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9. ABSTRACT

The research objective was to breed the milkfish, Chanos chanos, in captivity and to raise the fry from the egg. In the first year, eight very mature fish were captured and deemed ready for final spawning inducement. One fish (with eggs 0.818 mm in diameter) hydrated and was partially ovulated by two injections of 25 mg salmon gonadotropin SG-G100. The eggs were not fertilized. The injection procedure or excessive handling and sampling of three other fish resulted in early atresia (reabsorption) of the oocytes. Two fish died from the sampling and handling procedures, and two proved to be too immature with eggs below 0.6 mm in diameter. The preliminary results indicate that oocytes of 0.8 mm and above are at a critical stage (or beyond), and at which immediate hypophysantion is needed. Injection cannot be delayed. Excessive handling or stress at this stage causes rapid stresia of the oocytes. It appears that oocytes of about 0.7 mm are more suitable for reacting positively to injections. The size of an ovulated egg is about 1.2 mm in diameter. General husbandry methods have been developed for adults in captivity, and a diet formulated which appears to be acceptable. Safe handling systems have been developed using ice, and hypotheses made for the results of this treatment and general conditions of stress.

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5/17 Fritz / Gunning

AFG: Time chart on p. 23 is tied to objectives on pp 3-4. Maybe this is the kind of ?? achievable for research projects

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ANNUAL REPORT SUMMARY SHEET

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RESEARCH IN ARTIFICIAL PROPAGATION OF MILKFISH AID/ta-C 1189

Project Title and Contract Number

Colin E. Nash, Ph.D. Oceanic Foundation (Institute)

Principal Investigator and Contractor

Waimanalo, Hawaii 96795

Contractor's Address

1:13:75 - 1:12:78	1:13:75 - 1:12:76
Contract Period (as amended) From - To	Reporting Period From - To
Total Expenditures and Obligations Through Previous Contract Year	$153,628.47**
Total Expenditures and Obligations For Current Contract Year	$108,046.00

Narrative Summary of Accomplishments and Utilization:

A. SUMMARY

The program objective is to breed the milkfish, Chanos chanos, in captivity and to raise the fry from the egg, undertaking any research and development which may be necessary to attain that goal.

In this, the first year of the project, eight very mature fish were captured and deemed ready for final spawning inducement. One fish (with eggs 0.818 mm in diameter) hydrated and was partially ovulated by two injections of 25 mg salmon gonadotropin SG-G100. The eggs were not fertilized. The injection procedure or excessive handling and sampling of three other fish resulted in early atresia (reabsorption) of the oocytes. Two fish died from the sampling and handling procedures, and two proved to be too immature with eggs below 0.6 mm in diameter.

**Includes cost over-run of $11,674.47 above contracted $141,954.00.

The preliminary results indicate that oocytes of 0.8 mm and above are at a critical stage (or beyond), and at which immediate hypophysation is needed. Injection cannot be delayed. Excessive handling or stress at this stage causes rapid atresia of the oocytes. It appears that oocytes of about 0.7 mm are more suitable for reacting positively to injections. The size of an ovulated egg is about 1.2 mm in diameter.

The level and dose rate of SG-G100 used for mullet (20 μg/g body weight) appears too high for the milkfish. A dose of between 12 and 15 μg/g body weight is suggested at present.

A resident population of adults of varying ages, numbering over 50 fish, has been assembled. Some were brought from the island of Hawaii to Oahu by land and sea involving 18 hours of travel. All survived the journey. Consequently a small operating satellite field center has been established on Hawaii.

A total of 179 dead adult fish have been used for future compilation of age, weight/length, GSI, scale, and otolith data. All of the samples are stored. Determination of the GSI for Hawaiian fish indicated a rapid maturation of oocytes and spermatids in June, with a peak spawning period in July and early August.

Adult fish have been placed under photoperiod and temperature-regulated conditions to promote maturation out of season.

General husbandry methods have been developed for adults in captivity, and a diet formulated which appears to be acceptable.

The work on health care is proving to be very informative. Safe handling systems have been developed using ice, and hypotheses made for the results of this treatment and general conditions of stress. Autopsies on dead fish have revealed growths and evidence of heart attack. Techniques using commercially available human clinical test kits are proving useful indicators of stress; for example, the presence of hemoglobin and ketones in the mucus increases with stress.

Eye lens protein analyses are being undertaken to determine any different racial origins of milkfish.

B. PROJECT OBJECTIVES

The single objective of the project is 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-on 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. Increased 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.

Juveniles larger and healthier than those caught and distributed by the existing farming operators and expected products.

b. 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 factor.

3) Restrict receiving facilities on farms.

*An estimated stage differentiation.

C. ACCOMPLISHMENTS TO DATE

1. Broodstock Collection and Husbandry

a. Wild Fish

Although the milkfish, Chanos chanos, is an important component and subsistence product of the brackishwater coastal pond culture systems of the Philippines, Indonesia, and Taiwan, the indigenous fish to Hawaii generates little commercial interest. It is, however, abundant and is caught opportunistically with other species by the local fishermen and goes for consumption predominantly by the Hawaiians and Filipinos.

Commercial fishing equipment and methods are therefore not conducive to the capture and safe handling of potential broodstock fish. As a result new techniques and methods have had to be developed to give the fish the greatest chance for survival following capture.

A survey of the local fishery, fishpond operators, organizations, and private citizens who capture the milkfish was made to determine the best locations of adults and juveniles during the seasons, and the collecting gear and methods used. Figure 1 illustrates the prime locations for adults around Oahu. Sources of information have included the National Marine Fisheries Service (Honolulu); the State of Hawaii Department of Land and Natural Resources, Division of Fish and Game; past and present commercial fishermen; sportsfishermen; pond owners and operators; and individual fishermen who still use the traditional Hawaiian throw-nets. Outside the State, contact has been made with individuals in the Philippines, Fiji, and Tonga for information on their native techniques, fish behavior, and life history. Data received includes migration of adults at spawning, location and arrival of juvenile schools, food preferences and requirements, and natural behavior. Fishing methods, gear, and anecdotes have all been described.

A review of the survey indicates several significant factors which comply with our own observations and experiences. Primarily there was the universal comment that all the milkfish were not susceptible to handling, and that stress caused rapid death particularly if the fish had been captured with gill nets. Furthermore, the large size and strength of the fish prevented convenient handling and particular containers for transportation were necessary.

Notwithstanding these comments, the proximity of the natural spawning season in Hawaii (June through August) compelled an immediate intensive fishing effort to capture adults with existing boats and gear, but using the experience gained from many years of capturing the grey mullet. Although large adult milkfish are reported to frequent the offshore waters of Hawaii, the depths are beyond the design of the fishing gear available, and therefore a concentrated effort was made within the more sheltered waters of Pearl Harbor and Kaneohe Bay.

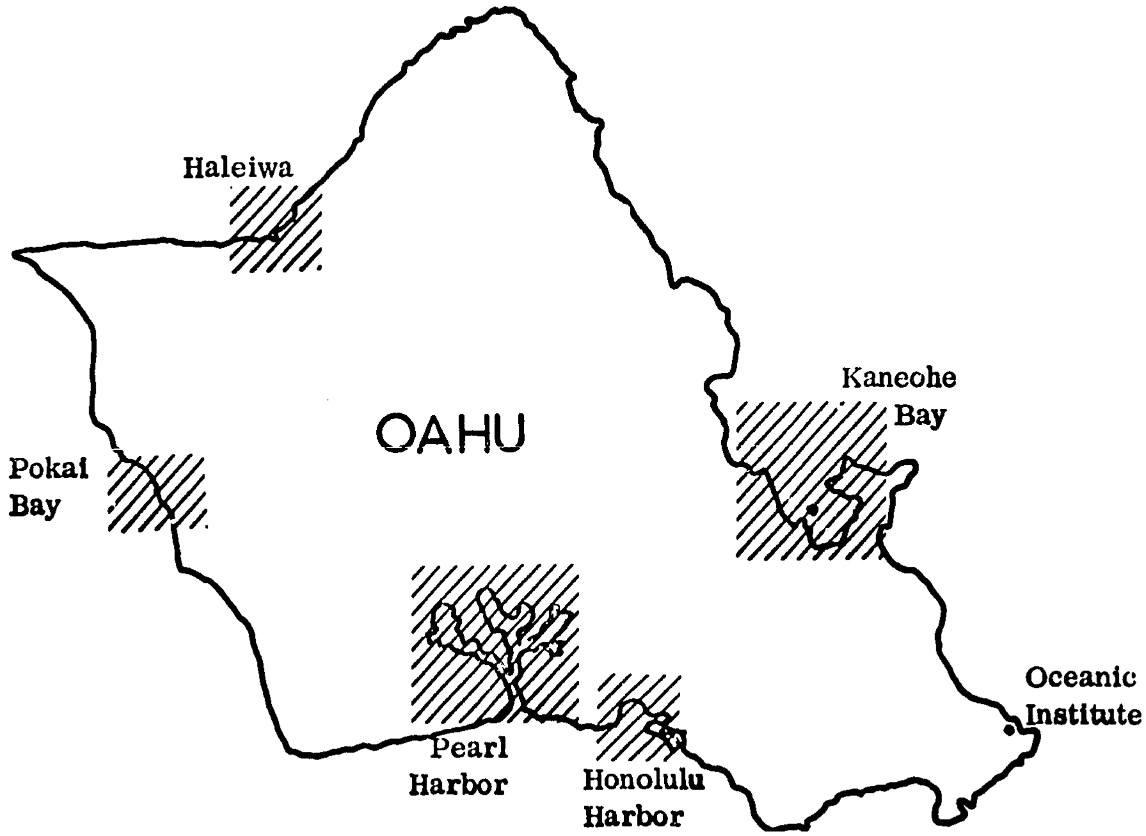

Figure 1. Adult milkfish fishing locations on Oahu.

