PRELIMINARY CAPTURE, HUSBANDRY AND INDUCED BREEDING RESULTS WITH THE MILKFISH, *Chanos chanos* (FORSKAL)

by

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Abstract

The program objective is to breed the milkfish, *Chanos chanos* (Forskal), 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 gonadotrophin 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.

Preliminary results indicate that oocytes of 0.8 mm and above are at a critical stage (or beyond) 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

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are stored. Determination of the CSI for Hawaiian fish indicated a rapid maturation of ococytes 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 and gastritis. 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.

Brookstock Collection and Husbandry

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 inteerest. 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 brookstock 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.

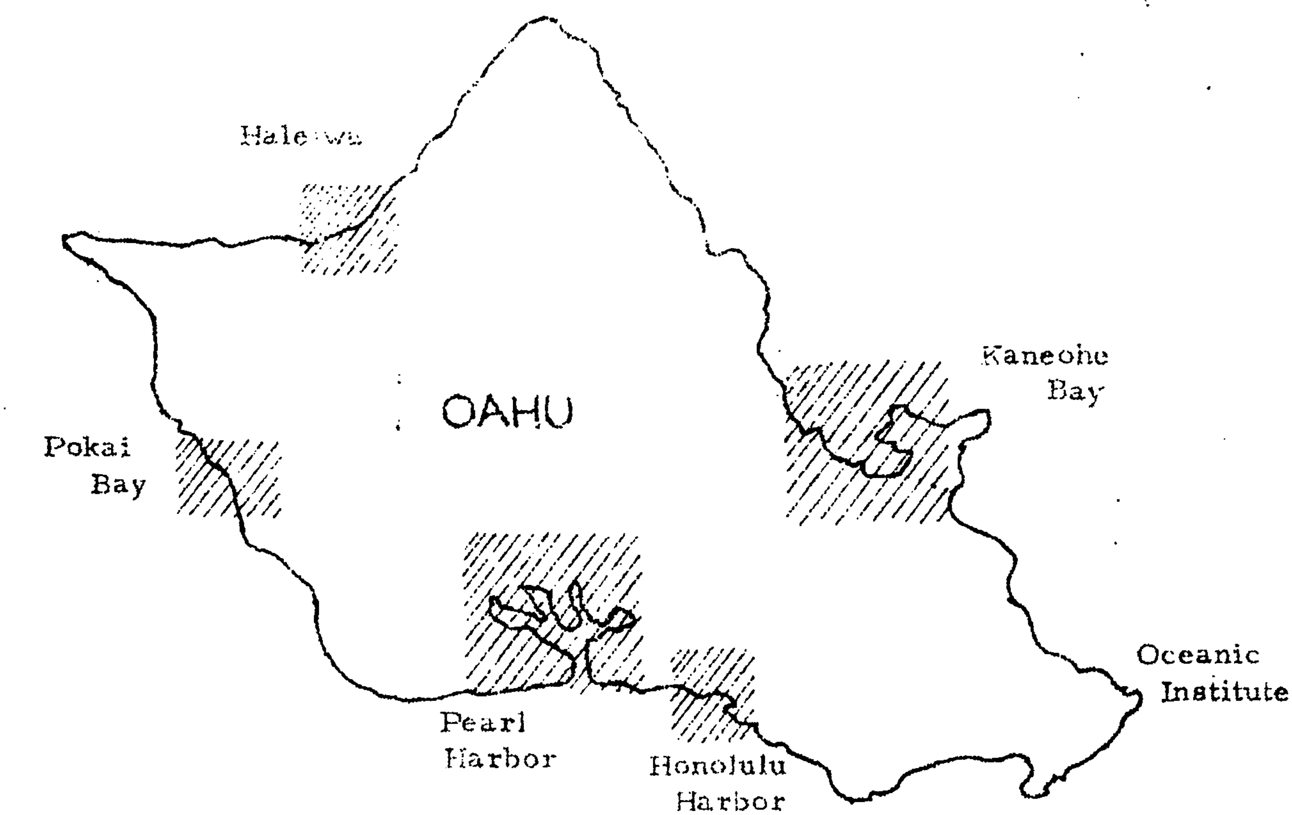

Fig. 1. Adult milkfish fishing locations in Oahu

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; sports-fishermen; 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.

At present over 50 live adult milkfish have been collected from the sea and are held as potential broodstock. The largest individuals weigh between 10 and 12 kg, and measure 1 -1.3 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 practised, 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 stronglyaerated water in the tank is reduced in salinity and temperature immediately to about 12 o/oo and 20 °C, respectively 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 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.

