.8	1716.0	F	1.7	0.100	I
I25	33.2	27.3	195.2	**			
2/21/78							
112‰							

Remarks: Seven milkfish adults captured from the sea were examined on February 20, 1978. The fork lengths ranged between 67.6 and 63.5 cm. All of them (1 female and 6 males) were immature.

*Maturity stage:

I	Immature
II	Yolk vesicle or maturing
III	Yolk globule or mature
IV	Ripe

APPENDIX II

Project Descriptions of "Research in Artificial Propagation of Milkfish, Year 05," TAC-1189

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).

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.

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.

6. Biomedical Program

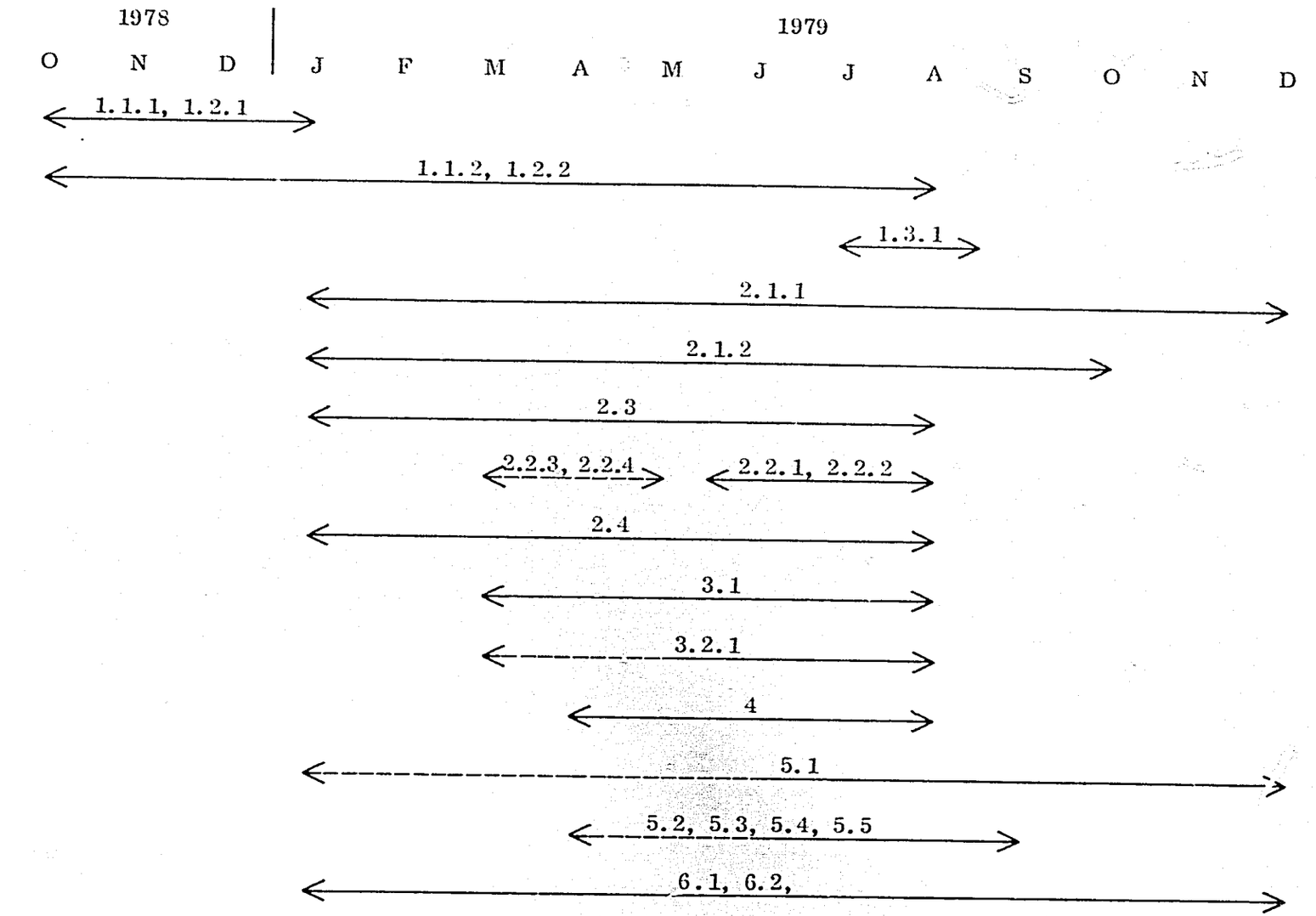
The Oceanic Institute's Pathology Laboratory will continue studies designed to aid in the husbandry and propagation of milkfish. Results of these experiments will be implemented as improved care and treatment techniques.