Access to fish in these waters requires several permits. Fishing within Pearl Harbor is severely restricted by the Navy, and all fishing, particularly out-of-season fishing, requires permits from the State of Hawaii Division of Fish and Game. Suffice it to say that the fullest cooperation was received by both military and State authorities for Institute staff or contracted fishermen to collect milkfish in these waters.

At present over 50 live adult milkfish have been collected and are held as potential broodstock. The largest individuals weigh between 10 and 12 kg, and measure 1 - 1.2 m in length. There were, in addition, a further 20 live adults which were lost one night due to storm damage to the tank's life support systems. The Institute has also collected about 125 large juveniles (1 - 2 kg) as a future broodstock being domesticated.

Successful capture of the large adults has been due to the careful procedures which have been practiced, modifying the methods used for mullet capture. The procedure utilizes two small 6-m boats and between 3 and 4 men. The net is a 200 x 8 m deep gill net of 6.6 cm mesh. Success with this monofilament net is related to water turbidity. In turbid waters, for example in Pearl Harbor, the net is invisible and the captured fish do not struggle much. In clear waters the net is readily visible and avoided by the fish. If driven into the net the fish struggle hard, often break the net, and either escape or damage themselves beyond recall. However, sometimes good fish are taken if the net is brought in quickly.

The fish are transferred by dip-net or pail to a large circular 750-liter holding tank on one boat. No more than five adults can be handled at once and the boat is driven quickly to shore. A flow-through water system pumps water into the holding tank and exhausts over the side. On shore the fish are transferred to a 1,200-liter circular tank with a firm cover, located on the back of a truck. The strongly aerated water in the tank is reduced in salinity and temperature immediately to about 18 ‰ and 20°C by the addition of a large ice-block. This treatment, together with the darkened tank, helps to counter, we believe, the physiological symptoms of stress. These, we hypothesize, are contraction of the blood vessels and the reduction of oxygen to the brain.

A final transfer to a shore-based tank of 16,000 liters is made, and newly captured fish are retained there for two weeks before removal to the permanent facilities. Treatment for injury is made at this time. A water-soluble antibiotic is used for treatment at a rate of 200 g/l until lesions or abrasions are healed. Reduced infection from bacteria can be accomplished by keeping the fish in lower salinity water. For details of health care studies see section C.3.

This effective but laborious technique has enabled the staff and contracted fishermen to capture in good condition fish up to 12 kg in weight. An estimate of the current success rate is about 50% of all fish captured. Most mortalities appear to be the direct result of physiological stress and not injury. All fish which do not survive capture or die later in captivity are autopsied and measured and weighed. Their gonads are preserved for histological examination and scales removed for growth and age studies. Preliminary analysis of the available data suggests that milkfish will be ready for spawning at about 4 years of age, and that natural growth in open waters is about 15 - 20 cm per annum.

b. Pond Fish

Among the many constructed and natural ponds which occur along the coastlines of the Hawaiian Islands, a small cluster of natural anchialine ponds was made available to us through a large development corporation. The ponds cover more than 5 acres in area. Biologically and geologically they are unique, occurring exclusively in recent lava flows and harboring both marine and brackishwater biota. The salinity varies but can drop to about 7‰ because of subsurface springs.

The site at Lahuipuaa on the Kona coast contains five irregular ponds, two of which are connected to the ocean by sluice gates. The others are not connected to the sea but at times join each other. Historically these particular ponds have been used for holding and raising fish, principally mullet, mulkfish, moi and other euryhaline species.

A first survey of the ponds was made in December 1974 to determine the abundance and distribution of fish in these ponds. The results showed a substantial stock of adult milkfish. Following discussions with the pond owners the stocks were safeguarded until a second visit was made prior to the milkfish breeding season in summer.

In June a census of the main Hopeaia Pond was made. Forty large milkfish were caught with seine nets and handled. A total of 34 large fish were marked for reference with Floy tags, anaesthetized with quainaldine sulfate, weighed, and measured. A sample of gonad tissue was removed through the oviduct with a polyethylene catheter using the standard sampling procedure developed for grey mullet. An additional three fish were killed and blood samples, eye lenses, and other tissues sampled for future examination.

All fish were returned to the ponds, except 24 which were relocated in a small pond for subsequent transportation to the Institute.

Hopeaia Pond is an irregular 1.25-acre pond not connected to the ocean. The maximum depth is about 1.5 m but many parts are shallow. The water is clear and slightly brackish (7 ‰) and seawater must penetrate through the ground into the pond at high tide. The bottom of the pond, which is mud, is thickly carpeted with the vascular plant, Ruppia maritima, which appears to be the principal food for both the mullet and milkfish which have been stocked in the pond in the past.

Although milkfish have been recorded in Hawaii and elsewhere as large as 30 kg and almost 2 m in length, little data is available as to the size and age at first maturity. Many of the adult fish in the Hopeaia Pond, while only 3 - 5 kg in weight and less than 65 cm in length, were in fact maturing. Many males were ripe with active spermatazoa, and the females in general showed advanced stage II and III oocytes--that is the yolk vesicle and yolk globule stages. While spawning is not anticipated in such low salinity waters, the ability of fish to mature under captive and pond conditions and at a size manageable in the laboratory is an important point for all future breeding work.

A third trip was made to the ponds in July to construct a small laboratory within an existing building on site. A small 1.5 kw generator was installed to run lights, water pumps, and air compressors. A plastic swimming pool was installed and filled continuously with oceanic water, discharging into the nearby pond.

Twenty additional large fish were collected, marked with tags as before, and weighed and measured. All fish not showing advanced signs of maturity were relocated in one pond for next year. Six mature fish were relocated in the swimming pool. The most advanced female was subsequently injected with SG-G100 to induce the final stages of spawning. Hydrating eggs were later released by this fish slowly over a long period, and these were not fertilized. Slow release following hypophysation in the mullet is an indication of premature spawning.

A fourth trip was made in August as part of a planned relocation of the larger tagged fish back to the Institute. The visit was timed to coincide with the shakedown cruise of the National Marine Fisheries Service vessel, the Townsend Cromwell, through the cooperation of the local laboratory Director. The fish were transported ten miles by road to the pier at Kawaihae in the 1,200-liter tanks. They were then transferred to the 3,800-liter fish transport tanks designed and used by the NMFS for tuna, and were connected to the ship's pumps for large-volume water exchange. The ship then sailed back immediately to Kewalo Basin in Honolulu and the fish were subsequently transported to the Institute by truck. The total time for transportation was 18 hours and not one was lost.

In addition to the volume of experience that has been gained in the capture and transportation of the larger milkfish, the availability of these resources has made it possible to undertake a great deal of laboratory work without jeopardizing future breeding adults. One invaluable result has been the better understanding of the female reproductory system. Autopsy of mature gravid females has shown that the eggs are released through a funnel rather than through the oviduct. This fact has demanded some change to the established sampling procedures developed for grey mullet, and a satisfactory technique has not yet been developed. The use of a cystoscope was contemplated to avoid actual sampling, but the dimensions of the available instruments were too great. This anatomical feature answers the questions of previous sampling difficulties experienced by ourselves and other workers.

In addition to the Lahuipuaa Ponds, further contact and informational exchanges were made with fishpond operators throughout the State. Some well advanced fish were obtained from the owner of the Molii Pond in Kaneohe Bay, about ten miles from the Institute. These fish were also safely transported back to the Institute tanks by truck.

A great deal of assistance was provided by private individuals in the State, particularly from the Hawaiians who still take milkfish by the traditional throw-nets. The lack of a mobile force to take advantage of their captured animals made this source of adults impractical to follow.

