BIBLIOGRAPHIC DATA SHEET

PN-AAJ-075

REVIEW OF BREEDING AND PROPAGATION TECHNIQUES FOR GREY MULLET, MUGIL CEPHALUS L.

PERSONAL AUTHORS - NASH, C. E.
SHEHADEH, Z. H.

CORPORATE AUTHORS - INT. CENTER FOR LIVING AQUATIC RESOURCES MANAGEMENT

1980. 88P.
(IN ICLARM STUDIES AND REVIEWS NO. 3)

ARC NUMBER - 639.3758.N248
CONTRACT NUMBER - AID/DSAN-G-0174
PROJECT NUMBERS - 9311124
SUBJECT CLASS - AM4081000000

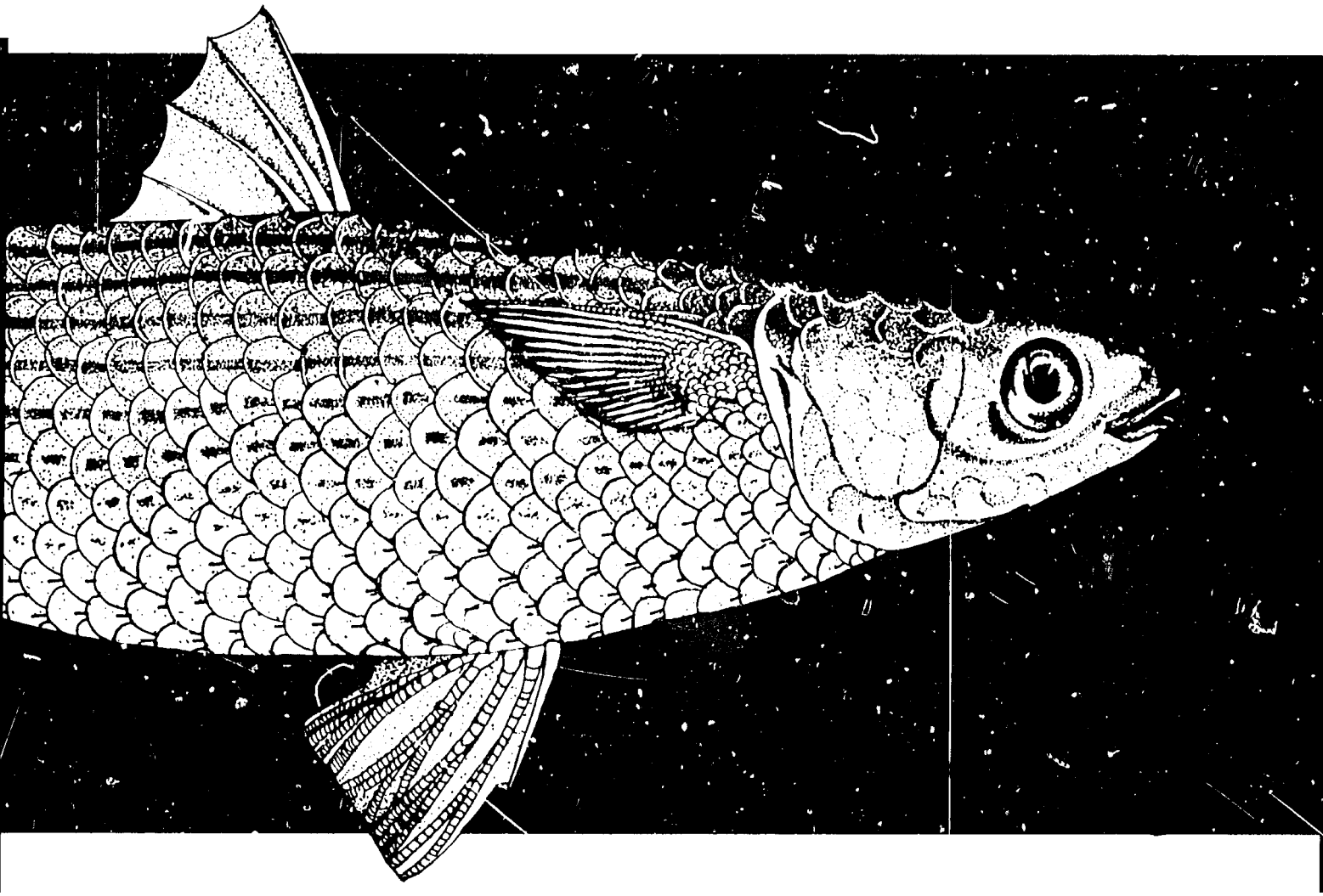
DESCRIPTORS -

FISHES	REPRODUCTION
AQUACULTURE	MULLET
BREEDING	HATCHERIES

ICLARM STUDIES AND REVIEWS 3

Review of Breeding and Propagation Techniques for Grey Mullet, *Mugil cephalus* L.

Edited by
Colin E. Nash
Ziad H. Shehadeh



ICLARM INTERNATIONAL CENTER FOR LIVING AQUATIC RESOURCES MANAGEMENT

Review of Breeding and Propagation Techniques for Grey Mullet, *Mugil cephalus* L.

Review of Breeding and Propagation Techniques for Grey Mullet, *Mugil cephalus* L.

Edited by

COLIN E. NASH
Kramer, Chin & Mayo Inc., Seattle, Washington, U.S.A.
and
ZIAD H. SHEHADEH
ICLARM

1980

INTERNATIONAL CENTER FOR LIVING AQUATIC RESOURCES MANAGEMENT
MANILA, PHILIPPINES

Review of Breeding and Propagation Techniques for Grey Mullet, *Mugil cephalus L.*

Edited by

C. E. NASH
Z. H. SHEHADEH

1980

Printed in Manila, Philippines

Nash, C.E. and Z.H. Shehadeh, Editors. 1980. Review of breeding and propagation techniques for grey mullet, *Mugil cephalus* L. ICLARM Studies and Reviews 3, 87 p. International Center for Living Aquatic Resources Management, Manila, Philippines.

ISSN 0115-4389

Cover drawing after H.C. Yang.

Preface

This review was prepared as a part of ICLARM's program on the controlled breeding and mass propagation of food fishes. It is a compendium of most of the available biological and engineering knowledge relevant to the breeding and mass propagation of the Mugilidae species, particularly the grey mullet, *Mugil cephalus* Linn. This knowledge has been accumulated over a period of 20 years with most major advances occurring within the last 10 years.

Chapters 4 and 5 deal exclusively with controlled breeding and mass propagation methods for the grey mullet. Reliable techniques are now available to stimulate maturation and induce breeding with pituitary homogenates, gonadotropins and steroids. Larval survival of 10-30% has been achieved on a small scale in Hawaii but mass production of fry is yet to be demonstrated. The present state-of-the-art can define most of the design and production limits of a large hatchery complex, but some key operating components are still undefined. These must be determined first at a pilot-scale production unit to make the management efficient and to define the cost:benefit ratio of a large hatchery.

The object of this publication is to describe current mullet breeding and rearing techniques in both biological and engineering terms. Armed with these data, fish culturists and engineers will be able to develop pilot-scale hatchery operations, adjusting and adapting available technology to local conditions.

The review is the joint effort of the following contributors:

Colin E. Nash
Guy N. Rothwell
Rosine M. Koningsberger
Ching-Ming Kuo

ZIAD H. SHEHADEH
Director General, ICLARM
May 1980

INTENTIONALLY
LEFT
BLANK

Contents

	Preface	v
Chapter 1.	**Introduction**	1
Chapter 2.	**History of Mullet Culture**	3
Chapter 3.	**Review of Natural Spawning and Induced Breeding**	
	Natural Spawning	7
	Induced Breeding	11
	Larvae and Larval Culture	28
Chapter 4.	**Hatchery Techniques**	
	Broodstock Collection	41
	Broodstock Maintenance	43
	Spawning and Fertilization	44
	Incubation and Hatching	50
	Larval Rearing	52
	Phytoplankton Culture	54
	Rotifer Culture	56
	Copepod and Amphipod Production	56
	Brine Shrimp Nauplii Production	57
	Staffing	57
Chapter 5.	**Hatchery Design**	
	Determining the Hatchery's Mission	61
	Sizing the Hatchery	61
	Site Selection	66
	Seawater System	66
	Materials and Construction	69
	Equipment	70
Chapter 6.	**Speculation for t**	77
	Acknowledgments	78
	References	79
	Appendix 1	85
	Appendix 2	87

Chapter 1. Introduction

The grey mullet species are representatives of a truly international group of fish in the family Mugilidae (order Mugiliformes). They make up an important and probably the most widely distributed commercial fishery in the world's coastal waters, and they are of significant importance, meeting the subsistence protein requirements of the peoples of the Pacific Basin, Southeast Asia, India, the Mediterranean and Eastern European countries, and in many parts of Central and South America. Mullet and mullet products contribute to small but valuable fishery economies in many European countries, the southern United States of America, Japan and Australia.

Most of the grey mullet species are fished to some extent, but the individual species with the greatest distribution and importance in the family is *Mugil cephalus* Linnaeus. Because of its biological behavior, size and nutritional quality, *M. cephalus* constitutes certain key continental shelf or inshore fisheries all over the world, and its protein value is utilized immediately by the local populace. Its life history and biological behavior place it in the key environmental niche at the interface between fresh and saltwater.

