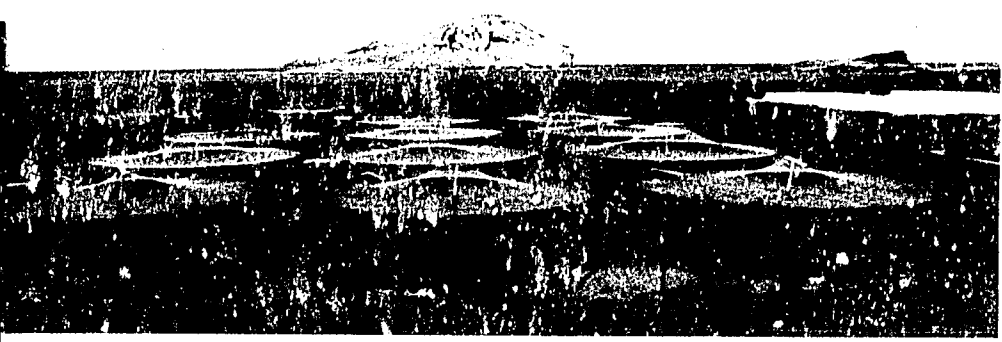


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PRODUCTION AND CULTURE OF MILKFISH

Proceedings for a workshop held at
Tungkang Marine Laboratory, Taiwan
April 22-24, 1985



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Oceanic Institute and Tungkang Marine Laboratory
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Washington, D.C.

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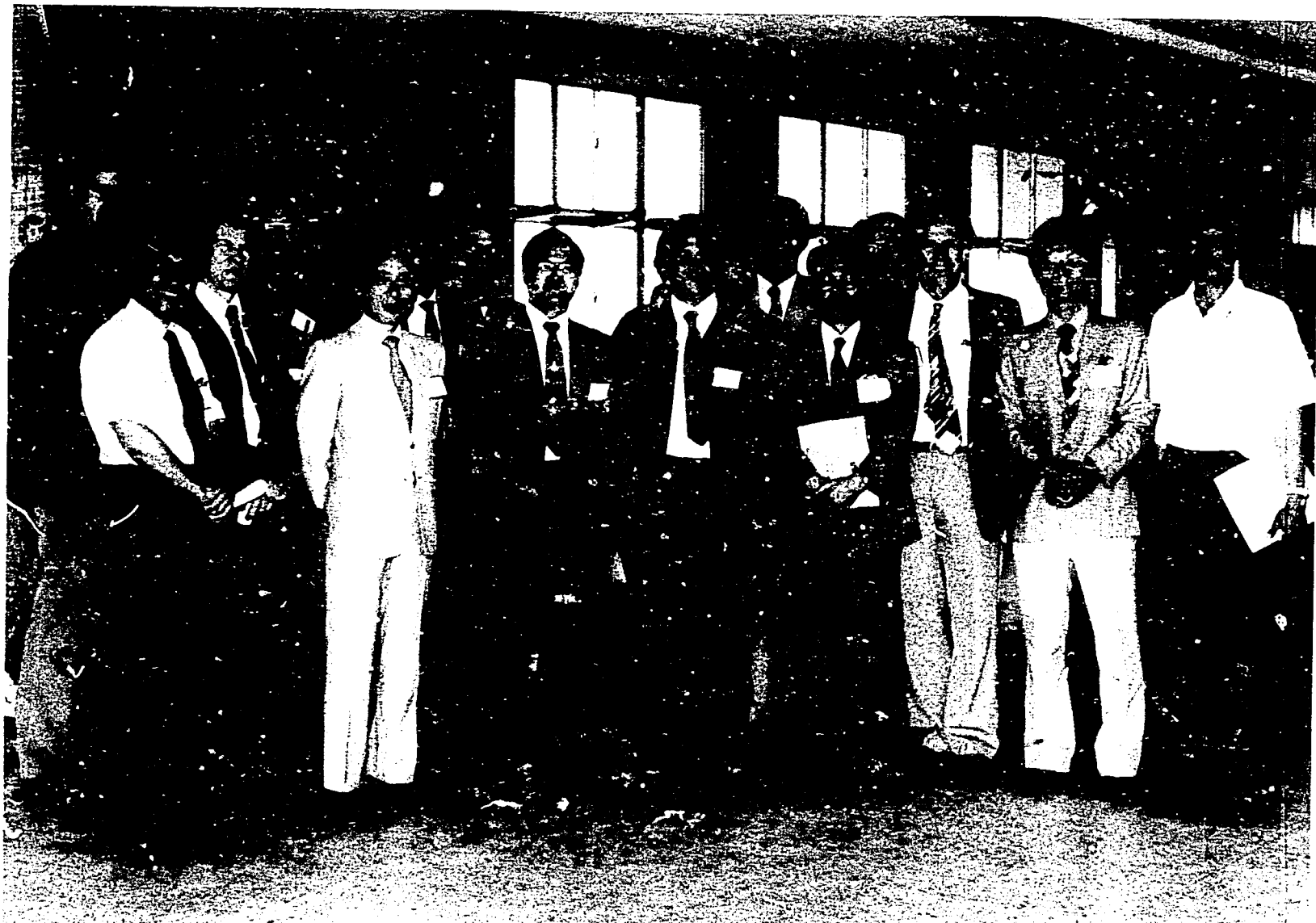
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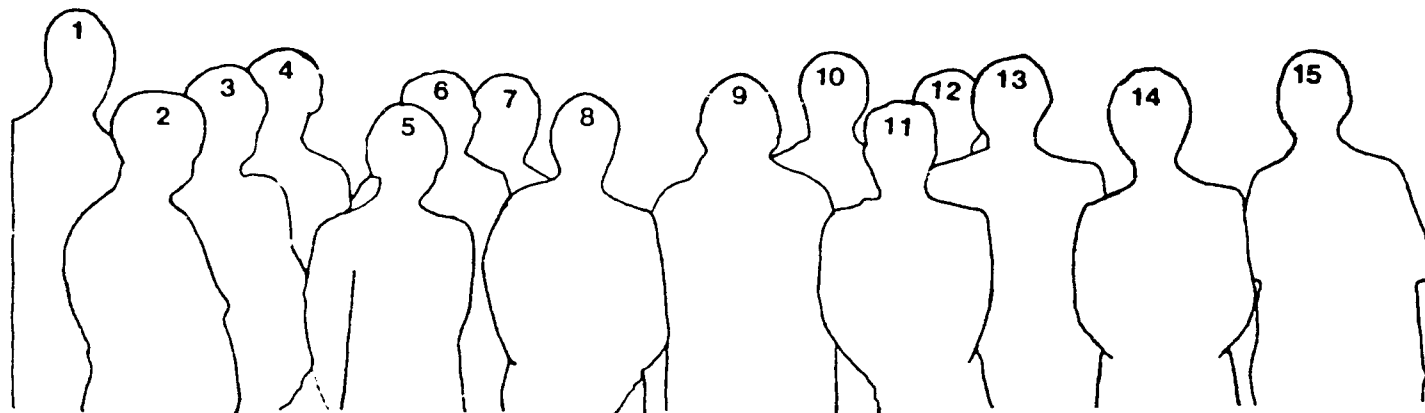
Cheng-Sheng Lee
and
I-Chiu Liao

Published By

The Oceanic Institute
and
Tungkang Marine Laboratory

Hawaii, USA
1985





Invited speakers and workshop organizing committee members

- | | |
|--|--|
| 1) L. Crim, Marine Sciences Research Laboratory,
Memorial University of Newfoundland | 11) I. Hanyu, Department of Fisheries, Faculty of
Agriculture, University of Tokyo, Japan |
| 2) I.C. Liao, Tungkuang Marine Laboratory, Taiwan | 12) C.M. Kuo, International Center for Living Aquatic
Resources Management (ICLARM) |
| 3) L. Trott, U.S. Agency for International Development,
Washington, D.C. | 13) J. Hunter, National Marine Fisheries Service,
California |
| 4) M. Gordon, Biology Department, University of
California at Los Angeles, California | 14) C.S. Lee, Oceanic Institute, Hawaii |
| 5) A. Kanazawa, University of Kagoshima | 15) L.T. Lin, Tung Hsing Fish and Shrimp Hatchery,
Taiwan |
| 6) T.J. Lam, Department of Zoology, National
University of Singapore | |
| 7) J. Wyban, Oceanic Institute, Hawaii | |
| 8) K. Fukusho, National Research Institute of
Aquaculture, Japan | |
| 9) C. Tamaru, Oceanic Institute, Hawaii | |
| 10) P. Bienfang, Oceanic Institute, Hawaii | |

Invited speakers missing from the photo
 S. Pamplona, Naujan Research Substation (SEAFDEC)
 F.H. Chen, Milkfish farmer, Taiwan
 C.C. Chiu, Milkfish farmer, Taiwan

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FORWARD

The proceedings include the papers presented at the workshop on the "Reproduction and Culture of Milkfish". This workshop was sponsored by the United States Agency for International Development (USAID) under cooperative agreement DAN-4161-A-4055-00 with the Oceanic Institute. The workshop was organized by Oceanic Institute in Hawaii and Tungkang Marine Laboratory in Taiwan. I wish to express my sincere thanks to Dr. I.C. Liao and his staff for making the arrangements and providing a meeting site. I also extend my warmest thanks to the invited speakers, the members of the Technical Advisory Group for the Milkfish Program and to all participants.

Milkfish has been cultured in Asia for centuries and is still one of the most important sources of animal protein. Despite years of research, the control of the reproductive process in this species has not yet been achieved. This presents a major obstacle that impedes further development of milkfish aquaculture. This workshop was designed to promote the exchange of knowledge among the world's experts and to accelerate progress in this area. Their presentations are summarized as follows:

Dr. L.W. Crim introduced the methodology for exogenous hormone administration. Many factors, including economics and convenience, influence the selection of a suitable method of

hormone application.

Dr. T. J. Lam reviewed the techniques for induced spawning in finfish developed since 1983. The hormonal and environmental approaches were discussed separately, however, and the best results can be achieved with a combination of the two. The operating cost and the resultant egg quality are the primary considerations for selecting the method of induced spawning.

Dr. C.M. Kuo presented a paper on the current technology of induced breeding of milkfish. Although significant progress has been made, a reliable and standardized technique has yet to be established. At present, the most commonly used hormone to induce final maturation of milkfish is human chorionic gonadotropin (HCG).

Dr. I. Hanyu discussed environmental factors in fish reproduction. He illustrated the effect that temperature and photoperiod have on the spawning seasons of fish using the rose bitterling and tabira bitterling as examples. The importance of these factors appear to vary within the spawning season.

Dr. C.S. Lee discussed environmental conditions which affect the reproduction of milkfish. Using ecological studies of spawning grounds, he showed that long daylight regimes and warm water temperatures promote final maturation. However, further research in this area is necessary to determine the optimum range of photoperiod and temperature in which milkfish will mature.

Dr. A. Kanazawa presented several studies in which the

protein, lipids, vitamins, minerals, and pigments were varied in formulated diets for fish. The results of these studies strongly indicate that the nutritional quality of prepared broodstock diets affect the egg quality and spawning performance. It was also pointed out that besides spawning and subsequent fertilization rates, hatching rates should also be included as a criteria to measure egg quality.

Dr. K. Fukusho discussed advanced techniques of larval rearing as practiced in Japan, including the production and nutritional value of rotifers, larval deformity, automatic feeding systems, and breeding programs. This information is valuable in designing milkfish hatcheries.

Mr. C.S. Tamaru discussed the population structure of milkfish in the Pacific and the methodology of research in this field.

Mr. S.D. Pamplona discussed milkfish farming in the Philippines including fry collection, fry rearing, growout, harvesting, and the status of research in this area.

Dr. I.C. Liao presented an overview of current milkfish culture methods in Taiwan. Included were shallow-water pond and deep-water culture systems, existing problems and possible solutions, followed by a discussion of future prospects.

Three milkfish farmers from Taiwan shared their valuable experience: Mr. Lin described the natural spawning of milkfish in his ponds and the broodstock management strategy that he

employs. Mr. Chen followed with a discussion on the traditional milkfish culture methods and Mr. Chiu on deep-water culture systems. In light of the new developments, further exchange of information between farmers and researchers is encouraged.

Dr. C.S. Lee et al. presented an easy-to-follow procedure for producing and implanting a slow hormone-releasing cholesterol pellet. The development of this technique will benefit research on breeding in many finfish.

In the preparation of these proceedings for publication, I would like to acknowledge the able assistance of Ms. A. Belanger and Mr. C. Lum.

C.S. Lee
Oceanic Institute
August, 1985

**REPRODUCTION and CULTURE
OF
MILKFISH**

**A Workshop at the Tung kang Marine Laboratory
Tung kang, Taiwan**

Schedule of Activities

April 22	Reproduction of Milkfish
	<u>Moderator: Crim</u>
8:30-9:00	Registration
9:30-10:00	Opening Remarks
	Mr. C.C. Young, President China Fisheries
	Greeting by the local mayor
	Dr. I.C. Liao, Director TML
	Dr. P. Bienfang, Vice President Oceanic Institute
	Dr. C.S. Lee, workshop organizer
10:00-10:15	Tea break
	Moderator: Dr. L. Crim
10:15-10:45	Methods for Acute and Chronic Hormone Administration in Fish - Dr. L. Crim
10:45-11:15	Induced Spawning in Fish - Dr. T.J. Lam
11:15-11:45	A Review of Induced Breeding of Milkfish - Dr. Ching-ming Kuo
11:45-12:30	Discussion: "Hormonal Control of Reproduction and Maturation in Milkfish"
12:30-2:00	Lunch
	Moderator: Dr. C.S. Lee

- 2:00-2:30 Environmental Factors in Fish Reproduction -
Dr. I. Hanyu
- 2:30-3:00 Environmental Factors in Milkfish Reproduction
- Dr. C.S. Lee
- 3:00-3:15 Tea break
- 3:15-3:45 Nutritional Factors in Fish Reproduction - Dr.
A. Kanazawa
- 3:45-4:00 Discussion: "Environmental Control of
Reproduction and Maturation of Milkfish"

April 23

The Biology and Culture of Milkfish

Moderator: Dr. I.C. Liao

- 9:00-9:30 Status of Marine Larval Culture in Japan - Dr.
K. Fukusho
- 9:30-10:00 Population Structure of the Milkfish Chanos
chanos, Past and Proposed Analysis -
Mr. C. Tamura
- 10:00-10:45 Milkfish Farming in the Philippines -
Mr. S. Pamplona
- 10:45-11:00 Tea break
- 11:00-11:45 Milkfish Culture in Taiwan - Dr. I.C. Liao
- 11:45-12:15 Discussion: "General Biology of Milkfish"
- 12:15-1:30 Lunch
- Moderator: Dr. C.M. Kuo
- 1:30-2:00 My Experiences in Artificial Propagation of
Milkfish - Mr. L.T. Lin
- 2:00-2:30 My Experience in Traditional Milkfish Culture -
Mr. F.H. Chen
- 2:30-3:00 Deep-water Pond System for Milkfish Culture-
Mr. C.C. Chiu
- 3:00-3:15 Tea break

3:15-3:30 Preparation of a Luteinizing Hormone-releasing
Hormone Cholesterol Pellet and Its Implan-
tation in the Milkfish (Chanos chanos Forsskal)
- Dr. C.S. Lee

3:30-4:00 Summary

4:00-4:10 Closing remarks

Dr. L. Trott, Senior Fishery Advisor of USAID

April 24

Field Trip to Commercial Milkfish Farms

OPENING REMARKS

C.C. Young

President of China Fisheries Association
Taipei, Taiwan

Good Morning!

Ladies and Gentlemen:

It is my great pleasure this morning to be here to participate in the opening ceremony of the Workshop on Reproduction and Culture of Milkfish held in the Tungkang Marine Laboratory. On behalf of the China Fisheries Association, I would like to extend my hearty welcome to honorable guests and friends coming from abroad as well as my Chinese colleagues. As Confucius' saying goes: "How happy to greet friends from remote places." I exactly share the same feeling this morning.

During my seven-year assignment as Vice Minister of the Ministry of Economic Affairs, I had many chances to visit the Tungkang Marine Laboratory. There were limited facilities and few staff at the time. However, I find a rather new atmosphere in this laboratory today not only because of the new buildings and wonderful facilities, but also because of the fantastic potential that the aquaculture scientists have expressed. My friends have been working hard to make this laboratory one of the most distinguished laboratories in the world, to become a laboratory doing research on milkfish and other aquatic animals, and also to become a training center for people coming from

Southeast Asia and even other countries. I realize that this workshop is part of the activities of an international cooperative research program and would like to congratulate the Oceanic Institute, the Aquaculture Department of the Southeast Asian Fisheries Development Center, and the Tungkang Marine Laboratory for such a successful beginning.

For centuries, the culture of milkfish has provided a source of protein for the people of Southeast Asia. In Taiwan, the traditional methods of shallow-water culture of milkfish are giving way to the more productive deep-water techniques. Such practices require a larger amount of investment in both time and capital. Both the traditional and the modern methods in milkfish culture, however, still depend on the wild stocks of milkfish fry. Fluctuations in the amount of available wild fry have continuously embarrassed the milkfish farmers both old and new. Such fluctuations can be economically devastating, especially to the farmers practicing the new culture methods. Obviously, a more reliable source of fry will provide a more stable industry.

The key for a more dependable source of milkfish fry is the control over the reproductive traits of the milkfish. However, failures in the demonstration of regulating that aspect of the milkfish's life history has continued to frustrate scientists from all over the world. It will probably take a combined effort of both minds and resources to solve the problems that have prevented success to date. In addition, the solution of those

problems associated with milkfish reproduction has many far-reaching aspects. Culturing of a desirable fish species has become a viable alternative to relying on the availability of natural stocks.

For all these reasons, it is especially gratifying for me to see the most respected scientists and the most experienced milkfish culturists gathering together in a cooperative effort to help solve the problems of reproduction and culture in milkfish. Once again, on behalf of the China Fisheries Association, may I extend my greetings and I wish you a fruitful and constructive workshop. Furthermore, I especially wish all of you good health and a pleasant stay in Taiwan.

Thank you.

GREETING SPEECH FROM THE LOCAL OFFICIAL

J.Y. Sheu

Mayor of Tungkang, Taiwan

Dr. Young, Dr. Lee, Dr. Liao, Honorable guests, Ladies and Gentlemen:

I am honored and pleased this morning to be here to participate in the Workshop on Reproduction and Culture of Milkfish.

First, I would like to welcome our honorable guests. Your presence has brightened our little humble town. Under the leadership of Dr. Liao in the last few decades, Tungkang Marine Laboratory has grown to be one of the distinguished research institutes in the world. We are honored and deeply appreciate the efforts and support of all the scientists and scholars involved. I hope more support will be provided by our friends, scientists, and scholars in the future.

Once again, I thank you for the support and wish all of you good health and a pleasant stay in Tungkang.

Thank you.

GREETING SPEECH

I-Chiu Liao

Director of Tungkang Marine Laboratory
Tungkang, Pingtung, Taiwan

Ladies and Gentlemen:

I appreciate the introduction I have just received from Dr. Lee, thank you. Mr. Young, our honorable president of China Fisheries Association, thank you for your opening remark.

Today we have many friends from the world, such as New Foundland, Canada, the United States, Japan, Singapore, the Philippines, Indonesia, Guatemala, St. Louisa, Ecuador, and local friends. It gives me great pleasure to see you all here today. I welcome you to this Workshop on the Reproduction and Culture of Milkfish. But before we begin, does anyone know how milkfish got its name? I have been asked many times by friends, colleagues, and guests, why it is called milkfish in English or Su-Mu Yu in Chinese. Well, I answer the English speaking people with this reply: milkfish doesn't produce milk and doesn't taste milky, but its ventral side is milky white, hence named "milkfish." If any one of you knows a better explanation, please let me know. In Chinese, however, milkfish got its name from something that happened a long time ago. About 300 years ago, General Cheng Cheng-Kung (Koxinga) landed at Anping Harbor and found that people were culturing one kind of fish that he did not know in mainland China. He asked: "What kind of fish is it?" or

"Su-Mu Yu?" in Chinese. People used his words to name the fish in order to honor him thereafter.

In the past 17 years since TML began, I have had many opportunities to work with milkfish. Milkfish is one of the major research items at TML. I remember when I first came here with my two colleagues and looked over the site that was to become TML, there were no pond and no building. But with the help of many supporters and colleagues like yourselves, TML has grown to become what it is today. We know that this could never have happened without your support, and so I would like to take this opportunity to thank you all for coming and sharing the fruit you have helped to create.

In fact, TML has gained the experience of organizing several international conferences or workshops recently, but this is the first time for an international conference on milkfish to be held at TML, or even in Taiwan. On behalf of the entire staff of TML, I would like to express our hearty welcome to all of you. Throughout your stay, please let us know if you need anything, and we will do our best to help. Again, welcome to TML and please enjoy your stay with us.

Thank you.

OPENING REMARKS

P. Bienfang
Vice President, Oceanic Institute

Milkfish is the most important cultured marine fish in the world. In Taiwan, Indonesia, and the Philippines, about 600 million pounds of milkfish are cultured annually. This represents about half of the production of all cultured marine fish in the world. In these three countries, up to one million people are employed in the milkfish industry.

Milkfish aquaculture has been practiced in Asia for centuries. Many of the traditional methods are still practiced today. For example, all of the juvenile fish needed to stock the ponds, the seed for this crop, are still gathered from the sea.

For this important industry to continue providing the peoples of Asia with inexpensive protein, a more reliable supply of seed needs to be developed. To do this, milkfish will have to be reproduced in captivity. The Oceanic Institute is committed to achieving this goal.

The Oceanic Institute is a private, nonprofit foundation located on Hawaii's main island of Oahu. The Oceanic Institute is dedicated to applied science in the areas of aquaculture and oceanography. 1985 marks the Institute's 25th anniversary. The Oceanic Institute's involvement in this program results from its internationally recognized leadership in marine finfish reproduction. Helping to improve the world's food producing capabilities

is the number one objective of the Oceanic Institute.

Modern science knows a great deal about animal reproduction. We have known how to reproduce terrestrial domestic animals for thousands of years. Indeed, controlled reproduction is a necessary first step in the domestication process. Few aquatic animals are easily reproduced. Despite many attempts, reliable methods for reproducing milkfish do not exist.

A cooperative agreement sponsored by the United States Agency for International Development (U.S. AID) has been initiated to solve this pressing problem.

In this program, the Southeast Asian Fisheries Development Center in the Philippines, the Tungkang Marine Laboratory in Taiwan, and the Oceanic Institute in Hawaii are collaborating to tackle this important problem of milkfish captive reproduction.

Research activities at all three labs will focus on the difficult task of reproducing milkfish in captivity. State-of-the-art techniques developed for controlled reproduction in other animals will be applied to milkfish.

The AID-sponsored program at the Institute encompasses five areas of activity. These are:

1. The state-of-the-art literature review and publication of a book presenting all that is known about milkfish.
2. The organization of an international workshop focusing on milkfish reproduction and culture.
3. The determination of the population structure of

milkfish in the Pacific Ocean.

4. The maturation of milkfish by feeding testosterone to the fish.
5. The controlled reproduction of milkfish by hormone implantation.

As the technology for reproducing milkfish in captivity is being developed, the Oceanic Institute will be training personnel from various interested countries in the state-of-the-art technology as it develops.

Milkfish aquaculture provides high quality animal protein to the peoples of the countries at a relatively inexpensive price-- it is truly the "People's Fish."

CLOSING REMARKS

Lamarr B. Trott

Senior Fisheries Advisor
U.S. Agency for International Development
Washington, D.C.

I am sure all of you will agree with me that this workshop has been a very worthwhile experience.

We all recognize the importance of milkfish, and we have heard presentations at this workshop that make us realize that we are on the cutting edge of science in addressing hormone implantation techniques to stimulate milkfish reproduction. Although we have heard that there is much to learn about the environment, nutrition, and basic natural history of milkfish, we are now much closer to the ability to develop hatcheries for this important food fish.

It is important to realize how we have been able to develop greater knowledge on milkfish reproduction. This is through sharing knowledge and working together, which is the primary purpose of this workshop.

You have heard two words referred to in discussing current milkfish research efforts -- collaboration and cooperation. The U.S. Agency for International Development has a cooperative agreement, not a grant, with the Oceanic Institute. The Oceanic Institute has a collaborative research design, developed with the Tungkang Marine Laboratory and SEAFDEC, to address the critical issues of milkfish spawning inducement, nutrition, environmental

concerns, and population determination. Not only is scientific knowledge being exchanged, but so are research scientists. An Oceanic Institute scientist will soon be working at the Naujan Laboratory of SEAFDEC in the Philippines, and SEAFDEC, and the Tungkang Marine Laboratory will have scientists working at the Institute in Hawaii. Very soon, training sessions will be established to pass on new developments.

Another very important aspect of this collaborative effort is to recognize the valuable contribution being made by milkfish farmers. This is one reason why this workshop was held at Tungkang, so that we can share information on successes and problems with those actually engaged in the milkfish industry. We are also proud of the accomplishments made by Mr. Lin, and as scientists are extremely pleased that he and other milkfish farmers were here to share their experiences with us. After all, it is ultimately the milkfish farmers that we are working for.

A final important aspect of this conference has been involvement of researchers from numerous countries - Canada, Japan, the U.S., Taiwan, the Philippines, Singapore, Indonesia - all working together on a common goal. As you are aware, the natural distribution of milkfish makes it available to the entire Indo-Pacific, not just southeast Asia. When hatchery techniques are developed, many nations can benefit from the milkfish. Then, it will not be called "the mysterious milkfish" as a SEAFDEC/IDRC film once called it.

I would like to say to all of you, keep up the good work. I would also like to recognize the tremendous effort by Dr. C.S. Lee of the Oceanic Institute in organizing this workshop, and recognize the effort of Dr. and Mrs. Liao and the entire staff of Tungkang Marine Laboratory who provided the beautiful facilities of the Tungkang Marine Laboratory and were truly excellent hosts. I also want to express our appreciation to the Tungkang Fishermen's Association for their participation in this conference and for the excellent meal last evening.

I am very pleased, as is the U.S. Agency for International Development, to have been part of this workshop. We look forward to another one, probably in the Philippines in October, and hope for announcements of even further advancements at that time.

Thank you.

METHODS FOR ACUTE AND CHRONIC HORMONE ADMINISTRATION IN FISH

By

Laurence W. Crim

Marine Sciences Research Laboratory
Memorial University of Newfoundland

ABSTRACT

Many methods are available for administering hormones to fish. The variety of methods include simple injections, hormone baths, incorporating the hormone into the feed, and more elaborate methods of controlled hormone release from sustained-release formulations which are implantable. The choice of methodology depends primarily upon economic and convenience considerations, while endeavoring to minimize the trauma associated with hormone treatment protocols. As improvements are developed in the area of sustained-released devices, more efficient hormone delivery protocols will be available in the future.

INTRODUCTION

It is well known that environmental cues mediate the cyclic secretion of hormone(s) which in turn regulate many seasonal physiological activities in teleosts such as growth, osmoregulation, and reproduction. Unfortunately, the culture of fish in captivity may prove disruptive to natural physiological rhythms and hormonal therapies must be found to overcome these barriers. Choosing a successful hormone therapy involves the selection of effective hormone formulations, the proper duration of hormonal treatment, and timing of the hormone administration according to appropriate physiological stages of receptivity. If, for example, it is desired to induce spawning in fish that are fully mature, an acute hormone schedule will likely be

satisfactory. On the other hand, relatively long-term hormone therapy may be necessary if one wishes to initiate sexual development in juveniles or advance seasonal gonadal recrudescence in adult fishes. Clearly, having a variety of different hormone delivery methodologies may prove useful in solving some of the problems arising from rearing fish under domestic conditions. The purpose of this paper is to draw attention to a variety of techniques allowing for convenient control of delivery of hormones in fish.

METHODS OF ACUTE HORMONE ADMINISTRATION

A common method of administering hormones is by injection of the material in solution or as a suspension (Olivereau and Olivereau, 1979) either intramuscularly (i.m.) or directly into the peritoneal cavity (i.p.) of fish. Injection of dissolved hormone produces a rapid increase in circulating hormone level; Crim and Evans (1976) demonstrated that plasma gonadotropic hormone (GtH) rises quickly and remains elevated above basal levels for several days following a single i.p. GtH injection in the rainbow trout. Furthermore, this study noted that the rate of disappearance of GtH from circulation varies directly with increases in temperature. The time between HCG injection and induction of spawning in gravid female goldfish is shortened by increasing temperatures (Stacey et al., 1979).

Another particularly convenient mode of hormone administration is the adding of hormone substances directly to

the aquarium water (Van den Hurk and Van Oordt, 1985) or to immerse fish eggs in a hormone bath (Goetz et al., 1979). A modified form of this idea, namely hyperosmotic saline bath immersion, was used by Antipa and Amend (1977) for introducing vaccines into Pacific salmon. The effectiveness of this technique for uptake of growth promoting substances in salmon was tested by Donaldson et al. (1979) but growth was only marginally stimulated. LHRH uptake from an anesthetic bath or directly from gill spraying has been demonstrated (Sherwood and Harvey, 1985), but these methods are inefficient and therefore uneconomical.

METHODS OF CHRONIC HORMONE APPLICATION

Injectable Preparations

Incorporating hormones directly into the diet represents another practical means of treating fish on a daily basis with hormone substances. Steroid hormones, methyl testosterone or estradiol-17 β , have been fed to fish producing all male or all female populations (Yamazaki, 1976; Fayamen and Shelton, 1978). Growth promoting substances have been administered via the diet (Donaldson et al., 1979). Generally the dietary route is not recommended for protein or peptide hormones because degradation by gut enzymes eliminates or seriously reduces biological activity. Surprisingly, dietary growth hormone supplements enhanced the growth of salmon presumably because of peptide breakdown products that retain some biological activity. In rats, spontaneous ovulation was prevented by feeding of a potent

GnRH antagonist (Nekola et al., 1982). However, high oral doses of the antagonist were necessary compared with injections of the GnRH antagonist parentally.

The period of biological effectiveness may be extended for injected hormone by incorporation of these materials into viscous or highly adsorptive liquid vehicles that delay uptake of the hormone or protect these substances from inactivation at the site of injection. Zinc phosphate suspension of a somatostatin analog increased about three-fold the duration of hormone action compared with administration of the analog in simple aqueous solution (Martin et al., 1984). The pituitary peptide hormone, ACTH, has been administered in a variety of depot vehicles including gelatin, zinc hydroxide, or phosphate and carboxymethyl cellulose (cited by Futaguchi et al., 1982); however, dispersates of zinc-ACTH preparations are stabilized by the addition of histidylhistidine, which improves the depot effects. Aida et al. (1978), adopting the principle of prolonged hormone release from a viscous liquid vehicle, treated fish with an emulsified solution of synthetic LHRH in Freund's adjuvant and ovulation was induced in the sexually mature plaice and goby. LHRH injections in saline were not effective. Modified liquid vehicles appear to extend hormone action in a few days, but for longer periods of sustained hormone release, implantation of solid hormone delivery devices are useful and have the advantage of eliminating a need for repetitive, stressful hormone injections over the course of

many days.

Prolonged hormone release (weeks, months, or even longer) from drug delivery devices for lipophilic and hydrophilic material have been reviewed recently (Graham and Wood, 1982; Vickery et al., 1984). A simple method for treating fish with steroid hormone was devised by Crim and Evans (1979) using moltant cocoa butter. The moltant hormone-containing cocoa butter was injected i.p. where it quickly solidifies forming a slow-release hormone implant. Cortisol in cocoa butter was administered to brown trout producing an elevation in plasma cortisol for a period of five weeks (Pickering and Duston, 1983). A similar methodology for testosterone implantation in rats was described by Gerrity et al. (1980) using hydrogenated soybean oil that is an injectable liquid at 41°C but forms a solid depot at mammalian body temperature.

An increased duration of delivery of peptides, proteins, and lipophilic substances has been accomplished by encapsulation of these substances into liposomes. Orally administered insulin in liposomes reduced blood glucose levels in the rat (Patel and Ryman, 1976). Liposomal GnRH evoked LH release from the rat pituitary gland (Kercret et al., 1983); when liposome entrapped human or salmon calcitonin was parenterally administered to rats (Fukunaga et al., 1984), the hypocalcemic action was prolonged and enhanced. Jackson and Franklin (1984) reported that lower doses of diethylstilbestrol and liposome delivery are required to

suppress prostate weight and plasma testosterone in the rat. To date, the present author is unaware of studies of any liposomal hormone delivery in fish.

Sustained release of LHRH analog by controlled delivery systems was discussed by Vickery et al. (1984). These formulations have the potential advantage of avoiding hormone overdose, preventing ineffective rises and falls in hormone level, and reducing the amount of hormone required to a practical level. Studies of reproductive cycles in rats and monkeys demonstrate that biodegradable microcapsules made from copolymers of glycolic and lactic acids release LHRH analog for a period of several weeks (Sanders et al., 1984).

Implantable Formulations

Relatively simple hormone implants may be made by incorporation of test substances into compressed tablets of cholesterol powder. Growth hormone was effectively administered to salmon in this fashion (Higgs et al., 1975), cholesterol pelleted formulations of pituitary extract (Robertson and Rinfret, 1957), and salmon gonadotropic hormone (MacKinnon and Donaldson, 1978) induced development of the testes in trout and salmon, respectively. A cholesterol matrix pellet suitable for delivery of LHRH analog was described by Kent et al. (1980) and this hormone methodology has been successfully applied to acceleration of the reproductive cycle of landlocked salmon (Crim et al., 1983a), rainbow trout (Crim et al., 1983b), and the

Atlantic salmon (Crim and Glebe, 1984). Weil and Crim (1983) compared various ways of administering LHRH analog to salmon and they concluded that a single hormone implant made from cholesterol or silastic elastomer was equally effective compared with frequent injections of releasing hormone in a liquid vehicle.

A membrane delivery system composed of silastic rubber (dimethylpolysiloxane) was described by Dziuk and Cook (1966) for the chronic administration of steroid hormones. This technique has the advantage that the hormone capsules are easily made in quantity (Moore, 1981), and a minimal tissue reaction occurs in response to the implant. The hormone delivery properties of these devices were outlined by Kincl et al. (1968) and Sundaram and Kincl (1968) and their studies demonstrated that diffusion of organic compounds through silastic membranes is limited by the surface area exposed to hormone material, wall thickness of the silastic membrane, and solubility of the hormone in diffusion medium (body fluids) or silastic membrane. In fish, Shelton (1982) indicated that testosterone is released from silastic capsules for periods of a year or longer and he has used this technology to produce monosex populations of the grass carp. Silastic capsules filled with testosterone are highly effective agents for triggering gonadotropic hormone production in the juvenile trout pituitary (Crim and Evans, 1982) and implantation of testosterone combined with LHRH analog therapy accelerates the

onset of sexual development (Crim and Evans, 1983).

Although low hormone solubility in silastic rubber would be expected for hydrophilic peptide substances, LHRH and TRH release from silicone implants in rats has been demonstrated (Lotz and Syllwasschy, 1979). A silicone elastomer hormone matrix implant containing relatively high LHRH analog content was used to suppress estrus in rats for periods in excess of eight months (Vickery et al., 1984) and successfully synchronized spawning in female Atlantic salmon (Crim et al., unpublished). Weil and Crim (1983) showed that LHRH analog implants made from silicone elastomer increase plasma gonadotropic hormone levels and advance the onset of spermiation in the landlocked salmon.

Hydrogels and biodegradable polymers release materials according to the characteristics of the drug-polymers composite (Graham and Wood, 1982). Hormone release occurs either because of swelling of the cross-linked preparation or because of simple hydrolysis or enzyme catalyzed dissolution of bioerodible devices. One advantage implantable devices offer is protection of the incorporated hormone from degradation over long periods of storage. For example, delivery and stabilization of prostaglandin E₂ was improved in this way (Embrey et al., 1980). This type of methodology appears to hold promise for sustained delivery of a variety of hormones including relatively large molecular weight materials such as proteins (Langer and Folkman, 1976). Using a polymer implant containing insulin, Creque et al.

(1980) demonstrated control of the blood glucose levels for one month in diabetic rats.

PERSPECTIVE

Many methods are available for administering hormones to fish. The choice of methodology depends primarily upon economic and convenience considerations while endeavoring to minimize the trauma associated with hormone treatment protocols. As improvement are made in the area of sustained-released devices, more efficient hormone delivery protocols will be available in the future.

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INDUCED SPAWNING IN FISH

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ABSTRACT

In the female fish, induced spawning usually is carried out at the end of vitellogenesis and involves the induction of final oocyte maturation (germinal vesicle breakdown), ovulation, and oviposition or stripping. There are two approaches to induction of spawning in fish: hormonal and environmental. In the hormonal approach, several chemical agents are available and intervene at different levels of the hypothalamus-pituitary - ovary axis. These include: a) pituitary extract (hypophysation); b) human chorionic gonadotropin (HCG); c) hypophysation + HCG; d) partially purified fish gonadotropin, e.g. SG-G100 (salmon); e) LH-RH and its superactive analogues (LHRH-A); f) LHRH-A + pimozide (a dopamine antagonist) - this combination is found to be highly effective in fish whose gonadotropin (GtH) secretion is under the control of both a hypothalamic releasing hormone and a dopamine-like hypothalamic release - inhibiting factor, e.g. goldfish; g) salmon GtH releasing hormone and its analogues; h) antiestrogens e.g. clomiphene citrate, tamoxifen, and cyclofenil, which stimulate GtH secretion; i) 17 α -hydroxyl - 20 Δ -dihydroprogesterone (17 α -20 Δ -P), a very potent inducer of final oocyte maturation, which appears to work best in combination with a priming dose of GtH, unless the starting oocyte stage is that of subperipheral or peripheral germinal vesicle; j) corticosteroids that also induce final oocyte maturation; and k) prostaglandins that induce follicular rupture (ovulation). The application of these various agents to induced spawning is discussed. There is a need to evaluate the various techniques in terms of cost effectiveness and egg quality (fertilizability, hatchability, early development, and larval rearing). There also is a need to standardize the procedure against such variables as age of fish, initial gonadal stage, hormone dosage, injection interval, time of injection, time to stripping from the last injection, season, and environmental conditions. In the male, spermiation or

seminal hydration may be induced using LHRH-A, HCG, androgens, or progesterone. In cases where the availability of ripe males and females is not synchronized, sperm cryopreservation may have to be carried out.