Apart from those fish taken by the Institute staff, all other fish, both alive and subsequently dead, were paid for at an advantageous rate to the collector. However, certain restrictions were made as to what was acceptable.

c. Juvenile Fish

Post-larval and juvenile milkfish were collected at various locations around Oahu for data on growth, behavior, nutritional requirements and disease, and also for trade with the pond operators in exchange for their larger fish. These juveniles should then become the resources several years from now.

The post-larvae, 12 - 16 mm in length, are almost transparent with pigment confined to the head and gut regions. They are usually found in the upper reaches of streams in warm shallow waters feeding on diatoms, organic sediments, and algae. Pigmentation of the body is complete after about three weeks of inshore life and the juveniles between 25 - 30 mm in length move downstream into deeper waters. During this period the fish can be captured easily with fine nets and transported with little loss of life. This is a distinct contrast to the handling of the older fish.

Juvenile recruitment has always been an indicator of the numbers and reproductive success of the adults at sea. Observations by Institute staff through the years indicate that the fish only have the one spawning period in the year (June to August), and that the local population, while not large, is probably sufficient to produce progeny annually sufficient to maintain this population.

Evidence indicates that actual spawning is cyclic within the spawning period and related to lunar periodicity. This is most apparent for observations of pond fish where there is access to ocean waters. Throughout the year, for example, the large fish in the Molii Pond in Kaneohe Bay are not seen or caught near the pond gates. However, coincidental with two consecutive high tides (and thus new moons), the largest fish moved through to the gates in an attempt to go to sea, presumably to spawn. Although these particular fish did not get out to sea, the first post-larvae were recorded in the area about four weeks later.

Light and temperature data of the environment where spawning is believed to occur is being collected to establish the controlled conditions for breeding fish on a year-round basis.

A final collection of juveniles was made in November. Many of these juveniles were released into ponds for future use in the project. A great deal of assistance has been obtained from the owners of the ponds around Oahu and on other islands, for both fish and the behavioral observations.

d. Husbandry of Captive Stock

Of vital importance is the maintenance of a healthy stock in captivity if the fish are expected to breed. Little is known of the qualitative and quantitative requirements of the milkfish diet.

In nature the milkfish is accepted to be a herbivore feeding on diatoms, organic material and various algae. In farm ponds in Southeast Asia and around Hawaii, the milkfish are known to feed directly on raw sewage. Large schools do congregate around the sewage outfalls. These areas are well known fishing locales but the fish are caught on the rod and line. The location is unsuitable for netting gear.

The more practical foods used for the herbivorous captive fish are modifications of the pelletized foods used for trout and catfish. Further less proteinaceous diets have been developed by the Institute for such fish.

All broodstock fish are usually retained in the larger swimming pool tanks or open dirt ponds lined with a butyl rubber liner. The pools and tanks are undisturbed to allow a natural growth of vegetation for the fish to browse on. This diet is supplemented with a prepared feed made in the laboratory. One such diet supplement being developed consists of the following ingredients:

Wheat middlings	55%
Cottonseed meal	14%
Soy bean meal	14%
Tuna fish meal	14%
Propylene glycol	1.4%
Visorbin (Vitamin B complex)	1.4%
Vitamin pre-mix	0.2%

The ingredients are milled to 0.5 mm in size and stored dry. When needed, the feed is mixed to a dough with water and fed at a rate of about 0.75 kg per day per 25 large fish. The approximate cost of the feed is 35 cents per kg.

e. Environmental Regulation of Captive Stock

A section of the Environmental Control Laboratory has been isolated for a number of adult fish to be subjected to controlled temperature and photoperiod regulation to encourage egg maturation out of season. The controls for the period have been set at 26°C and 18L/6D photoperiod. A total of six fish are being maintained under these conditions and fed daily with the prepared diet.

2. Induced Breeding

a. Data

During the year a month-by-month fishing effort was maintained for the capture of broodstock and for the accumulation of statistical data on the breeding cycle of milkfish in Hawaiian waters. The individual data is given in Appendix I. The summary data is detailed in Table 1.

Table 1. Monthly Gonadal Somatic Index (GSI)

	Males				Females			
Month	GSI	sd mean	sd	n	GSI	sd mean	sd	n
Jan	0.193	0.049	0.085	3	0.25	0.119	0.205	3
Feb	0.20	0	0	1	0.115	0.085	0.120	2
Mar	0.03	0	0	1	0.435	0.076	0.152	4
Apr	0.046	0.009	0.02	5	0.306	0.060	0.158	7
May	0.120	0.04	0.057	2	0.385	0.045	0.064	2
June	0.038	0.005	0.018	15	0.171	0.020	0.079	15
July	3.285	0.095	0.134	2	3.18	0	0	1
Aug*				0	1.345	0.299	0.597	4
Aug**	0.043	0.009	0.028	9	0.175	0.044	0.138	10
Sep	0.091	0.027	0.119	19	0.369	0.055	0.258	22
Oct	0.08	0.041	0.135	11	0.250	0.021	0.098	21
Nov	0.068	0.031	0.008	6	0.412	0.114	0.362	10
Dec	0.004	0	0	1	0.357	0.153	0.265	3
				(75)				(104)

*Early
**Late

The GSI is the most practical measure of maturity developed by fisheries biologists and can be expressed by:

$$GSI = \frac{w \text{ (weight in g for both gonads) } \times 100}{W \text{ (body weight in g.)}}$$

The GSI described in graphic form (Figure 2) clearly indicates the natural season in Hawaiian coastal waters commencing in June and terminating toward the end of August. July and early August are unquestionably the peaks of spawning activity in Hawaii. Although two individual spawning periods have been attributed to milkfish in the waters of Indonesia (or there are two separate races), and long single spawning periods are attributed to fish of India, the Philippines and Southeast Asia, in Hawaii and other Pacific Islands, for example Fiji, there is only one clearly defined and short period of two months.

Observations of fish within local coastal fishponds in May and June indicate the strongest desire of the fish to escape to sea. Large fish move toward the outlets of the ponds where they can be readily caught in the traps at the gates. These fish in fact proved to be an important contribution to mature broodstock.

The weight of the fish showing signs of maturation proved interesting. Historical records in the literature indicate that the smallest mature females are about 70 cm in length (about 4,500 g in weight, or 10 lb.). Mature female fish taken in Hawaii proved to be much smaller, even as small as 60 cm in length and weighing only 2,500 g. The scales of the fish have been taken but data on actual age has still to be produced.

Pond fish seem to mature slightly later than non-captives in the sea. This was certainly true for the grey mullet species.

b. Egg Sampling

The induced breeding methods developed by the Institute have always demanded two conditions. These are (1) an accurate knowledge of the exact stage of development of the eggs about to be induced, and (2) the use of a standardized hormone. Sampling and histological studies have, over the years, enabled an accurate development picture to be produced for the oocytes of the grey mullet. This development sequence has then enabled gross observed parameters to be used as the indicators for the level, dose rate, and time sequence of the standard hormone to be used.

The approach for the milkfish must be identical. Consequently the gonads of the broodstock female fish are sampled on a regular basis using the traditional techniques. A fine polyethylene cannula is inserted into the oviduct and a sample of oocytes is withdrawn by oral suction.

Unfortunately, sampling milkfish does not prove to be simple. The genital anatomy of the milkfish differs from that of the grey mullet and approaches more that of the salmon. The oocytes are released through a funnel from the gonad, enter the oviduct close to the genital pore, and are then expelled through the cloaca. As a consequence, the funnel does not provide the easy sampling passageway which is true of the oviduct of grey mullet, and egg sampling has proved difficult.