As the freshwater Chinese and Indian carps are part of the history of Asia, so the mullet have an established place in the archives of civilization from the arena of the Mediterranean. The mullet were well known and liked by the ancient Greeks and Romans and appeared in literature and sculptured friezes. They were abundant and obtainable from the shallow coastal waters of the northern Mediterranean Sea. Mullet later appeared in the collections and compendia of Agricola in the 15th century, and their descriptions preserved in the great natural history records of Gesner and Fuchs in the 16th century. The common mullet were therefore repeatedly fixed into the animal phyla by the pre-evolutionary naturalists whose numbers expanded rapidly after Linnaeus produced his more orderly taxonomic system in the 18th century. Undoubtedly, mullet were used to typify a marine fish for teaching the new and exacting branch of biology called taxonomy, which was popular at the time. Overenthusiasm may therefore have been responsible for the many species of mullet which were "discovered" around the world and named in the 19th century, and for the accusations against Linnaeus that he had confused all the Mediterranean and European species and given them one name. But, in retrospect, he was more correct in conservative recognition of new mullet species in his time.

M. cephalus is distributed abundantly throughout the subtropic regions and extends geographically approximately between the latitudes of 42° North and 42° South in all coastal waters (Figure 1). Because of this vast distribution there is still some confusion of the genera. Thomson (1966) accepted 13 genera and recorded 281 nominate species of mullets, of which he recognized 70 as valid and a further 32 indeterminate for lack of better descriptions. *M. cephalus* alone has at least 33 scientific synonyms, and the common names, such as "grey," "striped," "silver grey," "black," "sea" and "jumping" mullet (among others), are all freely interchanged. It is therefore essential that the common name

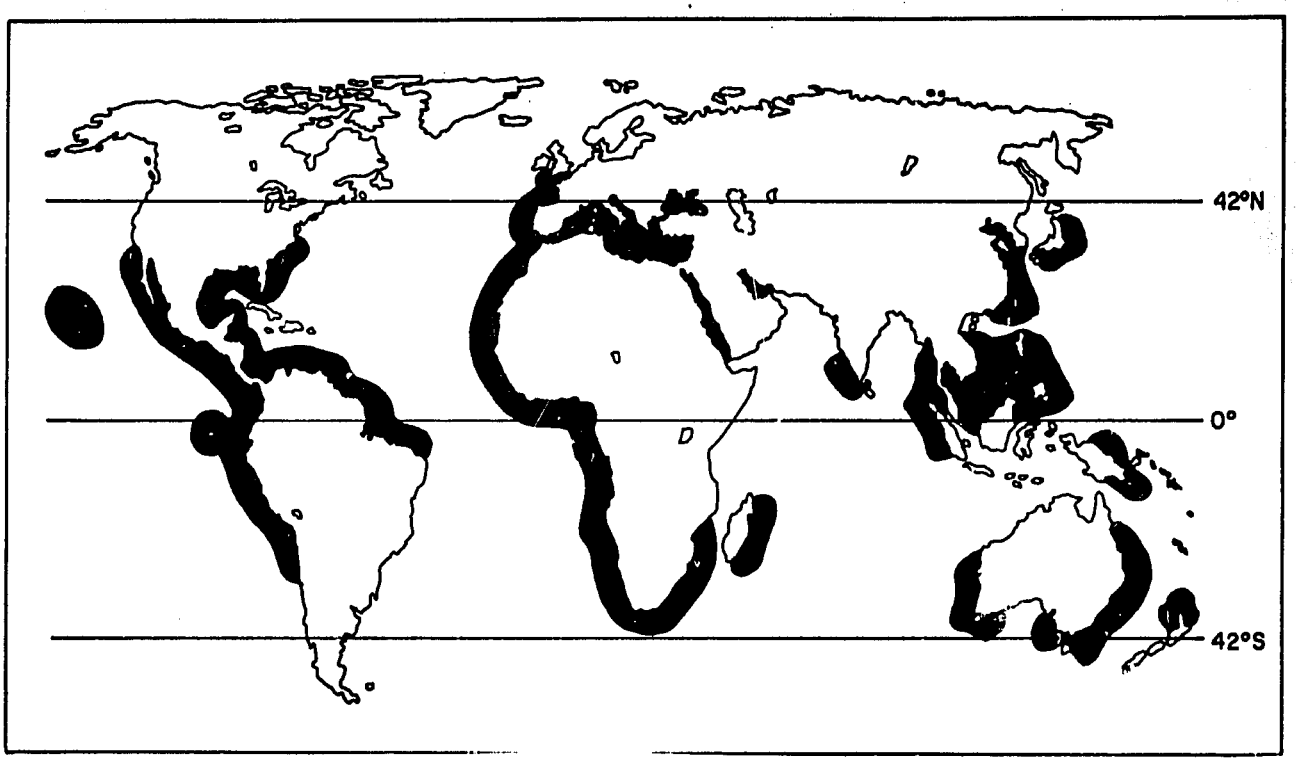


Fig. 1. Geographic distribution of *M. cephalus* (after Thomson 1963).

is frequently coupled with the scientific name in the literature of the Mugilidae.

The credit for first simplifying the taxonomy of a confused, largely uniform genera goes to Schultz (1946) who demonstrated that the differences between them were mostly identifiable by the diversity of the parts of the mouth and head. Microscopic examination of the tooth structure and in the patterns of dentition (Thomson 1975), the lips, the adipose eyelids and facial morphometrics are the most commonly used features. There are, however, continual proponents for (among others) osteology (Luther 1975), and muscle protein electrophoresis (Herzberg and Pasteur 1975). Added to the taxonomic picture, there is the evidence that races of mullet can be determined within regions by identification of the eye lens proteins (Peterson and Shehadeh 1971).

The environmental behavior, food preference, and feeding in the nearshore waters, together place *M. cephalus* and other mullet species at the important environmental interface between the land and the sea. With the exception of one or two species which have adapted entirely to freshwater conditions, the mullet are euryhaline and can be found in most coastal environments. *M. cephalus* is probably the most oceanic of all the species and is common to most of the islands of the Pacific and Indian Oceans. But it can also tolerate freshwater conditions for prolonged periods, as well as the hypersaline lagoons of Africa. It is most commonly found, and grows faster in, the brackishwater areas of the subtropics, and is the preferred fish for mullet farming.

Although in its early stage of development, mullet breeding and propagation is expected to produce strains of fish selected to meet the needs of fish farmers. The hatcheries will be the key to providing the farmer with high-quality seedstock, guaranteed to be highly viable on the farm and capable of efficient conversion of food for fast growth.

It has been said,

> "Give a man a fish, and he will have food for a day. Teach him to raise fish, and he will have food for the rest of his life."

Development of techniques to induce breeding and to rear grey mullet is an important step toward that goal.

Chapter 2. History of Mullet Culture

The farming of mullet (Mugilidae species) has been practiced for centuries, but the cultivation of this potentially invaluable source of animal protein for man has been small and nonintensive. Subsistence farming has been a tradition in the Mediterranean region, Southeast Asia, Taiwan, Japan and Hawaii, within fenced lagoons, creeks and swamps, and in manmade ponds (Figure 2).

Experimental activities in intensive mullet farming aimed at finding ways to optimize production, which is a necessary forerunner of any large scale operation, are more recent. Approaches of this increased effort to develop intensive culture differ from region to region because of traditional needs and practices.

In the Mediterranean region, especially in Italy, the traditional "valliculture" methods employed in farming mullet are now advanced (D'Ancona 1955; De Angelis 1969) usually in association with raising eels. In Egypt a successful stocking experiment was carried out at Lake Qarun in 1921 (Faouzi 1936) using *Mugil cephalus* and *M. capito*. No effort was made to optimize growth of the transplanted juveniles by supplemental feeding. A second transplantation was made of *M. cephalus, M. capito* and *M. saliens*. The latter was reported to spawn successfully in the lake, but the stock of the other two species had to be replenished each year with juveniles from the estuaries (El-Zarka 1968).

In the Soviet Union, experiments with mullet were carried out in the regions of the Black Sea and the Caspian Sea. In 1930 and 1934 *M. cephalus, Liza saliens* and *L. auratus* were introduced into the Caspian Sea (Thomson 1966). The transplantation of *L. saliens* and *L. auratus* was successful and their acclimatization, development and reproduction were studied in later years (Forceva-Ostroumova 1951; Babaian 1958). The work was followed by suggestions for further improvements in the region (Babaian 1960 and 1964; Chepurnov and Dmitriyev 1962). Apyekin and Tronina (1972) subsequently published the tentative results of experiments on the stimulation of the maturing and spawning of *M. cephalus, M. saliens* and *M. auratus*.

The mullet were introduced for a secondary crop in carp ponds in Israel in the 1950s (Perlmutter et al. 1957; Pruginin and Kitai 1957). Considerable research work was then performed on artifical feeding in ponds (Erman 1958), seasonal and regional variations in the spawning season (Abraham et al. 1966), histological studies of ovarian development in captivity (Abraham et al. 1968), culture at different stock densities (Yashouv 1966), and breeding and growth in captivity (Yashouv and Ben-Shachar 1967).

In Southeast Asia and the Far East, efforts to intensify mullet culture were centered in Taiwan, India and Hong Kong. Farming of mullet was also practiced in Japan, the Philippines, Indonesia and mainland China, but only as a secondary crop in the culture of other species, primarily carps. Research in these countries has been sporadic. High pond yields of almost 9,000 kg/ha were obtained in Japan farming mullet with eels, and fertilizing intensively with manure.