In the environmental approach, appropriate, but often undefined, environmental and social conditions are provided in a special spawning facility to induce natural spawning. Examples include milkfish (10 m diameter sea cage for five-year-old fish in the Philippines; big pond for 11-12-year-old fish in Taiwan; and tank for 12-year-old fish in Indonesia), sea bass, *Lates calcarifer* (spawning tank with running brackish water), goldfish (raising ambient temperature from 13°C to 20°C with provision of vegetation), and Indian carps (bunds where flooding is simulated). These and other examples are discussed. In some cases, it may be necessary to combine the hormonal and environmental approaches to achieve the desired effect.

INTRODUCTION

Induced spawning has been the subject of several recent reviews (Harvey and Hoar, 1979; Lam, 1982; Donaldson and Hunter, 1983; Scott and Sumpter, 1983; and others cited therein). It is not the intention of this review to repeat the details already reviewed, but to highlight briefly the salient points and concentrate on literature that has appeared since the above reviews.

Physiological Processes Involved

In the female fish, induced spawning normally is carried out at the end of vitellogenesis (i.e. tertiary yolk granule or globule stage) and involves the induction of germinal vesicle migration to the periphery (sometimes induced spawning may start from this stage), germinal vesicle breakdown (GVBD) or final

oocyte maturation, ovulation (follicular rupture), and oviposition; the last step may be replaced by stripping. In the male, the processes involved are spermiation (release of spermatozoa from the Sertoli cells into the tubules or lobules) (Baynes and Scott, 1985), thinning of the seminal fluid or plasma (seminal hydration, which should be considered separately from spermiation) (Baynes and Scott, 1985), and ejaculation (release of sperm to the outside) or stripping. These processes may not need to be induced as running ripe males are often available. However, in order to synchronize the spawning activities of the male and female, and/or to increase the quantity and quality of the milt produced, "spawning" induction in the male also may be carried out.

Approaches

There are two approaches to induction of spawning in fish: (I) hormonal and (II) environmental (Fig. 1). The two approaches may be combined to achieve the best effect. After treatment, the fish in each approach may be stripped for artificial fertilization or left for natural spawning. Whether the ovulated eggs are fertilized artificially or naturally, the fertilized eggs are incubated, and the hatched larvae raised to the fry stage in the hatchery. Larval rearing has been reviewed (Kuronuma and Fukusho, 1984); it will not be considered here.

I. HORMONAL APPROACH

This is based on an understanding of the neuroendocrine

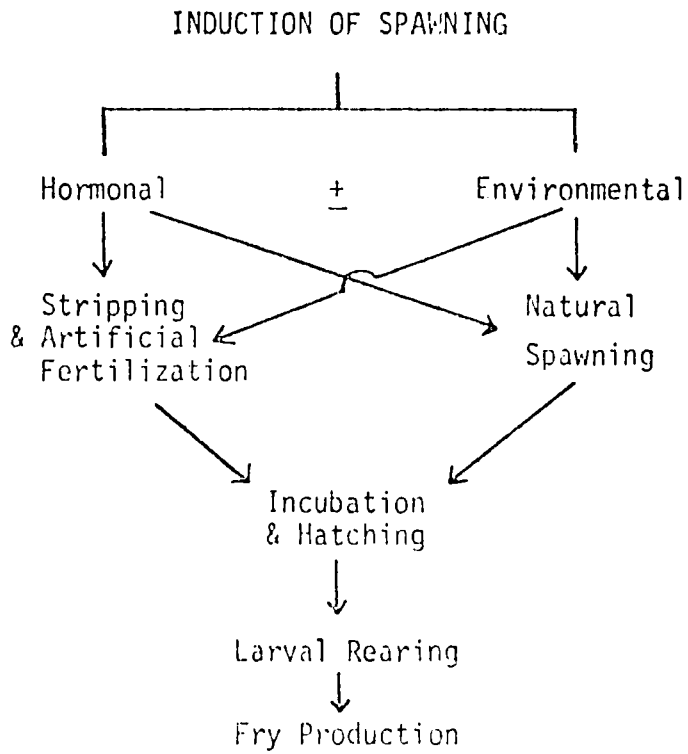


Fig. 1. Approaches to induction of spawning in fish.

control of oocyte maturation, ovulation and spawning behavior (see reviews by Goetz, 1983; Liley and Stacey, 1983; Stacey, 1983) in the female, and of spermiation and seminal hydration (see reviews by Billard et al., 1982; Scott and Sumpter, 1983) in the male. In general, our present understanding of the subject in the female may be summarized as follows (Fig. 2):

1. Stimulation of the release of gonadotropin-releasing hormone (GnRH) and/or inhibition of the release of gonadotropin release-inhibiting factor (GnRIF) causes the pituitary to secrete gonadotropin (GtH) into the bloodstream (Peter, 1983).

2. When a certain level of GtH is reached, a germinal vesicle migrates to the periphery and the theca and granulosa cells of the follicle are stimulated to secrete a maturation-inducing steroid (MIS) (Nagahama, 1983; Fostier et al., 1983; Goetz, 1983).

3. The MIS induces GVBD (resumption of meiosis) and also probably other associated events, e.g. hydration and coalescence of yolk granules or globules and/or of lipid droplets seen in some species. Evidence points to 17α -hydroxy- 20β -dihydroprogesterone (17α - 20β -P) as the MIS although corticosteroids have also been implicated (Goetz, 1983; Fostier et al., 1983). It is suggested that corticosteroids play only a supportive role: they may displace 17α - 20β -P bound to plasma protein and/or increase oocyte sensitivity to 17α - 20β -P (Jalabert, 1976).

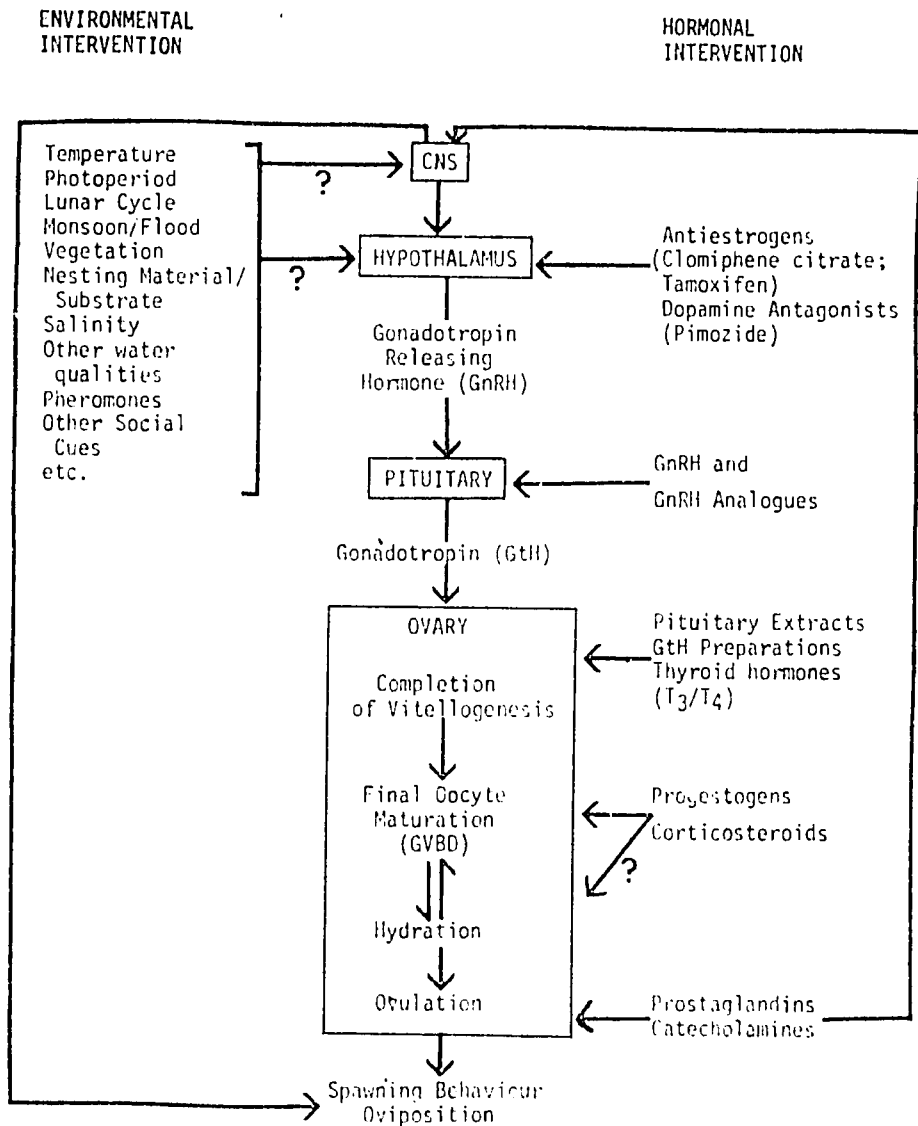


Fig. 2. Hormonal and environmental intervention in induced fish spawning.

may be used to induce spawning in fish, intervening at different levels of the hypothalamus-pituitary-ovary axis (Fig. 3).

The corresponding situation in the male is summarized in Fig. 4. The steroid mediation of GtH action on spermiation and seminal hydration still is not clear (Scott and Sumpter, 1983; Baynes and Scott, 1985). There is a good correlation between plasma levels of 17α - 20β -P and spermiation/seminal hydration in salmonids (Scott and Sumpter, 1983; Ueda et al., 1983; Baynes and Scott, 1985), but injections of 17α - 20β -P did not increase the volume of milt in rainbow trout (Scott and Baynes, 1982).

(a) Fish Pituitary Extracts (Hypophysation)

The use of pituitary extracts to induce fish spawning (hypophysation) has been practiced since 1931 when Houssay first introduced the technique. It still is widely used (see reviews by Harvey and Hoar, 1979; Lam, 1982; Donaldson and Hunter, 1983). The pituitary glands used usually are obtained from sexually maturing or mature donor fish that may be of the same or different, species. They can be used fresh or stored for subsequent use. The various storage methods are reviewed by Donaldson and Hunter (1983).

Hypophysation is a simple and practical technique but suffers from the disadvantage, *inter alia*, that the gonadotropic potency of the pituitary glands used often is unknown and is difficult to standardize. One possible solution is to use commercially available acetone-dried or lyophilized pituitary

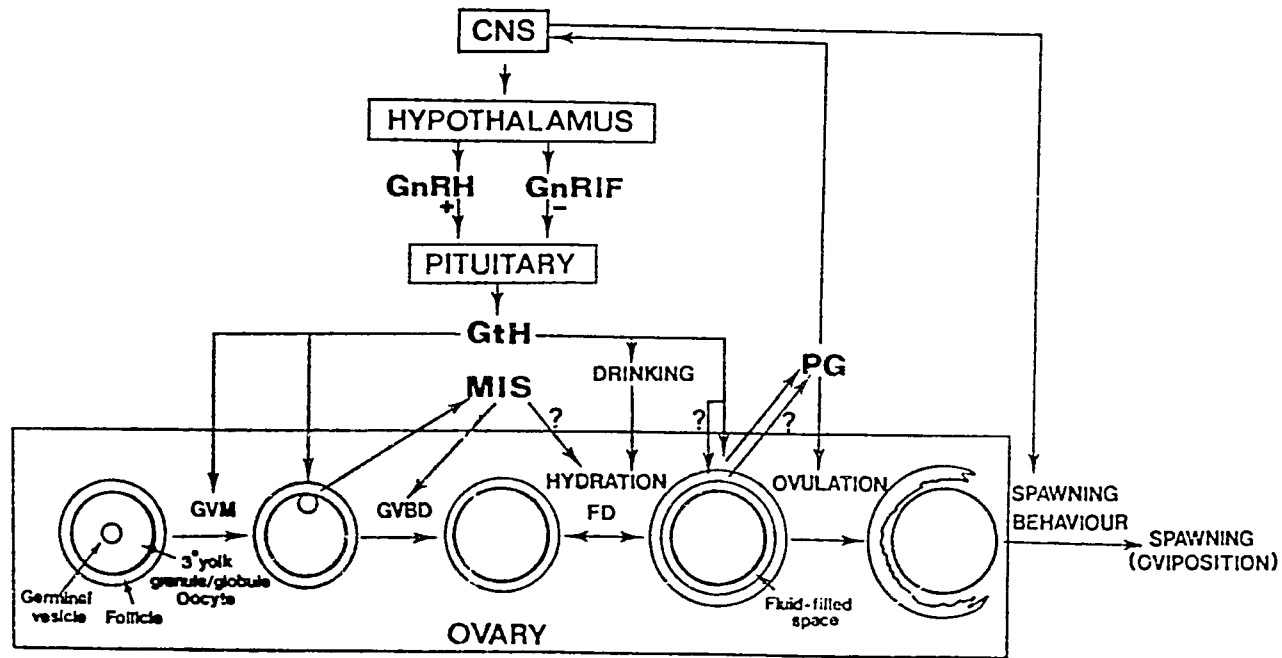


Fig. 3. Tentative schematic diagram on neuroendocrine control of final oocyte maturation, hydration and ovulation in fish (CNS = central nervous system; GnRH = gonadotropin releasing hormone; GnRIF = gonadotropin release inhibiting factor; GtH = gonadotropin; MIS = maturation-inducing steroid which is probably 17α -hydroxy- 20β -dihydroprogesterone (17α - 20β -P), although corticosteroids have also been implicated; PG = prostaglandin; GVM = germinal vesicle migration; GVBD = germinal vesicle breakdown; FD = follicular detachment; double-headed arrow indicates that hydration may occur prior to, during, and after oocyte maturation (GVBD).

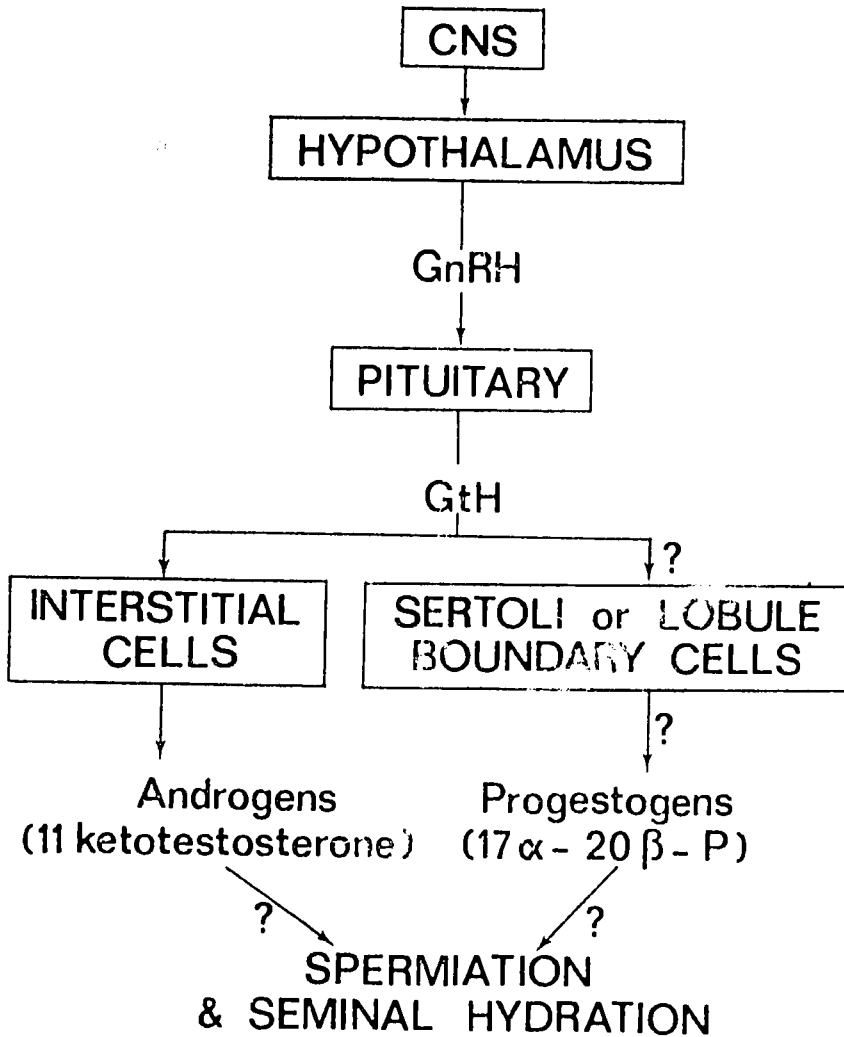


Fig. 4. Tentative schematic diagram on neuroendocrine control of spermiation and seminal hydration (see Fig. 1 for abbreviations).

powder that has been assayed for gonadotropin content (e.g. salmon pituitary powder from Syndel Laboratories Ltd., Vancouver, Canada).

Some recent studies using hypophysation are summarized in Table 1. It is interesting that a low dose of 1 mg carp pituitary per kg body weight induced oocyte maturation without ovulation in the golden perch, *Macquaria ambigua*. Whereas, a high dose of 15 mg/kg caused a decline in hatchability compared to 10 mg/kg, which appeared to be the optimal dosage in this species (Rowland, 1983); 5 mg/kg also induced 100% ovulation but the fertilization rate was more variable than with 10 mg/kg. Another point of interest is that in two species of catfish *Clarias macrocephalus* and *Bhamdia sapo*, the response time to ovulation was 13 h at around 27°C.

(b) Human Chorionic Gonadotropin (HCG)

HCG has been used successfully to induce ovulation/spawning in a number of species (see reviews by Lam, 1982; Donaldson and Hunter, 1983). The dosage required may vary in different species depending on how closely related the endogenous gonadotropin is to HCG (Lam, 1982). However, the dose-response relationship has not been studied in most cases. Two recent studies have addressed this problem (Rowland, 1983; Mollah and Tan, 1983). Rowland (1983) has shown that in the golden perch, *M. ambigua*, a low dose of 50 IU/kg could only induce oocyte maturation, while a high dose of 2000 IU/kg caused a significant reduction of

Species	Pituitary Used	Water Temp °C	Initial Gonadal Stage	Dosage (mg/kg)			Response Time (h)	Fertilization		Hatching Rate (%)	Comments	Reference
				First	Interval (h)	Second		Method	Rate (%)			
White sturgeon, <i>Acipenser transmontanus</i>	White sturgeon or common carp, <i>Cyprinus carpio</i> ; acetone-dried	12-15	Oocytes subperipheral (3.5 - 3.8 mm diam.)	1.2 - 1.5	12-24	2.8-3.5	?	Artificial	20-95	20-95	75% larval survival at 40 days	Doroshov <i>et al.</i> , 1983
			Oocytes Peripheral (3.8 - 4.0 mm diam.)	4 - 5	-	-						
				♂: 0.5-1.0								
Golden perch <i>Macquaria ambigua</i>	Common carp; acetone-dried	25 ±0.5	Oocytes 0.9 - 1.2 mm diam.	5	-	-	26-38 ^a	Natural	50-98	58.4	1 mg/kg induced only	Rowland, 1983
				10	-	-			(mean: 86.6)	+16.2 ^c	oocyte	
				15	-	-			(mean: 93.2)	+ 3.7 ^c	maturation (OM);	
				♂: 2 or 5 + 200 IU/kg HCG					(mean: 94.8)	-29.8	2 mg/kg induced 100% OM and ovulation, but only 60% spawning	
Asian catfish <i>Pseudorasbora parva</i>	<i>C. macrocephalus</i> (hemiplastic); acetone-dried	26-31	Oocytes 1.2 mm diam.	70	-	-	13 ^b	Artificial	?	72.0	+ 2.6 ^c	Mollah and Tan, 1983
				35	-	-	(1 at 14)					
				♂: not injected?			(1 at 16)		?	+ 3.8 ^c		
American catfish, <i>Ictalurus punctatus</i>	Characin, <i>Pseudorasbora parva</i> ; stored at 5°C in 100% ethyl alcohol	17-17	End of vitellogenesis (wild fish in spawning season)	0.75-6 ♂: 3-6	-	-	32 ^b at 17°C to 13 ^b at 27°C	Artificial	?	20.5 -70.4	0.37 mg/kg ineffective	Espinach Res <i>et al.</i> , 1984

a Time to natural spawning
b Time to ovulation
c Standard error

Table 1. Some recent studies on induced breeding by hypophysation.

hatching rate compared to 500 IU/kg, the optimal dosage (Table 2). In fact, 500 IU HCG/kg appeared to give better fertilization and hatching rates than 10 mg carp pituitary/kg (Tables 1 & 2), and Rowland (1983) has recommended the use of HCG for the induced spawning of *M. ambigua*. Mollah and Tan (1983) also have found HCG an effective substitute for fish pituitary extract in the induction of ovulation in *C. macrocephalus*, but in this case, the minimal effective dose was 2000IU/kg; 500IU/kg was ineffective, 1000 IU/kg partially effective, while doses of 2000, 3000 and 5000IU/kg were fully effective. The higher doses also did not adversely affect the hatching rate in this case.

HCG also has recently been used to induce spawning in milkfish, *Chanos chanos* (see review by Liao and Chen, 1984), curimbata, *Prochilodus scrofa* (Fenerich-Verani et al., 1984), white seabream, *Mylio berda* (Mok, 1985), and rabbitfish, *Siganus guttatus* (Juario et al., 1985).

(c) HCG and Hypophysation

A combination of HCG and hypophysation has been shown to be effective in inducing ovulation or spawning in several species (see reviews by Lam, 1982, 1984). However, a careful evaluation of this combination treatment versus HCG or hypophysation alone has not been done often. Recently, Rowland (1983) has shown that the combination treatment was not any more effective than either HCG or hypophysation alone in *M. ambigua*. Furthermore, milkfish, which were thought to require HCG plus hypophysation (see review

HCG (IU/kg)	Mean Oocyte Diameter Range (mm)	No. Fish/No. Injected			Mean % Fertilization ± SE	Mean % Hatching ± SE
		Oocyte Maturation	Ovulation	Spawning		
0	1.0 - 1.1	0/3	0/3	0/3	-	-
50	1.1	2/3	0/3	0/3	-	-
100	1.0 - 1.1	6/6	4/6	4/6	89.3 ± 5.3	70.8 ± 10.3
200	1.0 - 1.1	5/5	5/5	4/5	93.5 ± 2.2	75.0 ± 5.5
500	0.9 - 1.1	5/5	5/5	5/5	99.6 ± 0.4	87.4 ± 3.3
1000	1.0 - 1.1	5/5	5/5	5/5	98.0 ± 1.2	54.8 ± 14.8
2000	1.0 - 1.1	5/5	5/5	5/5	92.0 ± 4.6	44.2 ± 13.9

Table 2. Effect of a single injection of HCG on oocyte maturation, ovulation, spawning, fertilization rate and hatching rate in golden perch, *Macquaria ambigua*, at $25 \pm 0.5^\circ\text{C}$ (data summarised from Rowland, 1983).

by Lam, 1984), can be spawned by HCG alone (see review by Liao and Chen, 1984). However, this may be related to the condition or age of the fish: wild fish of unknown age were used in the former studies, while captive broodstock or 8-10 year old fish were used in the latter. In contrast, a single combined injection of homoplastic pituitary extract with HCG was more effective in inducing production of normal fertilizable ovulated eggs than single or multiple injections of pituitary extract alone in *Prochilodus platensis* (Espinach Ros et al., 1984b). Unfortunately, HCG alone was not studied.

(d) Partially Purified or Purified Fish Gonadotropin Preparations

Several partially purified or purified fish gonadotropin preparations are now available from research laboratories (see review by Donaldson and Hunter, 1983). Some attempts have been made to use some of these preparations to induce ovulation in fish (see reviews by Lam, 1982; Donaldson and Hunter, 1983). Though successful, they are at present not cost effective compared to crude pituitary extract or HCG.

(e) Luteinizing Hormone-Releasing Hormone (LHRH) and its Analogues

LHRH is effective in inducing gonadotropin release and ovulation in fish but its superactive analogues (LHRH-A) are more effective (see reviews by Lam, 1982; Donaldson and Hunter, 1983). There is increasing interest in the use of LHRH-A as an ovulating agent in cultured fish species: cyprinids (Fukien-Kiangsu-

Chekiang-Shanghai Cooperative Group, 1977; Jiang et al., 1980; Breton et al., 1983; Kouril et al., 1983; Billard et al., 1984a), Japanese eel, *Anguilla japonica* (Research Group of Eel Reproduction, 1978), salmonids (Donaldson et al., 1981; Van der Kraak et al., 1982; Crim et al., 1983a, b; Sower et al., 1982, 1984; Crim and Glebe, 1984; Billard et al., 1984b; Fitzpatrick et al., 1984), European sea bass, *Dicentrarchus labrax* (Barnabe and Paris, 1984; Zohar et al., 1984), and white sturgeon, *A. transmontanus* (Doroshov and Lutes, 1984). In most cases, the LHRH-A used is des Gly¹⁰[D-Ala⁶] LHRH ethylamide, although [D-Ser(But)⁶] LHRH ethylamide (Donaldson et al., 1981), [6-(D-2-naphthylalanine)] LHRH, des Gly¹⁰[D-Trp⁶] LHRH ethylamide (Weil and Crim, 1983; Crim et al., 1983a, b), D-Ser⁶ LHRH ethylamide (Breton et al., 1983) and [D-Ala⁶, Pro⁹-N-ethylamide]-LHRH (Lin et al., 1985) also have been used.

A recent development is the use of pimozide, a dopamine antagonist, to potentiate the ovulatory effect of LHRH-A. This is based on the premise that dopamine is, or can mimic, the GnRIF (Fig. 2; Peter, 1983) and that blocking the action of dopamine by pimozide would greatly potentiate the GtH release-response to LHRH-A (Crim, 1982; Chang and Peter, 1983a, b; Chang et al., 1983). To date, the LHRH-A + pimozide treatment has been shown to be highly effective in inducing ovulation in goldfish (Chang and Peter, 1983a; Sokolowska et al., 1984; Peter et al., 1984), common carp (Billard et al., 1983) rainbow trout and

brown trout (Billard et al., 1984b), African catfish (*Clarias gariepinus*) (de Leeuw et al., 1985), and loach (*Paramisgurnus dabryanus*) (Lin et al., 1985). The experimental protocols and data of some of these studies are summarized in Table 3. De Leeuw et al. (1985) have studied various dosages of pimozide + LHRH-A in a fixed ratio of 100:1 and found the minimal fully effective dosage to be around 5 mg pimozide + 0.05 mg LHRH-A per kg body weight (dosages of 2 + 0.02, 1 + 0.01 and 0.05 + 0.005 were ineffective). Lin et al. (1985) and Billard et al., (1984b) also have studied dose-response of pimozide + LHRH-A (Table 3).

Another recent development is the administration of LHRH-A by implantation using cholesterol pellet or silicone rubber implant (Crim et al., 1983a, b; Weil and Crim, 1983; Sokolowska et al., 1984; Billard et al., 1984b; Crim and Glebe, 1984; Crim, in this volume). This technique ensures a long-term delivery of LHRH-A without repeated handling of the fish, and makes it possible to induce maturation and spawning from an earlier gonadal stage (Crim et al., 1983a, b). Success recently has been obtained with the use of LHRH-A implant (cholesterol pellet) to spawn milkfish (C.S. Lee, pers. comm.).

A third development is the isolation and characterization of salmon GnRH (Sherwood et al., 1982, 1983). This peptide is effective in inducing ovulation in Pacific salmon but its analogues are more effective (Donaldson et al., 1983). This development opens up new possibilities of exploring various

Species (Stage)	Temp. (°C)	Injections(IP)/Implantation			No. Ovulated/ No. Used (Firm ⁴ , h)	References	
		Dose (mg/kg) µg/fish	Interval (d)	Dose (mg/kg)			
Goldfish, <i>Carassius auratus</i> (completed vitellogenesis)	18-20	LHRH-A ¹ (0.1)	1	P1H (10)	2/7 (24)	Sokolowska et al., 1986	
		LHRH-A ¹ +P1H (0.1+10)	1	LHRH-A ¹ (0.1)	7/8 (24)		
		P1H (10)	1	LHRH-A ¹ (0.1)	5/8 (24)		
		LHRH-A ¹ +P1H (0.1+10)	-	-	5/8 (24)		
		LHRH-A ² pellet ⁶ (125)	-	-	4/8 (24-48)		
		LHRH-A ² pellet ⁶ (125)	18	P1H	3/8 (30-50)		
		LHRH-A ² pellet ⁶ (125)	18	P1H	7/8 (7-29)		
Goldfish ("postvitellogenic")	20	LHRH-A ¹ +P1H (0.1+10)	3	LHRH-A ¹ (0.1)	7/8 (20)	Peter et al., 1985	
African catfish, <i>Catfish gariepinus</i>	25	LHRH-A ¹ +P1H (0.05+5)	-	-	9/9 (12.5)	De Leeuw et al., 1985	
Loach, <i>Piscesinotus dabryanus</i> (gravid)	14-22	P1H (1)	3	LHRH-A ³ (0.05)	5/10 (24)	Lin et al., 1985	
		P1H (1)	3	LHRH-A ³ (0.1)	7/10 (24)		
	20-24	P1H (1)	2.5	LHRH-A ³ (0.01)	5/8 (24)		
		14-17	LHRH-A ³ +P1H (0.01+1)	-	-		5/10 (24)
			(0.05+1)	-	-		10/10 (24)
	14-17	(0.1+1)	-	-	8/10 (24)		
		LHRH-A ³ +P1H (0.05+0.1)	-	-	4/8 (24)		
		(0.05+0.5)	-	-	8/8 (24)		
	(0.05+1.0)	-	-	10/10 (24)			
	Brown trout, <i>Salmo trutta</i> (4-yr-old at onset of spawning period)	4-7	P1H (1)	6	LHRH-A ¹ (0.01)		11/15 or 15/15 (5 days) (7 days)
(10)			(0.01)		13/15 (5 days)		
P1H+LHRH-A ¹ implant ⁷ (10+0.05)			-	-	7/10 or 10/10 (4 days) (8 days)		
(10+0.005)			-	-	3/10 or 10/10 (4 days) (8 days)		

¹ des Gly¹¹[D-Ala⁶]-LHRH ethylamide

² des Gly¹⁰[D-Trp⁶]-LHRH ethylamide

³ [D-Ala⁶, Pro⁹-N-ethylamide]-LHRH

⁴ Time after injection

⁶ Cholesterol pellet

⁷ Silicon rubber implant

Table 3

superactive analogues based on fish GnRH.

(f) Antiestrogens

Antiestrogens (clomiphene citrate, tamoxifen and cyclofenil) have been shown to be effective in inducing ovulation in several species of fish (see reviews by Lam, 1982; Donaldson and Hunter, 1983). They function by stimulating GtH secretion, presumably through abatement of the negative feedback effects of endogenous estrogens. As such, they probably would work best towards or at the end of vitellogenesis when blood estrogen levels are high. As blood estrogen levels subsequently drop rapidly during the subperipheral and peripheral germinal vesicle stages and the germinal vesicle breakdown stage (Fostier et al., 1978; Scott and Sumpter, 1983), antiestrogens probably would not work as well if injected into fish with oocytes at these stages (Lam, 1982). Another point to note is that excessive doses of the antiestrogens may be estrogenic and inhibit gonadotropin secretion instead (Breton et al., 1975).

(g) Progestogens

As stated earlier, there is good evidence that 17α - 20β -P is the maturation-inducing steroid (MIS) (Fig. 2). The steroid has been tested as an ovulating agent in rainbow trout, coho salmon, common carp, and northern pike by Jalabert and his collaborators (see reviews by Lam, 1982; Donaldson and Hunter, 1983). It is able to induce GVBD (oocyte maturation) at relatively low dosages of 2-3 mg/kg in these fishes, but is

unable to induce ovulation unless the fish (at the end of vitellogenesis prior to germinal vesicle migration) is primed first with pituitary extract or GtH (2 days before), or unless the fish already is at the subperipheral or peripheral germinal vesicle stage. Recently, Breton et al., (1983) investigated the possibility of using a low dosage of LHRH or LHRH-A to replace pituitary extract or GtH as a primer before 17α - 20β -P injection in the carp, but only limited success was obtained.

In the African catfish, *C. gariepinus*, two injections of 17α -hydroxyprogesterone (17α -P) at 3 and 5 ug/g respectively, spaced 4 h apart, induced oocyte maturation and ovulation (Richter et al., 1985). Eggs could be stripped within 12.5 h after the first injection, and most of the eggs could be fertilized and hatched normally. It is possible that 17α -P was converted into 17α - 20β -P by the catfish. 17α -P should be tested in other teleosts. At present, it has the practical advantage over 17α - 20β -P in being more readily available and cheaper (Richter et al., 1985).

(h) Corticosteroids

High dosages of corticosteroids (50-200 mg/kg) have been shown to induce oocyte maturation and ovulation in several species of fish (see reviews by Lam, 1982; Donaldson and Hunter, 1983). Deoxycorticosterone acetate (DOCA) was the most commonly used, although cortisol, cortisone, and corticosterone also have

been used (Hirose and Ishida, 1974; Babiker and Ibrahim, 1979). In fact, in the ayu, Plecoglossus altivelis, 10 mg per fish (40-50 gm) of cortisol was even more effective than 1000 IU HCG per fish (Hirose and Ishida, 1974). However, in goldfish, inhibition of secretion of cortisol and corticosterone by metopirone injections caused ovulation at $12 \pm 1^{\circ}\text{C}$ (Pandley et al., 1977). This was interpreted as caused by the build-up of 11-deoxycortisol and 11 β -hydroxylation by metopirone. In African catfish (C. lazera = C. gariepinus), DOCA was found to be an effective spawning agent by Hogendoorn (1979) but not by Richter and Van den Hurk (1982); in the study, only oocyte maturation was induced and not ovulation.

As in the case of 17 α -20 β -P, the efficacy of corticosteroids as inducers of ovulation may be improved by a priming injection of pituitary extract of GtH. In the grey mullet, Mugil cephalus, spawning repeatedly has been induced by an injection of 120 mg/kg DOC one day after a priming injection of 50 mg/kg carp pituitary homogenate, or 5 mg/kg SG-G100 (partially purified Pacific salmon gonadotropin), or 16,700 IU/kg HCG (Pullin and Kuo, 1980). Similarly, in the ayu (40-50 gm), 5 or 10 mg cortisol one day after a priming dose of 300 IU HCG induced ovulation effectively (Hirose and Ishida, 1974).

The role of corticosteroids in fish oocyte maturation and ovulation is not clear (Fig. 2). They are certainly effective in inducing oocyte maturation in vitro, though not as effective as

17 α -20 β -P (Goetz, 1983). As stated earlier, they may play a supportive role to 17 α -20 β -P and may be involved in oocyte hydration (Hirose and Ishida, 1974; Fig. 2).

(i) Prostaglandins

Prostaglandin may be the mediator of gonadotropin action on ovulation or follicular reapture (oocyte expulsion) (Fig. 2; Goetz, 1983). The few attempts to use prostaglandins to induce ovulation in fish have been reviewed by Lam (1982) and Donaldson and Hunter (1983). It may be useful to use protaglandin (e.g. PGF_{2 α}) to induce ovulation following induction of oocyte maturation by 17 α -20 β -P or corticosteroids (Lam, 1982). The timing of prostaglandin treatment *in vitro* or *in vivo* probably will be important if normal ovulated eggs are to be obtained, because there usually is a time lapse between oocyte maturation and ovulation in a normal spawning fish; 50 degree days (i.e. five days at 10^oC, etc.) in rainbow trout (Bry, 1981); five to nine hours in goldfish (Stacey et al., 1983); and about one day; in ayu (Hirose et al., 1983).

Prostaglandin also may induce female spawning behavior in fish (Fig. 2; Liley and Stacey, 1983). Thus, it may be possible to use prostaglandin (e.g. PGF_{2 α}) to induce ovulation as well as spawning behavior in fish following oocyte maturation (Lam, 1982).

(j) Which Of The Above Methods Is The Best In Terms Of Cost Effectiveness And Egg Quantity and Quality?

This question has not been addressed by investigators for any one species under the same conditions. Several of the above methods have been tried in golufish (see review by Lam, 1982), coho salmon (see review by Donaldson and Hunter, 1983), and African catfish (see Introduction in de Leeuw et al., 1985), but evaluation in terms of cost effectiveness and the quantity and quality of ovulated eggs has not been done. From the practical and economic viewpoint, HCG and pituitary extract still have the competitive edge at present, although other methods hold considerable promise, particularly LHRH-A with or without pimozide. In one study (Lin et al., 1985), LHRH-A + pimozide was shown to be more effective in inducing ovulation in loach than hypophysation. There should be more studies of the ovulatory efficacy of HCG in combination with corticosteroids and progestogens.