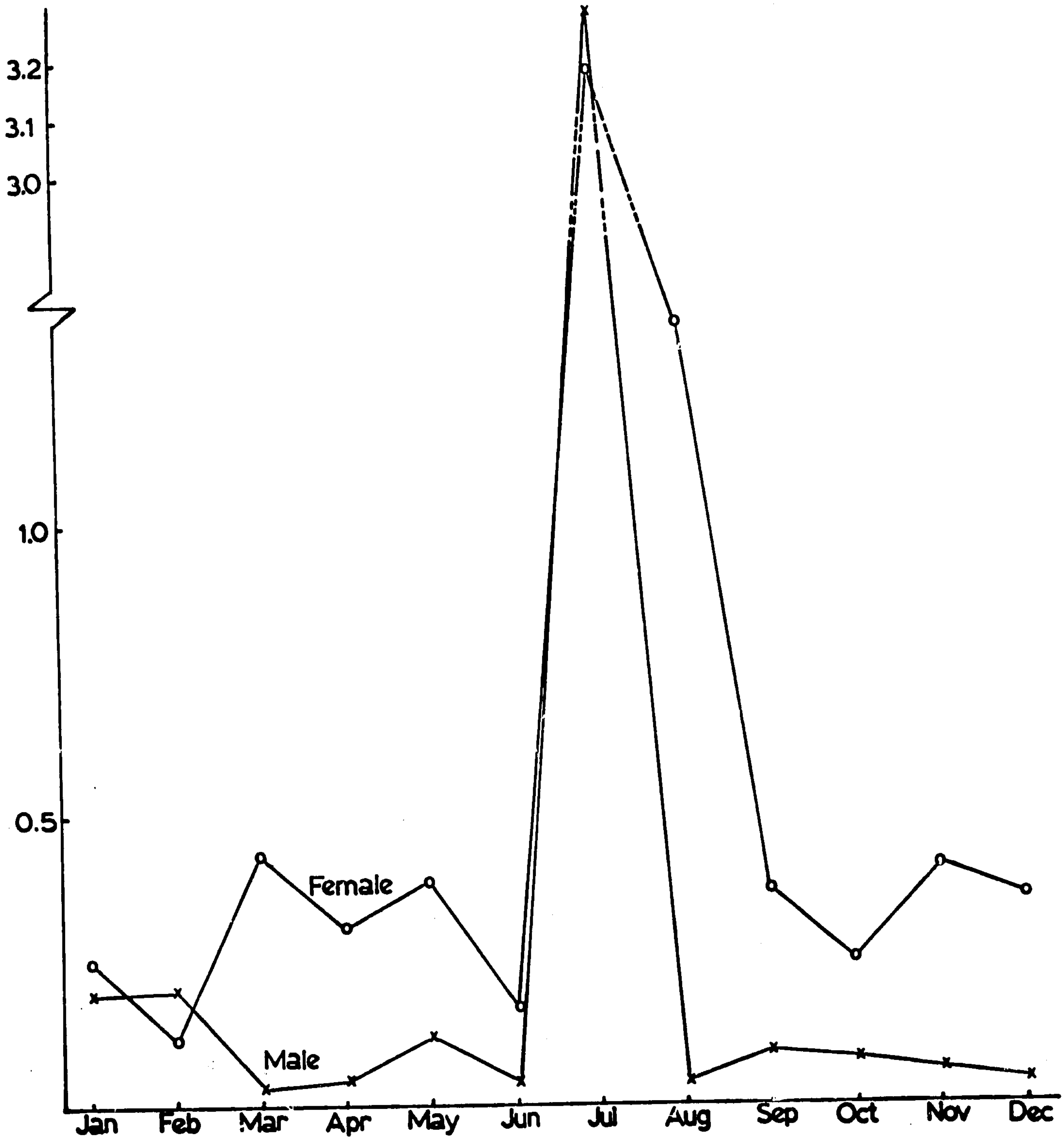

Figure 2. Monthly gonadal somatic index of the milkfish.

Egg samples can be obtained from the milkfish gonads, but are difficult to obtain. The fine cannula gets lost in the body, can rupture membranes and body organs, and many attempts may have to be made to obtain an egg sample--sometimes resulting in death of the fish.

It was hoped that the use of a cystoscope would make sampling unnecessary, and that an internal examination would prove sufficient. However, a fine enough cystoscope is not yet available.

Eggs that were sampled readily were measured, preserved, and examined histologically using standard procedures to determine vitellogenesis and to begin an assembly of an 'atlas' of egg development.

Egg diameter distributions of some fish before injection are illustrated in Figure 3.

c. Work on Mature Female Fish

Eight fish were deemed ready for final induced treatment in the summer. Background data of the fish is described in Table 2.

Table 2. Mature Female Fish

Fish number	Date	Egg diameter mm	Stage	Weight g
750702-1	7:8:75	0.876	IIIc	4,000
2	7:8:75	0.572	III	3,500
750715-1	7:17:75	0.815	IIIc	5,000
2	7:17:75	0.796	IIIc	4,750
3	7:17:75	0.545	IIIb	
750710-1	7:10:75	0.803	IIIc	6,750
2	7:10:75	0.545	IIIb	4,500
750723-1	7:23:75	0.818	IIIc	2,500

The stages of development used are those described by the Institute for the grey mullet. Stage IIIc is therefore the tertiary yolk globule stage, and IIIb the secondary yolk globule stage.

The standard hormone used is salmon gonadotropin SG-G100, prepared exclusively by the University Laboratories in British Columbia and designated BCR#2.

Data on the induced spawning tests is as follows in Table 3.

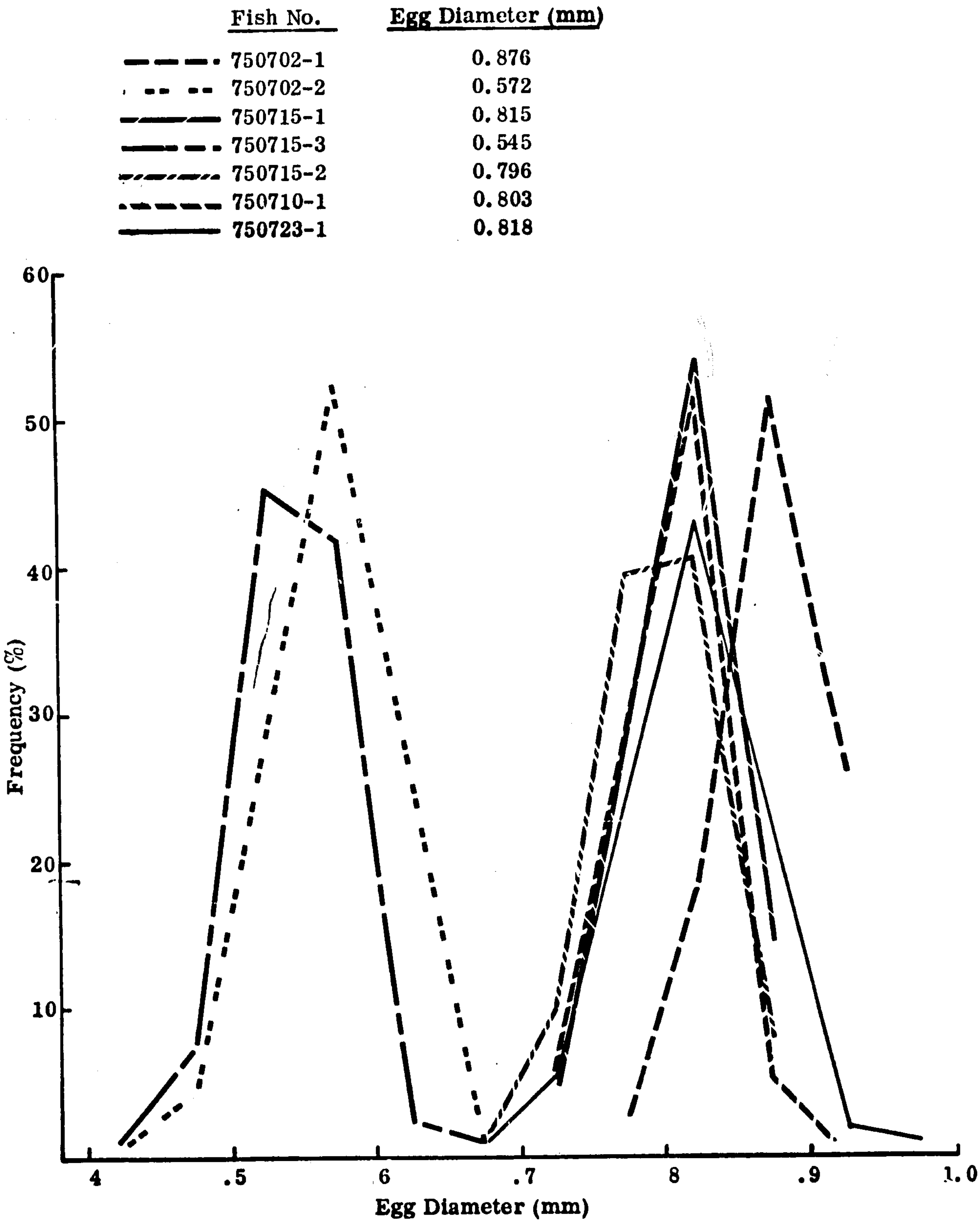

Figure 3. Egg diameter distribution.

Table 3. Injection Dose, Sequence, and Result

Fish number	Date	Egg diameter mm	Dose mg	Stage	Observation
750710-1	7:10	0.803	0	III	Mature
	7:14		15		
	7:15		30		
	7:16		40		
	7:17		0		Atretic
750715-1	7:17	0.815	0	III	Mature
	7:21		0		Atretic
750715-2	7:17	0.796	0	III	Mature
	7:21		0		Atretic
750723-1	7:23	0.818	25	III	Mature
	7:24		25		Hydrated
	7:25		0		Partially ovulated

Of the remaining four fish, two (750702-2 and 750710-2) proved to be too immature to be injected (egg diameters less than 0.6 mm); and two fish (750702-1 and 750715-3) died from repeated handling and difficulty obtaining egg samples.