There are two incidences of mullet rearing experiments in Japan. Between 1906-1909 at the Aichi Prefecture Fisheries Experiment Station, feeding experi-

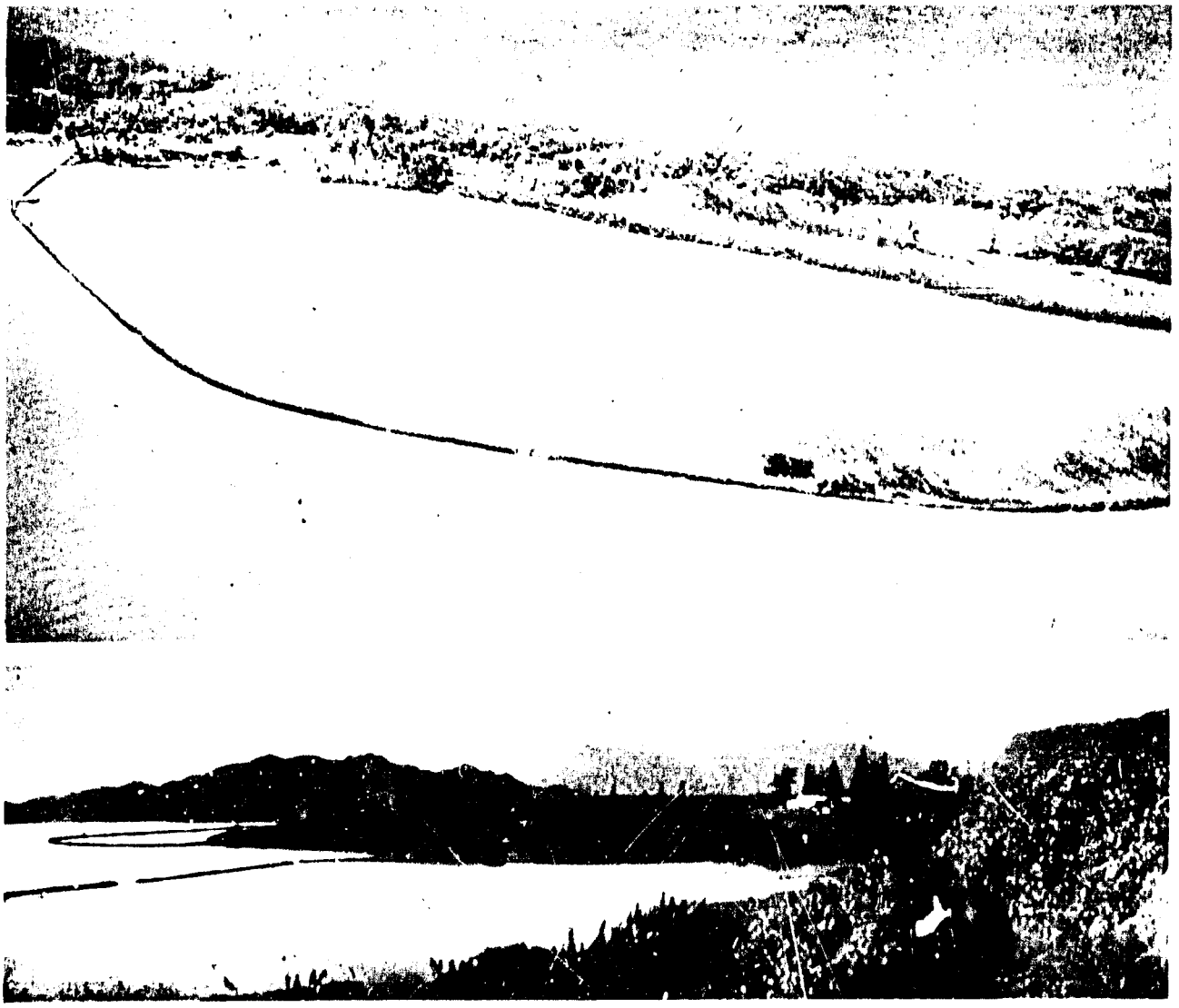


Fig. 2. Traditional Hawaiian fishponds (courtesy of the Oceanic Institute and the Bishop Museum).

ments with mullet juveniles were conducted and were repeated 40 years later (Tamura 1970). In the Philippines the possibility of raising mullet juveniles together with milkfish in the brackishwater ponds was first suggested by Adams et al. (1931). Although mullet were raised successfully with the milkfish for some time, a scientifically supervised rearing experiment was not conducted until 1953 (Blanco and Acosta 1958). Polyculture of mullet with milkfish and carp was again proposed but has yet to be intensified. In 1971 preliminary experiments on induced spawning of *M. dussumieri* were carrried out at the Marjan Sabalo Hatchery Experimental Station (Angelos 1971), indicating that research on intensive mullet culture in the Philippines had not been abandoned.

In Hong Kong intensive culture of mullet was imposed successfully on the traditional practice of carp culture (Bromhall 1954). The culturing method, developed empirically, used two pond systems stocked with *M. cephalus* as both the primary and the secondary crop (Lin 1940). The ponds were fertilized with organic matter and the fish given artifical food. Culling started when the fish were about seven months old and production was about 1,500 kg/ha. Chow (1958) conducted growth studies on pond reared mullet and found that their growth rate compared favorably with that of natural stocks.

In India, with its extensive estuarine waters in Kerala,

Bengal and Madras, mullet have been farmed from very ancient times. Research in intensive culture began in the 1920s when rearing experiments with young mullet were conducted in Madras at the Fisheries Department farm at Ippur (Campbell 1921; Hornell 1922) and at the Chingulpet Fort-moat fish farm (Gravely 1929). In the 1940s two further efforts were emphasized, namely, studying the feasibility of acclimating mullet juveniles to freshwater, and developing practices in polyculture. The first acclimation experiments were undertaken in Madras with *M. troschelli* and *M. wagiensis* (Devanesan and Chacko 1943; Job and Chacko 1947). This was followed by acclimatization studies of *M. parsia* in Bengal (Mookerjee et al. 1946) and of *M. cephalus* and *M. seheli* in Madras (Ganapati and Alikunhi 1949). Yields in Indian ponds were about 500 kg/hectare.

Several other reports on existing farming practices with their problems were published at the time. Improvements for the brackishwater farming of mullet in the Ganges Delta were suggested (Hora and Nair 1944). Basu (1946) advanced the adaptation of Chinese and Philippine practices for Bengal farms, and Pillay (1947) published his extensive examination of culture in Bengal, Madras and Kerala. The acclimation experiments led Panikkar (1951) to suggest that temperature and salinity tolerances of the mullet species be studied in detail, but little appeared in the literature until the report of Mohanty (1973). He recorded that juveniles of *M. cephalus* acclimated to freshwater were more tolerant of changes in conditions, and that tolerance varied with length.

The most significant development in India has been the undertaking recently of the artificial propagation studies at Kerala. Ovulation but not fertilization was achieved with *M. cephalus* (Sebastian and Nair 1973). However successful spawning and larval rearing were achieved by Sebastian and Nair (1974) with *M. macrolepis*.

Although the pond culture of Mugilidae is not practiced on a large scale in Korea, the grey mullet is one of the important food fishes in its southwest region. One instance of research deserves mention. Yang and Kim (1962) described an experiment to obtain and hatch eggs of the grey mullet and to rear the larvae, but the work does not seem to have been continued.

In Taiwan, at about the same time, the knowledge gained from centuries of pond culture was utilized to begin an intensive program on mullet culture. About 39% of the commercial catch of *M. cephalus* consisted of pond reared fish cultured in combination with carp in freshwater ponds and with milkfish in brackishwater ponds. Tang (1974) estimated that 10 million juveniles were required to support the pond culture of mullet each year. He pioneered work on the induced spawning of *M. cephalus* as he knew that these resources were not infinite.

In 1966 a comprehensive research program was established at the Taiwan Fishery Research Institute for intensive culture and mass propagation of juveniles. In subsequent years the techniques of induced spawning of mature fish caught during their seasonal migration were refined and improved, and survival of the larvae extended to 30 d. In 1973 the larval survival had increased to 19.35% and the production of juveniles had been established. A summary of a decade of work at the Institute was made by Liao (1974), and later (Liao 1977) he reported that two fish, which grew from fingerlings artificially propagated at the Tungkang Marine Laboratory, were successfully bred. From one of them, over 500,000 larvae were obtained and a yield of 1661 second generation fingerlings were produced, thus recording the first success in closing the reproductive cycle in captivity.

In the United States, the earliest mention of mullet farming was made by the U.S. Fish and Wildlife Service (Anon 1940) and attributed to Prytherch. A general article by Sharpe (1945) about the same farm quoted a yield of 5,000 pounds of fish per acre. Hiatt (1944) obtained information on the role of mullet in the food cycle of Hawaiian fish ponds. Lunz (1951) described preliminary experiments of mullet culture in brackishwater ponds in South Carolina. In Florida an experiment to raise the pompano, *Trachinotus carolinus*, inadvertently resulted in a yield of which *M. curema* and *M. cephalus* constituted the majority of fish (Johnson 1954).