The quantity and quality of the induced ovulated eggs have not been studied often. Yet these aspects are most important from the aquaculture point of view. Egg quality should be evaluated in terms of fertilizability, hatchability, and larval appearance and survival. Some recent studies, however, have considered egg quality and quantity. Crim et al. (1983b) found heavy mortalities in eggs taken from most of the rainbow trout (65%) induced to ovulate early by pelleted LHRH-A implant. Similarly,

Billard et al.(1984b) reported poor egg quality in rainbow trout induced to ovulate by LHRH-A + pimozide prior to the onset of the spawning season. However, similar treatment in brown trout at the onset of the spawning season did not result in any alteration of egg quality (Billard et al., 1984b; Table 3). Crim and Glebe (1984) also have found that implantation of LHRH-A pellet in Atlantic salmon (*Salmo salar*) 45 days prior to the normal spawning time induced ovulation of non-viable eggs, whereas a similar treatment about 28 days prior to the normal spawning time resulted in about one-half of the treated females producing eggs of good quality. Very early induction of ovulation (about seven weeks ahead of normal spawning time) in coho salmon, this time by salmon pituitary preparations, also has resulted in production of poor-quality eggs (Hunter et al., 1981). Thus, too early an induction of ovulation in salmonids may produce non-viable eggs, and this may not be related to the hormones used. Even early ovulation induced by photoperiod manipulation yielded smaller eggs than those of control fish (Nomura, 1962; Buss, 1980). However, even an acceleration of ovulation by a few days in coho salmon by LHRH-A has resulted in a lower fertilization rate compared to the control (Fitzpatrick et al., 1984).

In the African catfish (*C. gariepinus*), most of the ovulated eggs induced by 17α -P or LHRH-A + pimozide were fertilizable, producing normal larvae (Richteer et al., 1985; de Leeuw et al., 1985). In the golden perch (*M. ambigua*), egg

quality appeared to be related to the dosage of HCG or heteroplastic pituitary extract used (Rowland, 1983). High dosages of HCG (Table 2) or pituitary extract (Table 1) caused a drop in hatching rate. However, high dosages of HCG or homoplastic pituitary extract did not affect the hatching rate in the Asian catfish (C. macrocephalus) (Mollah and Tan, 1983; Table 1).

The above studies serve to underline the importance of considering egg quality in induced spawning work.

(k) Standardization

An induced spawning technique should as far as possible be standardized against variables such as age of spawner, initial gonadal stage, hormone dosage, time interval between injections, time to stripping or spawning from the last injection, time of injection during the day, time of year (season), and environmental conditions. These have been discussed by Lam (1982). Recent studies again have emphasized the importance of considering some of these variables.

Age. It may be more difficult to induce spawning in first-time spawners than in experienced spawners. In the rainbow trout, first-time spawners often produce eggs of a wider range of quality than older fish (Craik and Harvey, 1984b), and also are poorer sperm producers than older fish (Buyukhatipoglu and Holtz, 1984).

Initial gonadal stage. In the rabbitfish (S. guttatus), fish with oocytes less than 0.47 mm in diameter spawned only

after HCG injections, and the smaller the initial egg diameter, the more HCG (total dose) was required (Juario et al., 1985). On the other hand, fish with oocytes larger than 0.47 mm in diameter (characterized by germinal vesicle migration) spawned without HCG injection. In the rainbow trout, the median efficient dose of 17 α -20 β -P and salmon GtH on oocyte maturation *in vitro* decreases as the oocytes undergo germinal vesicle migration (end of vitellogenesis > subperipheral germinal vesicle > peripheral germinal vesicle), suggesting increasing hormonal sensitivity during germinal vesicle migration (Jalabert and Fostier, 1984). However, in the common carp, there was no correlation between the position of the germinal vesicle and the degree of response of the ovary to the injection of LHRH-A (Billard et al., 1984a).

Time to stripping or spawning. Recent studies have provided more evidence that egg viability declines rapidly because of overripening when stripping is delayed beyond a certain species- and temperature-dependent period of time after ovulation (Hogendoorn and Vismans, 1980; Mollah and Tan, 1983; Craik and Harvey, 1984a; Espinach Ros et al., 1984a; Richter et al., 1985). There also is increasing evidence that between the time when ovulation first occurs and the time when overripening sets in, there is an optimal time for stripping that gives the best fertilizability, hatchability and larval survival (Hogendoorn and Vismans, 1980; Richter et al., 1985). In natural spawning, there usually is a time lapse between ovulation and

oviposition; three to six hours in Japanese medaka, *Oryzias latipes* (Lam, 1982) and about four hours in mullet, *M. cephalus* (J. Kuo, pers. comm.). During this period, the ovulated eggs may undergo further hydration and other maturational changes (J. Kuo, pers. comm.).

The time to spawning in golden perch (*M. ambigua*) after a single injection of HCG and/or carp pituitary extract was dosage-dependent; sub-optimal dosages produced a longer latency period to spawning than optimal or above-optimal dosages (Rowland, 1983). In contrast, the ovulatory response latency to HCG injection did not appear to vary with dosages in the Asian catfish, *C. macrocephalus* (Mollah and Tan, 1983), although in the European catfish, *Silurus glanis*, the response latency to hormonal treatment was dosage-dependent (Fijan, 1975).

There is good evidence that the latency to ovulation or spawning after hormonal treatment is temperature-dependent (Lam, 1982; Espinach Ros et al., 1984a). The response latency also may vary with the type of hormone used; in *C. macrocephalus*, most of the HCG-injected fish ovulated 14-15 h post-injection whereas most of the pituitary-injected fish ovulated 13 h post-injection.

Time of injection. A recent study by Peter et al. (1982) adds to the evidence reviewed by Lam (1982) that there is a circadian variation in responsiveness of the ovary to GtH in fish. Thus, the time of injection may be of importance. However, injection of HCG at three different times of the day

(0600, 1800, 2400 h) in C. macrocephalus did not make a difference in the ovulatory response (Mollah and Tan, 1983); it is possible that critical times have been missed.

Season. In the golden perch, M. ambigua, induced spawning was less successful during the late spawning season than during the early and mid season, and this was related to the presence of atretic eggs in the late spawning seasons (Rowland, 1983).

Environmental conditions. Temperature certainly is an important factor to consider; it affects the injection interval, response latency, time to overripening, and ovarian responsiveness (Lam, 1982). Salinity appeared to affect the ovulatory response of milkfish (C. chanos) to hormones (see review by Lam, 1984). Other factors also may be important. Recently, Billard et al. (1984a) suggested that there are some undetermined environmental factors (not vegetation) present in a pond situation that may potentiate the ovulatory response of carp (C. carpio) to LHRH-A.

(1) Problems

Besides the problems of standardization, two problems that plague aquaculturists are concerned with induced spawning: (1) stress of handling, which may cause rapid atresia of the gonad, particularly in wild-caught fish, and (2) difficulty in inducing spawning in fish with oocytes slightly smaller than the "critical" stage (often defined only in terms of oocyte diameter and not in histological stage) (Lam, 1982, 1984). The recent

introduction of LHRH-A implantation suggests a possible way of solving or reducing these problems (see Crim, this volume and references cited earlier). The work of Dettlaf and Davydova (1974, 1979 a, b) in the sturgeon also has some bearing on the first problem: triiodothyronine restored the ovulatory response to hypophysation in fish subjected to cold or captivity stress. Thyroid hormones also may help with the second problem since they may enhance the effect of gonadotropin on steroidogenesis (Sen and Bhattacharya, 1981) and vitellogenesis (Hurlbert, 1977; Lam and Loy, 1985). The inclusion of thyroxine in the spawning injection has been tried with limited success in milkfish (Lam, 1984). It should be explored further, and triiodothyronine also should be tried.

(m) Induction Of Spermiation/Seminal Hydration

The relevant literature has been reviewed by Billard et al. (1982), Fostier et al. (1983), Donaldson and Hunter (1983), and Nagahama (1983) (see also Fig. 4). LHRH, LHRH-A, purified or partially purified GtH preparations, HCG, androgens and progesterone all have been used with success to induce spermiation/seminal hydration in fish (see review by Donaldson and Hunter, 1983; Crim et al., 1983a, b; Weil and Crim, 1983). Progesterone was shown to be more effective than androgens in goldfish (Billard, 1976) and northern pike (De Montalambert et al., 1978). As stated earlier, 17α - 20β -P did not influence spermiation and seminal hydration in rainbow trout (Scott and

Baynes, 1982). This should be studied further and 17β -P also should be tested.

II. ENVIRONMENTAL APPROACH

This is based on the premise that for each species of fish, there may be a specific, or a set of specific, environmental and/or social cues which trigger final oocyte maturation and ovulation leading to spawning behavior and spawning. The literature has been reviewed by Lam (1983) and Stacey (1984). In most species, particularly the tropical species, the specific spawning cues have not been identified; only suggestions have been made based on general observations without any experimental evidence. Despite this paucity of specific information, the environmental approach to spawning induction has been practiced successfully in several species. Examples may be grouped in accordance to the degree of specific environmental and/or social control exerted.

The least specific control is seen in the natural spawning of milkfish. In the Philippines, broodstock reared in 10 m diameter floating sea cages matured and spawned after reaching about four to five years of age (Marte et al., 1984). In Taiwan, milkfish maintained in ponds (filled with filtered sea water of 29-32ppt and aerated by paddle aerators) spawned naturally after 10 years, and subsequently every year (L.T. Lin as quoted by Kuo, 1984; Lin, in this volume). Spawning is preceded by two or three days of chasing courtship behavior (Kuo, 1984, reporting L.T.

Lin's observations). In Indonesia, captive broodstock more than 10 years old also spawned naturally in 6 m diameter tanks with running sea water (Vanstone, pers. comm.).

The next group of examples involves a slightly greater degree of control: broodstock is raised in one holding facility and, when sexually mature, is transferred to another especially-prepared holding facility for natural spawning. In Japan, broodstock of red sea bream (*Pagrus major*s) is maintained in floating net cages in the sea. When males are observed chasing females, the sexually-mature broodfish (three years old) are transferred to a land-based spawning tank with running sea water and an egg-collecting system. The tank has a capacity of 40-100 tons, and the broodfish are stocked at a rate of 1 kg fish per ton (100 fish in a 100-ton tank of 7 m diameter and 1.5 m in depth) with a ratio of 1:1 (Kuronuma and Fukusho, 1984; K. Fukusho, pers. comm.).

In Indonesia, several cultured freshwater species (carp, barb, gouramies and catfish) are spawned by a similar method (Lam, 1983). The spawning pond is first dried in the sun for several days until the bottom is completely dry and "cracking." Clean fresh water then is introduced in the morning followed by broodfish in the afternoon. In some cases, nesting or spawning substrates also are introduced, and running water also may be provided (Lam, 1983). A third example is the spawning of the Asian sea bass (*Lateo calcarifer*) in Thailand (Tattanon and

Maneewongsa, 1982; Maneewongsa, 1982). Broodstock is maintained in floating sea cages. Approximately one month before the spawning season (April-September), sexually-mature broodfish (three years old or more) of about the same size and age are selected from the cages and moved to a spawning tank on land. Round concrete tanks measuring 10 m in diameter and 2 m in depth are used, and each tank is stocked with approximately 24 spawners at a sex ratio of 1:1. Running sea water of 28-32ppt (average 30ppt) is supplied, or the sea water is changed every other day. Spawning activity occurs between 1900-2300 h on the first to the eighth day of full moon. Repeated spawning usually is obtained during the spawning season if good water quality is maintained in the spawning tank (dissolved oxygen not less than 6 ppm; pH 7.5 to 8.5; and temperature 28-34°C). Interestingly, natural spawning is successful only in the Songkhla and Satul Hatcheries located near natural spawning grounds and not in other hatcheries located away from natural spawning grounds. In the latter cases, natural spawning has to be induced by HCG injections (50-100 IU/kg). This underlines the need to identify specific spawning cues if the control of natural spawning is to be made reproducible.

There are a few examples of fish induced to spawn by more specific environmental manipulation. In India, gravid Indian major carps are induced regularly to spawn in bundhs where flooding is simulated (Sinha et al., 1974). In goldfish, gravid

fish can be induced to spawn by raising the water temperature, e.g. from 12°C to 20°C, and/or by placing vegetation in the tank (Stacey et al., 1979b). In the Atlantic silverside (*Menidia menidia*), spawning can be induced during the light period by a marked reduction in current velocity (Middaugh and Takita, 1983).

In all the above examples, only the terminal events (oocyte maturation, ovulation and spawning) are induced by environmental and/or social manipulations. In salmonids, the whole reproductive cycle (up to ovulation) may be accelerated by photoperiodic manipulations, e.g. by compressing the annual light cycle to six months (see review by Scott and Sumpter, 1983; also Elliot et al., 1984).

CONCLUDING REMARKS

Although the environmental approach to induced spawning is practical and usually gives good results as far as egg quality is concerned, the level of control involved cannot match that of the hormonal approach. Perhaps the two approaches can be combined to good effect. Sexually mature fish may be injected with the appropriate hormones and then placed in an environmentally and socially conducive tank to spawn naturally. In goldfish, a combination of a single HCG injection and environmental manipulation (temperature increase and presence of vegetation) is highly effective in inducing ovulation and spawning within 24 h (Stacey et al., 1979b; pers.comm.).

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A REVIEW OF INDUCED BREEDING OF MILKFISH*

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ABSTRACT

The increasing demand for milkfish fry has prompted investigations into artificial spawning of broodstock in captivity and mass propagation of the larvae in the last decade. The progress and present status of the artificial spawning of milkfish is reviewed.

The mature broodstock were established in various types of confinements, e.g. ponds, lagoon, concrete tanks and floating cages. Environmental conditions and associated factors in these confinements are summarized, and the importance of environmental factors and dietary requirements for the gonadal maturation of captive milkfish are briefly discussed. Attempts to induce gonadal maturation by hormone administration have produced limited success, but encouraging results in the gonadal maturation by the method of LH-RH analog implantation have recently been achieved.

Significant progress on the artificial induction of milkfish spawning by injections of gonadotropin preparations has been made, and the human chorionic gonadotropin (HCG) proved to be effective in triggering the final oocyte maturation. The injection dose and procedure which have produced successful spawnings, are summarized. However, a reliable and standardized induced breeding technique remains to be developed. The difficulties and problems in developing a reliable method for induced breeding are further discussed.

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The possibility of natural spawning of milkfish in captivity has been clearly demonstrated in recent years. The efficient use of mature broodstock and satisfactory spawning performances can be anticipated when the method of natural spawning is employed. Implantation of LH-RH analog cholesterol pellet has also produced promising results in the gonadal maturation and subsequent spawning of milkfish.

INTRODUCTION

Milkfish, *Chanos chanos* Forsskal, are widely distributed in tropical and subtropical areas of the Pacific and the Indian Oceans, ranging from 40°E to about 100°W, and 30-40°N to 30-40°S. They possess an impressive range of attributes that make them well-suited for culture in a wide range of environments. They are best described as herbivorous, euryhaline, hardy, fast-growing and disease-resistant. Milkfish are known as an important subsistence food fish of the brackishwater coastal ponds of the Philippines, Indonesia and Taiwan. Milkfish fry or fingerlings have also been used extensively as baitfish for the tuna fisheries of many Pacific islands.

The culture of milkfish in brackishwater ponds is the oldest culture practice in southeast Asian countries. It probably originated in Indonesia where milkfish farming dates back at least to the year 1400. With improvements in farming technology, production has reached 2,509 kg/ha in traditional shallow water ponds and 6,658 kg/ha in the deepwater culture ponds in Taiwan (Smith and Chong, 1984). In 1981, the total

milkfish production was 209,124 mt in the Philippines and 23,912 mt in Taiwan.

Despite the long history of milkfish farming in the Southeast Asian region, the culture of milkfish in artificial impoundments has been contingent on the availability of wild fry until 1984 when fry were produced in large quantities in captivity. The fry are collected exclusively from coastal areas during the natural breeding season, and the annual supply of fry for stocking the ponds is naturally inconsistent due to environmental conditions and fluctuations in yearly recruitment. Thus, a critical problem that faces the milkfish culture industry is the variable supply of fry to cope with the needs of industrial expansion and development.

The increasing demand for milkfish fry has prompted investigations into artificial spawning of broodstock in captivity and mass propagation of the larvae in the last decade. This paper is intended to present the current status of artificial spawning of milkfish.

BROODSTOCK

The mature milkfish that have been used for hormone-induced spawning are either captured from the sea or from fishponds which they have entered as juveniles. Captures of mature milkfish from the wild were recorded from Pandan, Hamtik and Tigbuaun of Panay Island (Philippines), Rangiro Atoll (French Polynesia) and others. However, most induced breeding attempts have used

captive broodstock, established in various types of confinements, e.g. ponds, lagoons, concrete tanks and floating cages (Kuo, 1982; Lam, 1984). Environmental conditions and associated factors in these confinements are summarized in Table 1. It appears that the milkfish are able to undergo sexual maturation in both natural and artificial environments and to attain sexual maturity at 5 years of age; males may reach maturity earlier, at around 4 years of age (Lam, 1984; Liao and Chen, 1984). Among the parameters concerned, it is difficult to single out the most important factor which triggers and influences the gonadal maturation. The salinity is not important since milkfish can mature in a wide range of salinity (8-106 ppt). Temperature and photoperiod are important factors for triggering and regulating the gonadal development in many teleosts, but information on the temperature-photoperiod requirements for milkfish maturation is still lacking (Lee, 1984; Marte et al., 1984). Maturing milkfish are generally collected during the warm season of the year, water temperature ranging between 25^o and 32^oC. The rapid gonadal development of milkfish prior to the spawning season seems to coincide with a rise in water temperature from about 25^o to 32^oC and with increasing photoperiod from 11 to 14 hrs of light (Marte et al., 1984). The temperature-photoperiod combination which is most effective in triggering the inception of gonadal development is far more important, but no information is available to date in this species.

Table 1. Occurrence of mature milkfish adults in captivity.

	Dirt Ponds				Lagoons (Christmas Island)			Concrete Tanks			Cage
	Lahaiipuaa Pond Complex (Kona, Hawaii)	Namalu Pond (Kauai, Hawaii)	O ₁ * (Oahu, Hawaii)	Tung Hsin Hatchery (Pingtung, Taiwan)	Blue Lagoon	Pelican Lagoon	Azur Lagoon	Tungkuang Marine Laboratory (Pingtung, Taiwan)	O ₁ (Hawaii)	Hsin Li Hatchery (Pingtung, Taiwan)	AQG, SEAFDEC** (Philippines)
Age (years)	4-5	> 5	5-7	> 9	4-5	4-5	6	4-5	6-7	8	5
Area	1.4 ha	10 ha	0.05 ha	750-1,000 m ²	~4 x 1 mile	~0.5 x 1 mile	?	8.5 ~12 m (d)	5.2 x 4.0 m	12 m (d)	81-100 m ²
Depth	~2 m	~19 m	1 m	1.3-1.5 m	~3 m	~3 m	?	1.2 m	1.3 m	2.0-2.5 m	3 m
Temperature (°C)	24-30	24-30	24-30	22-31	27-32	27-32	27-32	21-31	26	21-31	25-32
Salinity (‰)	8-12	34-42	32	29-32	102-106	76	80-100	14-30	22	33-35	25-38
Food Source (Benthic Algae)	Abundant	Poor	Poor	Poor	Very Abundant	Poor	?	Poor	Poor	Poor	Very Poor
Supplement Feed (% crude protein)	No	No	Ralston catfish chow	Eel feed (45%)	Artemia very abundant	No	No	?	Ralston catfish chow	Commercial feed (24%)	?
Stocking density	80-100 milkfish + 300 mullets	?	12 milkfish + 100-200 mullets	26-30	?	?	?	20-30	9-12	20	80-100
Maturity of broodstock	♀ YG*** (~0.75) ♂ Ripe	YG (~0.78) Ripe	YG (0.45-0.60) Ripe	YG (~0.8) Ripe	YG (~0.8) Ripe	YG (~0.8) Ripe	?	YG (?) ?	YG (?) ?	YG (~0.8) Ripe	YG (?) ?
Pond bottom	Mud	Sand and gravel	Clay	Mud	Thick layer of benthic algae	Sand and clay	?	Concrete	Concrete	Concrete	-

* O₁ : Oceanic Institute

** Aquaculture Department, Southeast Asian Fisheries Development Center

*** YG : Yolk Globule Stage

Diet and space requirement are also important factors influencing the gonadal development, but observations are not conclusive. In the dirt ponds at Tung Hsing Hatchery (Taiwan), mature broodstock have been established by nourishing them with a formulated eel feed containing 45% crude protein or with trash fish. The pond productivity was maintained by blue-green algae, maintaining a transparency of at least 20 cm. In contrast, mature milkfish were established by using a relatively low protein feed containing only 24% crude protein in a concrete tank at Hsin Li Hatchery (Taiwan).

The brine shrimp, *Artemia salina* was once introduced into the Isles Lagoon of Christmas Island in the mid-1970's and has flourished since then. The milkfish in this particular lagoon selectively feed on the brine shrimp, though abundant benthic algae are present. They are sexually mature at the average size of 1 kg throughout the year. Thus, the brine shrimp was once considered to be an important broodstock diet, but this conclusion is still questionable. In the Pelican lagoon, on the other hand, the milkfish feed exclusively on the benthic algae but reach sexual maturity at the same size. It is therefore concluded that the brine shrimp could perhaps be a good broodstock diet, but not essential. Studies on the environmental and nutritional requirements for maturation are potentially the most rewarding, since manipulation of these factors is the most practical and could lead to effective approaches to establish the

mature captive broodstock.

In parallel, attempts have been made to induce gonadal maturation by the hormone administration. The results obtained to date have not been encouraging (Lam, 1982; Lacanilao et al., 1984), except perhaps for the male (Lee et al., 1984). In the experiment of induced maturation by using the method of LH-RH analog implantation conducted at the Oceanic Institute in 1985, immature milkfish females reached a fully mature condition in one month and the spontaneous spawning of these implanted females was also obtained (C.S. Lee, pers. comm.).

INDUCED BREEDING

Artificial propagation of milkfish has been attempted by investigators at several research institutions since 1975. They are the Oceanic Institute (Hawaii), Aquaculture Department, Southeast Asian Fisheries Development Center (SEAFDEC-Philippines), Tungkang Marine Laboratory, Taiwan Fisheries Research Institute (Taiwan), and Brackishwater Fisheries Research Station (Bali, Indonesia). The subject has been reviewed by Kuo (1982) and Lam (1984), and the results of successfully induced breeding of wild and captive milkfish are summarized in Table 2.

The induced breeding technique, using gonadotropin preparations (mostly human chorionic gonadotropin, HCG) with or without acetone-dried piscine pituitary homogenate, is being widely used to reinitiate the final oocyte maturation process. The respon-

Table 2. Records of successful induced breeding of wild and captive milkfish.

Breedstock	Age (yrs)	Body weight (kg)	Initial oocyte diameter (mm)	Time of injection	Total dose (mgSPH or CPH + IU HCG)	Specific dose (mgSPH or CPH + IU HCG/kg fish)	Injection interval (hr : min.)	Time of stripping (hr : min. from last injection)	No. eggs stripped (Thousand)	Fertilization (%)	Hatching success (No. larvae recovered)	Reference	
Wild	7	7	7	2100 0530	60 + 4,000 90 + 6,000	7		8:30	18:30	?	?	+ (b)	Vanstone et al. 1977
	7	7	7	1200 2215 0630	60 + 4,000 90 + 6,000 90 + 6,000	7		9:15	8:30	?	?	+ (7)	
		6.5	0.75	0635 1840	42 + 2,800 42 + 4,200 1 + 0.5 ml vitamin B1	6.5 + 430.8 6.5 + 646.7 1 + 0.5 ml vitamin B1		12:06	10:50-11:50	7	39	+ (3,500)	Liao et al. 1979
	8	7.0	0.77	0830 1800	70 + 10,000 70 + 10,000	10 + 1,428.6 10 + 1,428.6		9:30	10:30	1,400	59.4	+ (26,492)*	Kuo 1980 and 1982, Zuero et al. 1979 and 1981
	7	10.0	0.74	7 7	100 + 10,000 100 + 20,000	10 + 1,000 10 + 2,000		12:00	10:00-11:30	7	26-28	+ (7)	Julio 1984
	5	4.1	0.73	0600 1800	100 + 10,000 100 + 10,000	24.4 + 2,439 24.4 + 2,439		10:00	12:00	7	7	-	Kuo et al. 1979
	6	6.5	0.74	0900 1500	160 + 10,000 100 + 15,000	15.4 + 1,538 15.4 + 2,308		9:00	4:00	7	7	-	
	6	4.8	0.81	1900 0900	25 + 2,500 25 + 2,500	5.6 + 656 5.6 + 656		15:00	6:00	7	7	-	
	7	4.0	0.76	7 7	40 + 5,000 40 + 10,000	10 + 1,250 10 + 2,500		24:00	10:00-14:00	7	9-32	+ (7)	Julio 1984
	Captive	9	5.0	7	11:00	0 + 6,000	0 + 1,250	-	15:20-15:50	600	20-60	+ (120,000)	Lin 1982 and 1984
10		7.0	7	16:00	0 + 10,000	0 + 1,429	-	8:00	800	65	+ (350,000)	Lin 1984	
10		6.0	7	16:00	0 + 8,000	0 + 1,333	-	18:00**	300	30	+ (30,000)		
10		6.0	7	8:00	0 + 8,000	0 + 1,333	-	24:00	200	20	+ (2,000)		
10		6.0	7	16:00	0 + 6,000	0 + 1,333	-	19:00	250	30	+ (20,000)		
10		6.5	7	18:00	0 + 6,000	0 + 1,231	-	15:00	600	30-60	+ (150,000)		
10		5.5	7	12:00	0 + 7,500	0 + 1,264	-	18:00	200	16	+ (1,200)		
8		3.5	0.6-0.8	18:00	0 + 3,500	0 + 1,000	-	16:33	-	7	+ (120)	Tsang and Hsiao 1979	

* Significant loss of hatched-out larvae during the process of the larvae transfer from incubation tank to rearing tank.
 ** Natural spawning following a single injection of HCG.

Table 3. Fertilization success of fresh and preserved sperm at various salinities.

Salinity (%)	Fresh sperm								Preserved sperm*
	5	10	15	20	25	30	35	40	35
Fertilization (%)	41.7	60.0	61.3	58.5	53.0	52.0	48.0	92.0**	53.6

* The 7-day-old sperm preserved at near-zero temperature was used.
 ** Only the suspended eggs were examined.

siveness of the breeder to the hormone treatment depends upon the physical condition of the recipient, the stage of maturity of ovarian oocytes and physiological acclimation to the spawning environment.

The results of induced breeding attempts have been varied and a standardized hypophysation technique has not been developed in this species. However, with the success in the induced-breeding attempts listed in Table 2, the following variations in dosages and procedures are indicated:

- . Injection dose
 - Piscine pituitary 0-48.8 mg/kg fish
 - homogenate HCG 1,000-4,880 IU/kg fish
- . Number of injections 1-3
- . Time of injections variable
- . Time interval between 8 hr 15 min - 24 hrs
- . Time of stripping from last injection 4 hr - 24 hr
- . Source of breeders wild and captive broodstock
- . Age of breeders 5 - 10 years
- . Fertilization 9 - 60%

Success in the artificial propagation of milkfish has been repeatedly achieved by using 8 to 10 year old captive broodstock. A single injection of HCG ranging between 1,000 and 1,429 IU per kg fish proved to be effective (Tseng and Hsiao, 1979; Lin, 1982 and 1984). The hydrated and fertilizable eggs were obtained by stripping followed by artificial fertilization.

The effective response of milkfish breeders to hormone treatments is determined by the following factors:

A. Sexual maturity of recipient

The maturity of the recipients is often represented by the developmental stage and the initial diameter of ovarian oocytes. It appears that the milkfish captured from seawater respond to the exogenous hormone treatments effectively when the ovarian oocytes are at the tertiary yolk globule stage (yolk-laden oocytes) and the initial oocyte diameter is larger than 0.66 mm, while for those captured from the brackishwater or from hypersaline conditions, an initial yolk-laden oocyte diameter of 0.72 mm may be required before they can be successfully induced to spawn.

The total amount of hormone required for spawning seems related to the advancement of the recipient's maturity; higher doses and more injections are required if the initial oocyte diameter is around 0.66 mm, and a lower dose (often by single injection) may be sufficient to induce spawning when the initial oocyte diameter is larger than 0.8 mm.

Furthermore, the milkfish is highly sensitive to handling stress and atresia of mature oocytes larger than 0.6 mm soon occurs if they are not given an injection of gonadotropin preparation within 26 hrs after capture.

The results of induced breeding attempts indicate that the females with oocytes 0.7 mm in diameter or above offer the

greatest chance for successful induced breeding. A priming dose of the gonadotropin preparation should be given immediately after capture to prevent the oocytes from atresia caused by handling stress, followed by a minimal number of injections of the gonadotropins in the ensuing period. A female with oocytes smaller than 0.7 mm is still in the maturation phase of ovarian development, and repeated low-level injections of gonadotropin preparation required to stimulate further development will certainly stress the fish acutely and may cause mortality. The responses of such fish to further hormone treatments are expected to be unsatisfactory, and it is therefore advisable to allow such females to undergo further natural vitellogenic development before injecting the hormone. Unfortunately, advancement of oocyte maturation by the method of hormone administration approach is not possible for fish which contain oocytes larger than 0.6 mm in diameter.

B. Physical conditions of recipients

The response of milkfish to hormone treatments is often influenced by the physical condition of the recipients. The method of capture and handling of mature breeders affects the extent of physical damage which, along with the physiological stress, can cause mortality of the fish. Minimizing physical damage to the fish is essential to obtain satisfactory results in spawning attempts. Careful capture with a seine net is the least injurious method. The fish are then handled in water-filled

plastic bags.

In the course of hormone administration for induced breeding, wild-captured breeders have often been unable to survive the stress of repeated handling and the physical damage from more than 3 hormone injections. However, captive breeders seem more capable of surviving handling stress than wild fish. The damage from handling results in skin and eye infections. The latter cause blindness in 24 hrs. It is therefore advisable that high doses of hormones should be given in, at most, two injections to complete oocyte final maturation, leading to the spawning within 24 hours.

C. Acclimation to spawning environment

The response of milkfish adults to the hormone treatments appears to be affected by the salinity change in the course of induced breeding attempts when such salinity adjustment is necessary for spawning. Both abrupt and gradual changes in the salinity imposed problems not only in the physiological stress, but in the ovulatory response of the hypophysated females. The method and injected hormone currently used seem most effective when the salinity adjustment is not required prior to the time of spawning.

PROBLEMS IN THE INDUCTION OF SPAWNING

Upon completion of final oocyte maturation, induced by the hormone injections, artificial fertilization is accomplished by manual stripping. The process often involves killing the

injected females and males. Determination of the proper time of stripping is critical and has proven to be extremely difficult. Lack of total synchronization of oocyte hydration and continued dribbling of both hydrating and fertilizable oocytes prior to the completion of the final maturation of all the ovarian yolk-laden oocytes contributes greatly to this difficulty. This has often resulted in a low rate of fertilization (up to 60%) and only a portion of total mature oocytes are recoverable.

The phenomenon of egg dribbling, both in non-hydrated and fertilizable eggs, is commonly observed when the spawning of the hypophysated females is approaching. This provides a useful indication on the progress of oocyte final maturation, but on the other hand, the dribbling has resulted in a significant loss of potential recruitment. The dribbling phenomenon has not been reported in any other finfish, and whether this peculiar behavior is a part of the normal final maturation of oocytes needs to be clarified.

The other obstacles to the success of artificial spawning through hormone treatment have been (1) the inability to capture fully mature females and males at the same time, (2) insufficiency of milt from males which are mature but not fully ripe, or (3) milt resorption after 2-3 days in captivity. These problems can be approached by the sperm preservation and the enhancement of spermiation through the administration of gonadotropin or androgen preparation. Attempts have been made to

preserve milkfish sperm at near-zero temperatures (0-4°C) and in liquid nitrogen (-196°C) (Kuo, 1980 and 1982; Hara et al., 1982). The milt was collected in sterilized capillaries or polyethylene cannulae and then stored at a temperature of 0-4°C. Viability of the sperm could be maintained for 7 days at this temperature (Kuo, 1980). Fertilization successes using fresh milt in salinities ranging between 5 ppt and 40 ppt and using 7 day old preserved milt at 35 ppt are summarized in Table 3. Improvement of short-term sperm preservation has been achieved through the addition of an extender. Glucose (0.2 M to 1.0 M) and NaCl (0.2 M to 0.8 M) were found to be suitable extenders for milkfish milt. The fertility of stored milt was maintained for 14 days.

Among various extenders examined, the milkfish serum gave the best results in terms of high motility and fertility of the preserved sperm (Hara et al., 1982). Satisfactory results in sperm cryopreservation were also obtained by using dimethyl sulfoxide (DMSO) as a cryoprotectant. Sperm preserved for 10 days in milkfish serum containing 12.4% DMSO yield higher fertilization and hatching rates than fresh milt alone (Hara and Tiro, 1984).

For biopsy of treated females, cutting along the ventral mid-line of the peritoneal cavity has also been employed to facilitate the collection of hydrated oocytes, which are then fertilized artificially. Hence, this kills valuable broodstock and the fertilization rate has always been lower than 60%.

Moreover, a high percentage of unfertilized eggs often causes problems in maintaining good water quality during the incubation and this can lower the hatching rate. Separation of fertilized eggs from the unfertilized eggs is necessary for improved hatching and this can be accomplished simply by the elevation of salinity of the incubation medium. A major portion of eggs collected at the salinity of 40 ppt constitutes as high as 92% fertilized eggs (Table 3).

The problems and difficulties which have hampered the progress of the development of a standardized technique for induced breeding are summarized as follows:

- limited ability of mature broodstock
- inability to capture the mature broodstock of both sexes at the same time
- atresia of mature oocytes resulting from handling stress during the process of capture and monitoring of ovarian maturity
- egg dribbling prior to spawning
- difficulty in determining the time of manual stripping for artificial fertilization
- milt resorption in captivity

Although significant progress in the artificial induction of milkfish spawning has been made in the last decade, a reliable and standardized induced breeding technique remains to be developed. Furthermore, the efficient use of mature broodstock

is still in doubt, if the technique of hypophysation and manual stripping are employed.

NATURAL SPAWNING

Spawning following hormone injection of 1,333 IU HCG per kg fish was recorded for the first time from pond-reared adults on July 20, 1983 (Table 2). 55% fertilization was obtained and 300,000 hatched larvae were recovered. Spontaneous spawning without hormone treatment has also been achieved with captive broodstock maintained in floating net-cages in the Philippines (Lacanilao and Marte, 1980; Marte, et al., 1984), but no fertilized eggs were recovered.

On October 6, 1983, a natural spawning of milkfish without prior injection was noticed at the Tung Shim Hatchery, Taiwan, in the course of a plankton collection in a 750 m² dirt pond, in which milkfish adults and seabass were stocked. Following intensive pond preparation, 30 and 50 10-11 year old milkfish adults were stocked in the 750 m² and 1,500 m² ponds, respectively. An unusual schooling behavior in this broodstock was noticed on April 5, 1984. Fish were moving vigorously along the edge of the pond and were found to have developed to a blastodisc stage. A half-million eggs were recovered by seine-net. The spawning occurred around 3 am and a fertilization rate of 55% was estimated. Hatching began at 9:30 am April 9 and was completed by 3-4 pm the same day. The water temperature was 27-

29°C and the salinity measured 34 ppt. A total of 210,000 larvae hatched and were reared successfully through the fry stage in 22 days. A survival rate of 26.1% was recorded for this particular spawning.

Natural spawning of these milkfish continued in the ponds. A total of 62 natural spawnings were recorded by September 21, 1984. More than 61.8 million eggs were recovered at an average fertilization rate of 50-60%, ranging between 10% and 95%. Under the difficulty of limited hatchery capacity at Tung Shin Hatchery, the fertilized eggs recovered were mostly dispersed to dirt ponds for the incubation and the subsequent process of larval rearing as well. A total of 4.4 million milkfish fry, ranging between 10 and 16 mm in size, were produced in 1984.

The same broodstock resumed their spawning on April 17, 1985. A total of 20 spawnings were recorded by May 7, 1985 and more than 20 million eggs have been collected.

A constant chasing courtship behavior was observed during the 2-3 days before spawning took place. Often the school of milkfish adults in the pond segregated into two or three smaller groups. The individuals within each group would interact with one another, displaying their own courtship behavior. Most spawnings occurred between midnight and 3 am. Synchronized egg development implied that only a single female among the group spawned each time. Sometimes spawning was also observed around mid-day. Spawnings were spread over the summer season in Taiwan, peaking

during the months of May through June.

Between January 25 and March 3, 1985, a total of 8 natural spawnings of 9-10 year old milkfish without hormone treatment were recorded in a broodstock pond at Gonol (Brackishwater Fisheries Research Station, Bali, Indonesia) (Poernomo et al., 1985). These observations clearly suggest the possibility of routine natural spawning of milkfish in ponds. However, the ecological characteristics of these ponds are not available and the environmental triggers for the spawning have not been identified. Temperature may be important (Wainwright, 1982; Marte et al., 1984 and Lum, 1983). It appears that 24°C is the minimum temperature required for milkfish spawning (Wainwright, 1982) and the time of spawnings is closely related to the water temperature (L.T. Lin, pers. comm.). Seasonal changes in temperature may also be important in triggering the inception of gonadal maturation. Studies on the ecosystem and its ecological parameters requires close attention.