The two fish detailed above (750715-1 and 750715-2) immediately went atretic, or began reabsorption of their eggs, following first handling; and the other fish (750710-1) went atretic during the injection sequence.

The best result was with the small female (750723-1) which was induced to hydrate and ovulate with two injections of 25 mg of hormone 24 hours apart.

From these preliminary tests it seems certain that eggs of 0.8 mm and above are at the critical stage or beyond, and at which immediate hypophysation is needed. Injection cannot be delayed. Excessive handling or stress as this stage causes the eggs to become atretic, or reabsorbed. It appears that eggs of about 0.7 mm are more suitable for reacting positively to injections. The size of an ovulated egg is 1.2 mm in diameter.

The first injection doses were made at the same level and dose rate as that developed for the grey mullet, namely about 20 μg/g body weight of the recipient. The milkfish which did hydrate (750723-1) underwent a very rapid late development. This suggests that the dose rate established for mullet may be too high for milkfish, and that about 12 - 15 μg/g body weight may be sufficient. This has some obvious economic advantages. The cost of injecting a mullet for spawning is about $80-120 per fish. At the same rate milkfish will be well over $500 per fish, because of their large size. The lower dose will give significant savings.

3. Health Care of Captive Fish

a. Stock Identification

The work in stock identification consisted of the collection and electrophoretic processing of eye lens nuclei from different fishes. The protein patterns that result from the processing may be used in a number of ways. One major use is to identify breeding populations of fishes. For aquaculture, the identification of breeding populations having characteristics such as stress resistance would be important. These populations would be more likely to thrive in captivity.

To date, about 100 pairs of lenses have been collected from milkfish and other different species, and processing is underway. For the purpose of visualizing the information from these patterns, a map is being planned. It will attempt to show the range, distribution, seasonal variations, migratory movements, etc., of fish populations as they are reflected from the patterns. The patterns themselves will be stored in a book for easy display.

b. Studies in Stress

(1) Occult hemoglobin in skin mucus of fish

The results of experiments showed that when fish are left out of the water, their skin mucus produces positive reactions to a commercial test ("stix") for free hemoglobin. Other studies confirmed that the hemoglobin appeared in response to stress and not other factors, e.g., new mucus production. These studies also demonstrated the specificity of these tests to hemoglobin, and not to other substances in the mucus.

(2) Ketone bodies in skin mucus

The experiments on free hemoglobin suggested the possibility that skin mucus could also reveal specific sources of stress such as starvation. An experiment was performed in which "stix" were again used, but this time to determine the presence of ketone bodies in the skin mucus of starving mullet (as all milkfish are being retained for breeding). These mullet did show positive reactions for ketone bodies as the starvation period lengthened. After feeding was resumed, ketone body reactions decreased.

These experiments on stress demonstrate the value of using skin mucus of fishes to quickly assess their health. They also show the ease of the technique of testing and reveal the promise of "stix" in diagnosing general and specific forms of stress.

Future work on stress may include the following: (1) working out the time relationships between the first detection of stress and subsequent appearance of pathological consequences. These consequences may include lipoblepheritis in the milkfish, abnormal behavior, or mortality itself; (2) investigating what effects certain conditions of containment may have in producing stress; and (3) examining other fish species for stress- and starvation-associated free hemoglobin and ketone body production, respectively. In all cases, stress studies can now be performed using a sensitive and quantifiable measure--the color change on a plastic strip ("stix")--without resorting to mortality and the waste of animals that it entails.

c. Survey of Naturally Occurring Pathology

Autopsies for naturally occurring diseases were routinely performed on fishes as their lenses were being collected. About 50 autopsies have already been performed on milkfish and other species.

4. Tag Development and Tracking

The conventional acoustic fish tag, a pinger which periodically emits a short pulse of high-frequency sound, has been in use for many years, with considerable success in lakes, rivers, and estuaries. For example, pingers facilitated much of what is known about the habits of salmon in our waterways.

The simple pinger fails in the open sea, however, because it provides only one dimension of the required tracking information, direction, leaving distance and depth almost impossible to estimate.

A less-known but very effective device, the acoustic transponder (Figure 5), is able to provide these missing dimensions, while at the same time making more efficient use of its batteries. Briefly, the transponder contains a sensitive acoustic receiver, and only emits its pulses upon hearing a coded signal from the tracking equipment. Range is derived from the round-trip travel time of the interrogating and responding signals. The responding signal may be coded to provide depth or other additional information.

In our case, we have established tracking requirements of one kilometer for range and 300 meters for depth in the open ocean, and lesser values in coastal waters, as adequate for navigation of small craft to keep a migrating milkfish in sight on the sonar screen. These requirements can be met by a commercially available high-resolution sonar set (Wesmar 200AB, Western Marine Electronics, Seattle, Washington), suitably modified, in conjunction with a transponding tag which can deliver one watt of acoustic power.

Adult milkfish we have dissected possess large, elongated swim bladders, extending over about half their body length. The acoustical target strength of such

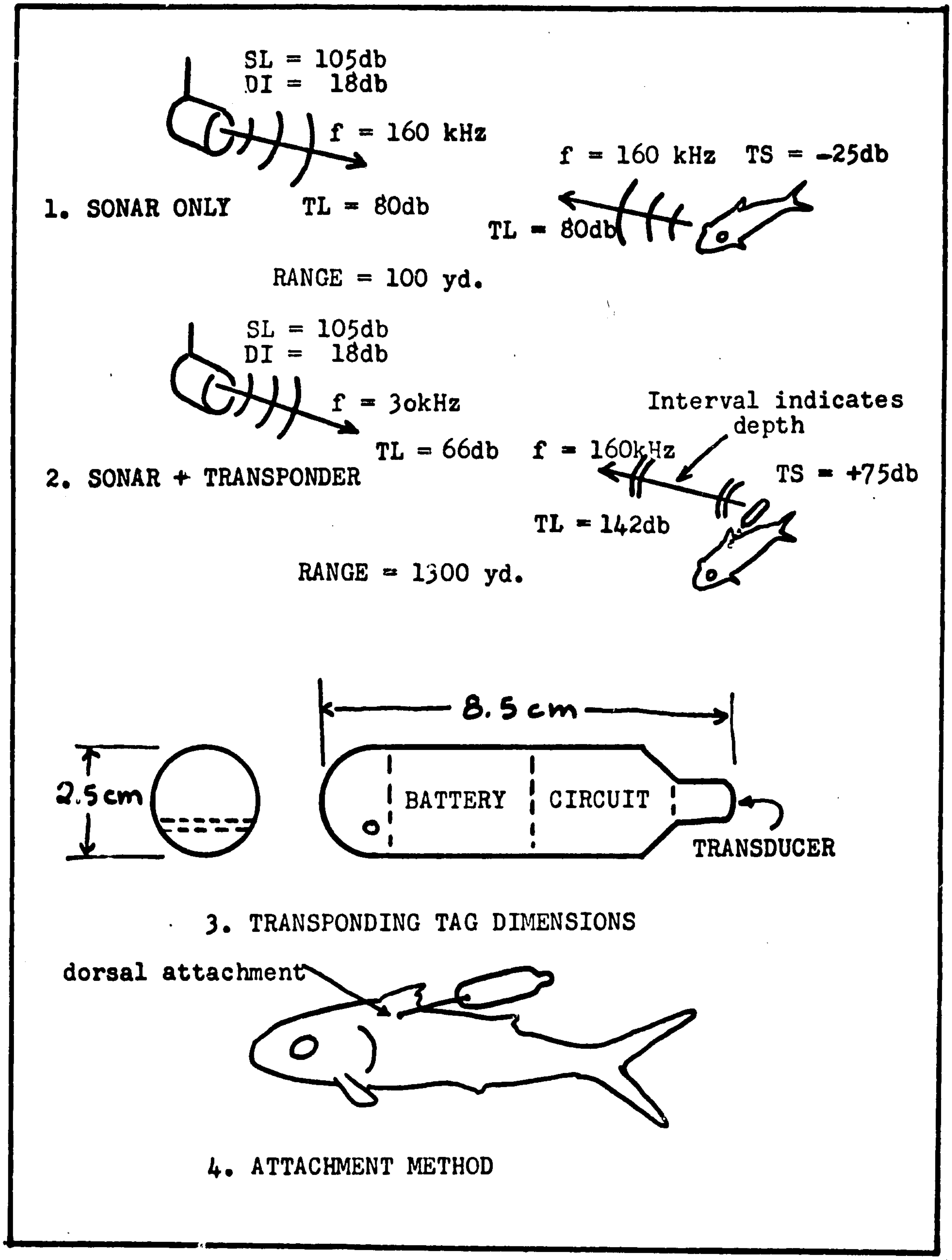

Figure 5. Tagging and tracking information.

animals is 20 to 25 db for a 70-cm adult. Our Wesmar sonar operates at 160 kHz, at an effective source level of 1,200 watts, with directivity index of 18 db with respect to an isotropic source. Taking the active sonar/passive target case, and allowing an echo signal to noise ratio of +6 db, the maximum allowable transmission loss is 87 db for tropical oceanic conditions. This much loss will occur in somewhat over 100 yards. For a school of, say, 100 fish, the range about doubles. Thus an untagged individual (or school) can be reliably detected only if it is less than 100 yards (or 200 yards) away.