At present, experimental work with Mugilidae is centered in Hawaii, Texas and Louisiana. In Hawaii, the Oceanic Institute successfully achieved artificial spawning of *M. cephalus* following induced breeding of captive broodstock (Shehadeh and Ellis 1970; Shehadeh et al. 1973b). Larval rearing efforts met with mixed success and individual survival figures (up to 25.5%) could not be guaranteed (Kuo et al. 1973a; Nash et al. 1974). In Texas mullet were raised successfully in ponds receiving the heated effluent from a power plant (Linder et al. 1974), and in Louisiana acclimatization experiments were conducted with mullet juveniles (Shireman 1974).

From this brief historical survey it is evident that the direction of the experimental research and development for the culture of the Mugilidae has, of necessity, been toward induced spawning and larval rearing. For centuries the farming of mullet, as with other species, has depended on natural resources for the annual replenishment of stock. However the annual spawning migrations of the adults and the strength of the year-classes are all subject to environmental biological factors about which

there is little agreement on importance. Present evidence indicates that these factors differ with locality, region and even between populations within the species. The natural availability of juveniles is therefore subject to unpredictable variations in occurrence and abundance. More importantly the juveniles are collected in those coastal and estuarine waters where pollution is threatening, or, in some cases, has already changed the ecosystem. The future availability of resources in such waters is becoming increasingly uncertain. Finally, improvement in fishing techniques particularly for schooling fish on their spawning migration, is affecting the natural population.

The literature is full of reasons for the depletion of natural populations and weakening year-classes. Yet there is an anomaly for the proponents of culture. Commercial fishermen consider that too many juveniles are removed by pond fishermen, thus depleting the resources of the natural fishery. Consequently, limits have been imposed on the number of juveniles of certain species which can be removed for intensive culture.

The foreseeable future of aquaculture is entirely dependent on the success of artificial propagation and larval rearing. Resources for the farmer have to be independent of the natural populations. If this is achieved, then genetics and selective breeding can benefit mullet farming, making the technology parallel to the development of the fast-growing strains and specialized breeds of intensive agriculture. The Mugilidae will have the advantage over many other families of fish because of the number of related subspecies which can be used to hybridize or breed particular strains for almost any geographic region or specific farming location.

The need for an intensive effort in mass propagation has been summarized by Oren (1971). He said: "In order to achieve maximum production of mullets under controlled conditions, one of the axioms is a steady supply of fry independent of conditions in the natural environment, natural spawning and migration of fry inshore. Only when such a supply is available will the most economic and most efficient way of utilization of the mullet, as a consumer of the first trophic layer, be channeled for the benefit of man." There is now sufficient data to justify the construction of pilot-scale hatcheries for mullet to apply present methods to specific regional and environmental conditions, and to demonstrate the techniques for the development of a modern, food producing, farming industry.

Chapter 3. Review of Natural Spawning and Induced Breeding

Natural Spawning

The success of an artificial propagation system for any plant or animal species depends primarily on the quality and quantity of broodstock resources. Large numbers of sexually mature individuals must be contained in good health under acceptable environmental conditions in order to reproduce and yield viable offspring.

Some essential external conditions for broodstock fish are suitable water quality, a nutritious diet, high standard of hygiene and limited physical disburbance. But most importantly, the broodstock must be exposed to the correct environmental parameters which influence the physiological changes in the pituitary gland and stimulate the gonads to seasonal maturity.

The endocrine system of all vertebrates forms the main link between the reproductive organs and the environmental regulators. Rhythmic regulators, such as temperature and photoperiod, mediated through the central nervous system, initiate neurosecretions which in turn regulate the activities of the pituitary gland. As one of many target organs, the gonads are influenced accordingly. The reproductive cycles are thus regulated intimately by the trophic hormones of the pituitary.

Many experiments with fish have used gonad development to interpret the effects of certain environmental regulators on the reproductive cycle. Among the factors concerned, temperature and photoperiod are the two most important which initiate pituitary activity for fish in temperate and subtemperate regions (Hoar 1959). The relative importance of each varies with different species of teleosts. Photoperiod has been reported as the dominant factor influencing the reproductive cycle of *Enneacanthus obesus, Notropis bifrenatus* and *Fundulus confluentus* (Harrington 1959), *Gasterosteus aculeatus* (Baggerman 1957), *Salvelinus fontinalis* (Henderson 1963) and *Oryzias latipes* (Yoshioka 1962). Temperature has been shown dominant for *Phoxinus laevis* (Bullough 1940), *Apeltes quadracus* (Merriman and Schedl 1941), *Gambusia affinis* (Medlen 1951) and *Couesuis plumbeus* (Ahsan 1966).

Henderson (1963) concluded for *Salvelinus fontinalis* that the influence of an environmental regulator varied with the stage of gonad maturation. The most responsive period to a regulator could also vary between males and females of the same species, and the gametogenetic process may be independent of environmental regulators at certain stages of maturity.

The reproductive cycle of most vertebrates is under the dual control of an internal physiological rhythm and an external seasonal rhythm. The refractory period or resting stage of the reproductive cycle is considered to be the time during which the two rhythms coincide and reinforce each other. As the fish are exposed to changing environmental conditions, such as photoperiod and temperature, the external rhythm begins to dominate. Its influence on the reproductive processes is transmitted by changes in the quantity of gonadotropin released from the pituitary gland.

The physical containment and maintenance of adult grey mullet in the most suitable conditions for survival

are not difficult problems. The fish readily become domesticated as testified by centuries of pond farming. They grow and are technically mature, although the final stages of gametogenesis are not completed and no incidence of total development and unassisted natural spawning in captivity has been recorded. At present these need the artificial stimulus of hormone injection to complete the breeding cycle.

In order to simulate the desirable environmental conditions for gametogenesis of captive broodstock, it is necessary to examine the conditions at locations where the grey mullet spawn in nature.

The natural spawning locations of grey mullet species, which have been described or deduced by many workers throughout the world, do not indicate specific and similar environmental patterns. The picture overall is confused both by misidentification of generic types observed and the variety of facts on which the spawning record is being made. For example, many reports are based on the collection of adult fish with ripe gonads—usually loose eggs; others describe small numbers of eggs or larvae in plankton tows and make predictions about the spawning location based on tidal movement and stage of development of the samples. Some reports describe inshore schooling and then a migration offshore, presumed to be for spawning; others describe schooling inshore for spawning. The location of spawning grounds for the mullet can only be described as controversial.

Anderson (1958) reviewed the records of many American workers who suggested the time and place of spawning of *M. cephalus* along the South Atlantic and Gulf coasts of the United States. From evidence of collection of larvae and the occurrence of juveniles on the coast from lower Florida to North Carolina, he believed that the striped or grey mullet spawned offshore over a broad area extending from about the 20 fathom line into the Gulf Stream. In contrast with the spawning of the silver mullet, *M. curema,* which began in early spring when water temperatures were rising over the Continental shelf (Anderson 1957), *M. cephalus* spawned during late fall or winter when water temperatures were falling.

Breder (1940) observed and described an aggregation of adult fish in the shallow creeks of the Florida coast and believed that spawning was taking place. Although he collected no eggs, his detailed description of the movements of the males around the females compares exactly with the observed spawning behavior of mullet in aquaria, and it must be assumed that the fish were attempting to spawn. The observations by Breder (1940) and Arnold and Thomson (1958) showed that *M. cephalus* forms large schools when spawning. The school is scattered into small groups, generally made up of one large female and a varying number of smaller and more active males.

Broadhead (1953), Dekhnik (1953), and Arnold and Thompson (1958) provided authenticated instances of *M. cephalus* spawning at sea in surface waters, but over deep water (50 fathoms in the Black Sea, and 750 fathoms in the Gulf of Mexico). Fitch (1972) described the capture of a ripe female over 40 mi off the Baja California coast.

Other more unusual spawning places have been suggested. Roughley (1916) believed spawning to occur in fresh water. Smith (1935), Breder (1940), Jacob and Krishnamurthi (1948) believed it took place in estuaries and tidal creeks, and Kesteven (1953) in the coastal surf zone of Australian waters.

Demir (1971) reviewed the information available on spawning of grey mullets in the North Atlantic. The spawning of *M. labrosus* was assumed to be April in British waters, as schools of adults were observed offshore and the juveniles entered tidal pools on the Channel coast and in Southern Ireland in July and August. Kennedy and Fitzmaurice (1969) believed the spawning period to last several weeks in Irish waters with May as the peak spawning month. Hickling (1970), in his contribution to the natural history of the English grey mullets, noted the spawning period of *C. labrosus* from January to April and that one of its spawning locations was near the Isles of Scilly. Two ripening *L. ramada* he found in autumn indicated a late spawning season. He did not find any *L. aurata* with active gonads. Le Dantec (1955) noted that genital activity for *M. labrosus* lasted from January through April for fish in the Biscay region.

In the Mediterranean, Caspian and the Black Seas, the spawning periods again varied from region to region. Yashouv and Berner-Samsonov (1970) produced an extensive review of the spawning seasons of five species of Mugilidae in the Black Sea and the Mediterranean. Hamis (1972) stated that the spawning areas of certain species of mullet were those places where the optimum conditions for the young were available. In the Black Sea, *M. saliens* spawned from late June to October, *M. cephalus* spawned from late May to late October, and *M. auratus* spawned from early June to early November. In the Mediterranean the spawning of *M. saliens* took place between May and October, *M. cephalus* from early May to September, *M. auratus* from early September to late December, *L. ramada* from early October to late December, and *M. labrosus* from early December to early April.