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, 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.

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 o/oo because of subsurface springs.

The site of Lahuipuaa on the Kona coast contains five irregular ponds, two of which are connected to the ocean of 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, milkfish, 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 1975 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 34 which were relocated in a small pond for subsequent transportation to the Institute.

Hopeaia Pond is an irregular 0.5 ha pond not connected to the ocean. The maximum depth is about 1.5 m but many parts are shallow. The water is clear and slightlybrackish (7 o/oo) and seawater penetrates 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 inlength, 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 m in length, were in fact maturing. Many males were ripe with active spermatozoa, 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 continously 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 1,500 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 fish 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 procédures 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 big. 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.

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, 13-16 mm inlength, 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 constrast 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 have only one spawning period in the year (June to August), and that the local population, while not large, is probably sufficient to produce progeny annually 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 are 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 other islands, for both fish and the behavioral observations.

Husbandry of Captive Stock

A 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 certain 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 rod and line. The locations are unsuitable for netting gear.

The more practical feeds used for the herbivorous captive fish are modifications of the pelletized feeds used for trout and carfish. 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 vegetations 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 E Complex)	1.4%
Vitamin pre-mix	0.2%

The ingredients are milled to 0.5 mm 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.

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.

Induced Breeding

Data

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

Table 1. Monthly Gonado-Somatic Index (GSI)

	Males				Females			
Month	GSI	sd mean	sd	n	GSI	sd mean	sd	n
Jan	0.193	0.049	0.025	.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	[illegible]	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	0.307	0.153	0.265	3
				(75)				(104)

*Early
**Late

The GSI is the most pr ıctical measure of maturity development by fishery biologists and car 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 (Fig. 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.

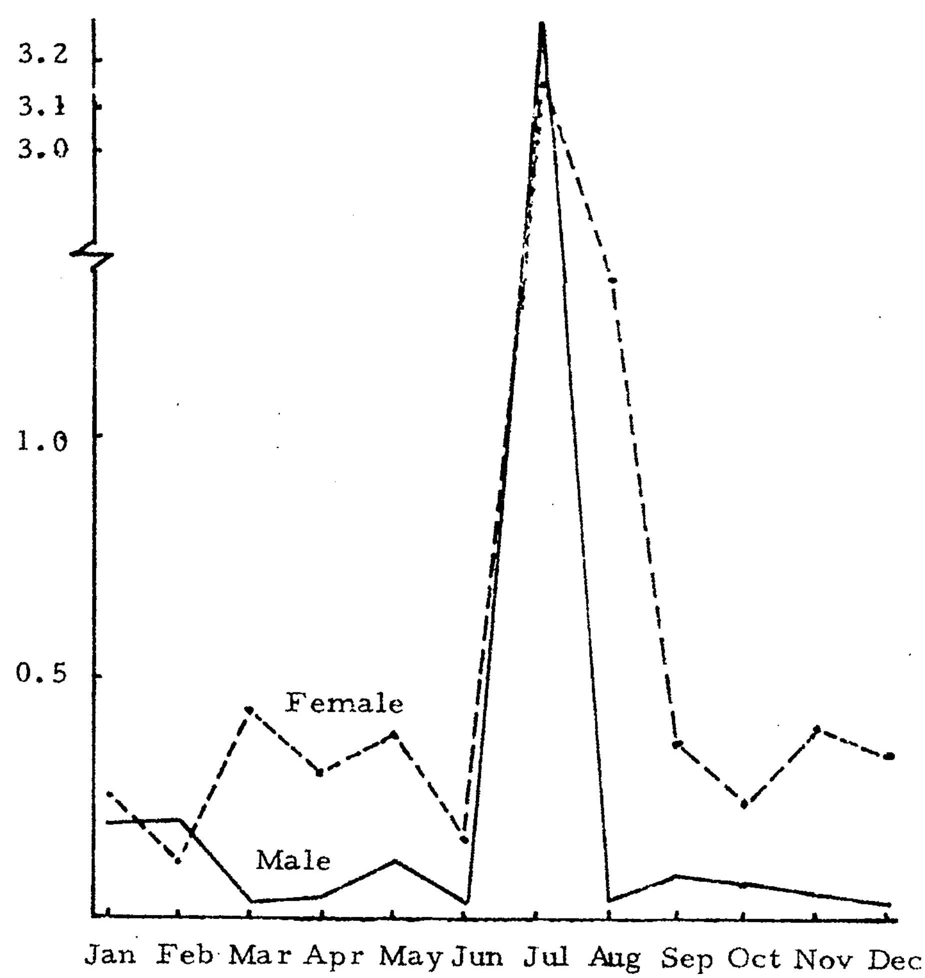

Fig. 2. Monthly gonado-somatic index of the milkfish

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 pond 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 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.