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ENVIRONMENTAL FACTORS IN FISH REPRODUCTION

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ABSTRACT

In the rose bitterling, *Rhodeus ocellatus ocellatus*, which continues cyclic spawning from spring through summer, gonadal maturation was induced by raising the temperature above 10°C in early spring. Gonadal regression at the termination of spawning was affected by shortening the daylength below 13L. This photoperiodic involvement developed during July, probably in connection with fatigue in the endocrine system. At a photoperiod over 14L, the regressed fish could soon be brought into recrudescence. In the spring-spawning tabira bitterling, *Acheilognathus tabira*, the lower limiting temperature for maturation was about 13°C, while the gonadal involution was caused by the temperature rising beyond 25°C in early summer. If the fish kept spawning under moderate temperatures, they were found to develop photoperiodism within a few months. During autumn, the spring-spawning goldfish could be rematured and suppressed under long and short daylengths combined with 24°C. At 16°C, however, the photoperiodic response was depressed and maturation could be completed regardless of daylength. Moreover, maturation cycles were repeated for many months. Patterns of environmental regulation of ovarian activity were compared, and common features were discussed.

INTRODUCTION

The majority of teleosts are seasonal breeders and each species has its own periods of breeding. Each species shows a series of regular, temporal changes in its reproductive activity, which are repeated annually, and therefore, make up the annual reproductive cycle. Thus, the breeding of the fish is so precisely timed that the fry are produced in conditions most

favorable for growth and survival. A number of investigations have been concerned with the role of the environment in regulating the reproductive cycle in teleost fish. However, it has often been pointed out that a comprehensive assessment of the regulatory role of environmental factors is not possible, because of the diverse experimental conditions adopted by different investigators, the lack of proper controls, and the equivocal results (Sundararaji and Vasal, 1976; Peter and Crim, 1979, among others).

Here, we do not intend to cover literature dealing with environmental control of teleost reproductive cycles. Rather, we would like to present our results in the same line of research using cyprinids as materials. Our approach is to first describe the annual reproductive cycle of a teleost in its natural habitat, and then to do rearing experiments, wherein the fish at different phases of the reproductive cycle are exposed to various photoperiod and temperature regimes, including those comparable to natural conditions.

RHODEUS OCELLATUS OCELLATUS

The spawning period of the rose bitterling, *Rhodeus ocellatus ocellatus*, extends almost six months -- from late March to mid-September. The female GSI (gonad weight X 100/body weight), shows a sudden rise in March, a plateau, and then an abrupt fall in September. Evidently, this fish matures when both temperature and daylength are increasing and regresses when day-

length decreases but the temperature change lags behind (Fig. 1).

Along with the accumulation of yolk in the ovary in March, the female ovipositor grows to about the height of the anal fin and begins cyclic elongation concurrent with cyclic ovulation. After oviposition, the ovipositor quickly shortens to the previous resting length. The fish continues the cyclic ovulation and oviposition throughout the breeding period. Toward the end of the period, the ovipositor stops elongating and dwindles, accompanying the ovarian involution. Thus, the gonadal condition can be estimated reasonably by watching the ovipositor.

Adult fish were collected from a natural habitat in the middle of January and maintained under several regimes of temperatures combined with photoperiods. Temperatures were either 10^o, 16^o, or 22^oC, and photoperiods were either 7, 9, 12, or 16L. These fish were fed mainly with tubifex worms. Freshwater mussels were placed in each aquarium as a spawning bed (Asahina and Hanyu, 1983).

Within four weeks of collection, GSIs increased rapidly at 16^o and 22^oC regardless of photoperiod, and some females started cyclic oviposition. There was no significant change in GSI at 10^oC. By raising the temperature to 13^oC, however, a rapid GSI increase and initiation of spawning were introduced in all of the photoperiod groups, i.e., 7, 9, 12, and 16 L groups (Fig.2). Apparently, gonadal maturation in the pre-spawning period depends

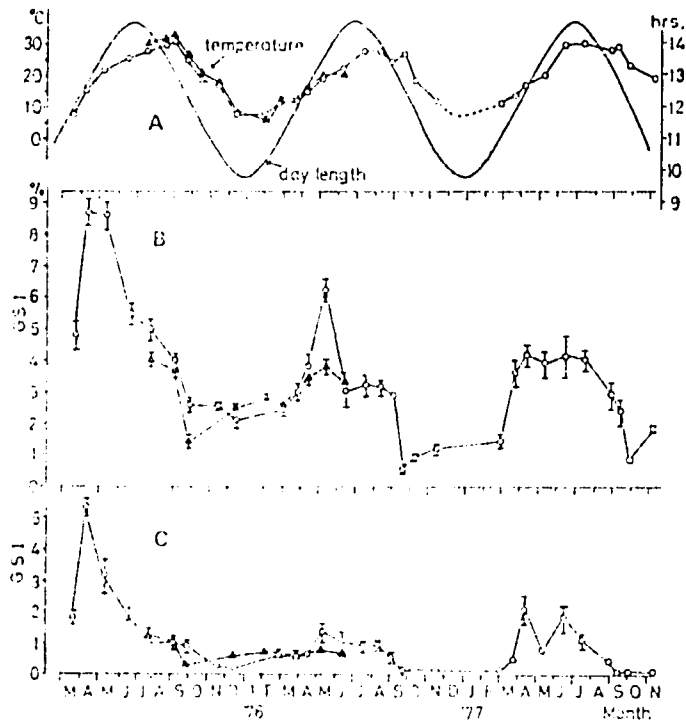


Fig. 1 Seasonal variations in day length and water temperature (GSI) of the female (B) and the male (C) *Rhodeus ocellatus ocellatus*. Each GSI value is the mean \pm S.E. of 20 individuals. -O-, Shintone Canal; -▲-, Kayanuma Pond. (Asahina et al., 1980)

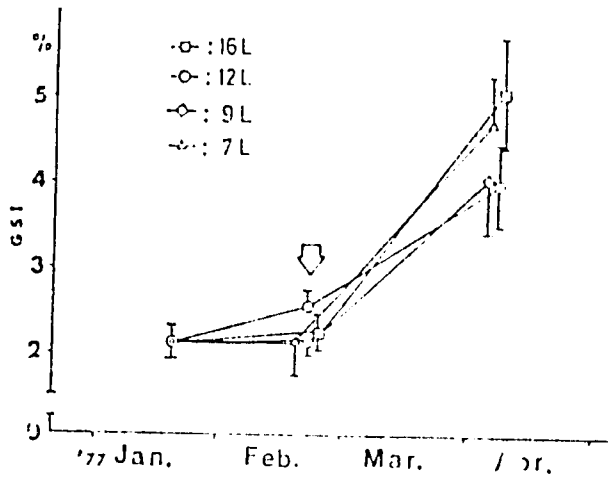


Fig. 2 Effects of low temperatures with various photoperiods on ovarian GSI (ovary weight x 100/body weight) in Rhodeus ocellatus ocellatus during the prespawning period. Fish were maintained at 10°C for 38 days, then the temperature was raised to 13°C (arrow). Experiment was started on Jan. 19, 1977. Fish were sampled on the 38th and 78th days thereafter. Symbols and bars, mean \pm standard errors. (Asahina and Hanyu, 1983)

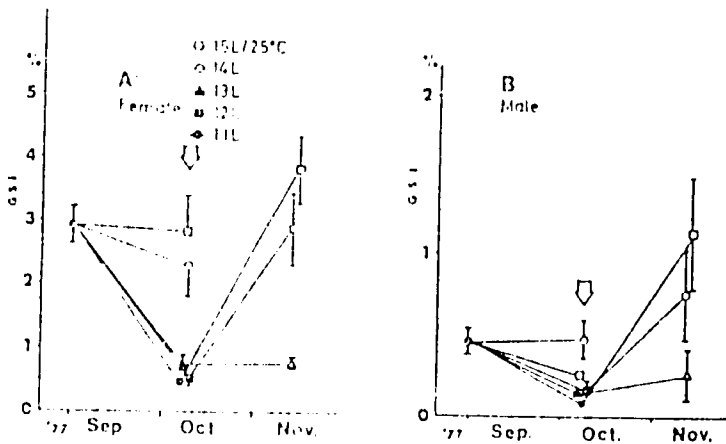


Fig. 3 Effects of various photoperiods on ovarian (A) and testicular (B) GSIs in Rhodeus ocellatus ocellatus during the late- and postspawning periods. Experiment was started on Sept. 1, 1977. Fish were sampled on the 36th and 60th days thereafter. In some experimental groups, photoperiods were changed on the 36th day (arrow). Temperature \pm standard errors (Asahina and Hanyu, 1983)

on the temperature rising above 10°C. Increasing daylength during this period seems to have little effect.

About half of the bitterling collected in August had regressing gonads. First, experimental fish were divided into five groups with different daylengths of 11, 12, 13, 14, or 15L. The temperature was kept at 25°C. Within a span of five weeks, the oviposition cycle resumed in both the 14 and 15L groups, whereas no oviposition occurred and female GSIs decreased significantly in the remaining groups. However, by an extension of daylength from 11 and 12L to 15 and 14L, respectively, ovarian rematuration was soon induced, oviposition commenced eventually, and GSIs increased markedly. In contrast, neither oviposition nor GSI increase was observed in the group kept at 13L throughout the experiment. Almost the same results were obtained from the males (Fig. 3).

These results indicate that during the course of the breeding period, photoperiodism becomes involved in the gonadal activity and this involvement manifests itself when the natural daylength comes to the critical level of between 13 and 14L. These results also tally with the fact that the natural spawning period of this species terminates abruptly in September, when the water is still warm.

Development of photoperiodism:

Experiments of a similar design were repeated once a month beginning in May and it was found that the photoperiodic response

first took place in August (Asahina and Hanyu, in press), which means photoperiodism developed in July. In another experiment, it was found that the bitterling, having completed rematuration under artificial long day regimes, became unresponsive to shortened daylengths. They continued cyclic spawning for some months even at shortened daylengths.

From these results, it is assumed that photoperiodic involvement develops along with fatigue in the system controlling the gonadal maturation of this species. It may be that the hypothalmo-hypophysial axis becomes tired after a certain period of exertion and passes into a state where it needs help or stimulation from long daylengths in order to maintain the high level of activity.

Decline of photoperiodism:

Responsiveness to photoperiod seems to be lost before the following spring. Possible roles of temperature in this phenomenon were studied using regressed fish caught in November. They were kept under the regimes of 5^o, 10^o, or 15^oC at 10L for nine weeks. Subsequently, each group was divided into two subgroups, 10 or 14L at 15^oC, and then reared for another five weeks.

At the termination of rearing, the gonadal rematuration was least advanced, but photoperiodic response, or the difference between the short and long daylengths, was unexpectedly most

distinct in the subgroups transferred from 5° to 15°C. In contrast, the gonadal rematuration was most advanced, but the photoperiodic response was least evident in the 15° to 15°C subgroups. Even the subgroup kept at 10L throughout the rearing had a notable increase in GSI (Asahina, unpublished).

Additional experiments revealed that rematuration under short daylength regimes proceeded with less hesitation at higher temperatures. Under 20° to 25°C combined with short daylengths, regressed fish could restore the gonadal activity and start spawning in two to three months. In nature, it appears that photoperiodic involvement becomes weak enough by mid-winter that the rising temperature in early spring takes over the decisive role, as an external factor, in the induction of gonadal maturation.

Based on these results, our interpretation of the photoperiodic involvement in the reproductive cycle of this species can be summarized as follows: long days help the control system maintain gonadal maturation when the control system becomes tired during the breeding period. Maturity cannot be sustained if daylength shortens below the critical level in late summer. However, the control system does not stay exhausted even with short days. It gradually recovers, despite the declining temperatures during autumn, and reaches a state where it can be stimulated to full functioning by rising temperatures.

Acheilognathus tabira

~~*Acheilognathus tabira*~~ is a spring-spawning bitterling. Judging from the ovipositor elongation, ovulation, and GSI, the breeding period of this species continues for two months only -- from late April to late June (Fig. 4).

Results from a rearing experiment conducted in early spring have also shown that rising temperatures are the only initiating factor of the breeding season in this species. The lower limiting temperature is about 13°C, although a slight difference exists between sexes (Shimizu and Hanyu, 1982).

Fish continuing cyclic oviposition in late May were exposed to several combinations of temperature (22°C, 26°C, or 30°C) and photoperiods (8, 12, or 15L). During the three-week exposure, both male and female GSI decreased greatly in the 30°C groups (12 and 15L), while GSIs and gonadal histology changed little in the 22°C groups (8, 12, and 15L). The decrease in GSI of females was less at 26°C than at 30°C. These results indicate that the termination of spawning period in this species is caused by the temperature rising beyond the upper limiting level of about 25°C (Fig. 5).

On the other hand, it was found that photoperiodism could develop in this species. When the fish kept under 22°C-15L for one to two months were transferred to 22°C-11L, they responded to this shortened daylength and showed rapid gonadal regression. Therefore, it may be possible for this species to terminate the

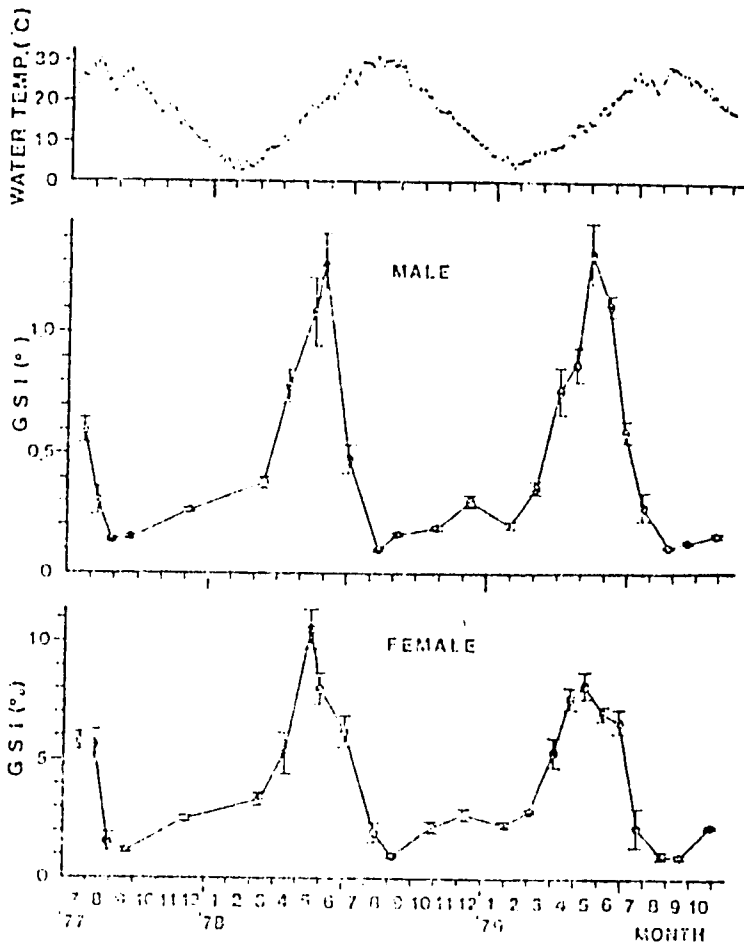


Fig. 4 Seasonal variations in water temperature at Lake Kasumigaura (upper), gonadosomatic index (GSI) of the male (middle), and of the female bitterling (lower). Water temperatures are from observation data by Freshwater Fisheries Experiment Station, Ibaraki Prefecture. Circles and bars indicate the means and the standard errors. (Shimizu and Hanyu, 1981)

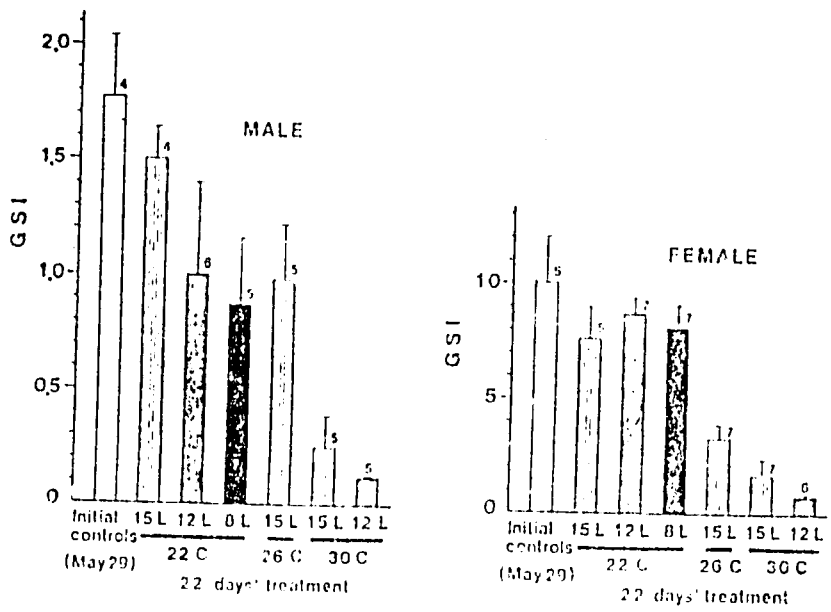


Fig. 5 Effect of photoperiod and temperature on GSI of *Acheilognathus tabira* in an early summer experiment (Exp. 2). Columns and bars indicate the means and standard errors, respectively. Figures on the tops are numbers of fish examined. (Shimizu and Hanyu, 1982)

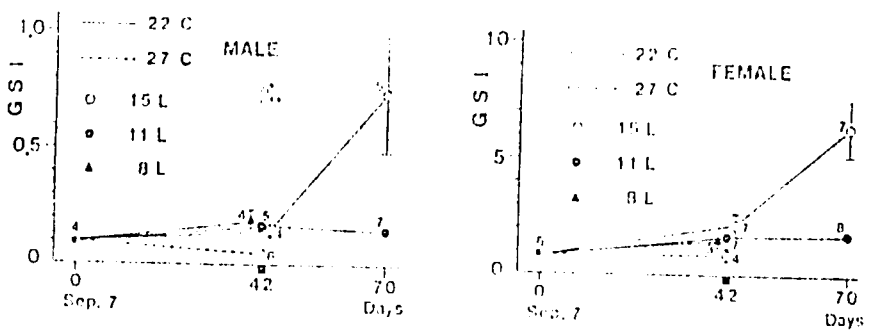


Fig. 6 Effect of photoperiod and temperature on GSI of *Acheilognathus tabira* in an early autumn experiment (Exp. 3). Symbols and bars indicate the means standard errors, respectively. Figures by symbols are numbers of the fish examined. The single asterisk denotes one male separated from others of 22°C-15L group because of its extremely high value of GSI. The double asterisks denote one male separated from others of 27°C-15L group for the same reason. (Shimizu and Hanyu, 1982)

spawning season in response to shortened daylength in some cool habitats of northern Japan.

Further, strong regressed fish with oocytes at perinucleolus stage in early September were maintained under regimes of 22°C-8L, 22°C-11L, 22°C-15L, or 27°C-15L. After six weeks, a slight increase in GSI and concomitant formation of both yolk vesicles and spermatocytes were observed in all the 22°C groups, whereas hardly any gonadal change occurred in the 27°C group. After another four weeks, however, only the 22°C-15L group revealed high gonadal activity with sharply increased GSI. The 22°C-11L group did not undergo any further changes. Nearly the same results were obtained in both sexes (Fig. 6). Therefore, it may be concluded that the initial stage of recrudescence is induced by the declining temperature in autumn, but further advancement of recrudescence is not readily made because of shortening daylength of this season. The latter situation is very similar to that of Rhodeus ocellatus in autumn.

Carassius auratus

The goldfish, Carassius auratus, mature and spawn in spring. According to Yamazaki (1965), this fish can be induced to full maturation in winter simply by raising the temperature. Yamamoto et al. (1966) found that the fish maintained full maturity indefinitely at 13° to 14°C. We also studied the goldfish, Comet variety, and the environmental effects on gonadal activity from

summer through winter (Razani, 1985).

During the summer, the ovaries of two- to three-year-old goldfish are regressed, possibly because of high temperatures, and the average GSI falls to a minimum value. The GSI gradually increases throughout autumn. After a minor increment in winter, it turns to a steep rise leading to full maturation. Ovarian histology shows only perinucleolus stage oocytes during August and early September, yolk vesicle stage in late September, and yolk globule stage oocytes in a few fish in October, with a majority in December.

The E_2 levels of the females correlated well with the changes of GSI and ovarian histology. But the T_4 levels and histological features of the thyroid usually showed an inverse relationship to the activity of gonads. The GtH levels were generally high in maturing and matured fish and low in immature fish. However, the changes in GtH levels under natural conditions seemed to have more correlation with the water temperature than with ovarian maturation.

The male goldfish moved into the post-spawning stage in July, and after completing regression in August, resumed maturation. They reached the spawning stage and started to milt in October. Spermiation continued until early December. In winter, spermatogenesis was arrested without falling into regression. Milting fish appeared again in March.

Gonadal responses to shifts of external condition:

At two-month intervals, fish were transferred from this natural condition to four different regimes of 24^o or 16^oC combined with 16 or 12L. When transferred to 24^oC in October and thereafter, the ovarian response differed remarkably between the two photoperiods. At 16L, the ovarian development was accelerated, except during summer, even to full maturation and ovulation. In contrast, ovarian activity was suppressed at 12L. Histologically, oocytes could not develop beyond the yolk vesicle stage and fell into atresia. This strong suppression seemed to be characteristic of the goldfish. Here again, the photoperiodic response in gonadal activity of the fish in the "off" spawning season was confirmed.

When transferred to 16^oC, however, the response of the ovary did not differ much between the two photoperiods. Ovarian development was accelerated at either 16 or 12L. In winter, the GSI of these photoperiod groups rose rapidly at almost equal rates. Hence, it is quite obvious that the strong photoperiodic response in the goldfish is limited to higher temperatures. We have studied this further and confirm that the suppressing effect at 12L is lost when the temperature is lowered below about 20^oC. Intermediate temperatures between 16^o and 20^oC appear to be most favorable for the gonadal development of the goldfish.

The male goldfish responded in a somewhat different manner. When exposed to 24^oC, the male fish matured rather quickly even

at 12L, and the minimal time needed for reaching milting condition was shorter in the 16L group than in the 12L group. In both groups, the fish appeared to pass readily into the post-spawning stage. Cool temperatures (16°C) combined with 12L or 16L played stimulative roles toward testicular maturation. After reaching milting condition, fish maintained active spermatogenesis regardless of photoperiod. But the speed for full maturation was slower under these regimes than under warm temperature regimes.

In an additional experiment conducted in late October, fish taken from the outdoor pond were brought under the regimes of 12, 13, 14, 15, or 16L at 24°C. Two months later, average GSIs of the female were elevated uniformly in the 14L, 15L, and 16L groups. About one-third of the fish in each group had started spawning. In contrast, GSIs were suppressed in 12L and 13L groups. The GSIs of the outdoor fish took the middle course. Accordingly, the critical photoperiod is between 13L and 14L in the goldfish.

Gonadal activities under constant conditions:

Long-term effects, rather than short-term, of external factors on the gonadal activity of the goldfish were studied in another experiment in which four groups, each consisting of 25 males and 25 females with individual markings, were subjected to 16°C or 24°C in combination with 12 or 16L. The 24°C regimes continued for one year and the 16°C regimes for 18 months, both

starting from June 27, when the breeding season was almost ending.

Under 24°C-12L, only a few fish, which had large and soft bellies prior to the experiment, spawned in the first month. All females completed their regressive stage by late July and remained immature thereafter. Under 24°C-16L, the females were stimulated to spawn during the first two months and then moved into the post-spawning stage. With the exception of three females, however, the remaining 22 fish stayed immature over the following months. Thus, the above-mentioned accelerative effect of this regime was transitory.

Under 16°C-12L, each fish spawned one to two times within the first two months and then stopped spawning. After a pause of about four months, spawnings resumed and each fish spawned two to three times. The majority of the females were immature and the remaining were in the maturing stages at the end of the experiment. In contrast, under 16°C-16L spawnings took place without noticeable interruption, and individual fish spawned two to six times during 18 months under observation. At termination, however, 12 out of 17 fish were immature, the remainder being in maturing stages.

On the other hand, most males kept under 24°C-12L completed two maturational cycles and commenced the third within a year. The first cycle was from July to November and the second from December to March, followed by a third. At termination, the

majority of the fish were in milting condition. These results show that testicular activity is hardly disturbed by lasting exposure to short daylength, which will suppress ovarian activity so severely as described above. Under 24⁰C-16L, however, the males were milting only within the first two months and then moved into the post-spawning stage. In the following months, a few fish milted but the majority remained without milting.

The males were milting continuously at 16⁰C regardless of the photoperiod. The amount of discharged sperm fluctuated considerably under both regimes.

DISCUSSION:

Based on our results and other available information, we can summarize the effects of external factors on the ovarian activity of goldfish in the "off" season as follows (fig. 7): the ovary can develop within a certain range of temperatures, and the formation of yolk takes place above the lower limiting temperature. But fully-mature fish are prevented from ovulation unless the ambient temperature rises beyond a certain level (ca. 15⁰C). Between this and a higher level (ca. 20⁰C), fish can repeat maturational cycles more easily in long rather than short daylengths. Beyond the higher level, the photoperiodic response becomes very distinct: the ovarian activity is accelerated, though transiently, at long daylengths, while it is suppressed at short daylengths. The suppressing effect, however, tends to

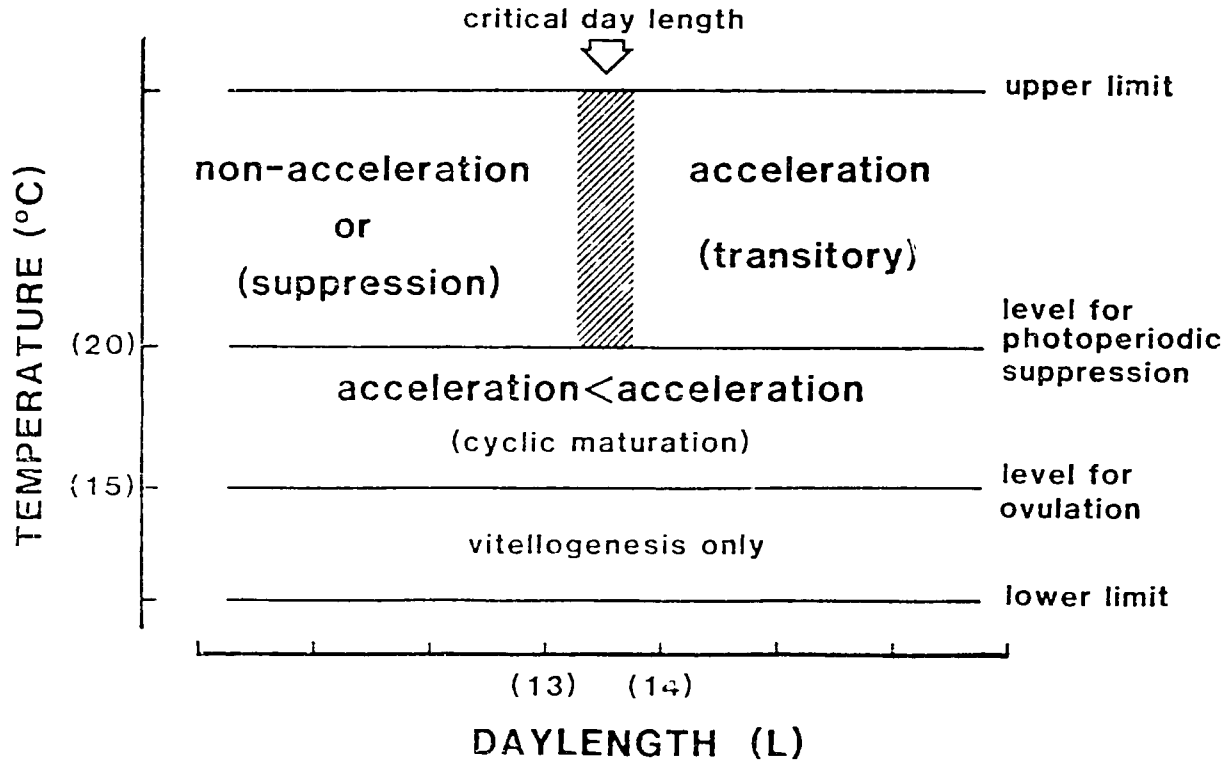


Fig. 7. Roles of temperature and photoperiod in ovarian maturation of cyprinids spawning in spring-summer. Terms and numerals in parentheses apply to goldfish.

disappear toward the beginning of the natural breeding season.

A similar pattern of environmental influence on ovarian maturation may be found in the bitterlings. As described above, *Rhodeus ocellatus*, can maintain or restore maturity under lengthened daylight and high temperatures at the end of the breeding season and thereafter. When development and manifestation of photoperiodic involvement are retarded at lowered temperatures, fish continue cyclic spawnings far into the "off" season with shortening daylengths (Asahina and Hanyu, in press). But temperature levels for suppression and ovulation have yet to be determined. Also, it should be remembered that the fish do not stay regressed and eventually start rematuration even under short daylengths and high temperatures.

Another spring-spawning cyprinid, *Gnathopogon elongatus*, has, in autumn, been shown to share gonadal responsiveness to long daylength combined with warm temperatures. The critical daylength for this response was also located between 13 and 14L (Lee et al., unpublished). This type of photoperiodic involvement seems to be common in the spring-spawning cyprinids. In addition, a small filefish, *Rudarius ercodes*, which spawns during spring and summer, could also be made to remature under artificial long day and warm temperature. The critical daylength was between 12 and 13L (Lee et al., 1984).

The intermediate temperature range to allow cyclic maturation in the goldfish may be of value from the viewpoint of

aquaculture. It is not yet known if the temperature range is common to cyprinids and other fishes. In this connection, the results obtained from carp may be worth mentioning. Right after the spawning season, female carp can remature and spawn under 24°C combined with long or short daylengths. At 16L, 12 of 15 fish spawned totally in three months and half of the spawners respawned in another three months. At 12L, two of 14 fish totally spawned twice, and eight spawned once within 10 months. These fish further retained maturity, but stopped spontaneous spawning. Spawning could be induced by some adequate stimulation (Davies, 1983). It should be clarified whether or not ovarian suppression by short daylengths does not occur in this species at higher temperatures.

It may be expected that this type of comparative study will contribute to better comprehension of basic principles in environmental regulation of gonadal activity, and also, to artificial control over reproduction of important fishes in aquaculture.

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**ENVIRONMENTAL FACTORS
IN THE REPRODUCTION OF MILKFISH**

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ABSTRACT

The environmental factors required for the maturation and spawning of milkfish are not yet defined. Possible effects of these factors, such as temperature, photoperiod, salinity, lunar cycle, space, tank shape, and stress, will be discussed in this report.

Long daylight regimes and warm water temperatures seem to be suitable for final maturation. In nature, water temperature determines the length of the spawning season. Although salinity does not affect gametogenesis, no spawning occurs below a salinity of 8ppt. The spawning cycle is related to the lunar cycle in nature but there is no relation under culture conditions. Maturation and spawning are not inhibited in a 25 m² tank.

INTRODUCTION

Timing and success of teleost reproduction are directly or indirectly controlled by environmental factors such as temperature and photoperiod. The importance of these factors to reproduction varies from species to species. Several papers have reviewed the related research (de Vlaming, 1974, 1975; Billard and Breton, 1979; Crim, 1982; Wootton, 1982; Lam, 1983). Environmental conditions influence gonadal activity in fish through the mediation of the hypothalamo-pituitary-gonad axis which results in endocrine fluctuation (Crim, 1982). Environmental factors served as proximate factors or as

~~zeitgebers~~ to reproduction of fish (Lam, 1983). Environmental cues for the reproduction of fish vary according to the spawning season (summer spawning or winter spawning) and different geographic locations (tropical, subtropical, or temperate regions).

Milkfish has a very wide geographic distribution from 40°E to about 100°W and 30-40°N to 30-40°S (Schuster, 1960), which encompasses tropical and subtropical regions. Winans (1980) indicated the existence of genetically distinct populations of milkfish in waters off Hawaii, the Philippines and several Pacific Islands. The environmental factors for maturation of milkfish may differ among these populations. This information suggests that the environmental conditions for reproduction of milkfish may be more complex than other fish species.

Maturation and spawning of milkfish have not been controlled. Although maturation and spawning in captivity have occasionally been achieved in the Philippines (Lacanilao and Marte, 1980), Taiwan (Liao and Chen, 1984; Lin, 1984), and Hawaii (Lee et al., unpublished), the most suitable conditions for reproduction are still undefined. There has been no systematic study of this problem.

In this paper, the environmental factors such as temperature, photoperiod, salinity, lunar cycle, space and stress, will be discussed in relation to their possible effects on reproduction in milkfish.

TEMPERATURE

Water temperature is one of the most important environmental factors affecting the reproductive cycle. Increasing water temperature induces early maturation of summer spawning species such as *Sillago sibama* (Lee, 1985). On the other hand, maturation of winter spawning species, such as mullet *Mugil cephalus*, is triggered by a decrease in water temperature (Kuo and Nash, 1975).

The effect of water temperature on milkfish reproduction is not known. However, some correlation may be proposed from the milkfish fry season. Liao et al. (1979), indicated the fry caught along the coast were about 10-15 days old. Spawning season was therefore two weeks earlier than fry season. Wainwright (1982) concluded that the fry season was initiated and ended by the rising and falling of sea surface temperatures, respectively. This implies that gametogenesis is initiated by the stimulation of cooler water.

However, milkfish matured in enclosed systems such as sea cages (Lacanilao and Marte, 1980), outdoor tanks (Liao and Chen, 1979; Tseng and Hsiao, 1979), indoor tanks (Lee and Weber, 1983) or ponds (Lim, 1984). There were no sudden changes in water temperature under these environments. The annual temperature ranges in these studies were 25° - 31°C in the cage, 21.4° - 30.7°C in the outdoor tank and around 26°C in the indoor tank. The annual temperature range under pond conditions was not

available. Experiments conducted on three-year-old immature milkfish in tanks showed no difference in response to hormone injection while the fish were kept at 28° - 32°C and at 23° - 26°C for 6 weeks (Lacarilao et al, 1984). Spermatogenesis was not initiated under either condition. Thus, the possibility that milkfish will not start gametogenesis unless they experience lower water temperature needs to be verified.

The length of the spawning season can be determined by the length of the fry season. Wainwright (1982) found a correlation between fry season and the average annual surface sea water temperature (Fig. 1). The area with a higher average surface water temperature has a longer fry season and a longer spawning season. The relationship between water temperature and the end of the spawning season is unclear, although a slight temperature decrease was observed at the end of spawning season in various locations. Fry season, which reflected the spawning season, faded away as water temperature decreased (Wainwright, 1982). Kumagai (1984) compiled available data and indicated that the milkfish fry season lengthens toward the South and is shorter in the North (Fig. 2). This information implies the length of spawning season is a consequence of water temperature.

Available information indicates that the minimum water temperature during the spawning season of milkfish is about 25°C (Liao and Chen, 1979; Wainwright, 1981,1982; Lin, 1984; Lee et al., unpublished). However, recent observations in Hawaii showed

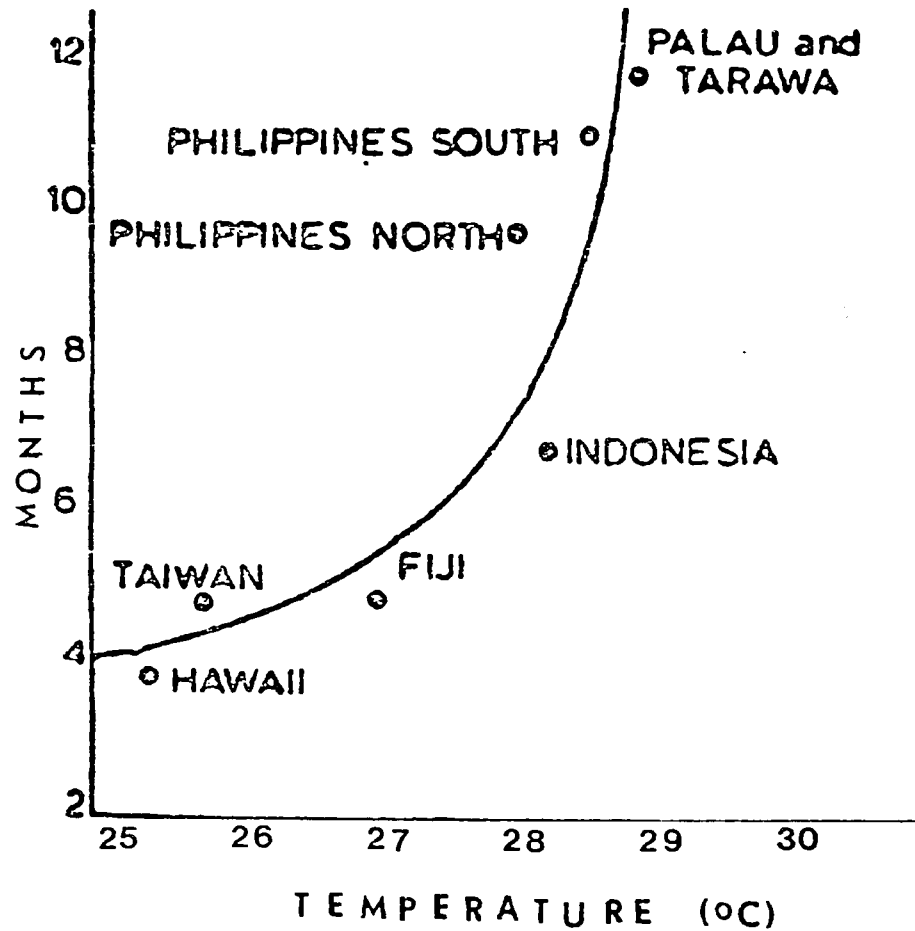


Fig. 1. Correlation between mean annual surface seawater temperature and length of fry season in various countries (after Wainwright, 1982).