Avanesov (1972) established that spawning of the grey mullet in the Caspian Sea occurred in a wide area beginning in the southern part at the end of May and extending northward. He recorded the most intensive spawning in the Turkmenian waters in the south in August, at a temperature of 25-29°C and about 7 mi offshore where the depth was 50-40 m. Spawning of

the long-finned mullet, he noted, occurred later at a considerable distance offshore where depths were over 400 m.

Wimpenny and Faouzi (1935) first recorded the shoaling migrations of *M. cephalus* and *M. capito* during the spawning period. The schools moving from the Delta Lakes of Egypt to the sea were entirely composed of spawning fish. Thong (1969) observed migrations of *M. auratus, M. labrosus* and *L. ramada* during egg laying from the coastal regions of northwest France into the open sea.

In the Pacific and Indian Oceans little information on spawning of Mugilidae is available except for *M. cephalus* which spawns in late winter in the tropics and subtropics. Some summer spawners were recorded by Sarojini (1958). Helfrich and Allen (1975) observed *Crenimugil crenilabis* spawning in dense schools of 500-1,500 individuals at night in summer in the shallow waters of Enewetok lagoon in the Marshall Islands. In tropical Bengal waters *L. parsia* spawned between January and March, and *L. cunnesius* from May onward in the time of the monsoon. Kurian (1960 and 1974) recorded the maturity of mullet in the ponds and lagoons of the Indian coast. He noted the different types of spawning migrations of *M. cephalus, M. tade* and *L. macrolepis,* but all were seaward or near to the estuaries. Patniak (1966) described the migratory behavior and maturation of *Mugil cephalus* in Chilka Lake. The breeding season appeared to last from October to December. Jhingran and Natarajan (1970), summarizing earlier work conducted and reported between 1957 and 1965, believed the season to be longer. They also noted that the larger fish over 32 mo old never returned from the sea to the lake.

Wallace (1974) recorded the distribution of *M. cephalus* in the high saline lake system of St. Lucia on the east coast of South Africa. He observed two seaward migrations in response to the changing hydrological and topographic features of the water system. No spawning was recorded in the lakes. Cervigón and Padrón (1974) observed *M. curema* in the high saline lagoons along the coast of Venezuela. They concluded that no spawning occurred in the lagoons but the adults migrated to the open sea and possibly for two spawning periods a year. Table 1 summarizes the spawning seasons of Mugilidae.

The records deducing the spawning locations from the number of eggs and larvae in plankton samples make no reference to the time of day at which spawning might occur. The observations of Dakhnik (1953) and Arnold and Thompson (1958) indicated that *M. cephalus* spawned at night at the water surface but over considerable depth. Anderson (1957) reported night spawning of *M. curema.* Houde et al. (1976) collected eggs of *M. curema* in surface nets hung from a dock in Florida.

Although much reference is made to water depth and location of spawning sites offshore., no workers report that the fish themselves descend deep to spawn. Hotta (1955) recorded very small larvae of *M. cephalus* in plankton tows from 100 fathoms deep near Japan, and Zviagina (1961) found eggs of *L. haematocheila* deep in Peter the Great Bay.

The incidences of mullet eggs and larvae being taken in plankton tows are few. Demir (1971) recorded the occurence of postlarvae of *M. auratus* and *M. labrosus* in British waters for the first time. Previously the eggs and larval stages of *M. labrosus* had only been recorded once by Sanzo (1936), and the eggs only of *M. auratus* by Sanzo (1931) and Vodyanitskii and Kazanova (1954). Although depth may not be important for the adults at spawning, it may be important for incubation and larval development.

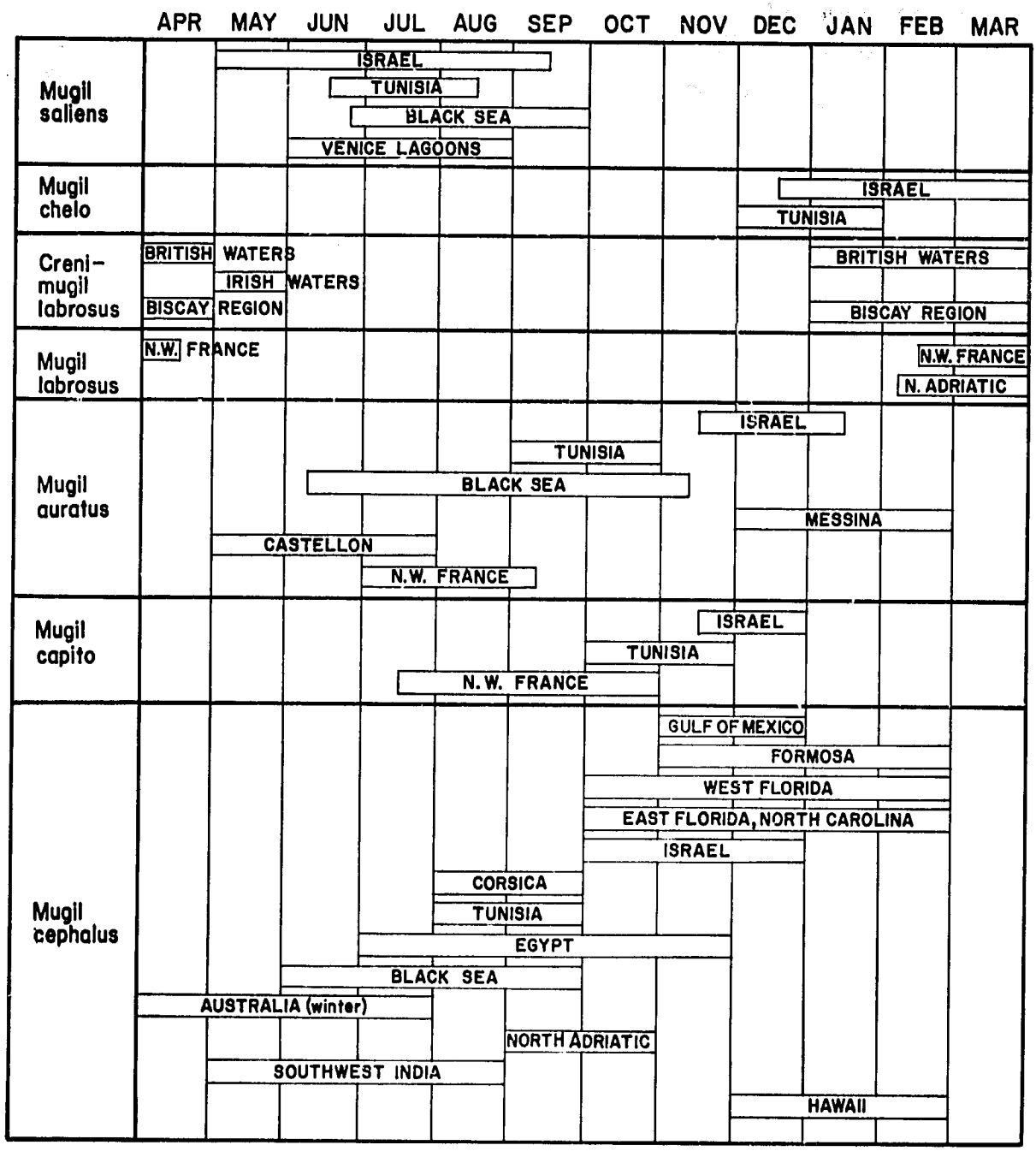
From histological examination of adults, Stenger (1959) believed that *M. cephalus* spawned more than once a year. Bromhall (1954), on the evidence of the size and distribution of the juveniles, concluded that there were two periods of spawning in the vicinity of Hong Kong and a lunar period apart. However, it is generally assumed under normal circumstances that most grey mullet species produce only one brood of eggs each year, and that some females only spawn in alternate years after their first maturity (Thomson 1955).

In summary, from the extensive and conflicting data and the possible misidentification by the observers, it is not possible to define a pattern in the environmental conditions necessary for successful natural spawning. Hamis (1972) was probably close to the truth when he stated that the spawning areas of certain species of mullet were those places where the optimum conditions for the young were available. More importantly, he should have included the optimum conditions for the incubation of eggs.

Photoperiod and temperature rhythms time the onset of gametogenesis and these rhythms, which differ from region to region throughout the world, influence the indigenous population of mullet species. Existing data on the influence of salinity, temperature and dissolved oxygen on the viability of the eggs of *M. cephalus* show that incubation and development occur to some degree within a wide range of these parameters. The results also show that there are optimum conditions for both.

Environmental data on the temperature of the water during spawning of *M. cephalus* indicate some adaptation from region to region, with temperatures recorded at 12-24°C. All records show a strong preference by the fish for oceanic water as the medium for incubation, with salinities of 32-35‰. It is interesting to note the observations of Wallace (1974), Cervigón and

Table 1. Seasonal spawning of some Mugilidae (after many authors) according to geographic region.