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 of fish 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 than 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 grey mullet, and egg sampling has proved difficult.

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 Fig. 3.

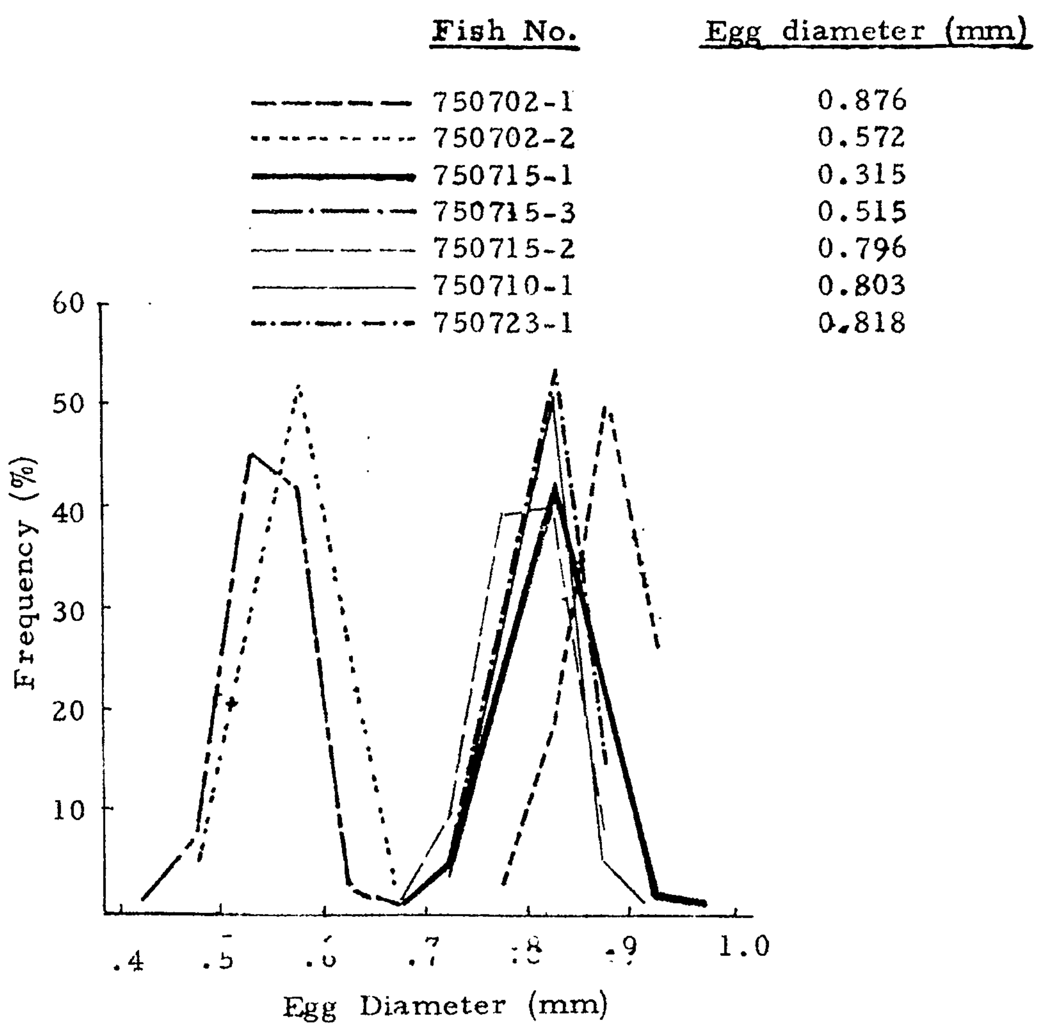

Fig. 3. Egg diameter distribution

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
750708-1	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 ECR#[illegible].

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

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	10	III	Mature
	7:21		0		Atretic
750723-1	7:23	0.818	25	III	Mature
	7:24		25		Hydrated
	7:25		0		Patially 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 while trying to obtain egg samples.

The two fish detailed above (750715-1 and 750715-2) immediately went atretic, or began reabsorption of their eggs, following initial 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 15 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, at which immediate hypophysation is needed. Injection cannot be delayed. Excessive handling or stress at this stage causes the eggs to become atretic, or reabsorbed. It appears that eggs of about 0.77 mm are more suitable for reacting positively to injections. The size of an ovulated egg is 1.2 mm in diameter.