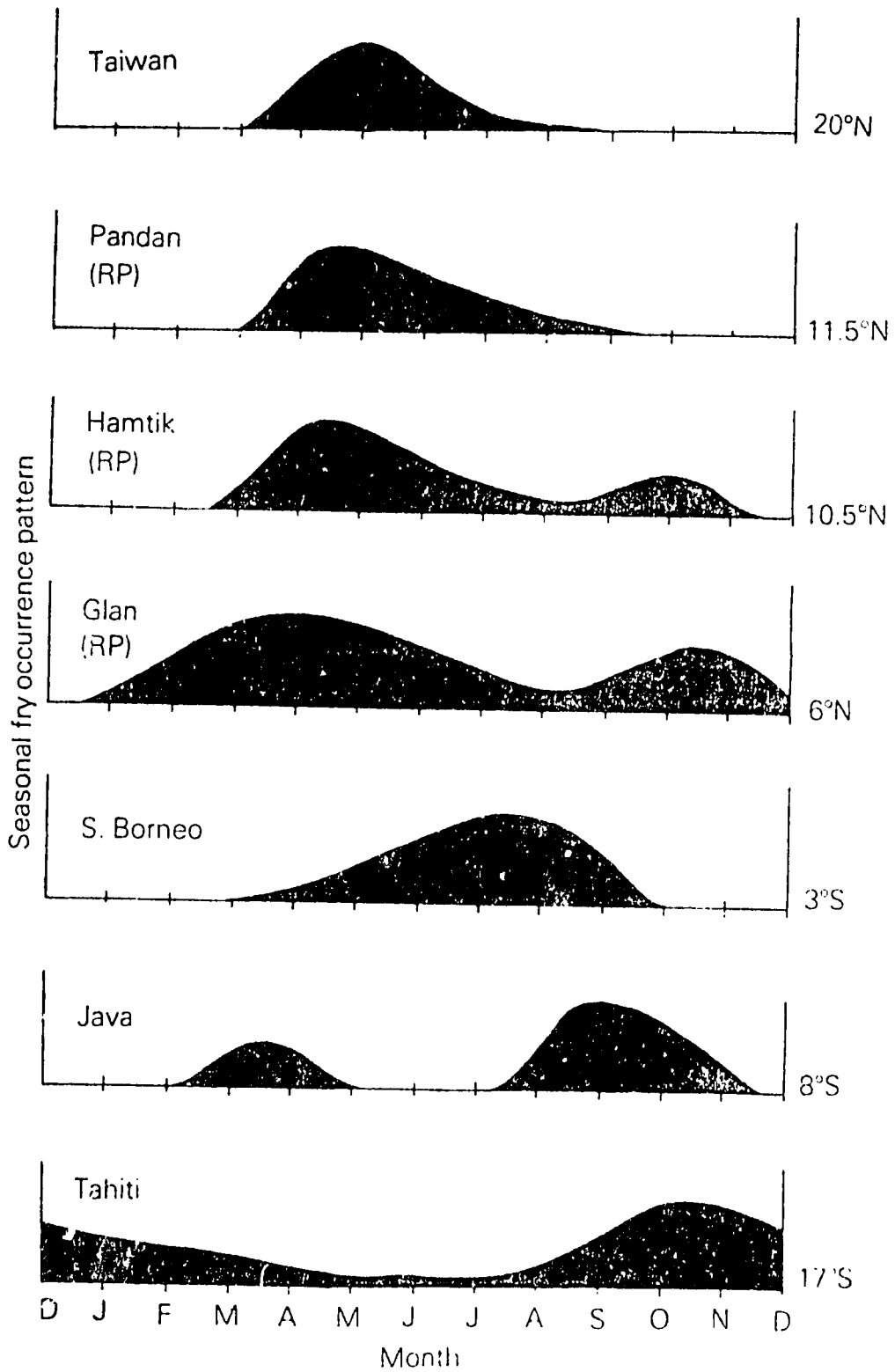


Fig. 2 Seasonal fry occurrence patterns at different latitudes (after Kumagai 1984)

milkfish with hormone implantations matured in a 6 meter diameter tank with water temperatures around 22°C (Lee et al., unpublished). The minimum water temperatures for final maturation may be changed by the condition of the fish.

PHOTOPERIOD

Photoperiod is another important environmental factor in the reproduction of fish. In general, winter spawning species need short light periods and summer spawning species need long light periods for initiating gonadal development. In milkfish, Lee and Weber (1983) tried to initiate the maturation by monitoring the photoperiod from 6L:18D to 12L:12D, and to 18L:6D. The fish were maintained under each photoperiod regime for three months, with the 18L:6D regime coinciding with the natural spawning season. They found 86% of the fish in the tank matured after three months under 18L:6D conditions. However, there was no control group in this experiment to verify that maturation success was induced exclusively by the photoperiod change.

The only light source in the above experiment was eight, six-foot fluorescent lights. Light intensity was 4.2×10^{14} to 1.1×10^{15} quanta/sec/cm² which is lower than natural sunlight. High natural light intensity did not inhibit the maturation and spawning of milkfish in sea cages (Lacanilao and Marte, 1980). Natural spawning also occurred in ponds where light intensity was decreased by phytoplankton. In Hawaii, maturation of milkfish occurred in outdoor tanks with diatom bloom (unpublished data).

Therefore, milkfish matured under both high and low light intensities. This suggests that light intensity may not be critical to the process of milkfish maturation. Kumagai et al. (1978) indicated that a change in the light intensity stimulated the maturation of milkfish.

The spawning of milkfish usually took place at night (Kumagai, 1981; Lin, 1984). Daylight, however, did not block spawning activity (Lin, 1984; Lee et al., unpublished)

SALINITY

Salinity affects the reproductive cycle of euryhaline fish such as grey mullet (*Mugil cephalus*). Although the mullet matured in a freshwater lake, natural spawning did not occur (Yashouv, 1969). Maturation of milkfish was reported under an extreme salinity range (Kuo et al., 1979; Crear, 1980). Mature females were found in the hypersaline pond (125 ppt) on Christmas Island (Crear, 1980). Natural spawning was also reported in this hypersaline pond. Kuo et al. (1979), on the other hand, obtained mature females from brackishwater (7-12 ppt in salinity) for induced spawning. In the Philippines, maturing milkfish migrated down to the sea from a freshwater lake (SEAFDEC, personal communication). No natural spawning was reported in brackishwater or freshwater. Mature males were found in both biota. Therefore, gametogenesis of milkfish occurs in a very wide range of salinities, from freshwater to 125 ppt. However, final maturation and spawning have not been observed in lower

salinities.

Artificial fertilization was achieved at a salinity of 5 ppt, while trials in salinities lower than 5 ppt. have not been attempted (Lee and Kuo, unpublished data) (Table 1). Lin (1984) indicated that fertilized eggs did not hatch at a salinity under 16.2 ppt and hatched larvae died at 16.2 ppt.

LUNAR CYCLE

Lunar involvement in spermatogenesis and vitellogenesis was not reported. However, the lunar cycle regulated the spawning rhythm of many tropical or subtropical marine fish (Johannes, 1978; Gibson, 1978; Schwassmann, 1980). Most fish species spawned on or around the new or full moon (Johannes, 1978). Milkfish were estimated to spawn during the first and last quarter moon, according to the appearance of fry in the wild during the full and new moons (Kumagai, 1981). Crear (1980) also indicated the correspondence of spawning activity with the lunar cycle on Christmas Island. Under culture conditions, spawning did not take place in correspondence with the lunar cycle (Lin, 1984).

OTHER FACTORS

There are many other factors that regulate reproductive activity in fish. Among them, space always is considered in the maturation of milkfish. No experiments have been conducted to demonstrate the influence of space on reproduction. For economic reasons, it is important to find out the smallest suitable

Table 1. Fertilization and egg size of milkfish at different salinities.

Salinity	5‰	10‰	15‰	20‰	25‰	30‰	35‰	40‰*	40‰**	35‰***
Fertilization Rate (%)	41.7	60.0	61.3	58.5	58.0	52.0	48.0	92.0	40.0	53.6
Egg Size (mm)	1.26 ±1.32	1.18 ±0.76	1.15 ±0.40	1.14 ±0.32	1.12 ±0.32	1.11 ±0.24	1.11 ±0.28	1.12 ±0.24	1.10 ±0.28	1.11 ±0.36

* Floating eggs only

** Sunk eggs only

*** Preserved sperm was used

facility for maturation of milkfish. Attempts to mature milkfish were carried out in facilities of various sizes (Liao and Chen, 1979; Lacanilao and Marte, 1980; Lee and Weber, 1983; Lin, 1984). The smallest maturation facility used was 25 M² and 1 m in depth at a stocking density of 10 fish (Lee and Weber, 1983). Recently, natural spawning of milkfish was observed in this tank (Lee et al., unpublished). The shape of the holding tank (round or rectangular) did not interfere with maturation or spawning of milkfish (Lacanilao and Marte, 1980; Lee et al., unpublished).

Stress is another important environmental factor in the maturation of milkfish. An efficient method of preventing stress has yet to be found. Handling stress inhibits the spermiation of male milkfish. Juario et al. (1980) used Durandron Forte "250" to overcome handling stress on male milkfish. Kuo et al., (1979) injected mature females with carp pituitary homogenate to prevent oocyte atresia induced by handling stress. Stress from salinity changes reduced the potency of exogenous hormones in milkfish (Kuo et al., 1979).

CONCLUSION

Based on current information (Table 2), the influences of temperature and photoperiod on the reproduction of milkfish are unclear. However, long daylight regimes and warm water temperatures (25^o C) seem to be suitable for milkfish maturation. In nature, the average water temperature affects the length of the spawning season. Salinity is not critical for gametogenesis.

Table 2. Summary of Important Factors at Various Milkfish Maturation Sites (Modified from Liao 1984)

Factors	Tank			Pond			Lagoon (Christmas Island)			Floating Net Cage Philippines	
	Taiwan	Oceanic Institute (Hawaii)	Oceanic Institute (Hawaii)	Kauai	Kona	Oceanic Institute (Hawaii)	Taiwan	Isles	Pulican		Azur
Age (yr)	M-4+, F-5+	6-7	7-8	?	?	?	10-11	4-5(?)	4-5(?)	6	5
Diameter or area	8.5-12 m round	5.2 X 4.8 m rectangular	6.5 X 6.7 m rectangular	10 ha	1.4 ha	0.05 ha	30 X 25 m rectangular	≈ 4 X 1 m/le	≈ 0.5 X 1.0 m/le	≈ 0.5 X 1.0 m/le	9 or 10 m
Depth (m)	1-2	1-3	1-2	?	1-2	1	1.3-1.5	≈ 3	≈ 3	≈ 3	3
Temperature (°C)	21-31 (annual)	25-27	25-26	24-30	24-30	24-30	25-31	27-32	27-32	27-32	25-32
Salinity (ppt)	14-30	32-34	32-34	38-42	8-12	32	29-32	102-130	60-76	80-100	25-38
Photoperiod (h light)	natural	artificial*	natural	natural (10.5-13.5)	natural (10.5-13.5)	natural (10.5-13.5)	?	?	?	11-14	
Food (% protein)	37-46	Ralston Catfish Chow (32.%)	Ralston Catfish Chow (32.%)	?	?	Ralston Catfish Chow (32.%)	rice bran, wheat flour, soybean meal eel food	benthic net # + <i>Actinula</i> saline	benthic net #	benthic net #	42
Feeding Rate	once to satiation	ad libitum	ad libitum	natural productivity	natural productivity	ad libitum	5% body wt.	natural productivity	natural	natural	2% body wt.
Stocking Density	20-30/tank	7-11/tank	14/tank	?	700/pond	12/pond (+100-200 mullet)	30/pond	low	high	high?	80-100/cage (0.5 kg/m ³)
Phytoplank- ton Bloom	Yes	NO	Yes	?	Yes	Yes	Yes	No	Yes	?	No
Flushing Rate	flow- through	≈ 4.9 gal/min	slow	low	moderate (upwelling)	moderate	adjusted as needed	nature	nature	nature	nature
Matured or Spawmed	matured	spontaneously spawmed	spontaneously spawmed	artificially spawmed	artificially spawmed	artificially spawmed	naturally spawmed	naturally spawmed	naturally spawmed	?	naturally spawmed
Reference	Liao & Chen 1979, 1984	Lee & Weber 1984, Lee	Loo et al. 1985	Nash & Kuo 1976, Kuo et al 1979, Kuo 1982 and personal communication			Liu, 1984	Kuo 1982, Vanstone 1982, Greer 1980 and personal communication			Lacanileo & Marte 1980, Murta et al 1984 & pers. comm.

* Fish subjected to 6, 12, and 18 h of light at different times.

Composed of halophilic bacteria, blue-green algae, diatoms, and fungi which cover the shell deposits.

Milkfish completed maturation and spawning in normal seawater but did not spawn in freshwater. The optimal salinity range for completion of normal reproduction is still unknown.

Stress on broodstock should be reduced. The lunar cycle influenced the spawning cycle in the wild, but not under culture conditions.

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NUTRITIONAL FACTORS IN FISH REPRODUCTION

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ABSTRACT

Although more than 300 species of fish have been cultured in various countries, the seeds of these fish have depended mostly on the larvae from wild fish. To establish the mass production of seed fish, it is necessary to obtain many good eggs and healthy larval fish.

It is likely that the nutritional quality of broodstock diets may affect the ovarian maturation, spawning, and egg qualities. Only a little information is available in this field, however. This paper presents the effects of proteins, lipids, minerals, vitamins, and pigments in diets on the quantities and qualities of eggs.

PROTEINS AND AMINO ACIDS

Takeuchi et al. (1981b), have studied the effects of low protein-high calorie diets (crude protein, 35%; 390 kcal/100 g) for a rainbow trout (3.5 g) on weight gain, feed conversion efficiency, survival rate, and egg quality. They have reared the rainbow trout with the test diets and a commercial diet containing 43-47% proteins for three years until the fish grew into adults, and have found that the test diets gave a similar nutritive value to the commercial diet in terms of the above nutritional parameters.

Watanabe et al. (1984a) also have examined the effects of broodstock diet on the egg qualities of red sea bream, and have

revealed that the group fed the low protein (36% crude protein) diet gave few floating eggs and few normal larvae (4% of hatched larvae) as compared to the group fed the control diet (51% crude protein); in the latter group 62% of the hatched larvae were normal in appearance (Table 1).

Kanazawa et al. (1985) have investigated the adequate protein sources of microparticulate diets for rearing the larval Ayu. They have made diets using feather meal, krill meal, white fish meal, bonito egg, yeast, soybean meal, etc., to give the essential amino acid (EAA) pattern similar to that of Ayu. The larval Ayu, fed several diets with the above EAA pattern, grew well as compared with the control group that was fed the live food during the long feeding period. The results presume that such a diet may be nutritionally adequate for the adult Ayu.

LIPIDS

(i) Essential Fatty Acids

Fish are incapable of synthesizing linoleic (18:2 ω 6), linolenic (18:3 ω 3), arachidonic (20:4 ω 6), icosapentaenoic (20:5 ω 3), and docosahexaenoic (22:6 ω 3) from lower units and require some of these highly unsaturated fatty acids (HUFA) as essential fatty acids (EFA). Kanazawa (1984) and Teshima (1985) have classified the EFA requirements of fish into Tilapia, rainbow trout, and red sea bream types (Table 2). Recently, Kanazawa and Alava (unpublished) have demonstrated that the milkfish Chanos chanos grew best on the diet containing 20:4 ω 6,

Table 1. Effects of Broodstock Diets on the Spawning and Egg Quality of Red Sea Bream. (After Watanabe et al., 1984a)

Diet	Control (crude protein 51%)	Low-protein (crude protein 36%)	Essential fatty acid deficient	Without supplemental phosphorus
Egg:				
Egg produced/fish ($\times 10^4$)	100.5	72.7	116.5	84.1
Abnormal egg (%)	30.7	70.7	93.7	67.9
Average number of oil globules	1.7	3.5	6.2	3.1
Hatched larvae:				
Rate of hatching (%)	69.4	23.6	0.9	26.3
Deformity (%)	23.3	84.1	--	75.5
Normal larvae (%)	62.4	3.8	--	6.2

Table 2. Essential Fatty Acid Efficiency of Fatty Acids on the Growth of Fish (after Kanazawa 1984 and Teshima 1985)

Species	18:2 ω 6	18:3 ω 3	ω 3-HUFA*
Rainbow trout	+	+++	+++
Chinook salmon	+	+++	+++
Coho salmon	+	+++	+++
Chum salmon	++	++	+++
Carp**	++	++	+++
Eel**	++	++	+++
Channel catfish	++	++	+++
<i>Lilapia zilli</i>	+++	+	+
<i>Lilapia nilotica</i>	+++	+	+
Plaice	--	--	+++
Turbot	--	+	+++
Starry flounder	--	--	+++
Red sea bream	--	--	+++
Black sea bream	--	--	+++
Opaleye	--	--	+++
Yellow tail	--	--	+++
Puffer fish	+	+	+++

* 20:5 ω 3, 22: 6 ω 3, 20:5 ω 3 + 22:6 ω 3, or a mixture of ω 3-HUFA.

** The addition of both 18:2 ω 6 and 18:3 ω 3 gives a higher efficiency than the single dose of each acid.

suggesting the EFA requirement similar to that in *Tilapia* and the insufficient bioconversion of 18:2 ω 6 to 20:4 ω 6. Since HUFA, such as 20:4 ω 6, affect growth and survival of the milkfish, the quality of eggs may be affected by the dietary status of mother fish and whether they were receiving ω 6-series of HUFA.

Watanabe et al. (1984d) have studied the effects of short period diets on spawning by rearing the mother rainbow trout (two years old) with an EFA-deficient diet for three months. The group fed the EFA-deficient diet had poor results in the number of eggs produced, the rate of eyed eggs, and hatching rate. The results were improved with the addition of 18:2 ω 6 to the diet. With the long-term feeding experiment over two generations, Yu et al. (1979) have demonstrated that the rainbow trout requires only 18:3 ω 3 as EFA. Watanabe et al. (1984) have revealed with a six-month feeding trial of the parent sea bream that the EFA-deficient diets gave a few buoyant eggs and many sunk eggs (about 75% or more). The feeding of corn oil as a substitute for squid liver oil in a basal diet resulted in a marked deterioration of egg qualities, and a few normal larvae (1.2% for corn oil diet, 53% for the basal diet).

Tropical fish *Siganus guttatus* laid eggs for more than five months if they received the diet with pollack liver oil (PLO), but did so for only two months if they received the diets without PLO (Hara, pers. comm.). The data suggest the inclusion of HUFA in diets is favorable to longer reproduction activity of *S.*

guttatus.

PHOSPHOLIPIDS

Generally, fish eggs contain a large amount of phospholipids. Kanazawa (1983) and Kanazawa et al. (1981, 1983a, b) have demonstrated that the larval Ayu, red sea bream, knife jaw, etc., necessitate dietary sources of phospholipids for their growth and survival (Fig. 1). Results also suggest that dietary sources of phospholipids have certain effects on both the larvae and parent fish. Watanabe et al. (1984c) have shown that the phospholipid and astaxanthin fractions of a krill meal were effective in improving the qualities of eggs.

MINERALS

The long-term feeding trials of rainbow trout also have revealed that the elimination of trace metals resulted in unusual spawning and inferior egg qualities in addition to poor growth and low feed conversion efficiency. In the case of the red sea bream, the production rate of buoyant eggs was lowered when receiving the phosphorus-deficient diet (Watanabe et al., 1984a).

VITAMINS

Vitamin E is the most important among the essential nutrients in relation to the development of reproductive organs. It also plays a role in relation to spawning and egg qualities, as also was observed in higher animals.

Takeuchi et al. (1981a) have reared the parent Ayu with the diets containing different levels of vitamin E for three months

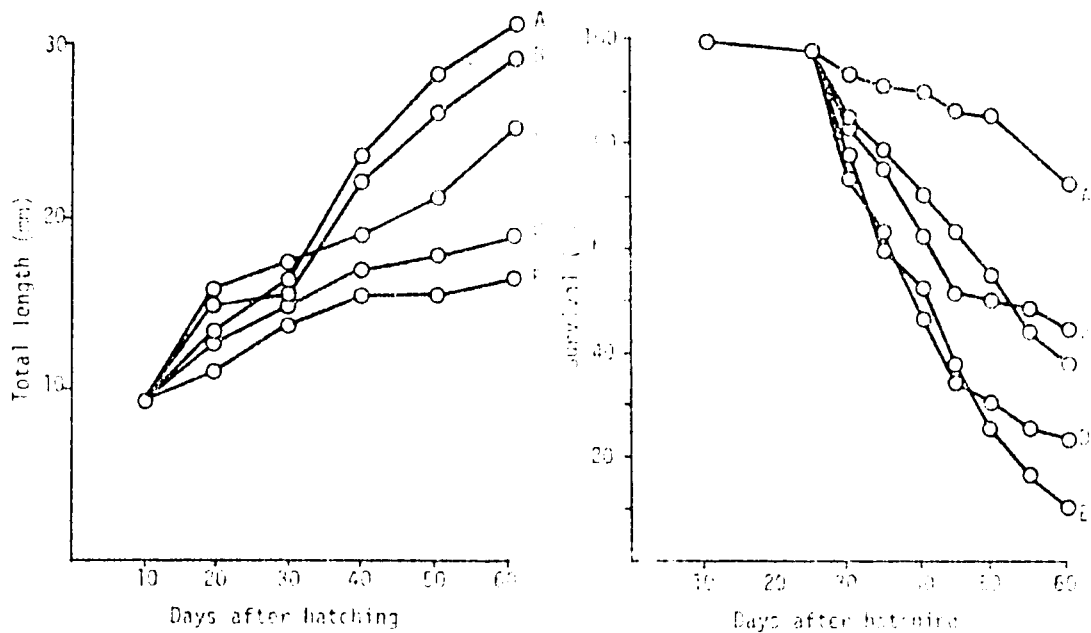


Fig. 1. Effect of Soybean Lecithin Levels on the Growth and Survival of the 10-day Larvae of Ayu

- A: Rotifer + Artemia nauplii
- B: 5% Soybean Lecithin
- C: 3% Soybean lecithin
- D: 1% Soybean lecithin
- E: 0% Soybean lecithin

(12) Kamazawa et al.)

before spawning and have estimated that the fish requires 3.4 mg of vitamin E in 100 g diets in terms of hatching rate and the survival of hatched larvae (Table 3). As for the carp, the 17-month feeding trials have shown that vitamin E deficiency in diets resulted in the retardation of ovarian development (Watanabe and Takashima, 1977). The gonad weight and gonadosomatic index of carp were 6.3 g and 3.3% for the vitamin E-deficient groups, and 68.1 g and 14.1% for the control group. The vitamin E-deficient and control groups were 6.3 g and 3.3% for the former, and 68.1 g and 14.1% for the latter.

PIGMENTS

Good egg qualities from the red sea bream have been obtained when the parent fish were fed the diets containing β -carotene + canthaxanthin, krill oil, or frozen supplements for two months before spawning (Watanabe, et al., 1984b)

In summary, egg quality is likely to be improved by changing the broodstock diet in the formula of proteins, lipids, minerals, vitamins, and pigments

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Table 3. Hatchability and Mortality of Fry Obtained from Ayu Fish Fed on the Diet Containing Different Levels of Vitamin E (after Takeuchi et al., 1981a).

Dietary vitamin E (mg/100g)	Eyed egg (%)	Hatchability* (%)	Mortality of fry (%)
1.04	46.6	16.4	57.4
3.42	59.0	46.3	27.9
10.57	61.3	36.7	39.1
39.14	63.2	39.1	22.3

* Calculated on the basis of fertilized eggs.

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STATUS OF MARINE LARVAL CULTURE IN JAPAN

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ABSTRACT

The technology for mass larval rearing of marine fishes has developed markedly in the last 15 years. 6.3 million juveniles of red sea bream *Pagrus major* and porgy *Acanthopagrus sblegeli* (12.1-16.0 mm total length - TL) were produced in a hatchery within a three-month period. The introduction and culture of the rotifer *Brachionus plicatilis* as a food organism is one of the most important factors in the rapid and successful development of this technology. This production of juveniles was supported by the production of 1.2×10^{12} rotifers or about 2.5 tons wet weight. Development of a spawn-taking method by producing natural spawns in tanks is another important factor. A female red sea bream of 1 kg spawned 2 million eggs in 1.5 months. Special attention to and improvement in environmental management for larval rearing and mass culture of rotifers promoted the development of the fry production technique. Therefore, large scale larval rearing (100-ton tank with harvest of 1 million juveniles of 10 mm in TL) and mass culture of rotifers (40-ton tank with harvest of 152.4×10^8 rotifers = ca. 45 kg for 18 days) has become possible. Several papers (Fujita, 1979; Uno and Hayashi, 1980; Smith and Hataya, 1982; Kitajima, 1983; Hirano, 1984; Kuronuma and Fukusho, 1984) have discussed the present status of technology for the mass seedlings in Japan.

This paper will discuss the technological development of mass larval rearing of red sea bream, one of the most successful species in Japan.

I. Introduction of a rotifer as the initial feed for larvae

The rotifer, *Brachionus plicatilis*, was originally a noxious zooplankton in eel culture ponds. It was isolated as a food organism, cultured and utilized for the feeding of finfish and crustacean larvae since 1965 (Hirata, 1979; Fukusho, 1983).

Introduction of the rotifer is considered to be a key factor in the development of mass production of marine fish fry.

Physiology, ecology, and the actual technique for mass culture of the rotifer are referred to in several papers (Kuronuma and Fukusho, 1984; Hirata, 1979; Fukusho, 1983).

II. Spawn-taking by natural spawning in tanks

During the initial stage, from 1965 to 1970, fertilized eggs were obtained by induced spawning of wild fish by hormonal injection. However, success was limited by poor egg quality.

In 1968, natural spawning of spawners in aquarium tanks was observed. Thereafter, natural spawning was attempted at many fish farming centers and hatcheries (Fushimi, 1984).

Three- or four-year-old fish (1-1.5 kg in body weight, 35-42 cm in length) are selected as spawners (Kitajima, 1978). With a stocking density of 1-1.5 kg of males and females per ton in tanks, the average number of eggs produced per female throughout the season was 2 to 3.5 million (Kitajima, 1978). The average number per female per day was 50,000 - 100,000. The total number of eggs was estimated from the wet weight of eggs collected; 1800 eggs weighing 1 gr.

The spawning tank is usually made of concrete, either round or square, 1-2 m in depth with a capacity of 30-100 tons. The shape is round or square and 1-2 m in depth. Spawned eggs are collected with a fine mesh net on the overflow outlet of the tank. Broodstock are usually held in cage nets, hanging from

rafts. Spawners are transferred to the spawning tanks when spawning behavior is observed.

Spawning season in tanks differs slightly among localities, and, in west Japan, runs from April to the beginning of June.

In recent years, gonad maturation has been accelerated by raising the water temperature. In this method, broodstock are cultured in concrete tanks with a recirculation system. This has produced from one to three early spawnings. The energy source for heating is usually oil and sometimes, solar.

Feeds for red sea bream broodstock have been investigated and the results of several experiments will be discussed in Dr. Kanazawa's paper in these proceedings.

III. Nutritional value of food organisms and improvements

Marine Chlorella spp. are taxonomically confused making identification of the species quite difficult. However, it is an essential feed for the mass culture of rotifers. Baker's yeast has been introduced as a substitute for Chlorella since Chlorella culture depends on weather conditions. Also, the liquid form is troublesome to feed to the rotifer (Hirata, 1979; Fukusho, 1983).

The food value of rotifers cultured with baker's yeast (BY-rotifer) was lower than that cultured with Chlorella (C-rotifer). Mass mortality was observed in the larvae (6 mm in TL, or 16-20 days after hatching) fed with BY-rotifer alone (Kitajima, 1978, 1983).

Chemical analysis revealed little difference in the amino

acid and mineral composition between BY- and C-rotifer (Kitajima, 1978, 1983). However, BY-rotifers contained fewer essential fatty acids for larvae of red sea bream, 23 highly unsaturated fatty acids (HUFA) such as eicosapentaenoic acid (20:5 n-3) and docosahexaenoic acid (22:6 n-3) (Kitajima, 1978; Watanabe, et al., 1983). The percentage of eicosapentaenoic acid in the fatty acids of C-rotifer was as high as 25% (Kitajima, 1978; Watanabe et al., 1983). The difference in the food value of both rotifers depended on the percentage of HUFA (Kitajima, 1978; Watanabe et al., 1983). Wild copepods, water fleas, and other live food organisms were analyzed for food value (Watanabe et al., 1983).

Based on this information, it has become common practice to enrich or improve the dietary value of rotifers by culturing with *Chlorella* for 12-24 hours before introduction into the larval rearing tanks. Furthermore, baker's yeast which is fortified with HUFA (as ω -yeast) has been developed and is commonly used at mass seedlings facilities (Imada, et al., 1979).

The rotifer consumption per larva was investigated and a reasonable food supply regime for larval rearing was established. The relation between the number (F) of rotifer consumed by one larva and its total length (L) in mm is represented by the following equation (Kitajima, 1978, 1983):

$$F = 0.3927 L^{3.676}$$

The number of rotifers required to raise 2 million larvae to early juvenile stage (10 mm in TL) is calculated on the basis of

acid and mineral composition between BY- and C-rotifer (Kitajima, 1978, 1983). However, BY-rotifers contained fewer essential fatty acids for larvae of red sea bream, 43 highly unsaturated fatty acids (HUFA) such as eicosapentaenoic acid (20:5 n-3) and docosahexaenoic acid (22:6 n-3) (Kitajima, 1978; Watanabe, et al., 1983). The percentage of eicosapentaenoic acid in the fatty acids of C-rotifer was as high as 25% (Kitajima, 1978; Watanabe et al., 1983). The difference in the food value of both rotifers depended on the percentage of HUFA (Kitajima, 1978; Watanabe et al., 1983). Wild copepods, water fleas, and other live food organisms were analyzed for food value (Watanabe et al., 1983).

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present data (Kitajima, 1978) and totals 57 billion with the maximum amount per day up to 8.4 billion.

IV. Occurrence of the lordotic deformity and a short history of solutions attempted

Lordosis, frequently observed (30-50% at the maximum) in hatchery-reared red sea bream and other species, is the most serious among several deformities including brachiospondylie, incomplete development of opercle bones and pug head (Kitajima, 1978). The lordosis with V-shaped vertebral column was found in fish with uninflated swim bladders (Kitajima, 1978). To solve the problem of lordosis, studies have been conducted in the areas of nutrition, environmental improvement and genetics.

Eggs from a hatchery where lordosis was prevalent were transferred to a second hatchery with limited cases. Newly hatched larvae were reared at both hatcheries. Deformed individuals were found in the first hatchery at the rate of 14.1-35.4%, varying among fish from different tanks with eggs from the same parent, but no cases appeared in the second hatchery. Thus, lordosis might not be the result of genetic differences but of undefined factors in effect before hatching. Some of these factors might include the physiological condition of eggs, and of breeders (Taniguchi et al., 1984). Lordosis was very common in hatcheries in Japan.

ω 3 highly unsaturated fatty acids (ω 3 HUFA), such as 20:5 ω 3, are important for the survival and growth of marine

fish larvae. Some rearing experiments suggest that the development of the swim bladder is affected by the quality of initial feeds. The groups fed on BY-rotifers tended to have a higher percentage of juveniles with uninflated swim bladders. By comparison, the groups that were fed C-rotifers or *w*-yeast had a lower percentage of abnormal swim bladders. Thus, nutritional improvement may reduce deformities (Fujita and Kitajima, 1978). However, the percentage of lordosis varies among the different fish groups with similar feed.

The influence of agricultural medicines, other poisonous substances and food organisms such as *Artemia salina*, were also considered but detailed investigations have not been conducted. The presence of phosphorus in sea water was considered an important factor in bone formation. Larvae were reared in various concentrations of phosphorus, but methodical results were not obtained.

Studies were made, in 1-ton tanks, to determine the optimum aeration level for larval rearing. Lordosis was scarce in the fish groups reared at 50-100ml/minute, while deformity appeared at a higher frequency with non-aeration or at more than 500 ml/minute. Moderate and uniform currents in rearing water led to a lower percentage of lordosis and a higher survival rate even without aeration, while the survival rate and percentage of fish with inflated swim bladders were low in tanks with non-aeration and no current. Thus, it was found that a moderate current,

suitable aeration, and rippling with weak showering of water at the mouth-opening stage are effective in reducing lordosis (Iseda, 1982). Furthermore, swim bladders were inflated for a short period at 3.5 mm in TL (five to six days after hatching in temperatures of 18-22°C). Accordingly, it was thought that abnormally-developed swim bladders were caused by a failure of air gulping at the surface, in the rearing tank (Iseda, 1982; Kitajima, et al., 1981).

Precise rearing experiments were conducted to examine this hypothesis. One tank was sealed with a layer of liquid paraffin and the other had an open surface as a control (Kitajima et al., 1981). More than 90% of the larvae in the control group had normal swim bladders on the seventh day after the initial feeding (4.2 mm in TL) but in the sealed tank, none was inflated (Kitajima et al., 1981). Thus, fish larvae must gulp air at the surface for initial swim bladder inflation (Kitajima et al., 1981). This mechanism is common in other species, such as porgy (*A. shlegeli*) and silver bream (*Sparus sarba*).

With the adaptation of improved environmental conditions discovered in these studies, lordosis has scarcely been found in hatchery-reared red sea bream. These conditions include: 1) supplying moderate current and aeration; 2) cleaning the surface to allow air to penetrate into the rearing water; 3) supplying water with moderate showering; 4) introducing newly-hatched larvae at a fairly low density; 5) selecting the individuals with

well-developed swim bladders; and 6) feeding rotifers with a high nutritional value.

V. Mechanization and automation in rearing system

There are several facilities which produce more than 1 million juveniles annually but the process is labor intensive. Mechanization of the rearing system is required to save manpower. An example of mechnization is automatic bottom cleaners classified into two types: sweeper and suction (Fushimi, 1984). The sweeper is used in round tanks, rotating on a shaft at the bottom and collecting sediments into a central drainage pipe covered with a fine mesh net. The suction type is used in square tanks and moves along the wide tank edge suctioning the debris and dead fish on the bottom and depositing it into a drainage canal.

An automatic feeder for micro formula feed is another labor-saving device. The Hiroshima Prefectural Fish Farming Center has introduced the feeders (Fushimi, 1984). Another feeder for rotifers has been developed and is employed at the Kagoshima Prefectural Fish Farming Center, where researchers designed a full-scale automatic rearing system (Fujita et al., 1982). The characteristics of this system follow (Fujita et al., 1982):

- 1) Raising juveniles to 30 mm TL in tanks
- 2) Producing 2 million juveniles
- 3) Feeding a regime of rotifer->brine shrimp->micro formula feed->minced meat.

4) Culturing rotifers with Chlorella

5) Arranging pipe lines to Chlorella tanks, rotifer tanks, and larval rearing tanks to supply feed automatically.

6) Computerizing the monitoring and recording of environmental conditions in various tanks to oversee daily routines for rearing.

7) Collating data for designing future production systems.

VI. Development and introduction of micro formula feed

Facilities for mass fry production have always been designed on the basis of rearing larvae by rotifers. A fairly large amount of tank capacity is shared for rotifer and Chlorella culture. Furthermore, the preparation of live food organisms is time consuming. Therefore, the development and introduction of micro formula feeds for larvae and juveniles could lead to a "revolution" of current larval rearing techniques. Larval rearing tanks must be modified for formula feeds feeding, and tanks for Chlorella and rotifers can be converted to rearing tanks for juveniles. Automatic rearing systems will be introduced, significantly decreasing the required manpower.

Micro formula feeds have been used experimentally in the initial period of larval rearing saving 20-30% of the rotifers required by the control group. Higher quality micro formula feeds have been developed in recent years using the new technology for preparing minute granules. Micro formula feed products are commercially available and are being used in some hatcheries.

VII. Introduction of foreign strains and breeding

Red sea bream has recently been introduced to Japan from Hong Kong and Korea, and is being cultured by aquaculturists. Taxonomically, they are the same species, but information on their genetic difference remains unknown. They exhibit the desirable red coloration and grow faster than the Japanese strain.

In red sea bream culture, wild species are used for aquaculture in virtually all cases. Experiments are being conducted on the hybridization between different species to create races or strains that grow more quickly and are more resistant to disease. Selection is one of the most successful methods in breeding of red sea bream. Selected populations (four to five generations) show nearly a 40% higher growth rate compared to wild populations.

Recently, biotechnology was applied to the study of the creation of triploids and all female individuals. Some researchers suggest that genetic management is quite important to maintaining the stock of the wild population, especially the fish ranching projects which liberate the artificially reared juveniles in coastal areas (Taniguchi and Tashima, 1978). Genetic degeneration resulting from the inbreeding of the artificial population, derived from few broods, may be happening in the sea (Taniguchi and Tashima, 1978).