Introducing a 1-watt transponder has the effect of increasing the apparent target strength of a tagged fish to +75 db, an overall increase of 100 db. Allowing the same echo signal to noise ratio as before, the maximum detection range is now 1,300 yards, (1,180 m), which matches well with the 1 km display range of the Wesmar sonar.

During this year we have acquired and installed the Wesmar sonar in our fishing boat; we have designed and tested the necessary modifications to the sonar, to provide an interrogation signal; we have designed and are testing a prototype transponder; and we are working with dummy tags on live adults.

As yet we have made no attempt to miniaturize the transponder circuit, because this will require tooling that is circuit-dependent. However, we anticipate no problems in this regard; the transponder's battery will be its largest component.

During the coming year we expect to miniaturize the tag and gain tracking experience. Hopefully, we may identify a spawning occurrence involving a school containing a tagged milkfish. It is more realistic to expect a lesser goal, namely, useful data on milkfish migration patterns during the spawning season.

D. DISSEMINATION OF RESULTS

The project is now at the end of its first year.

Information on the first attempts and results of induced spawning, suggesting the ideal size of the egg (0.7 mm) and possible dose level and rate of SG-G100 (12 - 15 μg/g body weight), were passed immediately to FAO, Rome, for inclusion in their quarterly FAO Aquaculture Bulletin. The same information was also communicated directly to the Southeast Asian Fisheries Development and Education Center, Aquaculture Center in the Philippines, where a milkfish propagation project is being supported by the International Development and Research Council of Canada.

Information on fish health care has been released and is in press for the Journal of Fish Biology. The paper is entitled "Occult hemoglobin in fish skin mucus as a possible indicator of early stress", by A.C. Smith and F. Ramos (listed as Oceanic Institute Contribution #123). References to the results with the milkfish are also being included in other manuscripts on fish health being prepared by Smith.

No personal overseas visits were made in the year under the auspices of the contract. A meeting between interested research organizations was planned for the year but this was pre-empted by the Philippine organizations to host both a national and then international conference on the milkfish. The national conference was held in July 1975, as planned, but the international conference was postponed from November 1975 to May 1976. Invitations to this international meeting have now been extended to both Drs. Kuo and Nash, and they will attend the meeting in May.

Under auspices of the International Center for Living Aquatic Resources Management, Dr. Nash toured Southeast Asia in April and May as an aide to the Director of that organization. Together they visited fisheries centers and talked with personnel in the Philippines, Indonesia, Malaysia, Thailand, Singapore, and Taiwan. Although the timing was too early for disseminating the results on the induced breeding, it was possible to describe and discuss the catching procedures which seemed to be more successful in Hawaii than in the Southeast Asian region. A major interest in milkfish propagation is developing in Taiwan at both the Tungkang and Tainan Fisheries Centers.

No feedback has yet been obtained. The Southeast Asian countries still have a strong desire to learn the induced breeding technology, and both SEAFDEC and the Inland Fisheries Department in Malaysia have sent trainees to the Institute for the mullet breeding season. The Institute has also produced a technical training film for distribution on the technology of rearing mullet and induced spawning procedures. Although several copies have gone to Southeast Asia, others have indicated their need for a copy but cannot afford one at this time (see Appendix II).

E. WORK PLAN FOR THE COMING YEAR

Reference should be made to Figure 4 to describe the work plan for the coming year.

The following events were started in the first year:

Objective 1. Establish broodstock in captivity.

Task a. Collect mature fish in season at sea,
b. Collect and grow-on immature fish,
c. Collect migratory fish in season,
d. Develop broodstock husbandry,
e. Hold, handle, and sample large fish.

Objective 2. Establish conditions for spawning.

Task a. Determine natural spawning conditions,
b. Simulate spawning conditions in laboratory,
c. Attempt spawning without hormones.

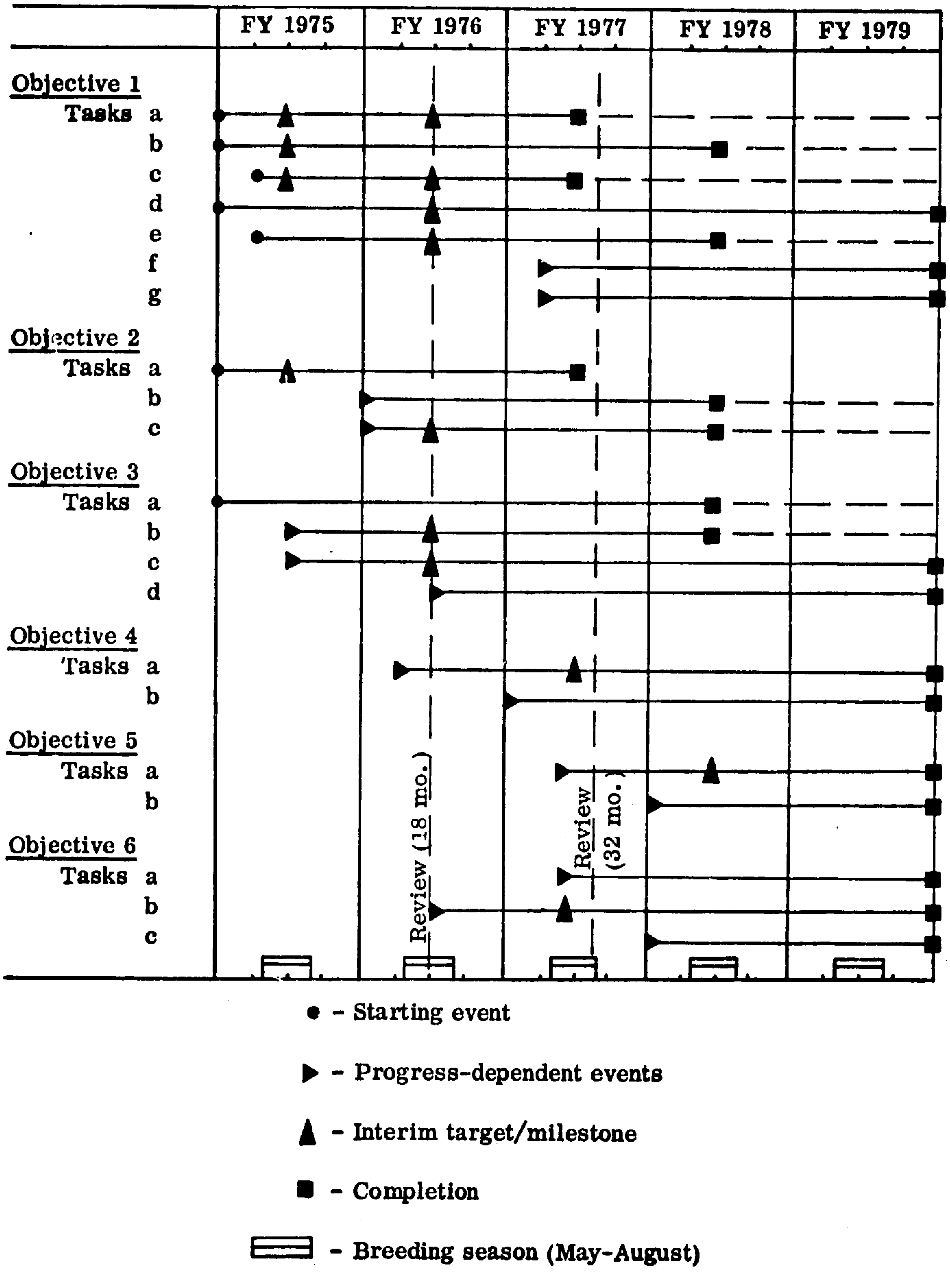

Figure 4. Project time chart [FY1 - FY2]

Objective 3. Induce spawning by hormone injection.

Task a. Determine optimum induced spawning procedure.

Objective 4. Improve survival of larvae in laboratory.

Task a. Nursery I development (with wild fish).

All these events will be strengthened in the second year. All new events will depend on progress during the year with the initial events.

All the initial target milestones under Objective 1 were achieved. The target milestone under Objective 2--to pinpoint the conditions at the natural spawning locations--was not achieved because equipment was not delivered until after the spawning season.