References consulted:

Australia	:	Thomson 1963	Gulf of Mexico	:	Arnold and Thompson 1958
Biscay Region	:	Le Dantec 1955	Hawaii	:	Kuo and Nash 1975
Black Sea	:	Hamis 1972	Irish Waters	:	Kenedy and Fitzmaurice 1969
British Waters	:	Hickling 1970	Israel	:	Yashouv and Berner-Samsonov 1970
Castellon	:	Belloc 1938	Messina	:	Sanso 1931
Corsica	:	Belloc 1938	N. Adriatic	:	Sanso 1936
Egypt	:	Paget 1923	N.W. France	:	Thong 1969
Florida East Coast/North Carolina	:	Anderson 1958	S.W. India	:	Kurian 1974
Florida West Coast	:	Broadhead 1953	Tunisia	:	Heldt 1948
Formosa	:	Tang 1964	Venice Lagoons	:	Gandolfi and Orsini 1970

Padrón (1974), and Zismann and Ben-Tuvia (1975), for fish which lived in hypersaline conditions but which migrated to normal oceanic salinities to spawn.

Kuo et al. (1974b) demonstrated that environmental manipulation of photoperiod and temperature increased the individual spawning frequency of adults, and that the spawning season could be prolonged throughout the year. The manipulations were all made in seawater of salinity 32‰. Their evidence indicated that the spawning behavior of the grey mullet species was not a strictly controlled and regulated act. In fact the evidence pointed to a loosely controlled behavioral response. Therefore the many controversial and apparently misleading observations on the spawning period and location will be authentic for that respective population at that specific time of year, and in that particular region.

It is concluded that the adults move to spawn in the nearest location which will provide the eggs and larvae with the highest chances for survival, specifically to oceanic water of salinities of 32-35‰. The distance of the migration may be short or long, depending on the local conditions of topography and tidal movements. For example, in Hawaii where there is no strong tidal movement but a coastal gyre, the adults move offshore beyond the coastal reef a distance of 1 or 2 mi; in Australia the fish move against the current many miles on both the west and east coasts before spawning (Thomson 1966), so that the long natural drift will return the juveniles back to the home estuaries several weeks later. Depth does not appear to be a vital factor for survival. Although the eggs have a natural buoyancy, are capable of surviving great pressures and can be found in deep water, the records do show that eggs and larvae develop predominantly in the upper ocean layers.

Induced Breeding

INDUCED BREEDING CONDITIONS

No record has been made of the Mugilidae spawning unaided in captivity or in artifical conditions. Successful fertilization of hand stripped eggs and milt from adults caught at sea has been claimed by Sanzo (1936) and Belloc (1938). Other successes have been reported with fish matured in large ponds. The chances for this practice to become the base of an artifical propagation unit are as yet too small to be considered further. Reliable results have been obtained from fish matured in captivity or captured at sea but induced to complete gametogenesis and spawn by the injection of hormones.

Suitable artificial conditions for holding the broodstock of grey mullet can only be described as those enclosures which permit the female fish to develop their oocytes beyond the tertiary yolk globule stage (stage III as described by Kuo et al. 1974b) and the males to complete spermiogenesis. Without hormone stimulation, females will not mature to the ripe stage (stage IV) prior to ovulation, but will undergo atresia (stage V) and degenerate.

The first report on the induced spawning of *M. cephalus* reared in captivity in freshwater ponds was made by Yashouv (1969). Liao (1974) summarized the work in Taiwan between 1963 and 1973 on the propagation of *M. cephalus,* using spawning fish collected from the sea and from fish contained in large saltwater ponds. For the broodstock the workers in Taiwan relied on strong uninjured male and female specimens selected from the catches of commercial fishermen during the spawning run along the southwest coast of Taiwan. The selected fish were placed in strong plastic bags filled with seawater and inflated with oxygen. Most of the mullet caught were of the IV-year class and measured about 32-50 cm in length and weighed 1.02-2.1 kg each. The specimens were all sexually mature with well developed gonads, but the eggs of the females were never fully ripe for natural spawning.

Pond stock were maintained in Taiwan in freshwater at first, with seawater added slowly over a 3-mo period prior to spawning. The fish were fed a special diet and also injected periodically with mullet pituitary glands and Synahorin. Pond reared fish were easier to spawn than wild stock as they were more docile and free from injury. Large tanks specially constructed for holding broodstock were made of concrete and measured 5 x 7 x 1.5 m deep. Seawater was circulated through each and all were aerated continuously. Liao et al. (1971) reported on the preliminary success with induced breeding of pond reared mullet, and later (Liao 1977) recorded the first incidence of producing second generation domestic stock from parents reared from hatchery stock four years earlier.

Shehadeh et al. (1973c) established broodstocks of *M. cephalus* in small, rubber-lined dirt ponds supplied with circulating seawater (Figure 3). In addition, the ponds contained a substrate of weighted polyethylene strips which increased the internal surface area and provided a stable supply of benthic diatoms, blue-green and filamentous algae. Three-year-old fish survived readily in such conditions and matured the following year. They were then successfully induced to spawn by hormone injection. The small ponds were excavated and lined with butyl rubber sheet (1mm thick) and had a volume of 26 $m.^3$ A food supplement was provided in addition to the available natural growth.

Sebastian and Nair (1974) reported on the collection and holding of *M. macrolepis* in preparation for spawning. Mature fish were taken from local brackishwaters in

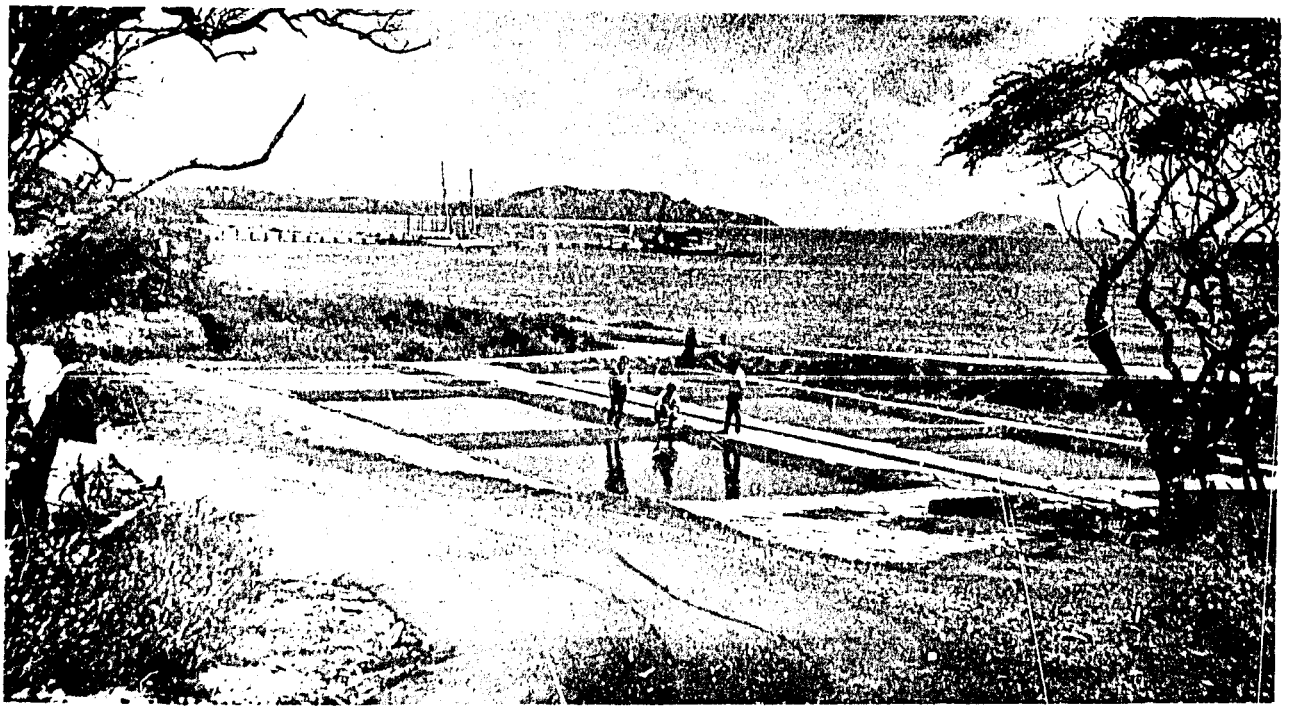


Fig. 3. Butyl rubber-lined seawater ponds for broodstock.

Chinese dip-nets and transported to small concrete tanks, 170 x 95 x 70 cm deep. The gravid female fish were 13-23 cm in length and 40-139 g in weight. Water in the tanks was changed intermittently.

Kuo et al. (1974b) described at length the holding of *M. cephalus* subjected to environmental manipulations of temperature and photoperiod. The time of onset of vitellogenesis was determined to be about 8 wk after exposure to a short photoperiod regime (6L/18D) at temperatures ranging from 17 to 26°C. The response of oocyte development to the retarded photoperiod regime was consistent and unrelated to any other preconditioning of photoperiod changes, including a simulated natural light cycle. The data also indicated that development of the oocytes was accelerated by constant exposure to a temperature of 17°C and a 6L/18D photoperiod, but that it was not completed as only limited yolk deposition occurred in the tertiary stage.

For *M. cephalus*, it is essential to maintain broodstock prior to spawning in full saline conditions (32-35‰). There is also evidence that the fish readily mature even after prolonged periods in captivity. Yashouv (1969) reported some success working with *M. cephalus* in freshwater, but the work of Hines and Yashouv (1971) on the increased activity of the spermatozoa of *M. capito* in seawater, together with the practical experience of others working with *M. cephalus*, indicate a preferential use of seawater for holding broodstock.