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POPULATION STRUCTURE OF THE MILKFISH CHANOS CHANOS,
PAST AND PROPOSED ANALYSIS

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ABSTRACT

The most widely accepted definition of a "species" for sexually reproducing organisms is that of individuals that can and do interbreed among themselves. In turn, this group of individuals remain reproductively isolated from other such groups. The term interbreeding implies that there is genetic uniformity within the taxon. However, organisms that exhibit a large geographic distribution have obvious geographic, environmental, and temporal barriers to gene flow. In fact, most organisms do not behave as a single panmictic group but exhibit some degree of genetic heterogeneity. This within species differentiation has been explained by the existence of demes, populations, or races. Defining the population structure of a wide ranging marine teleost, such as the milkfish, should provide insight into the forces that shape within species differentiation. Such information is highly relevant to understanding how new species originate.

The state of the art method used in determining the population divisions of a particular species is discussed. In addition, the past work on stock structure of milkfish is presented, followed by the proposed analysis to be carried out at the Oceanic Institute.

MILKFISH FARMING IN THE PHILIPPINES

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INTRODUCTION

Milkfish culture in the Philippines has been widely practiced as a good and profitable aquaculture business for a long time. Nearly 98% of the country's total production from fishponds could be attributed to milkfish farming (BFAR Statistics, 1981-1982). The culture of milkfish, or "bangus," in ponds started as early as the 17th century in the Philippines. Because of this, in the past, aquaculture has chiefly been known as milkfish culture in brackish water ponds.

In the mid-1950s, bangus farming in the Philippines achieved a major breakthrough (M. Lijauco, SEAFDEC). Bangus fish farmers discovered that the use of commercial agricultural fertilizers improved pond productions/yields by promoting the growth of filamentous algae. This discovery led the Filipino fish farmer to the frontier of scientific aquaculture.

Several years later, the benthic type of natural food complex, commonly called lab-lab, was found to be superior to the grass green algae as natural food for the milkfish. This

technique, which was patterned after the Taiwan practice, is believed to have revolutionized the Philippine milkfish farming system. Because of the prospect for higher production output per unit area, many bangus fish farmers across the country adopted this new technique with innovations to suit local conditions and practices.

These two major developments in the milkfish culture system resulted in an increased national average output from bangus fish farms (from about 500 kg/ha/yr in the late 1960s to 640 kg/ha/yr in 1976). The culture system has evolved into the fertilization technology level.

The use of organic and inorganic fertilizers, singly or in combination, has become a standard practice in pond soil and water conditioning, along with the use of pesticides and molluscicides to eradicate pond pests and predators. The hectarage of developed milkfish farms has increased as old and neglected ponds were improved and virgin swampland areas were converted into new fish ponds.

Statistics from the Bureau of Fisheries and Aquatic Resources (BFAR) show that the area occupied by brackishwater fishponds in 1982 covered 195,832 hectares, 5% of which was privately owned, and 49% was leased by the government. The fishponds were concentrated in the Ilocos Region, Central Luzon, Southern Tagalog, Western Visayas Regions, and the Zamboanga provinces. The total area of freshwater ponds, mostly privately

owned, was estimated to be 12,432 hectares in 1982. In all, the country's fishponds totalled 208,264 hectares.

Recent developments have broadened the scope of aquaculture in the Philippines. From the pond monoculture of milkfish, the aquaculture industry in the Philippines has graduated to pond polyculture of various species, such as carp, prawn, shrimp, siganids, catfish, and other aquatic crops.

The trend of development in recent years has become more technology-specific. Intensive and semi-intensive, rather than the traditional extensive farming system, are increasingly being adopted. New techniques on fertilization were introduced and practiced to maximize productivity. More organic agricultural waste products are used in ponds in place of imported pelletized fertilizers and chemicals. New stock manipulation techniques and polyculture systems have been accepted and practiced by fish farmers.

Much hope is pinned on the artificial and induced spawning of the "sabalo" and the advent of a hatchery system for milkfish. Simultaneously, milkfish farming in the Philippines is gradually advancing toward another frontier -- that of the feeding technology level.

In the near future, it is hoped that the nutritional requirement of milkfish in all its culture stages shall be determined and established and lead to the commercial production of economically viable pelletized feeds. Thus, a production

target of 2,000 kg/ha/yr easily could be achieved.

2. MILKFISH FARMING PROCEDURES GENERALLY PRACTICED IN THE PHILIPPINES

The milkfish farming system in the Philippines is heavily influenced or patterned after the methods practiced in Taiwan. However, because of the difference in the physical, climatic, and socio-economic conditions of the two countries, complete transfer of technology is not feasible. This led to the proliferation of innovative techniques, which were intermingled with the persistent traditional local practices and recent technological development in the country and other countries, resulting in what may be called the milkfish culture system in the Philippines.

Basically, the entire culture system could be divided into the following steps or phases:

2.1 Collection and/or procurement of seed for fry phase

Milkfish farming in the Philippines, just like in any other Asian country, is dependent on the supply of seed/fry from the wild. Santiago (1983) estimated that with 193,832 hectares of brackishwater ponds, 90% of which are stocked with milkfish and cropped twice a year, the quantity of fry needed by ponds would be 1.76 billion. For fishpens, the 15,000 hectares built and designated as fishpens in Laguna de Bay (although 34,000 hectares of fishpen was estimated to be in operation in 1983) would require 450 million additional fry. Total bangus fry needed for

ponds and pens therefore would amount to 2.2 billion.

It was further expounded by Santiago (1983) that the increased milkfish production targets, which can be attained through area expansion and intensification, would mean additional fry requirements. Shortage then would be a very serious problem. Fry gathering activity has to be intensified, and handling improved to minimize mortality. Experts at the recent international conference, held at the SEAFDEC Aquaculture Department main station in Iloilo, agreed that the quantity of fry being gathered from the sea still matches total requirement. However, they believed the distribution system should be made more efficient and the technology to keep the fry alive from the point of collection to the point of stocking should be improved.

2.1.1 Fry collecting grounds

The Philippines, an archipelago situated in the tropical area, is considered a natural fry ground. It has been observed that fry grounds are shallow sandy coasts, tidal creeks, and river mouths. The milkfish fry collecting grounds extend from the north in the Ilocos and Cagayan Region, along the western coast of Luzon and the Bicol Region, central, eastern, and western Visayas including Oriental Mindoro and Masbate, to as far as Mindanao and Jolo (Fig. 1).

Generally, it has been observed that the collecting season of fry starts in March and lasts until June or July. In some

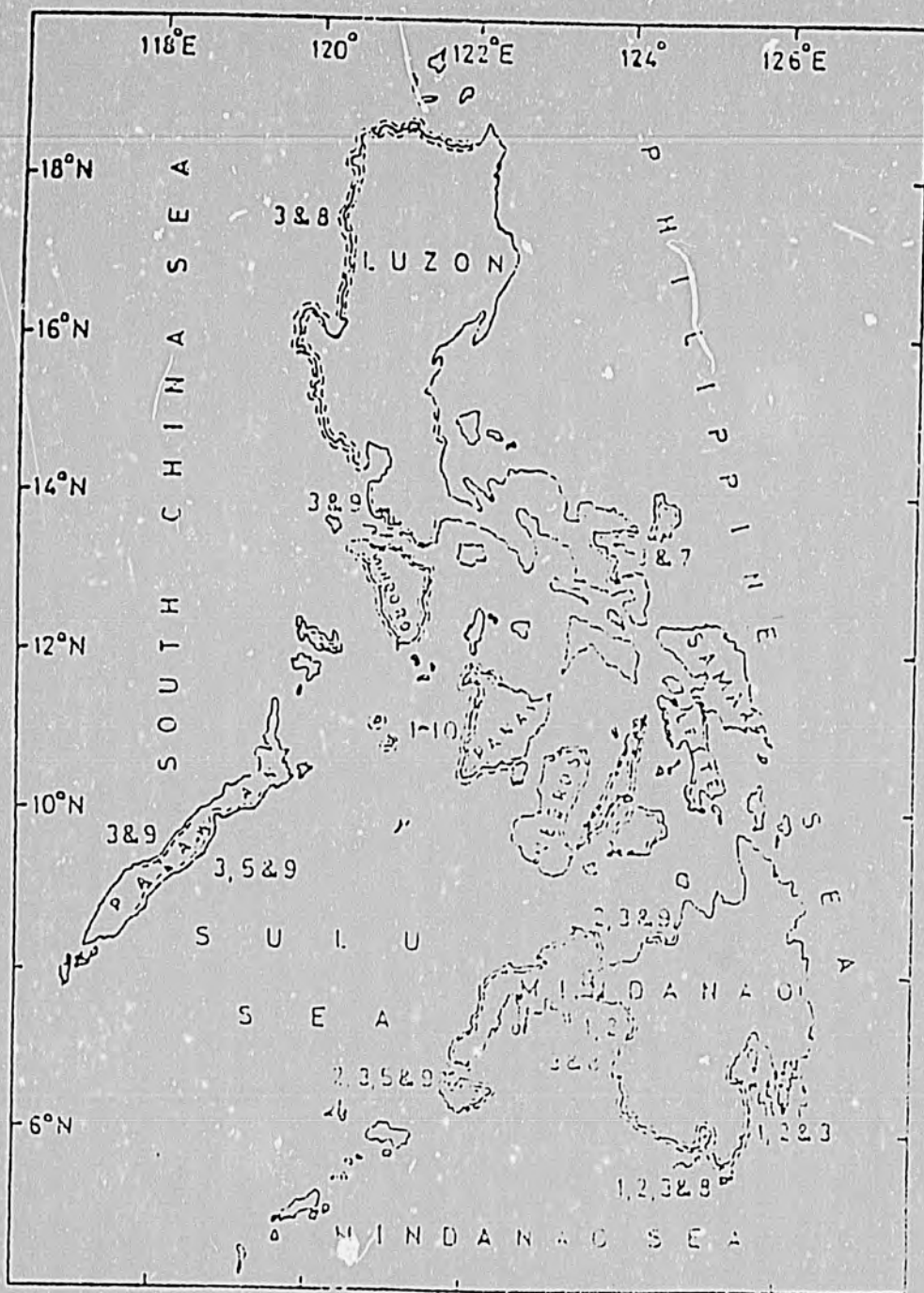


Fig 1 Milkfish fry grounds of the Philippines with the corresponding gears being used in each locality and their degree of exploitation. Single broken lines indicate exploited fry grounds and double broken lines indicate fully exploited fry grounds. Numbers 1-10 represent the catching gears being used in each locality as follows: 1- fry barrier, 2- skimming net, 3- tidal set net, 4- floating tidal set net, 5- push net, 6- push net with bamboo raft, 7- tow net with bamboo float, 8- tow net with double stick net, 10- fry seine net.

(after Villaluz et al., 1983)

areas, a second wave of fry occurs in October to November. The season usually is preceded by rainfall. A study conducted on Panay Island indicates the peak season of fry collection comes one to two days before the new and full moon. The monthly percentage catch (1980) of milkfish fry in the different areas of the Philippines is shown in Fig. 2.

2.1.2 Fry collecting gears

Collecting gear generally common to all grounds consists of stationary, pushed, or dragged net and bamboo contraptions operated in coastal areas with knee-deep to neck-deep water. Fig. 3 and Fig 4 show two samples of such collecting gear being used in the Philippines.

2.1.3 Storage and transport

Fry collected by fishermen generally are sorted at the beach area and stored in pots and basins awaiting purchase by fry dealers. Table 1 shows the various milkfish fry storage practices in the different localities of the Philippines. The stored fry are provided optimum care with daily water exchange and feeding of mashed hard-boiled egg yolk. Table 2 shows the milkfish fry transport methods in the different locations. It can be noted from the table that fry are generally transported in oxygenated plastic bags or styrofoam boxes for extra handling protection. Each bag is filled to about 1/2 to 1/3 full of diluted sea water with a stocking rate of 4,000 to 8,000 fry. The transport bag salinity range varies and is dependent on

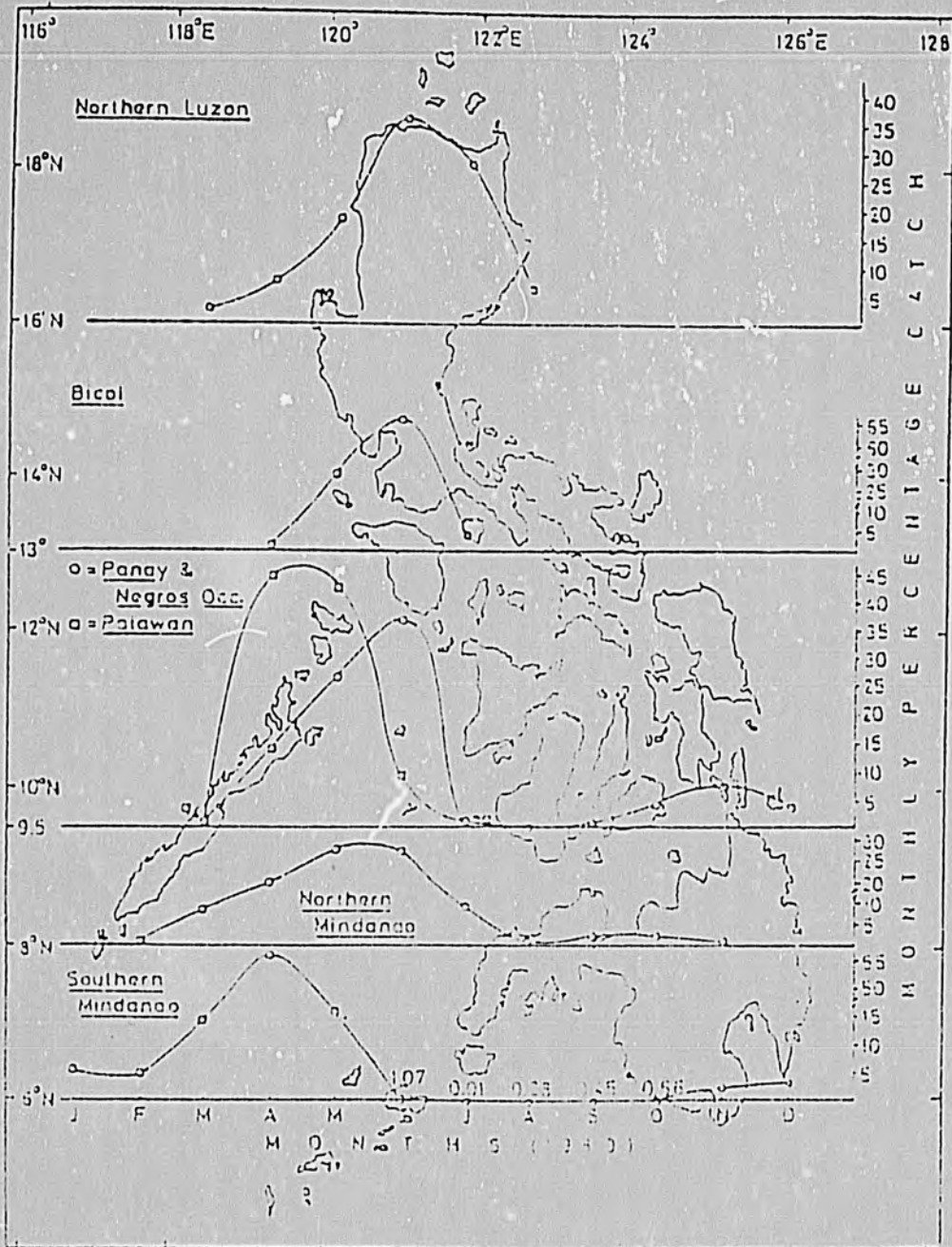


Fig. 2 Monthly percentage catch (1990) of milkfish in the different parts of the Philippines (after Villaluz et al., 1983)

Table 1 Milkfish fry storage practices in the different localities of the Philippines. 1/

Practices	Location							
	Northern Luzon	Bicol	Panay	Psalwan	Northern Mindanao	Southern Mindanao	Malabon*	
1. Container	1 a. plastic basin 1 b. earthen jar	1 a. plastic bin 1 b. earthen jar	1. plastic basin	1. plastic basin	1 a. plastic basin 1 b. earthen jar	1. plastic basin	1. plastic basin	
2. Water Depth	2 a. 5-6 cm 2 b. 5/8 filled	2 a. 7-8 cm 2 b. 2/3 filled	2. 5-6 cm	2. 5-6 cm	2 a. 5-6 cm 2 b. 2/3 filled	2. 7-8 cm	2. 5-6 cm	
3. Water Volume (liter)	3 a. 15-20 3 b. 10-12	3 a. 20-23 3 b. 18-20	3. 15-20	3. 15-20	3 a. 15-20 3 b. 18-20	3. 20-23	3. 15-20	
4. Salinity (‰)	4. 20-30	4 a. 24-29 4 b. 34-36	4. 18-20	4. 20-25	4 a. 14-20 4 b. 14-20	4. 5-10	4. 12-15	
5. Feed and feeding	5. egg yolk or flour every other day	5 a. egg yolk everyday 5 b. no feeding	5. egg yolk everyday	5. egg yolk everyday	5. flour or egg yolk daily	5. flour or egg yolk daily	5. egg yolk everyday	
6. Water management	6 a. complete change of water every other day 6 b. 5/8 of volume change daily	6 a. complete change of water daily 6 b. 5/8 of volume change daily	6. 5/8 of volume change daily	6. 5/8 of volume change daily	6 a. 5/8 of volume change daily 6 b. 5/8 of volume change daily	6. 5/8 of volume change daily	6. about 3 liters change daily	
7. Stocking rate (fry/container)	7 a. 6,000-8,000 7 b. 2,000-3,000	7 a. 5,000-5,500 7 b. 2,000-2,200	7. 3,000-6,000	7. 5,000-6,000	7 a. 3,000-6,000 7 b. 2,000-2,200	7. 4,000-6,000	7. 4,000-8,000	
8. Stocking density (fry/liter)	8 a. 300-500 8 b. 167-200	8 a. 217-275 8 b. 100-122	8. 150-180	8. 250-400	8 a. 150-400 8 b. 100-122	8. 174-300	8. 267-400	
9. Number of days in storage	9. 1-5 days 3 days average	9. 3-7 days 5 days average	9. 2-5 days 4 days average	9. 3-7 days 5 days average	9. 2-7 days 4 days average	9. 3-7 days 5 days average	9. 1-7 days 5 days average	
10. Mortality during storage %	10 a. 5-10 10 b. 5-6	10 a. 7-9 10 b. 3-5	10. 2-3	10. 4-5	10 a. 5-10 10 b. 5-8	10. 8-10	10. 2-3	

*After transport from different places in the Philippines

(after Villaluz et al., 1983)

Table 2 Milkfish fry transport methods in the different localities of the Philippines.

Methods	Location						
	Northern Luzon	Bicol	Panay	Palawan	Northern Mindanao	Southern Mindanao	
1. Destination	1. Bulacan and Metro Manila	1. Bulacan and Metro Manila	1.a. Iloilo 1.b. Manila	1. Metro Manila	1.a. Aboyan 1.b. Metro Manila	1. Metro Manila	
2. Mode of transport	2. Land	2. Land	2.a. Land 2.b. Land and air	2. Land and air	2.a. Land 2.b. Land and air	2. Land and air	
3. Transport time (Hours)	3. 8-14	3. 8-12	3.a. 2-4 3.b. 4-8	3. 3-4	3.a. 5-6 3.b. 2-5	3. 5-10	
4. Container of plastic bag	4. Pandan bag	4. Pandan bag	4.a. Pandan bag 4.b. Styrofoam (40 x 40 x 40 cm)	4. Styrofoam boxes (30 x 45 x 30 cm)	4.a. Pandan bag 4.b. Styrofoam boxes (30 x 30 x 30 cm)	4. Styrofoam boxes (30 x 45 x 30 cm)	
5. Size of plastic bag (cm)	5. 50 x 83	5. 55 x 76	5.a. 55 x 76 5.b. 30 x 60	5. 55 x 76	5.a. 65 x 76 5.b. 30 x 60	5. 51 x 75	
6. Water volume (liter)	6. 8-10	6. 8-10	6.a. 8-10 6.b. 3-4	6. 4-5	6.a. 8-10 6.b. 3-4	6. 3-4	
7. Salinity (‰)	7. 14-18	7. 12-22	7. 14-20	7. 12-16	7. 10-16	7. 2-4	
8. Stocking rate (fry/bag)	8. 4,000-6,000	8. 4,000-5,000	8.a. 5,000-6,000 8.b. 6,000-8,000	8. 4,000-5,000	8.a. 5,000-6,000 8.b. 6,000-8,000	8. 4,000-5,000	
9. Stocking density (fry/liter)	9. 400-750	9. 400-625	9.a. 500-750 9.b. 1,500-2,000	9. 500-1,250	9.a. 500-750 9.b. 1,500-2,000	9. 1,250-1,333	
10. Mortality-%	10. 4-5	10. 3-5	10.a. 2-3 10.b. 3-5	10. 4-6	10.a. 5-8 10.b. 2-4	10. 4-6	

(after Villaluz et al., 1983)

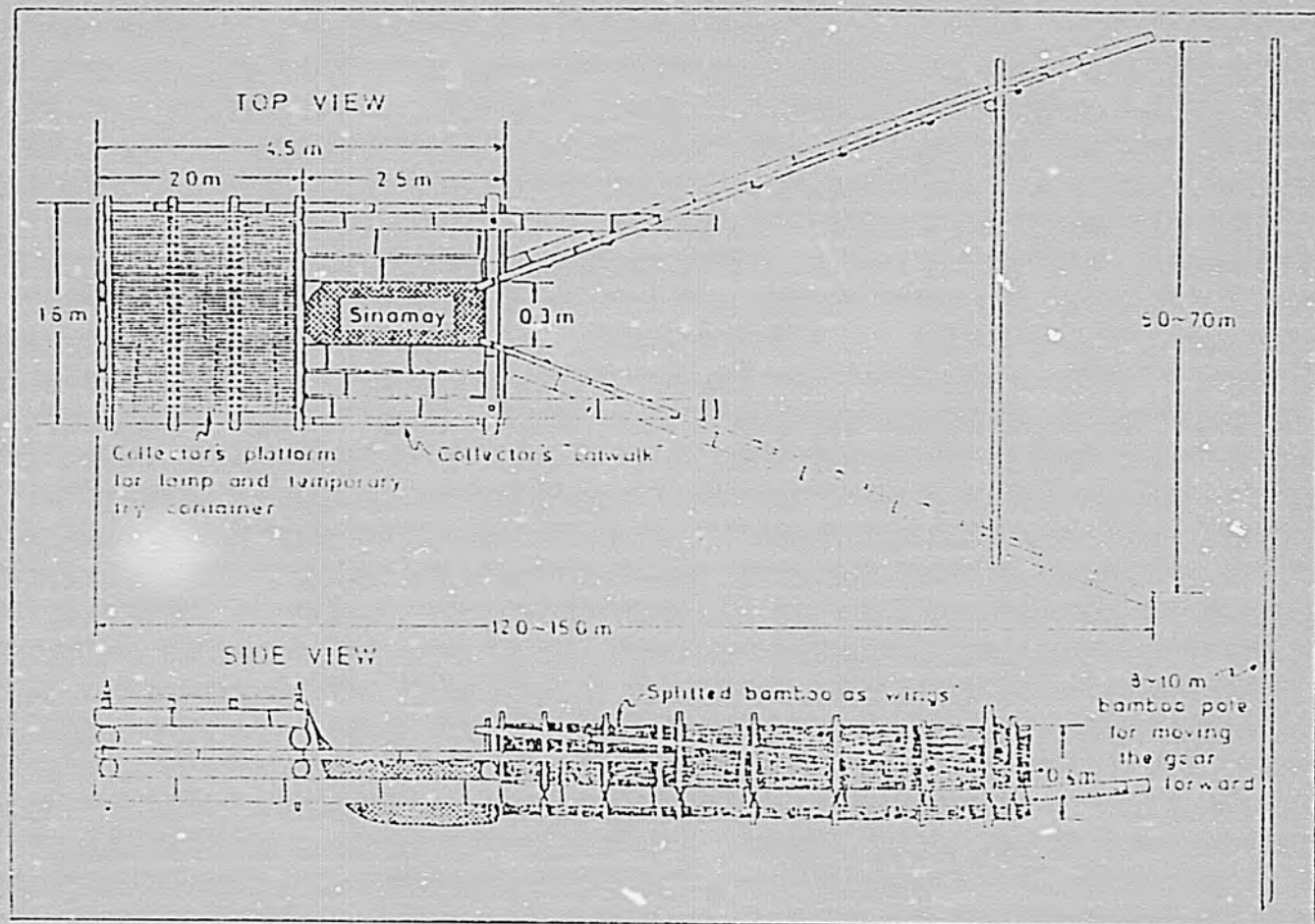


Fig. 3. Fish net with bamboo raft: its structure and dimensions
(after Villaluz et al., 1983)

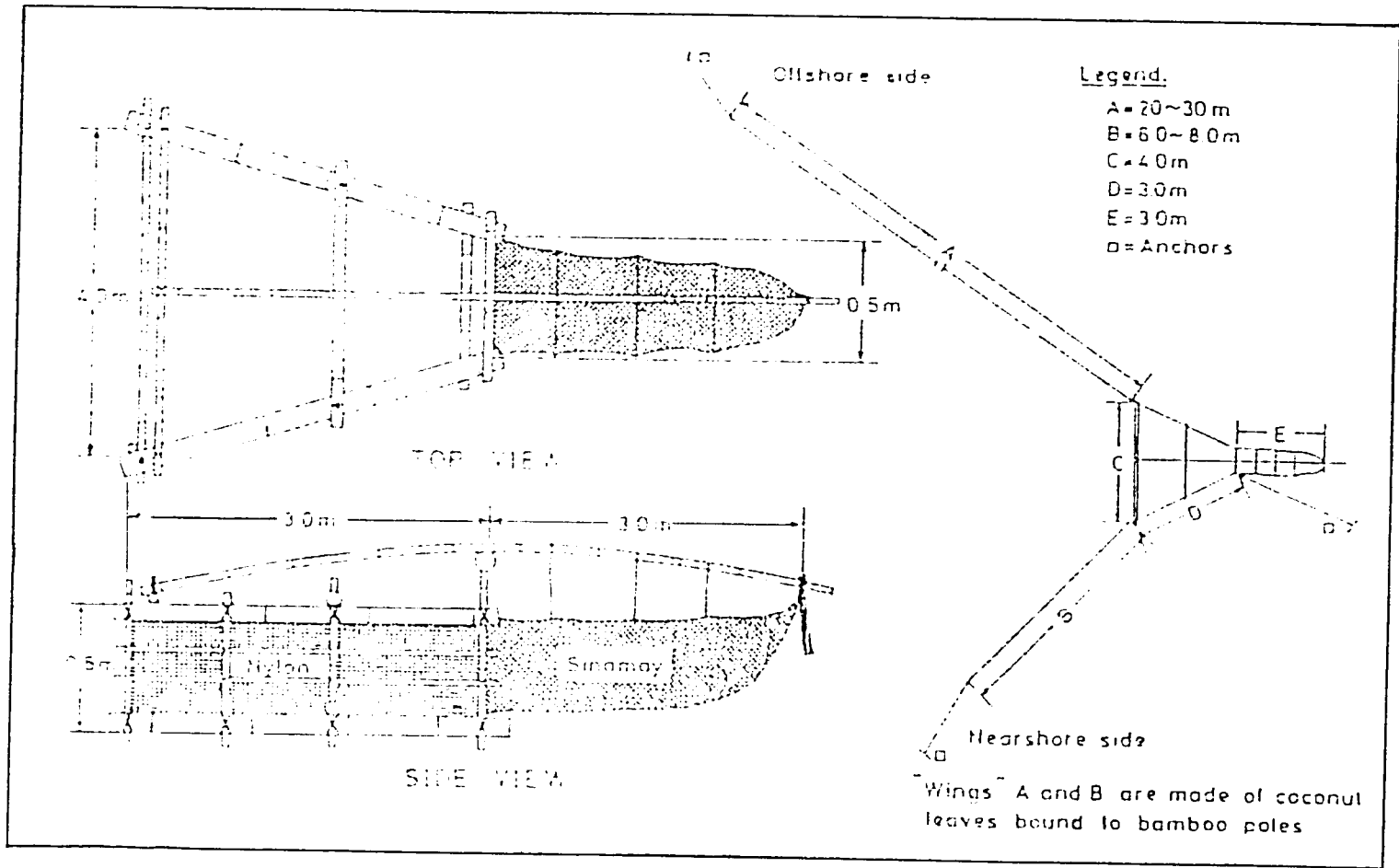


Fig. 4. Floating fish trap: its structure, dimensions and how to set it up (after Villaluz et al., 1983)

location.

2.2 Fry rearing

Milkfish fry collected from the wild generally measure from 10-15 mm in length and weigh from 0.002 to 0.006 mg. Milkfish farm operators normally procure the whole year's fry requirements in a single lot during the peak season when the price is low. Others prefer buying in separate lots depending on the availability of pond space. The common practice or system of rearing milkfish fry to fingerlings 1-2 g in weight is to use nursery ponds, which are built specifically to receive and rear the fry for a period of 4-6 weeks. Ordinarily, the nursery pond size ranges from 1,000 to 4,000 square meters. Prior to the arrival of the stock, the ponds are thoroughly prepared to insure optimum care and highest possible survival.

During stocking, the fry are acclimated gradually to the existing pond salinity level to prevent salinity and temperature shock if the transport bag water salinity differs significantly from that of the pond. Ordinarily, acclimation is done in plastic basins under the shade for about 4-6 hours. The normal practice is stocking the fry during the colder part of the day, in the late evening or early morning.

Some fishpond operators have adopted a modified method of pre-stocking the fry in a small acclimation pond built within the nursery pond proper at a rate of 5,000/m². The stock are fed daily with patches of lab-lab or mashed egg yolk when they are

observed to be weak. After about a week, the fry are released into the pond proper, breaking some sections of the dike. By this time, the fry should have grown larger and developed scales. The acclimation pond is provided with shade to keep water temperature low especially during hot sunny days.

2.3 Culture and production in grow-out ponds

Three methods of culturing/producing milkfish in grow-out ponds are being practiced in the Philippines. These culture systems are primarily determined by the feeding scheme being adopted, stocking, and the method of stock transfer in ponds.

2.3.1 The extensive farming system or otherwise called the Straight Run Method

This is considered the traditional way of milkfish farming and still dominates the scene with fish ponds running to several tens, and, in some cases, hundreds of hectares. A recent survey estimated the country-wide average farm size to be 16 hectares. The ponds utilized by this method is characterized by haphazardly oriented ponds and dikes, unlevelled pond bottoms, and relatively large rearing compartments. The fish stay in the same compartment from the time they are stocked to the time they are harvested. The primary food of the cultured fish is lab-lab, a highly-nutritious mixture of plankton. The lab-lab is grown during the pond preparation, and the fish are stocked when the pond has sufficient growth of the lab-lab. Ponds are fertilized to grow a sufficient amount of this food. Harvesting of stocked

fish is done at one time.

2.3.2 Mixed-size group culture or multi-size stocking method

This method essentially is a form of the stock manipulation technique. The pond is stocked with fingerlings of different sizes and are grown to marketable sizes in the same pond for as long as the feed is sufficient. The ponds are also fertilized to grow natural foods. Selective harvesting is done after the first 45 days and every 15 days thereafter. Two culture periods are possible in this method, which is claimed to produce from 2,200-2,700 kg/ha/yr. The technique, however, has three major limitations:

2.3.2.1 Selective harvesting by gill nets entails extra cost for additional manpower;

2.3.2.2 The quality of harvested fish is relatively poor as gilled fish bear marks and often are scaled; and

2.3.2.3 Uncertainty of available fingerlings of desired size at the appropriate time.

2.3.3 Modular Pond Method

This is a complicated but highly productive type of culture method that has gained wide popularity among milkfish farmers in recent years. This sometimes is referred to as the "1-2-4 technique," or progression system. This method was first seen in practice at large-scale farms in the province of Pangasinan. The method consists of a series of grow-out ponds linked to one another and performing as a unit, thus dividing the whole farm

into sub-units or modules. The system is termed progression because the stock are transferred in succession from one pond to the next until harvest. Fish and pond size progressively increase. Correspondingly, density decreases after every transfer. Culture period in the pond lasts from 30-45 days, and any pond vacated is immediately prepared to receive an incoming stock. The area or dimensions of the various ponds increase in the proportion of 1:2:4 for nursery, transition, and rearing ponds, respectively. This culture system has many variations. Some have finishing or catching ponds and others have two rearing compartments. A full accounting of the stock in each pond is a clear production advantage of this method. It is also known to have a higher cropping index, and more crops (as many as six) can be produced per unit area per year.

2.4 Milkfish culture and production in freshwater fishpens

Milkfish culture and production using fishpens have contributed greatly to the total milkfish production in the Philippines. The large-scale culture in Laguna Lake fishpens adds a new and unique dimension to the world of aquaculture. Portions of Laguna Lake are enclosed using semi-permanent structures and stocked with milkfish fingerlings at densities 10-12 times higher than those in brackishwater ponds. The growth rate of the fish was observed to be very high. The fish attain marketable size in 5-6 months feeding only on the natural planktonic life of the lake water. Studies have shown that the

yields obtained range from 2-4 tons per hectare to as high as 10 tons per hectare. This unusually high production output per unit area is attributed to the unusually rich and unique environmental conditions in Laguna Lake.

It was reported and recorded that in 1976, about 47,000 metric tons of milkfish were cultured and produced in more than 7,000 hectares of fishpens in Laguna de Bay alone. Ten years (1983) after the first fishpen was set up in the lake, 34,000 hectares have been established, and, have created socioeconomic problems within the Bay town areas. The areas for capture fisheries shrunk significantly and conflicts arose between the small fishermen and the big fishpen operators.

Despite being a profitable business enterprise, the fishpen business in the Philippines now is beset with many problems. Typhoons and other natural and man-made calamities could damage or destroy fishpens. The cost of nets, cords, and construction material has more than doubled over a 10-year period. The net effect was that only the wealthy capitalist could profitably engage in the fishpen business. The small fishermen were inevitably dislocated or displaced by the economics of scale. Ecologically, there has been increasing fear of an eventual and total ecological imbalance. Periodic occurrences of mass or near-mass killing of milkfish stocks in the fishpens have been experienced and reported.

3. HARVESTING

Harvesting may be partial or total depending on the culture or production method that is being adopted or practiced. The decision to harvest often is dictated by economic and operational considerations, such as:

- 3.1 prevailing market price;
- 3.2 phase of the tide;
- 3.3 weather conditions;
- 3.4 state of the food supply in the pond; and
- 3.5 desired size

Exceptional cases of premature harvests occur following mass kills and other natural calamities.

4. POST HARVEST HANDLING

It is estimated that about 90 % of the catch or yield from milkfish ponds and fishpens go to the domestic or local market as fresh fish. A small volume is marketed as marinated or smoked. The major domestic marketing outlets are concentrated in the Metro Manila area and a few other population centers. Transporting the produce to these centers takes from a few hours to about two days depending on the mode of transportation used. Handling of the catch for marketing includes pre-chilling, packing, and actual transporting.

The harvested fish are pre-chilled immediately or immersed in ice water. This is done to accomplish the following: 1) serve as a convenient killing medium, thus preventing excessive

physical damage and resulting in attractive-looking and good-quality fish; 2) slow down autolysis, or enzymatic breakdown activities; and 3) remove blood, slime, dirt, and bacteria from the skin of the fish, thus minimizing further deterioration and eventual spoilage.

Chilling of harvested bangus to 0°C is not practiced because a recent study on handling, icing, and transporting found no advantage to icing immediately after harvest. Icing requires only about 450 kilograms of ice to a ton of fish in two hours of immersion as against 900 kilograms of ice and four hours of immersion in the chilling. Where cold storage and ice plants are available, fish farmers are sure to produce good quality fish that will reach the market.

5. PROBLEMS AND CONSTRAINTS OF MILKFISH FARMING IN THE PHILIPPINES

Santiago (1983) stated that fishpond acreage and production gradually increased until the late 1960s when the areas devoted to culture increased 20% and production went up 52% within three years. Since the early 1970s, however, such spectacular growth has not been sustained. What were the problems?

The milkfish industry is highly dependent on the supply of bangus fry collected from the wild. Other constraints, according to Dr. Santiago, include:

- 1) high death rate of milkfish fry during collection, storage, transport, and rearing;

- 2) high cost of feeds, fertilizers, pesticides, and other inputs;
- 3) prevalence of parasites, diseases, and pollutants;
- 4) lack of financing and credit; and
- 5) lack of marketing, processing, and distributing facilities.

6. RESPONSE OF RESEARCH

To increase production per unit area and to solve the problems of inadequate supply of fry, it was reported by Santiago (1983) that researchers at the SEAFDEC, Aquaculture Department, have focused their studies on four objectives:

- 1) increase the supply of milkfish broodstock;
- 2) generate technology for the artificial propagation of fry;
- 3) refine techniques of wild fry and fingerling collection, storage, and transport; and
- 4) improve and standardize milkfish culture technique.

Furthermore, SEAFDEC researchers, aware that our milkfish industry is dependent on the supply of fry from the wild, have directed their efforts to breeding milkfish in captivity in order to supplement wild fry supply. The first step undertaken was to capture wild adult milkfish or "sabalo." A massive effort to catch sabalo began in 1977 and is still continuing at the Naujan Research Station, where migrating Lacustrine sabalo from Naujan Lake are collected alive and acclimated for induced maturation

and eventual spawning in plastic/canvas tanks.