6.1 Sex identification

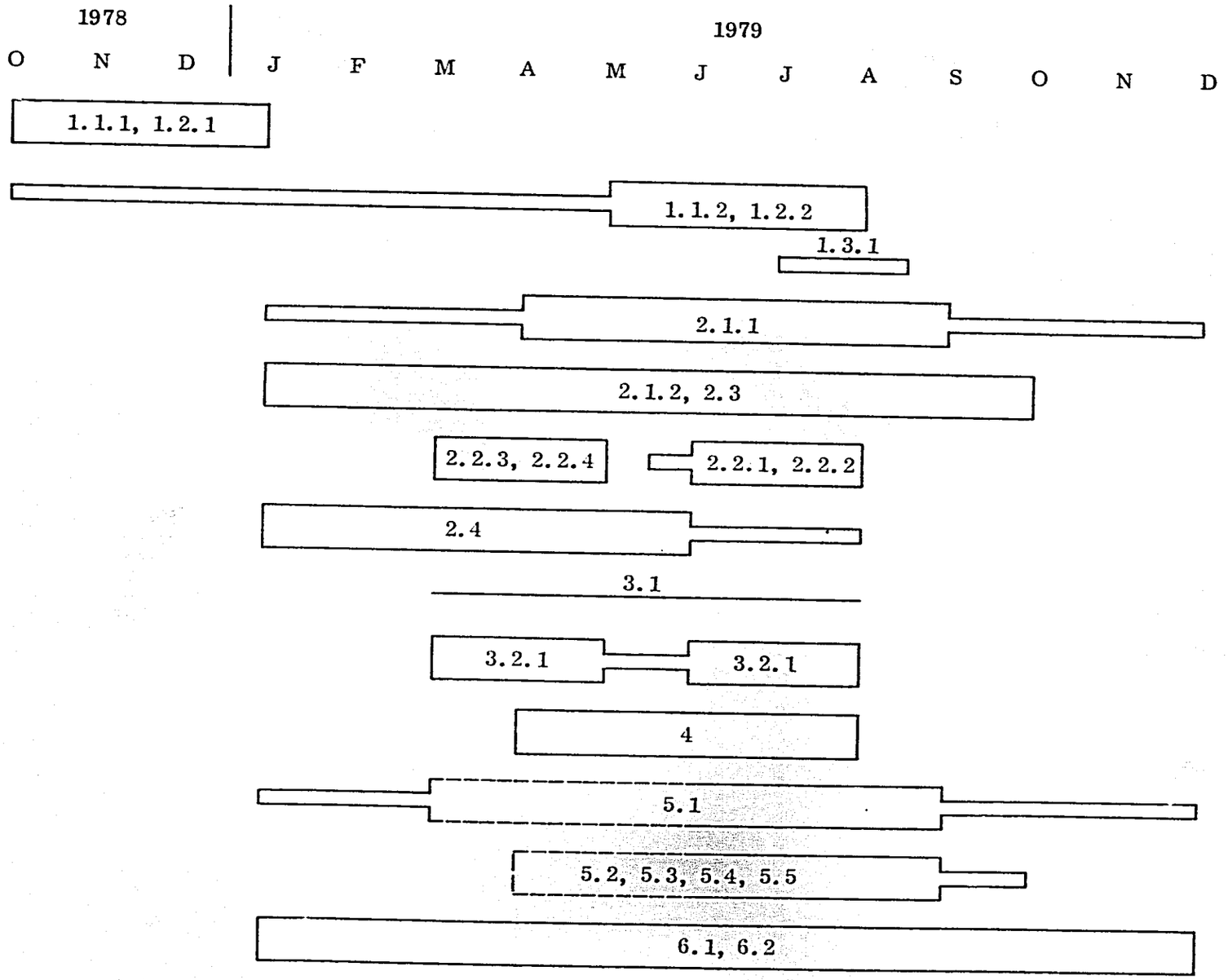
Mucus and blood serum from milkfish specimens are being tested to develop a simple and reliable method for sex identification. Such a method will allow rapid sexing of milkfish with a minimum of handling stress. This work is a continuation of program begun in 1978.

6.2 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.



TIMETABLE OF PROGRAM



LEVEL OF ACTIVITIES