The first dose of injection was 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 optimum. 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, becauce of their large size. The lower dose will give significant savings.

Health Care of Captive Fish

Stock Identification

The work in stock identification consisted of the collection and electrophoretic processing of eye lense 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 likely to thrive better 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 population as they are reflected from the patterns. The patterns themselves will be stored in a book for easy display.

Studies on 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 case of the technique of testing and reveal the promise of 'stix' in dianosing 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 lipoblepherities 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 not 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.

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.

Milkfish have been found to have a high incidence of a grossly and histologically identifiable gastritis. Of 60 individuals autopsied, this condition was found in 67%. With respect to location of capture, the percentage of afflicted fish ranges from 38% to 100%. Other parameters for example fish size, sex, etc., are currently being evaluated for possible relationship to the stomach condition.

The well known 'nervousness' of the milkfish may be a manifestation of the stomach irritation, or its cause. The role of this condition in the long-term health and survival of the animal is currently being evaluated. For example, does the stomach lining sometimes bleed, causing, as it does in humans, anemia or even mortality if enough blood is lost to lead to hypovolemic shock? A possible preventive is also being considered.

There is evidence that a wide variety of conditions--e.g. stress, microbial disease, trauma, malignancy, etc.-- can lead to clot formation in vivo. It has been speculated that should such clots form, from whatever cause, and lodge in blood vessels to vital organs, for example the heart, it could result in the death of fish.

To explore this possibility in milkfish, the hearts and ventral aortas from four individuals were autopsied. The ventral aorta from one of three of these fish, all of which had been caught by hook and line and stored frozen prior to autopsy, revealed several 'old', organized clots. Because of their size, number and location, they almost certainly interfered to some extent with the outflow of blood from the heart. Most dramatic, however, were the hearts from the remaining two fish in this group and a fourth fish, which died of unknown causes after thrashing about in a pond for approximately one hour. These hearts each contained a fairly fresh clot that was solidly impacted in the conus arteriosus. There was no question that this clot completely sealed the outflow tract from the heart, and that these (or any other) fish could not survive such an event.

Studies continuing on more animals are strengthening the hypothesis of in vivo clotting from a variety of conditions, as mentioned, resulting in at least morbidity (first milkfish above) or the final cause of death (remaining three milkfish above). The conditions that initiated clot development in the first and fourth milkfish of this study' are not clear; but hypoxia-induced ventricular fibrillation, resulting in blood stasis in the heart, is probably the cause of the clots in the other two ocean-caught fish, and may also have been involved in forming the clot in the pond fish. In many cases, the conditions initiating clot development may not be evident, but they may be considered or academic concern because the clots once formed become the principal problem. Therefore, effort is being directed at evaluating tests from human clinical laboratory medicine to detect clotting in its earliest stages, and of ways to stop its further propagation in the live animal.

Immune Status of Milkfish

A possible quick and easy method, adapted from human clinical laboratory medicine, was tested for assaying the presence and concentration of protective antibodies in milkfish. The method, known as radial immunodiffusion (R.I.D.), consists of applying samples of extract--in this case, from milkfish lens nuclei (an easily obtained and stored source)--on a gel containing antiserum against antibodies. Positive results are produced in the form of rings of precipitate around the sites of sample application. In the milkfish, some variation in concentration of precipitate among individuals was observed, but results were all clearly positive. They suggest that R.I.D. can be applied as practicable method in milkfish culture for:

a) Following the chemical immunie response of individuals in both health and disease (possibly using blood or skin mucus for this), and

b) Differentiating milkfish stocks with varying degrees of antibody production. Those with the highest titers may be most useful for pond culture.

Hypersensitivity Responses to Milkfish Serum

The common Hawaiian sea cucumber, Holothuria cinerascens, responds with severe contractions of its body wall musculature to contact with diluted milkfish serum. This response appears to be immunologic in nature, and desensitization is possible leading to improved health and longevity. Follow up studies are attempting to evaluate the sea cucumber/milkfish serum system for investigating immunologic responses, stress resistance, and longevity in an invertebrate generally considered ancestral to fishes and other vertebrates, including man. Application and benefits to the latter group are anticipated from this basic research.

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 Fig. 4, 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.