To improve the present survival rate of 40% from the time the fry is caught in the wild to the time they attain fingerling size, SEAFDEC, AQD, conducted studies on stunting techniques, salinity preferences, and freshwater acclimation. Techniques were developed to hold milkfish fry for 14 days without aeration and to transport fry at lower temperatures and salinities with survival rates of more than 90%.

SEAFDEC researchers also have demonstrated that higher survival rates of fry reared to fingerlings in brackish water nursery ponds can be obtained through supplemental feeding with rice bran, use of nylon nets as additional substrates to increase natural food, and gradual acclimation of fry from sea water to the salinity of the water in the nursery pond.

A method was also developed to acclimatize fry from seawater to freshwater with minimum mortality. This step was observed to reduce mortality during transport.

Progressive fish farms and SEAFDEC researchers began to look for alternatives such as increasing productivity per unit area without additional increases in production input. An important advance in this direction has been the introduction of a modular pond system (Lijauco, 1983b). Using the modular system and lab-lab method, SEAFDEC, Department of Aquaculture, has demonstrated that an annual yield of 2 metric tons per hectare can easily be attained.

To generate additional income for our milkfish farmers, optimum stocking densities for the polyculture of milkfish and prawn also were determined by our SEAFDEC researchers. The possibility of integrated farming of milkfish, prawn, sea bass, and tilapia also has been demonstrated.

In summary, Santiago (1983) expressed that research at the Department has brought us closer to the ultimate goal of completely domesticating the milkfish. By such feat, we should then be able to manipulate its biology, and entirely control its reproduction and growth. By then, we can say that science finally has brought the milkfish from the wild to the farm.

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MILKFISH CULTURE IN TAIWAN*

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ABSTRACT

Continuous modification for almost 300 years gives milkfish culture in Taiwan a traditional style of its own. It is called the shallow-water pond culture system, or fertilizing culture method, in which the farmer grows benthic algae by treatment of tillage, fertilization, and sunning. This method fully utilizes a balanced ecosystem in the milkfish ponds.

Another culture style has recently been developed and improved. It is called the deep-water pond culture system, or feeding culture method, in which the unit productivity may reach four to five times that of the traditional style. Owing to the higher profit in the modern style than in the traditional one, the former is becoming more and more popular. Nevertheless, the total national production is increasing rapidly and the imbalance of supply and demand results in a reduction of profit, which comes as a big shock to the milkfish farming industry.

A brief introduction and comparison of the above-mentioned culture systems are made to investigate their advantages and disadvantages. The prospects for milkfish culture in Taiwan are also briefly described.

INTRODUCTION

The history of milkfish culture development in Taiwan is beyond investigation. However, the beginning of the practice probably dates back 300 years (Chen, 1952).

The reasons milkfish became a cultured species at such an

*Contribution B No. 35 from the Tungkang Marine Laboratory

early date and has remained an important species for so long are listed as follows:

1. Because of the high fecundity of the spawners, supply of fry is abundant. Fry are easy to identify and are caught in season.
2. Fry are easy to handle as they are hardy and euryhaline. The tolerance and adaptability of milkfish to salinity is extremely high and they survive a wide range from 0 to 158 ppt (Crear, 1980).
3. Benthic algae grown from fertilization ponds is a suitable food for milkfish, which are basically herbivorous but can also be omnivorous. In addition, the growth rate of milkfish is much faster than that of many other herbivorous fishes. Because of these characteristics, large commercial culture of milkfish was possible long before the development of artificial feed. Moreover, production of artificial feed is economical because inexpensive ingredients are adequate.
4. Stocking density for milkfish culture tends to be high since the milkfish is not cannibalistic. Polyculture with other species has also proved feasible. Hence, the economic value per unit surface area of milkfish culture pond is high.
5. The milkfish's resistance to disease is high, and the high survival rate of milkfish ensures a reasonable harvest.
6. Although milkfish is bony, it is highly palatable. This delicious food fish can also be used as baitfish for the tuna longline industry.

After more than 300 years' development, a standard year-long routine for the traditional shallow-water pond culture system or fertilizing culture method, has been developed. Recently, a modern, deep-water pond culture system or feeding culture method, has been established. The unit productivity of the modern style is as high as four to five times that of the traditional style, resulting in a production of 12 metric tons per hectare (Huang, 1981).

Although milkfish culture in Taiwan does have certain problems, which will be discussed in this paper, milkfish is a desirable species for aquaculture. Management of the traditional style of culture has resulted from centuries of trial and error. Ecological principle is applied throughout, resulting in the most economical management methods. For Third World countries, with problems of animal protein deficiency, milkfish culture in the traditional style is an ideal method of helping to relieve this severe crisis. If the quality of milkfish can be improved in the future by genetic engineering or selective breeding, the prospects for milkfish culture are extremely good.

CULTURE STATUS

In general, the management methods of milkfish culture in Taiwan can be divided roughly into the traditional shallow-water pond culture system and the modern deep-water pond culture system.

1. Shallow-water pond culture system

The size and shape of the milkfish ponds and methods of culture vary with the local topography, climate, preference and traditional family concept of the fish farmers.

Ponds usually are constructed on the tidal flat where tidal fluctuation can be used to maintain the water level. The soil is preferably silty loam, but not necessarily, as fertilization may improve the soil (Chen, 1971).

Dikes, canals, nursery ponds, overwintering ponds, and production ponds are constructed in different sizes and arrangements for ease of management (Tang, 1962; Lin 1968) (Fig. 1; Table 1). Maintenance of bottom soil, cultivation of benthic algae, mixed stocking, and selective harvesting are the general techniques used for the shallow-water pond culture system. Production of 2,000-2,500 kg/ha per year has been achieved in Taiwan (Chen, 1976; Huang, 1974).

A general layout and construction of shallow-water milkfish ponds in Taiwan are as follows (Tang, 1962; Lin, 1968):

a. An outer dike is used for protecting tidal land (usually 3-5 meters high, 1:2.3-3.0 slope, 3.7-5.0 m wide on top), inner dikes for water supply and drainage (1.7 m high, 1:1.5-2.0 slope, 1.5-3.7 m wide on top), and small dikes for distinguishing the different ponds (0.8-1.0 m high, 1:1-1.5 slope, 0.5-0.7 m wide on top).

b. Water supply and drainage facilities are comprised of a

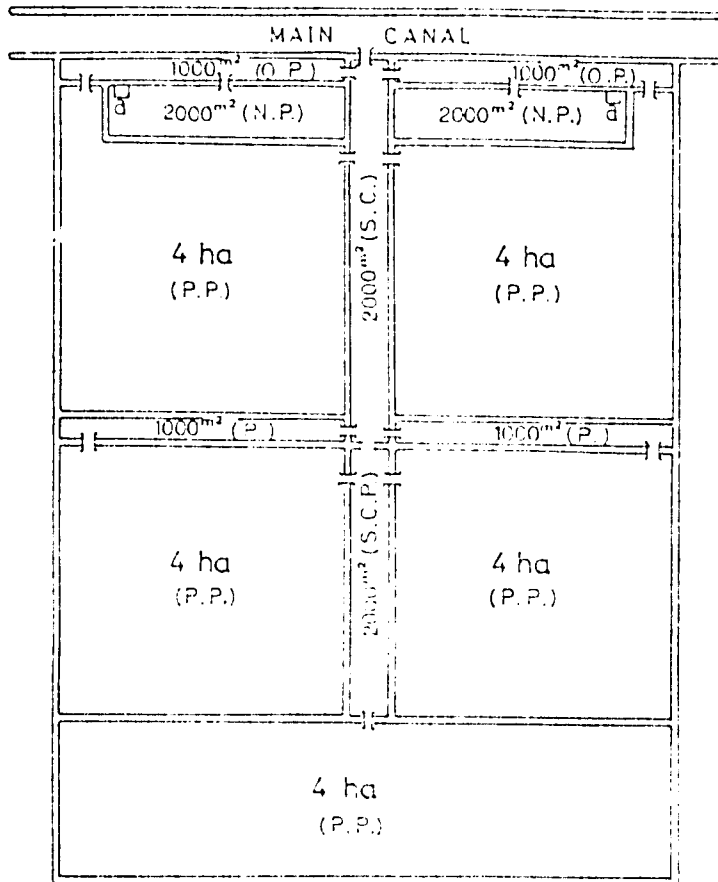


Fig. 1. Schematic sketch of a pond system of 21.2 ha.

O.P., overwintering ponds (for use in November to March) and passageways (in April to October); N.P., Nursery ponds; P., passageways; S.C., Subcanal; S.C.P., subcanal and passageway; P.P., production ponds; a., acclimatization pools (after Lin, 1963).

Table 1 Types of ponds and canals found in a pond system of 20 hectares or over (after Lin, 1968)

Type of ponds and canals	Area (ha)	Approximate % in total area
4 to 6 production ponds 3 to 5 hectares each	20.00	94
2 overwintering ponds	0.20	1
2 nursery ponds	0.40	2
4 passageways and refuges	0.40	2
1 subcanal	0.20	1
Total	21.20	100

Note: For farms of 100 ha total area, a layout of five similar systems should be used, consisting of ponds and canals as described above.

main canal (the canal that connects the sea, usually 12-15 m wide, dependent on tidal condition), subcanal (6 m wide, 1.5 m depth), fish passage ways, and water gates (Fig. 1).

c. Nursery ponds, wintering ponds, and production ponds (Fig. 1) are used to attain marketable size of the milkfish. The production ponds are 3-5 hectares in area. They are usually longer in the east-west direction than north-south with a 1-3:10,000 incline to the water gate. Nursery ponds are 2.5-3.0% the area of production pond in size and are located near wintering ponds. Wintering ponds should be built where water exchange is convenient. They are canal-shaped with 1.5 to 2.0 m in depth and equipped with wind shelter on the northern side.

The general practices of shallow-water pond culture system apply techniques such as: drying of tillage and fertilization of bottom soil to grow benthic algae as food for the milkfish; keeping water level around 30-40 cm deep; and mixing stocking and selective harvesting. With diligence and the experiences of generation after generation of Taiwanese farmers, annual production is as high as 2,000-2,500 kg/ha per year (Huang, 1974). This is much more than that of the Philippine Islands and Indonesia, although Taiwan suffers a three-month cold winter period each year.

The techniques and management of shallow-water milkfish culture system in Taiwan involve several steps:

a. Preparation of the ponds (November to March)

Pond maintenance, or the "winter treatment," in which the processes of drying, fertilizing, and flooding are repeated and cultivation of the benthic algal mat are performed. Before the stocking of the fingerlings, tea-seed cake, or tobacco waste, is used to kill pests and predators (Chang, et al., 1977). When water is re-introduced to the ponds at 30-40 cm in depth, the ponds are ready for stocking (Lin, 1968).

b. Stocking and stock manipulation (April to August)

Multiple-size stocking is performed from April to August. Fry and fingerlings of different sizes are stocked repeatedly. The general stocking pattern practiced in Taiwan is 4,000-5,000 overwintered fingerlings of 5-100 g body weight per hectare plus 5,000-8,000 new fry (Lin, 1968). Fish are first stocked in nursery ponds, then production ponds. Depending on the preferences of the fish farmers, stocking rates of some milkfish ponds are low at 4,000 fingerlings/ha to promote faster growth of the fish.

c. Maintenance of water condition and growth of food organisms (April to October)

Good water condition has to be maintained after the fish are stocked in the ponds. Organisms such as blue-green algae and diatoms are grown and maintained for feeding the milkfish. Fertilization to enhance the growth of the algal mat and supplemental feeding with rice bran or peanut cake is necessary to sustain the growth of the fish.

d. Control of pests, predators, and diseases

Predators, competitors, and pests in the milkfish ponds must be eliminated before stocking the fingerlings. Screen nets in the water gate are needed to prevent their entrance.

e. Selective harvesting and marketing (June to October)

Selective harvesting with different mesh size gill nets is performed upon demand of the market. The wholesale price recently has been about U.S.\$ 2/kg.

f. Overwintering (November to March)

Water temperature of milkfish ponds sometimes drops to 10°C or below when the cold front occurs during the three-month winter periods in Taiwan. Overwintering ponds to protect the fish from the chilly northerly wind need to be constructed. Water temperature is maintained by a wind shelter and/or water heaters (Ting, 1978). Stocking density is determined depending on the size of the fish, depth of the overwintering ponds, and other environmental factors. The optimum stocking density is less than 1.3 kg/m³ (Ting, 1978; Lin, et. al., 1981; Lin, 1931).

Fig. 2 summarizes the year-round management routine of the traditional shallow-water pond culture system.

2. Deep-water pond culture system

In the middle 1970's, a deep-water pond culture system was developed because of 1) pressure of limited land utilization; 2) limited manpower supply in rural areas; 3) relatively low market price for other freshwater fishes when compared with milkfish; 4)

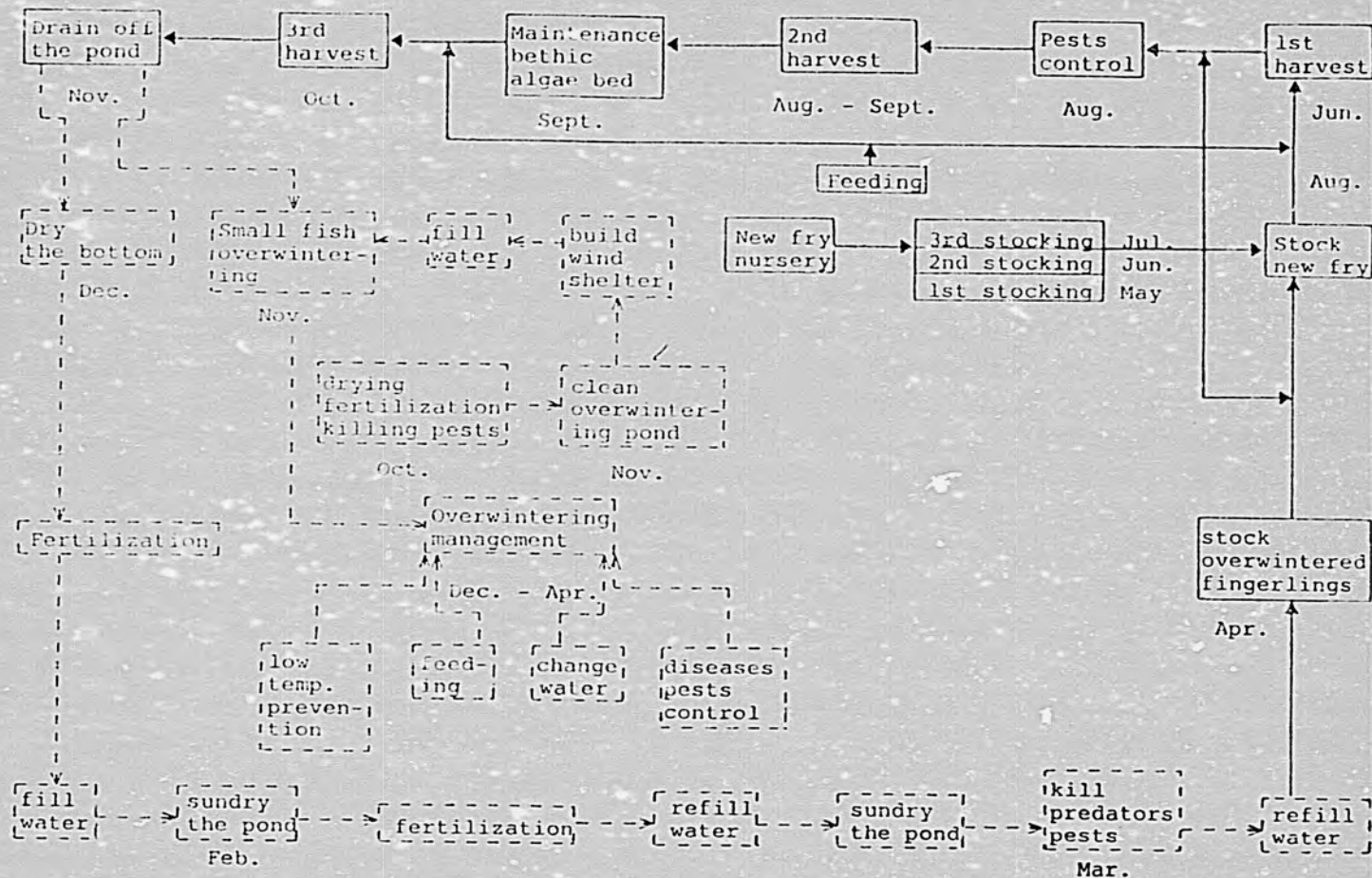


Fig. 2. Flow chart for traditional shallow-water milkfish culture system. Dash line shows preparation stage and solid line shows rearing and production stage (after Chang, 1977).

availability of formulated feeds; and 5) elimination of the need for overwintering ponds because of the stable water temperature (Chen, 1981).

Ponds are deepened to allow an increase of the stocking density and productivity of up to four to five times that of the traditional style. In general, 25,000 or more fish/ha are stocked resulting in an annual production of up to 12,000 kg/ha (Huang, 1981).

The general management practices are outlined as follows:

a) Pond preparation is similar to that of the traditional style. Liming and fertilization may be applied depending on the soil condition and water management.

b) Milkfish fingerlings of 1.5 cm total length are first stocked at 12,000/ha after April. A second stocking of 13,000/ha is performed after a selective harvesting.

c) An automatic feeder and two paddle wheel aerators are installed for each hectare of pond for feeding artificial feeds and aeration. Milkfish fingerlings are domesticated to feed on the pelleted feeds. Feeding quantity is controlled to maintain good water quality.

d) Milkfish over 500 g of body weight are harvested selectively by gill nets to decrease the stocking density and to obtain maximum growth of the fish.

e) Milkfish smaller than marketable size are left in the production ponds after the November harvest. They continue to

grow to marketable size and are harvested between March and May, whenever the market price is high.

The major constraints of the deep-water pond culture system are the availability of fry, disease outbreaks, and the high investment risk.

Table 2 summarizes and compares the major characteristics of the two culture styles. Some of the typical scenes as well as facilities of both culture styles are shown in Plate I.

Table 3 summarizes and compares the advantages and disadvantages of the two culture styles.

EXISTING PROBLEMS, POSSIBLE SOLUTIONS AND PROSPECTS

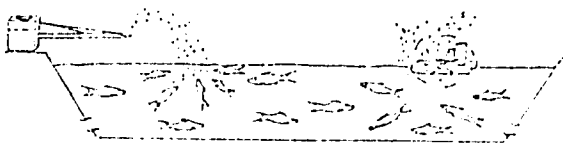
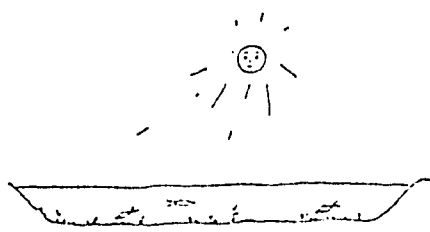
Although the culture techniques and productivity of milkfish culture in Taiwan already are the most advanced among culturing countries, several problems need to be solved by future studies.

1. Seed supply

The technique of artificial propagation is primarily established (Lam, 1984; Liao and Chen, 1984; Lin, 1984), yet massive production of fry still is unlikely in the near future. Since wild fry are still a major source of seed and the demand is far beyond the supply in Taiwan, import of fry from the Philippines and Indonesia is necessary at the present time.

On the other hand, if seed supply can be totally dependent on artificial propagation, once the technology is established completely, the present system of wild fry fishery will be ruined. During the transition period, the livelihood of the

Table II The traditional and modern style of milkfish culture in Taiwan



Traditional style

(shallow-water pond culture system;
fertilizing culture method)

- * Tilling
- * Fertilizer added
- * Sun - Benthic algae growth
- * Some supplemental feeding
- * Needs overwintering facility

Modern style

(deep-water pond culture system;
feeding culture method)

- * High carrying capacity
- * Artificial feeds
- * stocking density 5 times higher
- * Good for overwintering

300 years tradition:

- Maximum yield: 2-2.5 MT
- Depth: 30-40 cm
- No feeding
- No aeration

New development:

- Maximum yield: 8-12 MT
- Depth: 2-3 m
- Automatic feeder
- Paddle wheel aerator

Table III The advantages and disadvantages of traditional and modern style of milkfish culture in Taiwan

TRADITIONAL STYLE (shallow-water pond culture system; fertilizing culture method)	MODERN STYLE (deep-water pond culture system; feeding culture method)
<p>Advantages</p> <ul style="list-style-type: none">* A satisfactory culture method by utilizing a balanced natural ecosystem* Feed-saving and energy-saving* Healthy fish, almost no disease <p>Disadvantages</p> <ul style="list-style-type: none">* Maintenance of water quality is very sensitive and experience-dependent* Maximum yield is limited to a relatively low level* Better growth rate can only be obtained by lowering the already low density* Overwintering facility is needed	<p>Advantages</p> <ul style="list-style-type: none">* Less attention to water preparation and maintenance* Much higher stocking density (5 times or over)* Much higher yield (4 times or over)* Fast growth* No need for additional overwintering facilities. Continuous production in winter months when high price can be guaranteed <p>Disadvantages</p> <ul style="list-style-type: none">* Comparatively expensive facilities and formulated feed* High investment risk

EXPLANATION OF PLATES

- 1-1 Overwintering facilities used in the traditional shallow-water pond culture system: an overwintering pond with wind shelter made of bamboo and straw (left), and a nursery pond (right).
- 1-2 Dried bottom of production pond after harvest (Dec. - Apr.): it should be completely dried until the bottom cracks.
- 1-3 A typical scene of traditional milkfish pond in summer: shallow production pond (depth: 30-40 cm) and piled straws for overwintering on the banks.
- 1-4 Selective harvesting by gill net during summer (Jul. - Aug.): milkfish are harvested when body weight is above 300 g.
- 1-5 and 1-6 Facilities used in the deep-water pond culture system: two paddle wheelies and one automatic feeder are used for each hectare of pond.
- 1-7 The size of ponds in the deep-water pond culture system is one to three hectares with the mean production of eight to twelve metric tons per year.
- 1-8 Taiwanese way of milkfish packing: milkfish are carefully packed in bamboo baskets.

large population of fry fishermen and fry dealers is a social problem to be solved.

However, in the foreseeable future, a steady supply of fry is good for the industry because a stable, and probably reduced, price will encourage the fish farmers. The production of hatchery fry may also benefit the baitfish culture farmers tremendously as the baitfish business may become more promising and profitable. The year-round mass production of fry in the future probably will produce enough fry for both local and overseas requirements to balance supply and demand in Taiwan and other countries.

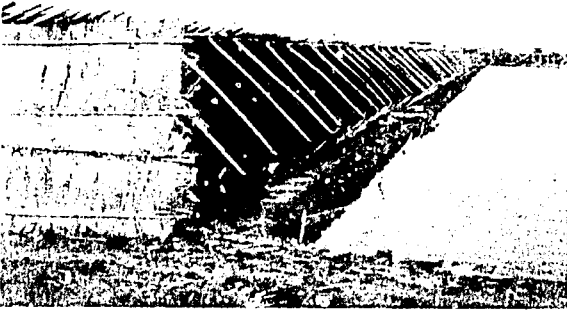
Third World countries, where fry fishery is not available, may be able to adopt the technology of milkfish culture when a steady supply of fry is possible through artificial propagation.

2. Improvement of culture techniques

The annual production of 2,500 kg/ha of the traditional shallow-water pond culture system is impressive. Yet, the possibility of further improvement must be investigated. Many problems still exist for the newly-developed deep-water pond culture system. Owing to fluctuating management, production by this system is not stable. A new culture system may be desirable, based on a combination of the two systems. A marked improvement may result when the merits of the two systems are fully exploited in a combined system.

3. Improvement of overwintering

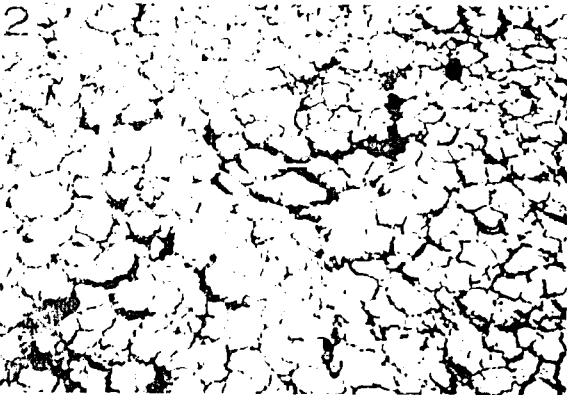
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5



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3



7



4



As the traditional shallow-water pond culture system is still an important system for milkfish culture in Taiwan, an overwintering facility is still essential. Hence, improvement of the facility is necessary to reduce the high mortality rate during overwintering. Further improvement of management techniques may eventually eliminate the process of overwintering.

4. Improvement of artificial feeds

Artificial feed is the only food source for fish culturing in the deep-water pond culture system. The nutritional value of the feed and the nutritional requirement of the fish need to be investigated. A less expensive feed is also desirable.

5. Pest prevention

Many different kinds of pests exist in milkfish ponds. Tilapia is always an extremely frustrating problem for milkfish farmers. However, milkfish and tilapia behave differently when they swim against a water current and therefore can be separated during the inflow and outflow of the pond water (Huang, 1974).

6. Prevention of diseases

Disease is seldom a problem for milkfish. However, outbreaks of disease do occur during the overwintering period when stocking density is high. Precautions must be taken to prevent large losses (Lin, 1982).

7. Coordination of production and marketing

The unit productivity has increased four to five times since the development of the deep-water pond culture system. This

rapid increase in production seems to create a problem of overproduction, and the imbalance of supply and demand results in a profit reduction. Since production cost is high for this system, price drop caused by overproduction results in operating losses for many fish farmers. Big losses may discourage some of them and thus cause a shift to other businesses. Overproduction and price drop clearly cause damage to the milkfish industry.

Moreover, the younger generation in Taiwan, like the younger generations of most other countries, refuses to consume bony fish. The bony milkfish therefore is not considered as a high class fish. Various food processing methods, such as bone removal, canning, smoking, and pickling, may be worth testing to encourage consumption of milkfish and develop a tourist fishery for overseas visitors. The potential problems of market shrinkage and overproduction demand more studies on production, processing, and marketing of milkfish.

CONCLUSIONS AND RECOMMENDATIONS

Milkfish has been, and is seen, as a very important culture species in Taiwan. It is a desirable culture species for the tropical and subtropical areas, and not only is it an important food fish, but it is also a good baitfish for the tuna longline fishery. It has the potential to help solve the problem of animal protein deficiency in Third World Countries.

Although the present production satisfies the market demand in Taiwan, improvement of the technology for milkfish culture

is still desirable. For the further progression of the milkfish industry, recommendations are listed as follows:

1. The establishment of artificial propagation, including the technology for spawner breeding, hormone manipulation, and larval rearing, will help solve the problem of seed deficiency.

2. Studies on the aquaculture engineering of milkfish ponds need to be encouraged. In exploiting the merits of the shallow-water and deep-water ponds, a new pond system may be developed. A method where ecological principle can be fully applied with high unit area productivity probably is the most desirable system.

3. Studies on selective breeding and genetic engineering of milkfish to develop quality strains are advisable.

4. Studies on socio-economic evaluation are necessary to coordinate production and marketing. Studies on processing and marketing are necessary to promote consumption of milkfish. Educating the people about the value of milkfish may ensure a future market for milkfish culture. Promotion of the concept, "a bony fish is not necessarily a bad fish," will encourage the people to enjoy more species of fish, especially the delicious milkfish.

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**MY EXPERIENCE IN ARTIFICIAL PROPAGATION OF MILKFISH -
STUDIES ON NATURAL SPAWNING OF POND-REARED BROODSTOCK**

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ABSTRACT

A broodstock of 110 10- to 11-year-old milkfish was reared with a formulated diet. The body weights ranged from 3.0 - 7.5 kg. The dimension of the three broodstock ponds was 750 m² or 1,000 m² by 1.3 m - 1.5 m in depth.

The natural spawning and fertilization of eggs occurred 62 times between April 8 and September 21, 1984. The number of eggs collected from all of the spawns were estimated to be 61,836,000. Fertilization rates from individual spawning events ranged from 10% to 95%. The total number of hatched larvae from all of the spawns was estimated at 27,765,000. After a rearing period of 13-24 days, approximately 4.4 million fingerlings, ranging from 1.0-1.6 cm in length, were obtained.

Larvae were reared in outdoor rearing ponds. The highest survival rate was estimated at 76.2% with an average of approximately 17.7%.

Maturation, spawning, and massive production of larvae was shown to be feasible and practical for milkfish reared in outdoor earthen-bottom ponds.

INTRODUCTION

After decades of research by scientists and aquaculturists from many different countries, the basic techniques for artificial propagation of milkfish is primarily established. Vanstone et al. (1977) and Chaudhuri et al. (1977) succeeded in inducing the breeding of wild spawners by hormonal treatments. Artificial spawning, fertilization, and hatching were first

accomplished with wild spawners. Liao et al. (1979) reported the production of 2,859 fry by induced spawning of spawners captured at sea. Juario et al. (1979) and Kuo (1982) also reported successful production of milkfish fry with a good fertilization rate. Hsiao and Tseng (1980) achieved induced spawning by using broodstock from rearing ponds. Success in breeding pond-reared milkfish was also demonstrated in 1982 and 1983, producing 120,000 and 503,000 hatched larvae, respectively (Lin 1982; Lin 1984).

Natural spawning and fertilization of milkfish in my fish ponds was first discovered on October 6, 1983, indicating the possibility of natural spawning in a captive environment. Controlled environment and artificial feed are capable of allowing gonadal maturation of spawners. The feasibility of large-scale larval production was demonstrated in the three experimental ponds in which natural breeding occurred in 1984. The successful rearing of larvae in outdoor ponds simplified the procedures of artificial seed production of milkfish. This is an important technological breakthrough in the artificial propagation of milkfish.

MATERIALS AND METHODS

1. Pond size and stocking number (Nov. 1983 - Sept. 1984).

Data for the three experimental ponds are listed in Table I.

2. Management of pond water

Filtered seawater was pumped through the coastal sandy

Table I Surface area and stocking number of the three experimental ponds

Pond	Surface area (m)	Water depth (m)	Stocking number	Sex ratio ♀:♂	Age	Body weight (kg)
A	25 x 30	1.30	30	1:1	11	4.5-7.5
E	25 x 30	1.50	30	1:1	10-11	4-6
F	30 x 34	1.50	50	1:1	10-11	3-5

layer. During the culturing period of the broodstock, water color was green. When transparency of the water dropped to less than 20 cm, a large quantity of water was changed. The water also had to be changed after a heavy rain. Salinity of the water was maintained at 29-39 ppt. Paddle wheel aerators were used to increase and dissolve oxygen and maintain circulation of the water.

3. Feed

The feeds used were rice bran, wheatmeal, soybean meal, and formulated eel feed.

4. Source of broodstock

Wild fry with total lengths of 1.2 - 1.5 cm were caught off the Southern coast of Taiwan in 1973 and 1974. They were poly-cultured with shrimp and then monocultured in milkfish ponds in 1976. They were inspected for gonadal development and milkfish with gonads were selected as broodstock. The broodstocks were distributed among three ponds in 1983.

5. Estimation of egg number

Beakers were used to sample hydrated eggs for counting. The average of several samples was obtained as the standard count to estimate the total egg number.

6. Larval-rearing ponds

a. Indoor ponds: 3.5 x 4.5 x 1.0 m

b. Outdoor ponds: modified shrimp ponds at sizes from 150

to 3,000 m².

All rearing ponds were equipped with an aeration system.

RESULTS

1. Natural spawning of broodstock

The water quality of the broodstock ponds was well-maintained from January to March 1984. So, no massive water exchange was performed. Salinity increased to 36-39 ppt because of evaporation. Water temperature was recorded to be above 25°C in the mornings. From April on, lower salinity was maintained by frequent water changes or rain. The spawning conditions from the three ponds were as follows (Fig. 1):

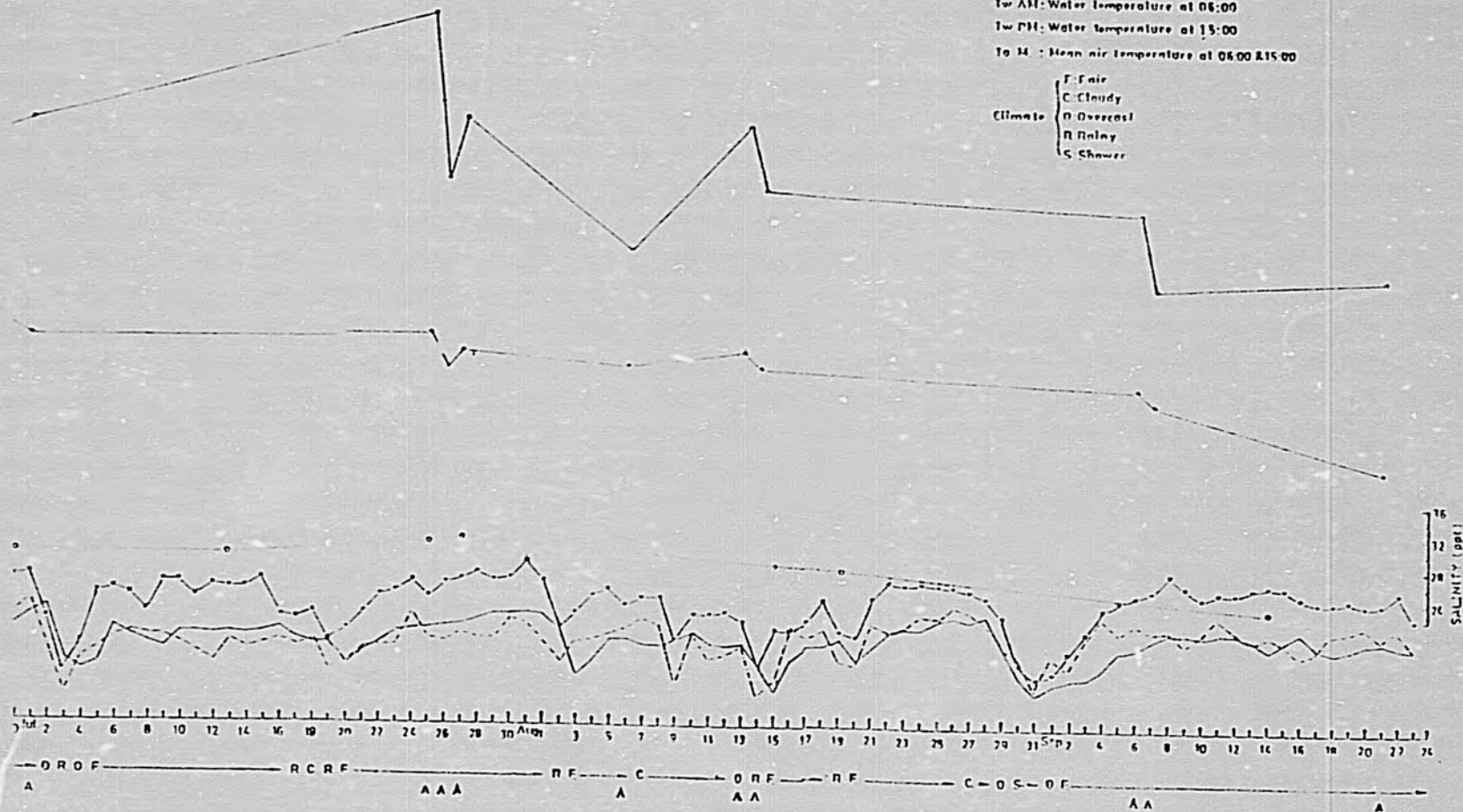
a. Pond A

Spawners began circulating along the sides of the pond and the feeding activity decreased from April 5, 1984. Based on the developmental stage of the eggs, first spawning was estimated to be about 3 a.m. on April 8. Eggs were suspended in water. As water was circulated by paddle wheel aerator, eggs were collected by a small set net. A total of 500,000 eggs were collected. The average fertilization rate was 55%, resulting in 210,000 hatched larvae. After 24 days, 54,800 fry with total lengths of 1.0 - 1.65 cm were produced.

The second spawning occurred on April 29. Thereafter, frequent spawning took place. The number of eggs collected and the fertilization rate increased gradually. The peak of the spawning period was between May and June. Reduction in spawning

To AH: Water temperature at 06:00
 To DH: Water temperature at 15:00
 To M: Mean air temperature at 06:00 & 15:00

Climate
 F: Fair
 C: Cloudy
 O: Overcast
 R: Rainy
 S: Shower



Designed & drawn by Chang, L. C.
 Tung Shing Aquaculture Hatchery

frequency began after July, and the last spawning was on September 21. During the later stage of spawning, both quantity and quality of the spawned eggs decreased. There were 33 spawnings with 39,560,000 eggs collected in this pond (Table II).

b. Pond E

The pond water was very clear throughout the year because of proper biological treatment (filtration of water by mussels). First spawning was discovered under strong sunlight at 10-11 a.m., April 15, and 400,000 eggs were collected. The fertilization rate was only 20%. A total of 20,000 fry were hatched and produced. The second spawning occurred on May 3. Both egg quantity and fertilization rate increased gradually thereafter. The frequent spawning period ended on June 29. The total number of spawnings was 23 with 16,670,000 eggs collected (Table III).

c. Pond F

The broodstock in pond F were small and their condition was not as good as those of the two other ponds. Spawnings occurred between May 15 and June 19. Total number of spawnings were six, with 5,606,000 eggs collected (Table IV).