The procedures to be used will be exactly those initiated during the first year. The strongest emphasis must still be placed on the collection of fish. Although many fish are now in captivity and almost filling the available facilities, very many more fish are necessary. The two limiting factors to an early success for this project are the numbers of fish available, and the number of people available. There is, for example, a large backlog of egg samples and gonads still to be prepared histologically and analyzed. This data is important for developing the oocyte atlas. No attention is being given at all to the males in terms of their reproductive physiology. There is also a large backlog of fish scales and otoliths which will indicate the exact age of the fish being used. These jobs require competent but junior personnel.

The plans and proposals for working in the LDC's will be confirmed at the forthcoming international milkfish conference in Manila in May. It is hoped that a fish tag suitable for the milkfish will have been developed, and that a field trial can be planned for the next fish-breeding season. A suitable lake-sea migratory fishery will be selected in the Philippines and fish trapped en route to the breeding grounds will be tagged and released. Their movements will then be tracked at sea and the spawning grounds determined. Environmental programs to determine the conditions and parameters will then be designed for study by the nearest resident research group.

Permitted Budget for 1976 set by the Contract

1.	Salaries (man-months)	46,060	(39 1/4)
2.	Fringe benefits	3,915	
3.	Consultant	0	
4.	Travel and transportation	5,500	
5.	Capital purchases	4,500	
6.	Vehicle and supplies	5,342	
7.	Publications	250	
8.	Overhead (85% of #1 and #2 above)	42,479	
	Total	$108,046	

A fiscal report for Year 01 is included in this report.

The 01 report shows a cost over-run of $11,674.47. It must be anticipated that a cost over-run will occur again in 1976 because of the necessary demands in time and supplies, if the backlog of material is to be analyzed, and if the greatest advantage is to be taken of each year.

F. MINORITY PERSONNEL AND WOMEN

The complement of staff involved in the project, either in full- or part-time capacity, numbers twelve. Of these twelve, two are female, both Oriental. Both are technicians on the project, being responsible for laboratory work.

The male staff includes one Japanese, one Chinese, one Hawaiian, one Jew, and the rest Caucasian. Their duties include all work from Principal Investigator to Technician and Fisherman.

In addition to the resident staff, the project involves local fishermen hired by results, and they constitute Orientals and local Hawaiians.

Of the total Institute staff, some of whom provide the administrative and clerical services associated with the grant as overhead, eleven of the thirty are female.

G. SMITHSONIAN SCIENCE INFORMATION EXCHANGE, INC.

The information for the Smithsonian Science Information Exchange follows.

SMITHSONIAN
SCIENCE INFORMATION EXCHANGE, INC.
1730 M STREET, N.W. PHONE 202-381-5511
WASHINGTON, D.C. 20036

FORM APPROVED
BOB NO 105 R0002
EXPIRES 11/76

SIE NO.

NOTICE OF RESEARCH PROJECT

SUPPORTING AGENCY:

U.S. Agency for International Development

AGENCY'S NUMBER(S):

Contract No: TAC-1189
and/or
Control No:

TITLE OF PROJECT:

Research in Artificial Propagation of Milkfish Year 02

PRINCIPAL INVESTIGATOR, ASSOCIATES School or Division Department

Colin E. Nash, Ph.D.

RECIPIENT INSTITUTION:

Name and Address: Including Zip Code.

Oceanic Institute
Waimanalo
Hawaii 96795

PERIOD FOR THIS NRP:

Start Date: 1:13:75
End Date: 1:12:78
Annual Funding: $108,046.00

SUMMARY OF PROJECT: Be brief-200 word maximum: (Include Objective, Approach, Current Plans and/or Progress)

Induced breeding techniques using salmon pituitary gonadotropin SG-G100, and other hormones, are being used to stimulate maturation, hydration, and ovulation of the milkfish, Chanos chanos, Forskal.

Preliminary results indicate levels of 12 - 15 μg/g body weight in two injections of SG-G100 induce hypophysation of oocytes 0.7 mm in diameter and above. Ovulated oocytes are 1.2 mm in diameter. Excessive sampling and handling of adult females carrying Stage III oocytes of 0.8 mm in diameter causes atresia.

Resident populations of adult broodstock can be maintained healthily in captivity. Techniques using available clinical test apparatus are being developed to indicate stress conditions; for example, the presence of hemoglobin and ketones in the skin mucus increases with stress. Eye lens proteins are being examined for evidence of racial groups of milkfish.

APPENDIX

Appendix I: Preliminary milkfish data for GSI determinations

Appendix II: Film flier for "The Induced Breeding and Rearing of the Grey Mullet"

Month	Fish No.	Sex	Weight (gm)	Fork Length (cm)	Gonad Weight (gm)	GSI
June 6906	1	F	3,604	67.5	5.2	0.14
June	2	M	4,428	69.5	2.0	0.05
June	3	F	3,684	66.5	7.4	0.20
June	4	M	4,910	72.5	2.0	0.04
June	5	M	4,053	68.5	1.0	0.02
June	6	M	3,684	65.0	2.4	0.07
June	7	M	4,428	70.0	1.9	0.04
June	8	M	4,081	68.5	2.0	0.05
June	9	F	4,081	68.5	3.0	0.07
June	10	F	3,855	70.0	9.5	0.25
June	11	F	3,740	69.0	3.8	0.10
June	12	F	3,544	64.5	3.7	0.10
June	13	M	4,512	76.5	1.0	0.02
June 6917	1	M	5,339	70.5	0.5	0.01
June	2	F	5,453	71.5	7.5	0.14
June	3	F	5,453	71.0	11.0	0.20
June	4	M	4,657	67.5	1.5	0.03
June	5	M	5,000	71.0	1.5	0.03
June	6	F	5,453	77.0	12.5	0.23
June	7	F	4,885	68.0	10.5	0.21
June	8	M	5,000	72.5	1.0	0.02
June	9	F	4,345	66.5	7.0	0.16
June	10	F	4,345	66.0	2.0	0.05
June	11	M	4,544	67.0	2.0	0.04
June	12	F	4,572	65.5	6.5	0.14
June	13	F	4,657	68.5	4.0	0.09
June	14	M	4,572	66.0	1.5	0.03
June	15	F	2,229	67.0	8.5	0.38
June	16	F	4,345	65.5	3.0	0.07
June	17	F	4,572	68.5	7.0	0.15
Aug. 7524	4	?	1,164	42.0		
Aug.	5	?	2,061	51.5		
Aug.	6	?	2,115	53.0		
Aug.	7	?	1,304	43.5		
Aug.	8	?	1,622	47.5		
Aug.	9	?	1,992	47.5		
Aug.	10	?	2,659	56.0		
Aug.	11	F	2,704	57.0	2.5	0.09
Aug.	12	F	3,555	62.5	4.2	0.12
Aug.	13	F	3,588	62.0	6.2	0.17
Aug.	14	M	4,903	71.0	2.2	0.04

Month	Fish No.	Sex	Weight (gm)	Fork Length (cm)	Gonad Weight (gm)	GSI
Aug. 7531	15	M	3,290	59.0	1.0	0.03
	16	M	2,801	59.0	0.7	0.03
	17	F	3,670	63.0	3.1	0.08
	18	M	2,145	52.0	0.2	0.01
	19	F	7,518	83.5	108.0	1.44
	20	M	4,358	65.0	2.3	0.05
	21	F	4,899	71.0	27.1	0.55
	22	M	2,754	57.0	2.5	0.09
	23	F	3,397	61.5	6.5	0.19
	24	M	2,218	52.5	0.1	0.01
	25		799	37.0		
	26		574	33.0		
	27	F	3,509	63.5	5.8	0.17
Aug. 7520	2	F	10,150	91.5	97.0	0.96
Aug. 7521	3	F	7,515	82.0	62.0	0.83
Aug. 7522	4		1,164	42.0		
	5		2,061	51.5		
	6		2,115	53.0		
	7		1,304	43.5		
	8		1,622	47.5		
	9		1,922	47.5		
	10	M	2,659	56.0	2.0	0.08
	11	F	2,704	57.0	2.5	0.09
	12	F	3,555	62.5	4.2	0.12
	13	F	3,588	62.0	6.2	0.17
	14	M	4,903	71.0	2.2	0.05
Sept. 7508	1	F	2,965	56.0	12.0	0.41
	2	M	2,940	57.0	0.6	0.02
	3	F	864	49.0	0.2	
Sept. 7522	1	M	4,726	64.0	24.0	0.51
	2	M	2,280	51.5	1.6	0.07
	3	F	4,358	64.0	20.2	0.46
	4	F	3,959	62.0	17.0	0.43
	5	F	3,460	60.0	11.8	0.34
	6	F	3,082	58.0	10.0	0.32
	7	F	3,086	57.5	17.5	0.57
	8		1,969	57.5		
	9	F	2,347	53.5	6.1	0.26
	10	F	1,453	44.5	7.2	0.50