Tracking requirements of one km for range and 300 m for depth in the open ocean, and lesser values in coastal waters, are 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 soner 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 dissected show large, elongated swim bladders, extending over about half their body length. The acoustical target strength of such animals is 20 to 25 db for 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 (90.77m). 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 (90.77 m) or 200 yards (181.54 m) away.

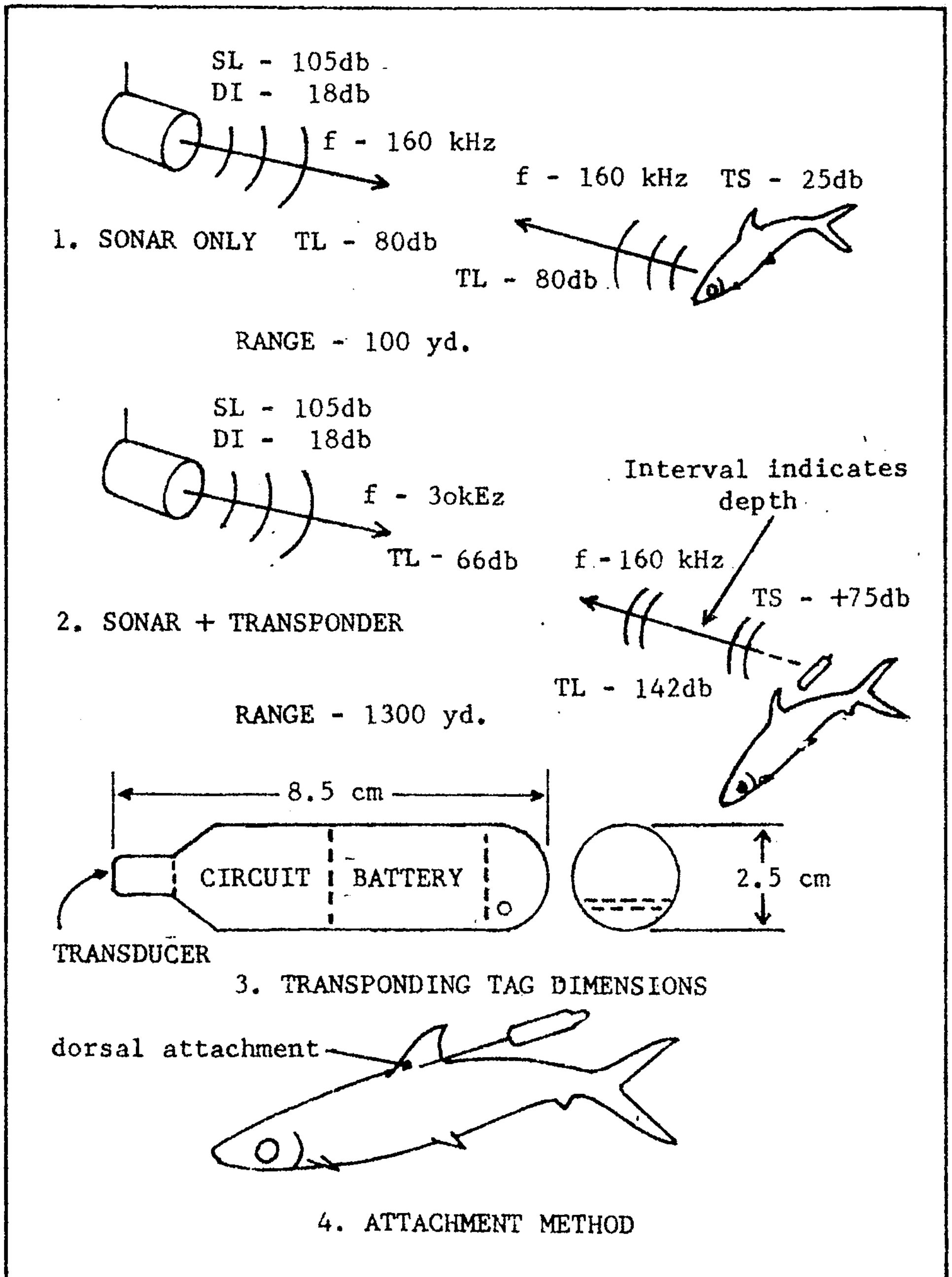

Fig. 4 Tagging and tracking information

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.

The Wesmar sonar has been installed and modifications designed and tested to provide an interrogation signal. A prototype transponder has been designed and dummy tags fitted to live adults.

No attempt has been made yet to miniaturize the transponder circuit, as this will require tooling that is circuit-dependent. However, no problems in this regard are anticipated and the transponder's battery will be its largest component.

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