Ambient temperature and photoperiod conditions regulate the normal seasonal maturation of captive stocks. But the feasibility of breeding throughout the year, or of extending the breeding season by manipulation of the photoperiod and temperature regimes, has been proved and will produce greater use and increased efficiency of propagation facilities.

FECUNDITY

Thomson (1963) reviewed a number of papers which reported the fecundity of *M. cephalus* in terms of total egg numbers. He quoted estimates of 1.2-2.8 million eggs per fish. Sebastian and Nair (1974) estimated the fecundity of *M. macrolepis* at 1.2-4.0 million eggs per fish depending on size. Hickling (1970) and others listed the fecundity of several species of grey mullet as follows:

Species	Thousand eggs/kg	Author & Year
L. cunnensis	15-57	after Sarojini (1958)
A. forsteri	126-650	Thomson (1957)
L. parsia	200-600	Sarojini (1957)
C. labrosus	372-745	Hickling (1970)
L. ramada	581-1243	Hickling (1970)
M. cephalus	1200-2800	Thomson (1963)
M. cephalus	3600-7200	Nikolskii (1954)
M. cephalus	1572-4774	Grant and Spain (1975)

Kuo et al. (1973a) determined the fecundity of *M. cephalus* to be about 648 eggs/g body weight of three-year-old fish. Nash et al. (1974) quoted 849 eggs/g for

older individuals. Liao et al. (1972) reported 0.7-1.9 million per fish.

MORPHOLOGY AND QUALITY OF EGGS

The morphology of eggs before fertilization was described for several of the Mugilidae by the early naturalists, most of whom made reference to the characteristic large oil globule. The availability of fertilized eggs at all stages of development from induced breeding has resulted in full descriptions for *M. cephalus* by Sanzo (1930), Tang (1964), Yashouv (1969), Liao et al. (1971), Kuo et al. (1973a) and Tung (1973); Sanzo (1936) for *M. chelo* and *M. labeo* (1937); Anderson (1957) for *M. curema;* Perceva-Ostroumova (1951) and Dekhnik (1954) for *M. saliens* (see Figure 4).

Fertilized eggs of the Mugilidae are spherical and transparent. The surface of the egg shell is smooth and unsculptured. The yolk appears unsegmented and there is predominantly one large oil globule making them extremely buoyant. The eggs are not adhesive.

Eggs from *M. cephalus* and *M. capito* were observed by Yashouv and Berner-Samsonov (1970) to have more than one oil globule but which subsequently developed and hatched. During development they observed the droplets to merge. On hatching the larvae had one oil globule (rarely two) located in the yolk sac.

Sanzo (1936) described eggs of *M. chelo* with one large and several smaller globules, and Perceva-Ostroumova (1951) noted the same for *M. saliens.* Kuo et al. (1973a) stated that the frequency of multiple oil droplets in eggs of *M. cephalus* increased with the manual pressure of artificial stripping. Spontaneous release of the eggs by the females produced eggs with a single oil globule and Nash et al. (1974) considered that to be normal and desirable. Although the small oil globules were observed to coalese during development, the survival of eggs which initially contained multiple oil droplets was always low.

Yashouv and Berner-Samsonov (1970), in an extensive contribution to the knowledge of eggs and early larval stages of Mugilidae, reviewed data of egg and oil globule diameters in samples from *M. saliens, M. cephalus, M. capito, M. auratus* and *M. chelo* at a variety of locations. They included some data from the synopses on *M. cephalus* prepared by Thomson (1963 and 1966). The comprehensive data revealed a wide range of diameters reported for the same species in different locations.

Kuo et al. (1973a) reported the mean egg diameter of fertilized eggs of *M. cephalus* as 930 μ, with a range of 880-980 μ. The single large oil globule had a uniform diameter of 330 μ.Tung (1973) quoted a mean egg diameter of 0.89 mm for the same species, and oil globule diameter of 0.39 mm. Nash et al. (1974) specified a mean egg diameter of 0.93 mm.

A question posed by many workers culturing either Mugilidae or other species by induced breeding is whether the quality of individual oocytes is inferior to those produced in nature. Induced breeding does accelerate final development and therefore the egg and subsequent embryo may be deficient in certain biochemical constituents necessary for total development.

Bromhall (1954), Sarojini (1958), Erman (1961), and Hickling (1970) examined the gonads of several species of mullet, including *M. cephalus,* and observed that their state of development was often consistent with a seasonal production of more than one batch of eggs, possibly within a period of a month. Kuo et al. (1973b) demonstrated that *M. cephalus* could be induced to spawn more than once a year. It is possible that artificially induced spawning, with its positive climax and total release of oocytes from the ovary, may be an unnatural forced reaction, impeding complete development of the embryonic stages.

Little work has been accomplished on the biochemical composition of the oocytes before and after spawning. Kuo (unpublished data) examined changes in the biochemistry of eggs of *M. cephalus* during hydration and changes in mean egg diameter, water content and osmolarity. He showed that both soluble (glucose) and insoluble (glycogen) carhohydrates gradually decreased through hydration with a distinct drop prior to spawning. Total lipids increased from 16 to 25%. The major polar lipids were cholesteryl-esters and triglycerides. The proportion of free fatty acids was small. Palmitoleic acid ($C_{16:1}$), palmitic acid ($C_{16:0}$) and oleic acid ($C_{18:1}$) were major fatty acids, with decreasing amounts of myristic acid ($C_{14:0}$), linoleic acid ($C_{18:2}$) and some higher fatty acids containing 20, 22 and 24 C atoms. He recorded a conspicuous increase in the amount of soluble nitrogen-containing ninhydrin compounds. There was a general increase in amino acids, particularly the neutral ones, such as alanine, serine, leucine and isoleucine. There was some change in the level of total protein but less pronounced than that of the soluble nitrogen containing compounds.

Kuo (unpublished) also showed the preovulatory eggs were isotonic to seawater and the ovulatory eggs became hypotonic due to the rapid intake of external water during hydration. In the four-hour postovulatory period, a remarkable increase in the osmolarity adjusted the osmotic pressure from hypotonic to isotonic to seawater at the time of spawning. During this process, there is a net increase in egg electrolytes (Na, K, Ca, Mg, Cl) although concentrations decrease due to increase in egg water content. No comparable data exist for the analysis of eggs from *M. cephalus* developing naturally without induced treatment.

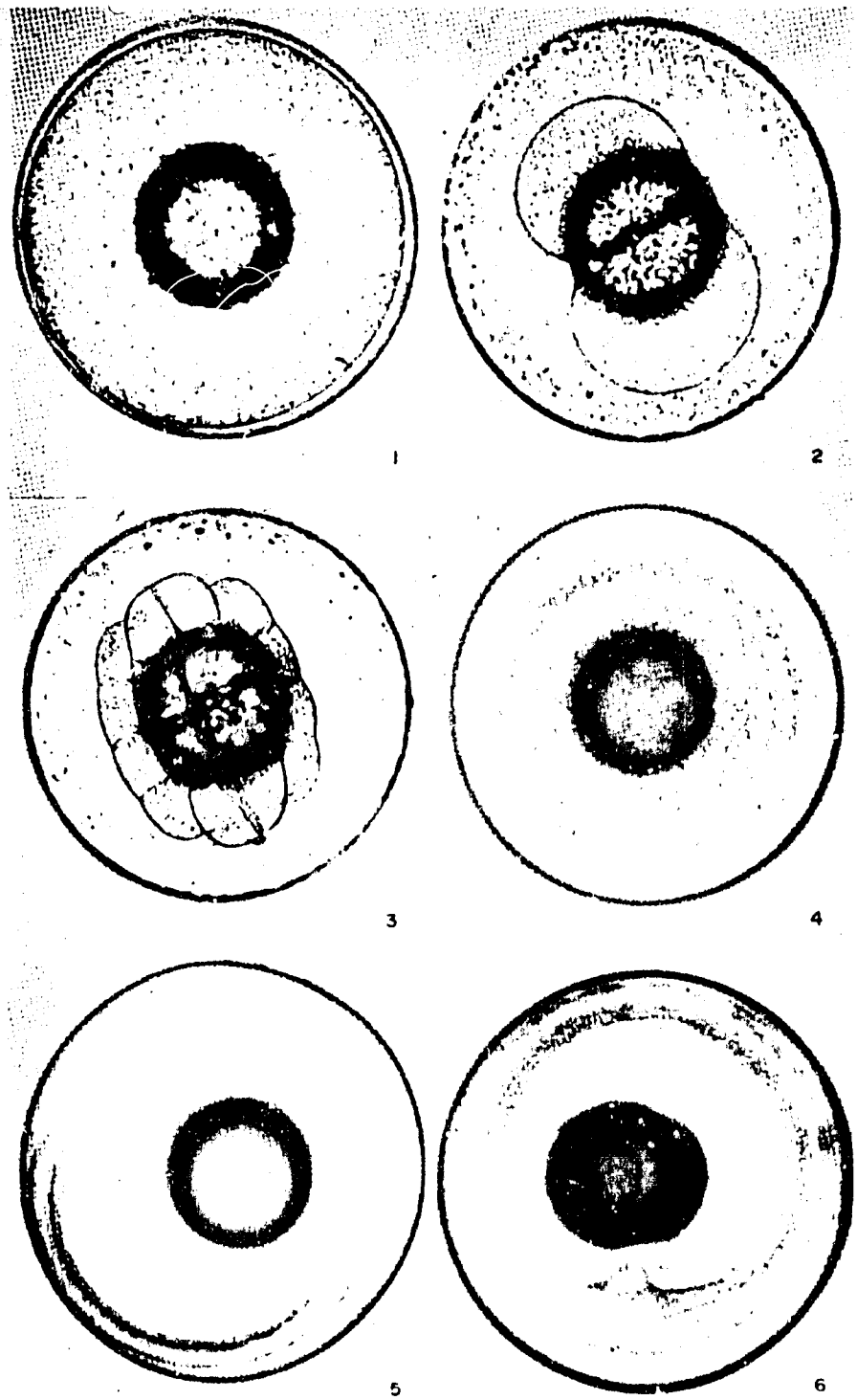


Fig. 4. Development of eggs: (1) Fertilized egg (x60). (2) 2-cell stage (x60). (3) 16-cell stage (x56). (4) Blastula (x56-57). (5) Gastrula (x59-60). (6) Gastrula (x59-60).