Month	Fish No.	Sex	Weight (gm)	Fork Length (cm)	Gonad Weight (gm)	GSI
Sept. 7522	11	F	6,515	80.0	6.5	0.10
	12	F	6,074	73.0	4.1	0.07
	13	M	5,450	72.5	10.0	0.18
	14	F	4,370	6.7		
	15	F	7,275	80.0	66.2	0.91
	16	M	6,105	74.0	6.1	0.10
	17	F	2,925	58.0	2.1	0.07
	18	M	3,350	60.0	1.6	0.05
	19	F	7,810	81.0	68.5	0.88
	20	M	2,395	52.5	0.6	0.03
	21		1,805	46.0		
	22		2,495	53.5		
	23	M	2,235	51.5	0.5	0.02
	24		2,770	57.0		
	25	M	2,395	60.0	0.3	0.01
	26	F	2,082	60.0	3.0	0.14
	27		2,285	54.0	0.4	0.02
	28	M	2,590	59.5	1.0	0.04
	29	F	2,850	59.5	1.0	0.04
	30	F	4,335	67.0	16.8	0.39
	31	F	2,225	58.0	3.4	0.15
	32	M	1,900	50.5	0.4	0.02
	33	F	1,535	46.5		
	34	F	1,435	44.0		
	35	F	690	34.5		
	36	F	760	36.0		
	37	M	760	34.0		
	38	M	595	33.5		
	39	M	720	35.5		
Sept. 7523	1	F	5,734	69.5	32.2	0.56
	2	M	4,613	70.0	2.5	0.05
	3	F	6,067	72.0	47.0	0.78
	4	M	4,683	69.0	3.0	0.06
	5	M	4,768	71.5	5.7	0.12
	6	M	5,243	73.0	2.9	0.06
	7	M	5,736	71.0	15.0	0.26
	8	F	4,820	71.0	17.2	0.36
	9	F	2,066	54.0	1.6	0.08
	10		1,518	49.0		
	11	F	4,103	70.0	12.1	0.30
	12	M	2,285	57.0	0.7	0.03

Month	Fish No.	Sex	Weight (gm)	Fork Length (cm)	Gonad Weight (gm)	GSI
Oct. 7504	1	M	3,224	62.0	0.7	0.02
	2		2,908	57.0		
	3		2,105	52.5		
	4		2,679	56.0		
	5	F	3,330	64.5	6.3	0.19
	6	M	4,330	67.0	2.4	0.06
	7	M	4,536	69.5	2.6	0.06
	8	M	5,030	70.5	4.0	0.08
	9	F	4,233	66.5	6.2	0.15
	10	M	5,998	72.5	3.2	0.05
	11	F	4,920	71.0	15.9	0.32
	12	F	6,457	74.5	27.2	0.42
	13	M	6,911	80.5	32.8	0.48
	14	F	8,950	82.5	49.0	0.55
Oct. 7509	1		557	33.5		
	2	M	2,840	58.0	0.2	0.01
	3	F	3,470	64.5	11.1	0.32
	4	F	3,368	68.5	7.5	0.22
	5	M	5,457	79.5	2.8	0.05
Oct. 7511	6	M	3,258	61.5	1.0	0.03
	7	F	3,428	61.5	3.2	0.10
	8	F	3,620	60.5	6.2	0.17
	4	M	2,860	58.0	0.6	0.02
	5	M	2,636	56.0	0.5	0.02
	9	F	3,331	62.0	3.8	0.11
	10	F	4,537	69.0	3.3	0.07
	11	F	3,683	64.5	5.4	0.15
	12	F	3,065	58.5	1.4	0.05
	13	F	2,650	57.5	2.0	0.08
	14	F	3,720	62.5	0.7	0.02
	15	F	4,090	70.0	1.3	0.03
	16	F	4,185	64.5	13.7	0.33
Oct. 7518	1	F	6,634	73.0	27.2	0.41
Oct. 7521	1	F	5,219	70.5	17.8	0.34
Oct. 7525	1	F	4,154	70.0	10.8	0.26
	2	F	5,550	74.0	22.3	0.40
Nov. 7506	1		2,157	54.0		
	2	F	5,510	73.0	3.1	0.06
	3		2,180	53.0		
	4	F	8,760	81.0	90.5	1.033
	5	F	4,311	70.0	17.6	0.41
	6	F	7,721	82.0	64.2	0.83

Month	Fish No.	Sex	Weight (gm)	Fork Length (cm)	Gonad Weight (gm)	GSI
Nov. 7506	7	F	3,656	74.0	29.2	0.80
	8	M	4,162	68.0	2.1	0.05
	9	F	3,870	65.0	10.4	0.27
	10	F	5,298	69.0	24.3	0.46
	11	F	5,735	73.0	3.3	0.06
	12	F	5,594	72.0	4.2	0.08
	13		7,927	50.0		
	14	M	3,492	67.0	2.2	0.06
	15	M	4,656	66.0	2.7	0.06
	16	M	4,860	72.0	3.0	0.06
	17	M	4,758	70.0	3.2	0.07
	18	F	3,668	73.0	4.2	0.12
	19	M	3,275	60.0	1.6	0.05
Dec. 7502	1	F	6,847	77.5	29.8	0.44
	2	F	5,705	72.0	32.5	0.57
	3	M	5,692	75.0	2.3	0.04
	4	F	6,702	79.0	4.3	0.06
Jan. 7504	1	M	7,500	77.0	10.0	0.13
	2	F	6,040	75.0	28.0	0.46
	3	M	5,600	76.0	9.0	0.16
Jan. 7511	1	F	5,228	74.8	2.4	0.05
	2	M	4,219	66.0	12.1	0.29
Jan. 7521	1	F	5,725	72.5	13.5	0.24
Feb. 7522	1	F	4,405	69.0	9.0	0.20
	2	F	3,466	61.5	1.0	0.03
	3	M	3,205	62.0	6.5	0.20
Mar. 7501	1	F	3,720	64.0	8.0	0.22
	2	M	2,907	59.5	1.0	0.03
	3	F	5,741	73.0	26.0	0.45
Mar. 7519	1	F	5,330	72.5	26.5	0.50
	2	F	4,996	73.5	28.5	0.57
	3	F	4,686	71.0		
Apr. 7504	1	F	6,407	78.0	34.0	0.53
	2	F	5,566	71.0	9.5	0.17
	3	M	5,716	73.5	3.5	0.06
	4	M	3,350	60.5	2.0	0.06
	1	M	6,275	77.0	3.5	0.06
	2	F	5,083	69.0	18.5	0.36
	3	M	4,345	65.0	1.5	0.03
	4	F	3,540	62.0	4.5	0.13
	5	F	4,490	67.0	6.5	0.14

Month	Fish No.	Sex	Weight (gm)	Fork Length (cm)	Gonad Weight (cm)	GSI
Apr. 7504	6	M	4,400	65.5	0.8	0.02
	7	F	6,786	75.0	28.5	0.42
	8	F	5,466	70.0	21.5	0.39
May7506	1	M	6,880	83.0	5.3	0.08
	2	F	6,389	76.5	27.7	0.43
May7516	1	F	5,960	77.7	20.5	0.34
	2	M	5,764	72.0	9.5	0.16
Aug. 7510	1	F	7,693	75.0	165.5	2.15
Aug. 7520	2	F	10,150	91.5	97.0	0.96
	3	F	7,515	82.0	62.0	0.83

THE OCEANIC INSTITUTE

THE INDUCED BREEDING AND REARING OF GREY MULLET

Technical Film on the Culture of the Grey Mullet, Mugil cephalus

The Oceanic Institute in Hawaii has prepared a technical training film on the induced breeding and rearing of the grey mullet. It describes the culture techniques and procedures developed by Drs. Ziad H. Shehadeh, Ching-Ming Kuo, and Colin E. Nash for spawning adults and rearing juveniles in the hatchery.

Primarily this is a training film for fisheries scientists and technicians in the developing countries where the grey mullet is an important subsistence food fish. However, the procedures are also of interest to all fisheries scientists who use induced breeding techniques or who attempt to mass-propagate marine and brackishwater species of fish.

The film is very suitable for university departments providing classes in marine biology and zoology. A special edition will be available shortly for a more general audience.

Sections in the film deal with a history of farming mullet, broodstock capture and holding, induced spawning procedures, spawning behavior and fertilization, egg incubation, larval rearing with larval food production, and juvenile handling. One excellent sequence illustrates the release of eggs by the female and fertilization behavior by the males. Very few fisheries biologists have ever seen this event.

All pertinent information is covered visually and on the soundtrack. The film is distributed with a copy of the text in English. It is hoped that copies of the film in French, Spanish, and Chinese, with appropriate texts, will be available shortly.

The copyrighted 23-minute 16mm color film, with soundtrack, was produced by Ahuimanu Productions. It can be purchased from the Oceanic Institute, Waimanalo, Hawaii 96795, U.S.A. for U.S. $300.

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