Depending on the egg diameter, which varies greatly, a liter of hydrated eggs usually numbered $700,000 \pm 100,000$. In 1984, the total number of eggs collected from the three experimental ponds was 61,836,000. Of these, 6,700,000 eggs were given to other aquaculturists. The remaining 55,136,000 eggs hatched into

Table II Continued (II-1)

Spawning time	Weather ^a	Water temp. (°C)	Salin. (ppt)	Egg diameter (mm)	No. of eggs (1,000) ^b	Fert. rate (%)	Fry hatched (1,000)	Hatch. rate (%)	Fry produced	Surv. rate (%)	Pond area (m ²)	Rear- ing days	TL when harvest	Remark
Jun. 4, 2:00	R/F	30-30.9		1.15-1.21	700	92	600 ^d							water change after hatching, aeration inadequate, morning of June 5: all died
Jun. 9, 3:00	F	29.9-32.7	30.6	1.12-1.19	850	82	600	86						released
Jun. 10, 0:30	F	29.7-32.1	30.7	1.14-1.18	1,870/1	88.2	1,000		263,475	26.8	1,000	11	11-14.5	June 24: DH
Jun. 11, 1:00	F	30-33.1		1.15-1.2	900	60	50 ^d							larvae used for dirt expt.; fert. eggs stop develop. at late stage, hatching rate low
Jun. 13, 2:00	F	30-32	30.75	1.15-1.21	1,540/1	85.7	1,140	86.3	446,520	39.2	700	16	10-15	
Jun. 20, 2:30 3:00	F/S	29-31.9	29.2	1.15-1.22	2,640/2	30	1,550	73.1	360,930 ^d	20.6 ^d	500 ^d	15 ^d	11-14.5 ^d	July 6: DH
Jun. 21, 2:00	F	30-31.8		1.1-1.18	250									for expt.
Jun. 22, 1:00	F	29.6-32	29.2	1.1-1.2	2,200	80	600	34	99,810	16.6	200	15	12-15	July 8: DH
Jun. 23, 3:00	F	30-32		1.14-1.2	2,300	61								fert. eggs released
Jun. 24,	R	29-30.7			few									few; typhoid attacked, eggs not collected
Jun. 29, 3:00	F	29-32.6	29.4	1.15-1.2	600	90								fert. eggs released
Jul. 1, 2:00	F	30.7-33.2		1.1-1.18	660	80	200							larvae released
Jul. 25, 0:30	F	30-32		1.15-1.21	2,200	80	150	55	603,140	63.5	300 150			Aug. 9: DH 950,000 = 600,000 - 300 m ² , 457,340, 76.2% (surv rate); 350,000 - 150 m ² 145,800, 41.7% (surv. rate)

Table II Continued (II-2)

Spawning time	Weather ^a	water temp. (°C)	Salin. (ppt)	Egg diameter (mm)	No. of eggs (1,000) ^b	Fert. rate (%)	Fry hatched (1,000)	Hatch. rate (%)	Fry produced	Surv. rate (%)	Pond area (m ²)	Rear- ing days	TL when harvest	Remark	
Jul. 26	F	30.2-32.9	30.7	1.15-1.2	330	60									fert. egg released
Jul. 27, 1:00	F	30.2-33.1		1.15-1.2	660	70	320	70	163,790	51.2	250	17	11-15		fert. eggs moved to fish pond for hatching
Aug. 6, 2:00	F	29.5-32		1.13-1.18	150	60	70	67							for diet expt.
Aug. 13, 0:30	F	29.1-30.5		1.1-1.18	600	70	60	0.14							most fert. eggs died, hatching poor
Aug. 14, 1:00	R	27.9-28		1.1-1.18	300	60									fert. eggs moved to NKIMT for expt.
Sept. 6, 4:00	F	29-32.4			250	50	20	16							fert. eggs died
Sept. 7, 3:00	F	29.6-32.6	22.3		100	40									fert. eggs died
Sept. 21, 6:00	F	29.6-32			120	0									almost all cloudy eggs fert. failed

Key for Table II, III, IV

a: weather: F = fair; C = cloudy; O = overcast; S = shower; R = rain

b: eggs collected/estimated number of females spawned

c: number is the total or average count of Apr. 29 and Apr. 30 (pond A)

d: number is the total or average count of pond E and pond A

e: number is the total or average count of May 6 and May 7 (pond E)

f: number is the total or average count of pond E and pond F

Table III Record on natural spawning and larval rearing of milkfish in Pond E, Tung Hsing Fish and Shrimp Hatchery in 1984

Spawning time	Weather ^a	Water temp. (°C)	Salin. (ppt)	Egg diameter (mm)	No. of eggs (1,000) ^b	Fert. rate (%)	Fry hatched (1,000)	Hatch. rate (%)	Fry produced	Surv. rate (%)	Pond area (m ²)	Rear- ing days	TL when harvest	Remark	
Apr. 15, 10:00-10:30	O/F	25.2-27	40	1.10-1.15	400	20	20	25	0	0					
May 3, 0:30	O/F	27.8-30.1		1.16-1.2	600	50	200	66.7	33,520	41.8	16 x 2	22	10-16	May 26: DH	
May 4, 2:00	F	28.2-30.7		1.1-1.2	700	60	600 ^d	66.7 ^d	228,000 ^d	39 ^d	600 ^d	19 ^d	11-14.5 ^d	May 24: DH; reared in outdoor ponds	
May 6, 2:30	F			1.15-1.2	500	20	30								
May 7, 2:20	F	27.7-29	36.5	1.15-1.21	1,600/1	80	200	71.4	47,000 ^c	20.4 ^c	300 ^c	17 ^c	11-14.5 ^c	rest. eggs 280,000 reared; 1,120,000 released	
May 14, 1:00	F	30.1-32.1	38		1,000	89	670	75.2	212,090	31.7	200	21	10-13.5	June 5 and 6: DH	
May 15, 0:10 2:10	F	29.9-33			3,520/2	85	2,520		381,880	19	1,500	16	11-14	360,000 hatchlings released	
May 16, 0:40	C/O	30.1-30.3		1.18-1.2	550	90	380							larvae released, eggs 700,000/1	
May 25, 0:20	F	28.4-31	36.7	1.12-1.21	1,500	80	975							larvae released	
May 26, 0:30	F	28.7-31.9		1.14-1.21	1,820/1	85	1,200	77.6			400			heavy rain destroyed all larvae in outdoor ponds	
May 26, 23:00 (May 27)	O	29.2-30.7		1.15-1.2	800	80	540	84.3		30-40	16 x 3			released, rest reared indoor	
May 28	S	28.5-28		1.15-1.2	500	60	150		3,810 ^d					May 29: fry released 15,000; pond water salin. only 19 ppt, raise to 24 ppt by sea-water; low surv. rate	

Table III Continued

Spawning time	Weather ¹	Water temp. (°C)	Salin. (ppt)	Egg diameter (mm)	No. of eggs (1,000) ^b	Fert. rate (%)	Fry hatch ^d rate (1,000)	Hatch. rate (%)	Fry produced	Surv. rate (%)	Pond area (m ²)	Rear- ing days	TL when harvest	Remark
Jun. 2	F	29.1-32.4		1.14-1.2	660	67 ^d	430 ^d		139,790 ^d	32.5 ^d	300	16	11-14.5	June 19: DH
Jun. 3	S/F	29.9-32.3		1.14-1.2	450		50 ^d							
Jun. 4	R/F	30-30.9			100	80	600 ^d							water change after hatching, aeration inadequate, all died the morning of June 5
Jun. 9, 2:30	F			1.14-1.18	250	85	150	75						released to Mr. Lee
Jun. 10, 2:00	F	29.7-32.1		1.14-1.18	330	70	100							
Jun. 11, 1:30	F	30-33.1		1.14-1.18	200	60	50 ^d							larval used for diet expt.; fert. eggs stopped develop. at late stage, hatch. rate low
Jun. 15	F/S	29.9-30.9	31		300	80	50	20.8	140,000 ^f	56 ^f	300 ^f	15 ^f	11-14.5 ^f	July 1: DH
Jun. 20, 2:00	F/S	29-31.9		1.10-1.2	440	75	200	65	360,930 ^d	20.6 ^d	500 ^d	15 ^d	11-14.5 ^d	July 6: DH
Jun. 21, 1:00	F	30-31.8		1.15-1.18	150									for expt.
Jun. 23, 1:00	F	30-32		1.1-1.2	200									fry released
Jun. 29, 0:00	F	29-32.6			100	70								fert. eggs released

Table IV Record on natural spawning and larval rearing of milkfish in Pond F, Tunq Hsing Fish and Shrimp Hatchery in 1984

Spawning time	Weather ^a	Water temp. (°C)	Salin. (ppt)	Egg diameter (mm)	No. of eggs (1,000) ^b	Fert. rate (%)	Fry hatched (1,000)	Hatch. rate (%)	Fry produced	Surv. rate (%)	Pond area (m ²)	Rear- ing days	TL when harvest	Remark
May 15, 3:00	F	29.9-33			2,420/2	10								all cloudy eggs, discarded
May 26, 0:10	F	28.7-31.9		1.15-1.2	2,200/2	80	1,400	79.5			500			heavy rain destroyed all larvae in outdoor ponds
Jun. 5, 1:30	R/O	28.9-29.2			few									
Jun. 6	F/S	27.2-29.9			286	56	40	25						No. of eggs too few, not collected
Jun. 15	F/S	29.9-30.9			500	65	200	47	140,000 ^f	56 ^f	300 ^f	15 ^f	11-14.5 ^f	July 1: DH
Jun. 19	F	30-31.4			200	60	40	33.3	6,830	17	16	18	10-14.5	reared in indoor concrete tanks

27,765,000 larvae. Out of this number of hatchings, 2,980,000 were distributed to other aquaculturists. The remaining 24,785,000 larvae were reared in the indoor or outdoor ponds. After 13-24 days, 4,388,285 fry with 1.0 - 1.85 cm total length were produced. The average larval survival rate was 17.7% (Fig. 1; Table II, III, and IV).

2. Larval rearing

Results of the experiments in 1983 showed that newly-hatched larvae are capable of surviving in outdoor ponds despite strong direct sunlight or rain (Lin, 1984). Hence, most larvae were reared in outdoor ponds with a small number in indoor ponds in 1984. The result of the rearing is as follows:

a. Indoor ponds

Stocking density was maintained at 4,500-6,000/m². The larvae grew to a marketable fry size (TL 1.0-1.5 cm) in 24 days in water temperatures of 25.9-28°C. When the water temperature was between 30-32°C, only 17-18 days were needed. Fry quality was poor and a variety of sizes was obtained. The total survival rate was between 17% and 41.8%.

b. Outdoor ponds

Stocking density was maintained at 1,500-2,000/m². The larvae grew to marketable fry size in 21 days at water temperatures from 26.1-30.5°C. Only 13-15 days were needed when the water temperature was 28-33°C. The average duration of larval rearing was 15 days. The fry were uniform in size and in

very good condition. The highest survival rate was 76.2% with an average of 17.7%. Heavy rain killed all the hatched larvae from the May 26 spawn.

DISCUSSION AND CONCLUSION

The reproductive biology of milkfish has always been an important topic for research. A large amount of money and effort have been expended on broodstock production by either harvesting wild spawners or culturing adult fish. The techniques of hormone-induced maturation and artificial propagation developed in recent years contributed mainly to the knowledge of the academicians. However, large scale commercial seed production is still an unrealized goal. The bottleneck is the availability of suitable matured spawners, which restricts the reproductive research of milkfish.

Milkfish spawners (age 9-10) in my experimental ponds were injected with hormone nine times, which resulted in six induced spawnings in 1983. Although more than 500,000 larvae were hatched, only about 80,000 survived to the fry stage (Lin, 1984). However, the percentage of spawners reaching maturation in captive environments has increased. Spawners were discovered to spawn naturally in ponds on October 6 of the same year. Propagation of milkfish therefore was simplified.

The frequency of natural spawning and number of eggs collected in 1984 were remarkable, indicating that an artificial captive environment is not necessarily restrictive to the

maturation and propagation of milkfish. Moreover, the reproductive cycle of milkfish is better understood.

Upon inspection of the fertilized eggs collected, development of each hatch of eggs was found to be uniform. The spawning activity of the milkfish can therefore be determined to occur in a very short time. However, several days of courtship and chasing took place before and after the spawning. According to the examination of gonadal tissues, diameter, and developmental stages of fertilized eggs, spawning of each milkfish in the pond seems to be divided into several different times. Each spawning usually was performed by a single female. Occasionally, two to three fish spawned together in one day. The frequency of two or three days of consecutive spawning was high in the same pond. Pheromone(s) released into the water during the spawning of the fish probably stimulated the spawning of other females. Thirty-three spawnings occurred in pond A with only about 15 females (Table II). Also, 23 spawnings occurred in pond E with a similar number of females (Table III). This fact indicated the possibility of several spawnings by a single female in a spawning season (April to September). Whether a female finished her spawn in several consecutive days, or eggs developed rapidly in the ovary after the first spawn is still uncertain. More studies are necessary to understand the spawning behavior of milkfish.

The consecutive spawnings indicated that gonad maturation of

milkfish was not uniform among the stock. According to the spawning frequency and quantity of eggs collected, the spawning period began in April, with the peak in May to June, and ended in August to September. Water temperature and weather were factors affecting the spawning period. This spawning period of pond-reared milkfish corresponds with the fry collecting season along the coast of Taiwan. It is remarkable that spawners from different sources spawn during the same season.

Fertilized eggs from natural spawning were collected for large-scale production of fry. The set-net employed was very efficient in collecting all the eggs within two to three hours without disturbing the spawners in the ponds.

The adaptability of milkfish fry is high. Large-scale production of fry can be achieved in small concrete tanks or large outdoor ponds with simple management. The fry produced were supplied to milkfish grow-out ponds in Southern Taiwan in 1982, 1983, and 1984. In comparison, the fry's survival rate, growth rate, and ability to resist cold temperatures were proven to be better than that of wild fry. Therefore, the economic value and potential of pond-produced fry are expected to be high.

During the preparation of this report (April to May 1985), the same stock of broodstock began to spawn consecutively. The number of eggs spawned was directly proportional to an increase in body weight of the spawners. The improved larval-rearing

technique resulted in a better larval survival rate.

With well-organized commercial management of the larval production industry, wild fry may be replaced by hatchery-produced fry as the major source of milkfish seed in Taiwan in the future.

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MY EXPERIENCE IN TRADITIONAL MILKFISH CULTURE

By

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The traditional shallow-water milkfish culture is practiced according to the seasons in the Chinese Agricultural Calendar. There are 24 "seasons" in a year, with each season lasting 15 days. The first day of each season is given a name to describe the weather or a natural condition associated with it.

"Spring begins" (February 4) marks the beginning of the working year. During this period, the ponds are prepared. This is followed by fry stocking, which begins at "Brightly clear" (April 5). Overwintering fry are the first to be stocked, and new fry are stocked after "Sowing rain" (April 20).

Depending on the growth of the fish and the prevailing market situation, fish may be harvested during the period from "Summer solstice" (June 21) to "Hoar frost" (October 23). Overwintering ponds are prepared between "Autumn begins" (August 5) and "Cold Dew" (October 8). At Hoar frost, fish that are ready for overwintering are placed in their respective overwintering ponds. All preparations are completed before "Winter solstice" (December 22).

Improvement of the traditional practices of shallow-water milkfish culture are discussed. Stocking density and fish production have fluctuated annually, and increases in production have resulted in the drop of fish prices. Hence, the management of milkfish culture should be adjusted accordingly. Suggestions focused on the improvement of the marketing and processing of the milkfish are presented.

During the several hundred years' history of milkfish culture in Taiwan, a special yearlong routine of management has been established. This traditional management method was developed through the personal experience and talents of the fish farmers. In order to improve this system for modern situations,

a thorough understanding of the system is necessary. This paper introduces the general practice of this traditional milkfish culture system in Taiwan.

The Taiwan fish farmers follow the Chinese Agricultural Calendar for the culture practice. There are 24 "seasons" in a year. Each "season" lasts for 15 days, resulting in a 360-day year. Names are given to the first days of the seasons and describe the weather, natural condition, or recommended agricultural practice at the particular time of year.

Brackish or sea water in shallow ponds are used in the traditional practice. The Tainan and Kaohsiung areas in southwestern Taiwan are the locations for most of the traditional milkfish culture grounds.

The working year begins on "Spring begins" (Feb. 4), or after the Chinese New Year. When the weather warms, stocking preparations begin. During the 60-day preparation period, good conditioning of the ponds has to be developed. Hence, debris and disintegrated benthic algae in ponds and ditches are removed, with the material possibly being used to repair eroded and damaged dikes. Fertilizer, which often consists of rice bran, is applied if the bottom soil is meager. When fertilization is adequate, a rich layer of benthic algae can be grown on the bottom of the pond.

As "Brightly clear" (April 5) approaches, fingerlings from overwintering ponds are transferred to grow-out ponds. After

stocking all the large and medium overwintered fingerlings (50-100g BW/fish and 10-50g BW/fish), small overwintered fingerlings (3.5-10g BW/fish) may be stocked, if necessary, depending on the availability of pond area.

The first group of new fry is collected for rearing for the next year beginning at "Sowing rain" (April 20). Fry of all groups of stocking for the following year are purchased or collected at "Grain fills" (May 21) to "Grain in ear" (June 6).

New fry collected this year are stocked for rearing into juveniles for next year's first and second stocking at the period from "Summer solstice" (July 21) to "Mild heat" (July 7).

The busiest time of the year comes at "Autumn begins" (Aug. 15), when preparation of overwintering ponds begins. Some of the work involved include cleaning and replacing of old bamboo, removing debris in ponds and ditches, and fertilizing and sunning of ponds. All of the work should be done before "Cold dew" (Oct. 8).

All the overwintering fish are transferred into the overwintering pond at "Hoar frost" (Oct. 23) to prevent mass mortality caused by the freezing Northerly wind.

At "Drizzly snow" (Nov. 23), the overwintering pond should be stocked with overwintering fish, and all dead algae floating at the corners of the grow-out ponds should be sunk to the bottom. With this, all the work for the year is finished and a ceremony is performed to mark the occasion.

"Winter solstice" (Dec. 22) is sometimes considered the beginning of the new working year, with the first job being the preparation of bamboo for the replacement at mid-autumn. Sunning of the pond bottom also is performed. During the period from "Mild cold" (Jan. 5) to "Severe cold" (Jan. 20), outbreaks of fish disease are common. Precaution, therefore, should be taken at this time. Table 1 summarizes the yearlong routine of traditional management.

In recent decades, the stocking rate and productivity of milkfish culture have changed tremendously. Table 2 summarizes the variation of stocking rate and productivity of milkfish culture in Taiwan during the last 40 years. The cost for fry was high as 6,000 more fry were needed 40 years ago and 4,000 more fry at 20 years ago in stocking. The cost for fertilizer and management also was higher at those times. Progress in recent years has reduced the production cost and increased profitability.

Improved culture techniques and fertilizer have caused a tremendous increase in production, and the expansion of culturing acreage and the increase in production have resulted in competition among fish farmers. Therefore, the price of fish actually has been reduced. In order to adjust to this situation, management needs to be altered. So far, reduction in stocking rate has reduced cost in seed and other areas.

The popularity of the milkfish has been on the decline in

Table 1 Year-round routine of traditional management on milkfish culture

Season	Stocking	Harvest	Works to be done
Mild cold (Jan. 5) Severe cold (Jan. 20)			Taking precaution for outbreak of disease
Spring begins (Feb. 4) First rain (Feb. 19) Bugs awaken (Mar. 5) Vernal Equinox (Mar. 21)			<ol style="list-style-type: none"> 1. Sunning, fertilization 2. Removal of debris in ditches, repairing of dikes 3. Preparation of nets and tools 4. Getting ready for stocking
Brightly clear (Apr. 5) Sowing rain (Apr. 20) Summer begins (May 5)	Stocking of over-wintered juvenile First stocking of new fry		
Grain fills (May 21) Grain in ear (Jun. 6)	Second stocking of new fry		
Summer Solstice (Jun. 21) Mild heat (Jul. 7) Severe heat (Jul. 23)	Distribution for the first group of new fry	Harvest begins depending upon the growth of the fish and the prevailing market situation	
Autumn begins (Aug. 7) Limit of heat (Aug. 23) White dew (Sept. 8) Autumnal Equinox (Sept. 23)			<ol style="list-style-type: none"> 1. Changing bamboo for overwintering ponds 2. Clearance of overwintering ponds from debris, sunning, and fertilization 3. Soil management 4. Elimination of dead algae
Cold dew (Oct. 8) Hoar frost (Oct. 23)	Placing fry for the first two seasons next year into overwintering ponds	Harvest period ends	
Winter begins (Nov. 7)			
Drizzly snow (Nov. 22) Severe snow (Dec. 7)			<p>Finishing all works Celebration</p>
Winter Solstice (Dec. 22) Mild cold (Jan. 5) Severe cold (Jan. 20)			<p>Time for preparation of bamboo</p> <p>Selection of suitable bamboos for the use of overwintering ponds next year</p> <p>Burying bamboos to improve durability</p>

Table 2 Stocking rate and production per unit hectare of traditional milkfish culture ponds

	Stocking rate (fish/ha)	Average harvesting size (kg)	Production (kg)	Market price (NT\$/kg)
40 years ago	10,000	0.15	1,500	16.67
20 years ago	8,000	0.20	1,560	33.33
Recently	4,000	0.40	1,560	58.33-66.67

recent years. The traditional breakfast of milkfish soup has been replaced gradually by "convenient foods," and the bony milkfish has discouraged the new generation from consuming this high-quality food fish. To prevent the potential problem of market shrinkage, improvement in processing and marketing is necessary. One way to improve the situation is to employ the bone-removing machine currently being used in the Philippines.

In conclusion, traditional management of milkfish culture needs to be improved and adjusted to suit the modern society.

DEEP-WATER POND SYSTEM FOR MILKFISH CULTURE

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ABSTRACT

For several hundred years, milkfish culture in Taiwan has been performed by building shallow ponds (water depth 30cm) along the coast. Seawater is introduced into the ponds and benthic algae are grown by application of rice bran as fertilizer and sunlight.

An improved modern culture system recently has been developed by taking advantage of the euryhaline characteristics of milkfish. Fresh water is used to culture the fish in 2m deep ponds. Aerators and artificial feed are applied. Hence, the unit stocking density is allowed to increased to 3 to 4 times. This modern culture method has proven to be a great success.

INTRODUCTION

Although milkfish culture in Taiwan has been utilized for several hundred years, the deep-water pond culture system has been in use for only about a decade. The improved modern system, however, increases the milkfish production tremendously.

According to the statistics of the Taiwan Fisheries Bureau, the total production of milkfish in 1976 was 26,800 metric tons, of which 26,600 metric tons were produced in shallow-water ponds. Only 200 metric tons were produced by the deep-water pond culture systems. However, in 1983, out of the total production of 37,000 metric tons, 9,000 metric tons were produced by the deep-water pond culture methods. Production in shallow-water ponds has achieved almost no growth at 28,000 metric tons, while the deep-

water pond system has accomplished a growth of 45 folds.

Factors initiating the use of the modern method are as follows: 1) Milkfish is the most popular food fish in Taiwan. 2) The development of artificial diet for milkfish and the automatic feeder save time and labor. 3) The expected profit is higher than that in culturing other species.

Most of the ponds for this culture method were modified by adding paddle wheel aerators and automatic feeders to freshwater ponds (water depth 2 meters). Some of the ponds were developed by excavation and division of traditional ponds (water depth 30cm, 4-5ha per pond) into 1-ha deep ponds, with the addition of automatic equipment.

Because of the increase in depth and volume of water, as well as the aeration equipment, the unit stocking rate was raised from the traditional 6,000-8,000 ha/yr to 20,000-25,000 ha/yr. The annual production, therefore, increased from 2,000-2,500kg to 8,000-12,000kg.

In order to illustrate the management practice of the deep-water pond culture system, the production procedures of a deep-water milkfish farm (four 1-ha ponds) are described in the following.

PREPARATION

1. After harvesting at the beginning of the year, sunning of the ponds is performed. No fertilization is needed, but lime is applied to eliminate benthic pests. The bottom and the walls

of the ponds are flattened by a tractor every other year.

2. Two paddle wheel aerators and one automatic feeder are installed in each pond.

Stocking

After "Brightly clear" (April 5), ponds are stocked with 1.5cm fry at a rate of 12,000/ha. The second day after stocking, a small amount of artificial diets (prescribed for fingerlings) are used to domesticate the fry for feeding. After three to four days, feedings can be done regularly.

MANAGEMENT

1. Feeding is done in the early morning and afternoon. The feed is placed in the automatic feeder, which can adjust the rate and time of feeding. The bottom of the feeding area is monitored one hour after feeding to determine the optimum feeding quantity. Fish are caught once or twice every month to measure the body weight, which also is a good guideline in determining feeding quantity.

2. Ponds are checked every day at 11 p.m. and 3 a.m. to prevent oxygen depletion.

3. When the fish grow to 200g, the artificial diet for growout should be used.

HARVEST

1. In late July-early August, at least one thinning harvest is needed to adjust the density in the pond. The second batch of fry is also stocked at this time. Fish from the first stocking

should have been harvested in late August so that fry from the second stocking can attain optimal growth.

2. Harvest is done by gill net.
3. Most fish weigh approximately 500g when harvested.
4. With the above-mentioned stocking density and management, 10 tons can be harvested each year.

In the months following November, it becomes very cold in Taiwan. Most milkfish cultured in shallow water ponds are transferred to over-wintering ponds. From November to May, the price of milkfish rises, resulting in a good opportunity for fish farmers who use the deep-water pond culture system to earn high profits. But vibriosis and the cold weather, caused by a Siberian cold front, can cause mass mortality. Measures to reduce loss caused by this problem are needed. One solution could be the use of underground water to warm up the water during a cold spell. Another possibility would be an early harvest, which can be performed when signs of vibriosis is observed.

PREPARATION OF A LUTEINIZING HORMONE-RELEASING HORMONE
CHOLESTEROL PELLET AND ITS IMPLANTATION IN THE MILKFISH

(CHANOS CHANOS FORSSKAL)

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Milkfish in captivity for undetermined reasons usually do not proceed through their normal reproductive cycle and spawn naturally. This phenomenon is very common among fish that are reared in captivity. The environmental cues that normally mediate the reproductive activities of fish are not present in culture conditions. A variety of hormonal therapies have been employed to overcome the barriers that prevent the production of gametes and spawning in captive fish.

A common practice for inducing gonadal maturity in fish is to utilize a series of hormone injections such as described for the Japanese eel (Yamamoto, et al., 1974). Hormonal preparations usually consist of the desired chemical messenger being dissolved in a fluid, (i.e., normal saline, phosphate buffered saline, etc.) that is osmotically compatible with the internal environment of the recipient. However, when such a solution is injected it usually dissipates rapidly within the body and only provides a

surge in the amount of circulating hormone for a brief period (Crim and Evans, 1976, Crim, 1985). For the circulating level of the desired hormone is to be kept elevated, a series of injections is required. This procedure is relatively inefficient because it consumes time and it inevitably increases the stress on the individual because of the additional handling necessary for each injection. The "stress factor" has been reported to be one of the factors which can block the normal reproductive cycle of fishes (Billard et al., 1981). Obviously, an increase in stress would compromise the efficacy of any hormone treatment. This is especially true for the milkfish which appears to be very sensitive to handling. For example, oocytes from wild spawners undergo atresia if they are not given a hormone injection (either carp pituitary homogenate, salmon pituitary homogenate or human chorionic gonadotropin) within a few hours after capture (Juario et al., 1984; Kuo, 1985). Likewise, milt resorption is observed within 2 - 3 days of captivity (Juario et al., 1980). To make matters worse, wild milkfish usually do not survive the handling for more than 3 injections (Kuo, 1985). Some of these problems may be alleviated by using pond reared fish although many problems persist (Lam, 1984). Clearly, a procedure that can deliver the desired hormone at the required circulating levels and minimize the stress due to handling would be extremely advantageous.

Recently, there have been several methods developed which

address the problems associated with a multiple injection protocol. These new techniques employ the use of hormone pellets or pumps which release a particular quantity of the chemical message over a long period of time (Crim, 1985). A specific example of a slow releasing hormone pellet is one that incorporates the luteinizing hormone-releasing hormone, (LHRH), into a cholesterol matrix. This pellet, formulated by Kent et al., (1980), was designed to control the release of this neuropeptide in animals.

The LHRH's molecular structure has been described and subsequently followed by the production of synthetic LHRH analogues which are several to hundreds of times more potent than the native neuropeptide (Coy, et al., 1975). These LHRH analogues, (LHRH-A), have been pelleted and used successfully to advance the spawning period of the Atlantic salmon (Crim, et al., 1983a, Weil and Crim, 1983), and spring-spawning rainbow trout (Crim, et al., 1983b). When milkfish were treated with a similar LHRH-A cholesterol pellet, a higher percentage of gonadal maturation was reported in treated fish and approximately a month before its normal reproductive season (Lee, et al., 1985).

The following is a procedural guide to first, produce a cholesterol LHRH-A pellet, second, biopsy milkfish gonads to monitor changes in ovarian development, and lastly, implant the LHRH-A cholesterol pellet into the milkfish. The procedure is the result of the ongoing research focused on inducing gonadal

maturation in the milkfish conducted at the Oceanic Institute, Hawaii.

I. Preparation of pelleted hormone

Synthetic LHRH analogue, (des-Gly¹⁰-[D-Ala⁶]-LHRH ethylamide), was purchased from Sigma Chemical Company, USA. Two milligrams of LHRH-A was dissolved in 0.3ml of 50% ethanol. The solution was then mixed with 190 mg of cholesterol, (USP grade), until a paste-like consistency was obtained. The paste was then dried in an incubator set at 35°C. The resulting dried powder was then mixed with 10 milligrams of cocoa butter which serves as a binder. This particular step requires that the mixture be kneaded thoroughly with a wooden stick for the uniform production of pellets. Finally, the mixture is then packed into a pellet using a plexiglass mold. The resulting pellet produced weighs approximately 23 milligrams and has an average length and diameter of 5.5 millimeters and 2.4 millimeters respectively (Plate I). A single pellet that is produced in this manner contains 200 micrograms of LHRH-A. Each pellet is kept refrigerated in a 400 ml polyethylene microcentrifuge tube.

The dosage of the pellet may be varied in two ways. First, the LHRH concentration can be altered during the initial mixing of the bioactive peptide with 50% ethanol. The second is by changing the size of the cholesterol pellet. This requires that the plexiglass mold used to make the pellet be altered by simply changing the size of the hole in which the pellet is packed.

There is considerable flexibility offered to the investigator as far as altering the dosage of the pellet by taking advantage of either method or a combination of both. Characteristics of the pellet, i.e., releasing rate, longevity, shelf life, etc. are all topics that are currently under investigation.

II. Handling of milkfish

Milkfish are transferred from tank to tank by placing them in long plastic bags filled with seawater. Transferring milkfish in this manner minimizes the trauma to their mucous and epidermal layers. For the *in vivo* monitoring of their gonads and the implantation of the hormone pellets, milkfish are placed in a round fiberglass tank that contains seawater with the fish anesthetic 2-phenoxyethanol at a concentration of 0.3 milliliters per liter. When an individual fish becomes disoriented and goes "belly up", (after approximately 7 - 8 minutes), a series of operations are performed on each individual fish.

Step. 1. The fish are individually identified by a particular fin clip. The identification of each fish allows the investigator to follow the response of individuals to the hormone treatment. In addition, there is now evidence that milkfish spawn more than once during their spawning season (Lee et al., 1985, Lin, 1985). In light of this new information it becomes necessary to identify each fish in order to gain insight into some very basic questions such as, how often does a single female spawn throughout the season, what is the spawning frequency for

each individual female and how does the hormone treatment affect the rematuration process, etc.

Step 2. The stage of gonadal maturity for both males and females needs to be assessed. In the female, a sample of ova is retrieved by use of a polyethylene cannula which is inserted into the urogenital pore (Shehadeh, et al., 1973) (Plate II). With a little suction, a biopsy of the ovary can be obtained. Eggs that are obtained are then fixed in 10% formalin and their size frequency distribution is measured. By monitoring the qualitative changes in the eggs (see Plate III) and the changes in their size frequency distribution through time, one can determine the effects of the hormone pellets on ovarian development. For the mature male, pressure on the belly can cause sperm to ooze out of the cloaca. For a male in a lesser state of maturity, sperm needs to be cannulated, and for an immature male, no sperm can be obtained at all.

Step 3. If needed, a blood sample can be drawn from the caudal vein using a 6 cc luer lock disposable syringe equipped with a 1 1/2 inch 20 gauge hypodermic needle (Plate IV). Blood samples of 3 ml/fish/month have been routinely taken with no apparent adverse effects on the maturation of these individuals. The serum that is obtained from the blood samples provides an opportunity to monitor the *in vivo* fluxes in circulating hormone levels, quantities of soluble protein, qualitative changes in proteins, etc.

Step 4. The implantation of a cholesterol pellet containing LHRH-A begins by placing the pellet into an implanter equipped with an 11-gauge needle. The area of implantation is between the lateral line and the dorsal fin. At the implant site, a scale is removed with a scalpel handle and a very small incision (2-4 mm) is made with a sterilized scalpel blade. The needle of the implanter is pushed into the muscle via the small incision to a depth of about 2 cm (Plate V). The pellet is then injected into the muscle and the implanter is withdrawn from the tissue. The intramuscular location of the pellet is away from any of the vital organs and thus reduces the chance of serious injury during the implantation procedure. This is in contrast to placing the pellet within the peritoneal cavity. When placed in the peritoneal cavity, the pellet is in direct contact with the internal organs and is also free to move within the cavity (Plate VI). There have also been reports of pellet loss when placed in this particular part of the fish's anatomy (Crim, pers. comm.). With the particular implanter that is being used, there are no sutures required and the injection site heals completely (including the scale) within a month. The time required for the implantation alone is approximately 15 seconds. If an individual fish goes through all of the steps mentioned, the time to completion is about 10 minutes.

III. Recovery of the fish

After completion of the operation, the fish are placed into

their respective tanks. It is required that some assistance, in the form of fresh seawater passed over the gills for at least a minute, be given to revive the fish. The fish usually will assume a normal swimming posture within minutes after return to their tanks.

This procedure for the implantation of LHRH-A cholesterol pellet and the staging of sexual maturity has been performed on well over a hundred fish, and repeatedly conducted for four consecutive months with only the loss of three individuals. Fish recover very quickly and resume feeding within two days. In addition, preliminary results of the ongoing research for inducing maturation of gonads in milkfish using this procedure have been extremely promising (Lee et al., 1985).

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Plate I. A LHRH-A cholesterol pellet and the device used to implant the pellet into milkfish to induce maturation.

Plate II. An anesthetized milkfish undergoing a gonadal biopsy utilizing a polyethylene cannula. The biopsy is necessary to determine the state of maturation of the milkfish gonads.

Plate III. The egg of a milkfish retrieved via the cannulation procedure. This particular ovum is typical of a tertiary yolk globule stage, and measures 850 um in diameter. When the ova attain this stage, the female may be induced to spawn using the various techniques that are available. (Magnified 63x).

Plate IV. A blood sample being drawn via the caudal vein from an anesthetized milkfish. The average volume of blood taken is 3 ml per fish per month with no apparent adverse effects on the maturation of individuals.

Plate V. The intramuscular implantation of a hormone pellet.

Plate VI. An x-ray photograph of a milkfish showing the relative locations of intramuscular and intraperitoneal implants.

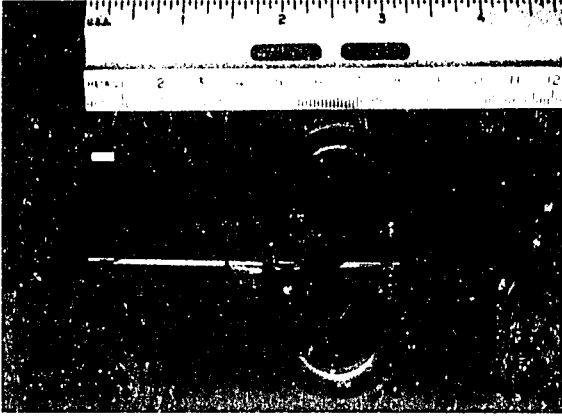


Plate I



Plate IV



Plate II



Plate V

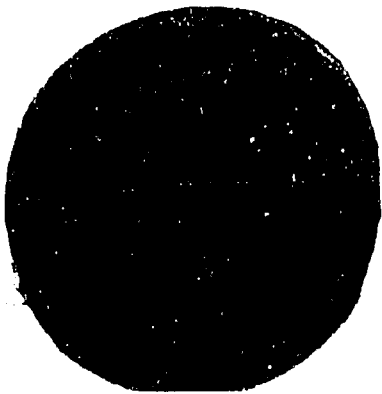


Plate III

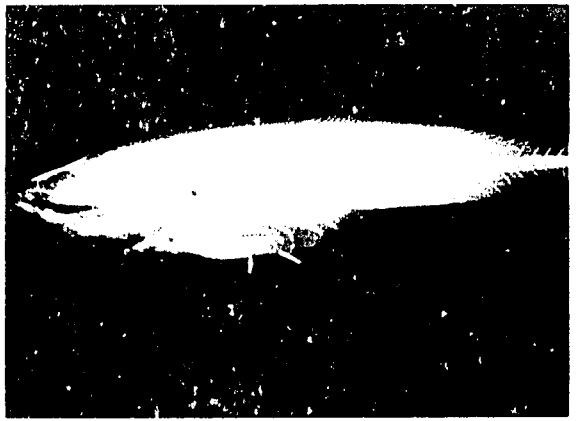


Plate VI