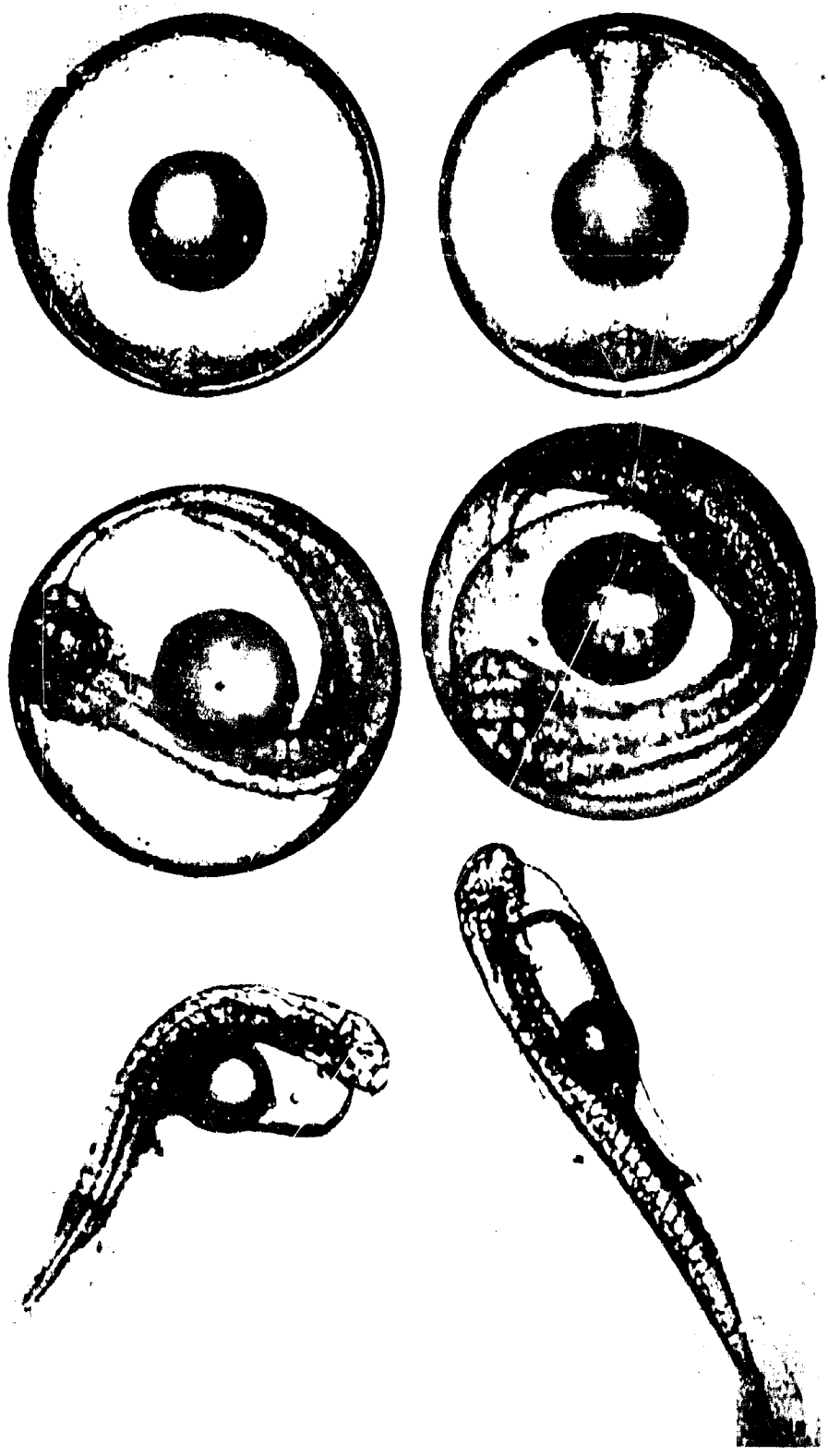


Fig. 4. (cont.). (7) Neurula (x57-58). (8) Developing embryo (x54-60). (9) Developing embryo (x54-60). (10) Developing embryo. (11) Hatching (x32). (12) Newly hatched larva—lateral view (x36). After Kuo et al. 1973a.

Although the need to identify the biochemical composition of normal eggs of Mugilidae still exists, artificial propagation of certain species following induced breeding has been reasonably successful in Taiwan, Hawaii, Israel and India. It is improbable that the quality of eggs produced by external hormone stimulation is inferior. The failure of the larvae to survive in large numbers is probably due to subsequent mishandling by poor techniques.

SPAWNING BEHAVIOR

The induced breeding techniques developed by Shehadeh and Ellis (1970) and Shehadeh et al. (1973b) permitted observation of natural spawning behavior following injection of regulated doses of purified salmon pituitary gonadotropin. The final courtship and spawning behavior described by them was similar to that observed by the others many years before.

For the final stages of the present induced breeding techniques with natural spawning, two or three males are placed in an aquarium with each recipient female about two hours before spawning. A female will usually spawn some 12 hr after receiving the second and last injection. The males become more active as hydration in the female progresses, indicated by distention of the belly and frequent excretion of calcium deposits.

The prelude to spawning is violent quivering by the male fish which are lying parallel to and facing the same way as the female, or which turn to touch the cloaca. The first release of a small number of ripe eggs stimulates the males to liberate spermatozoa. The female then responds with an explosive and continuous release of eggs (see Fig. 24, p. 49).

Although male fish were once given exogenous hormone treatrnent to finalize maturity, the practice was found unnecessary for spawning in the natural breeding season. If males are needed for spawning out of season, then spermiation can be induced readily by the injection of 17-alpha methyltestosterone, HCG or SG-G100 (Shehadeh et al. 1972).

SELECTION OF STOCK

The effectiveness of the hypophysation technique for spawning depends ultimately on the selection of suitable recipient fish at the proper stage of ovarian development. For species of fish which undergo normal gonad development but fail to spawn in captivity, identification of this stage is critical.

To date, selection of recipients has been largely subjective. External anatomical characteristics have been described and used, e.g., depth and fullness of belly, color and state of swelling of the cloaca, softness and resilience of the belly, roughness of pectoral fins or presence of head tubercules. More complex descriptions include the microscopic appearance of oocytes (Sundararaj and Goswami 1969), histological structures of eggs (Chen et al. 1969), or other histological data. Other physiological parameters associated with sexual maturity, such as elevated plasma proteins and calcium concentration, have been used but are of little practical use. Shehadeh et al. (1973a) described a method for the assessment of ovarian maturity *in vivo*, which was accurate and reliable and could replace all subjective methods for Mugilidae.

Kuo et al. (1974a) described standard procedures developed and applied regularly to induce spawning of *M. cephalus* under controlled conditions. Methods for determination of the stages of egg development and required dosage of partially purified salmon gonadotropin (SG-G100) for spawning were illustrated and emphasized so that the procedures could be readily used by other culturists (Figures 5, 6 and 9). The potency of the gonadotropin was described by Donaldson et al. (1972) as 1 mg equivalent to 2,150 IU human chorionic gonadotropin (HCG).

Liao et al. (1971), from experience gained spawning *M. cephalus* freshly taken from coastal waters, recommended a first injection within 1 hr after stocking in the tanks and again within 24 hr. The total dosage for each fish was 2.75-5 mullet pituitary glands mixed with 20-50 rabbit units of Synahorin. Vitamin E was also injected in the fish. The fish were later stripped and the eggs fertilized by the dry method after careful observation for the correct spawning time. The best response was judged by distention of the belly 10 hr after the first injection, a loose and soft belly, and finally the spontaneous release of eggs when the spawner was handled.

Anatomical characteristics are not always the most reliable indication of maturity, particularly for fish held captive all year round. Many females with soft and enlarged bellies can be in the early stage of oocyte development even during the natural breeding season. Enlarged abdomens are often the result of engorged intestines and accumulation of visceral fat.

Ovarian maturity, that is the stage of development of intraovarian oocytes, is most accurately obtained by the method of Shehadeh et al. (1973a). Intraovarian oocytes are removed *in vivo* from an unanaesthetized female through a polyethylene cannula. The cannula is inserted into the oviduct for a distance of 6-7 cm from the cloaca and the oocytes sucked orally into the tube by the operator as the cannula is withdrawn. Oocyte samples from the mid-portion of the ovary are the most representative and sampling error is minimized by avoiding the extremities.

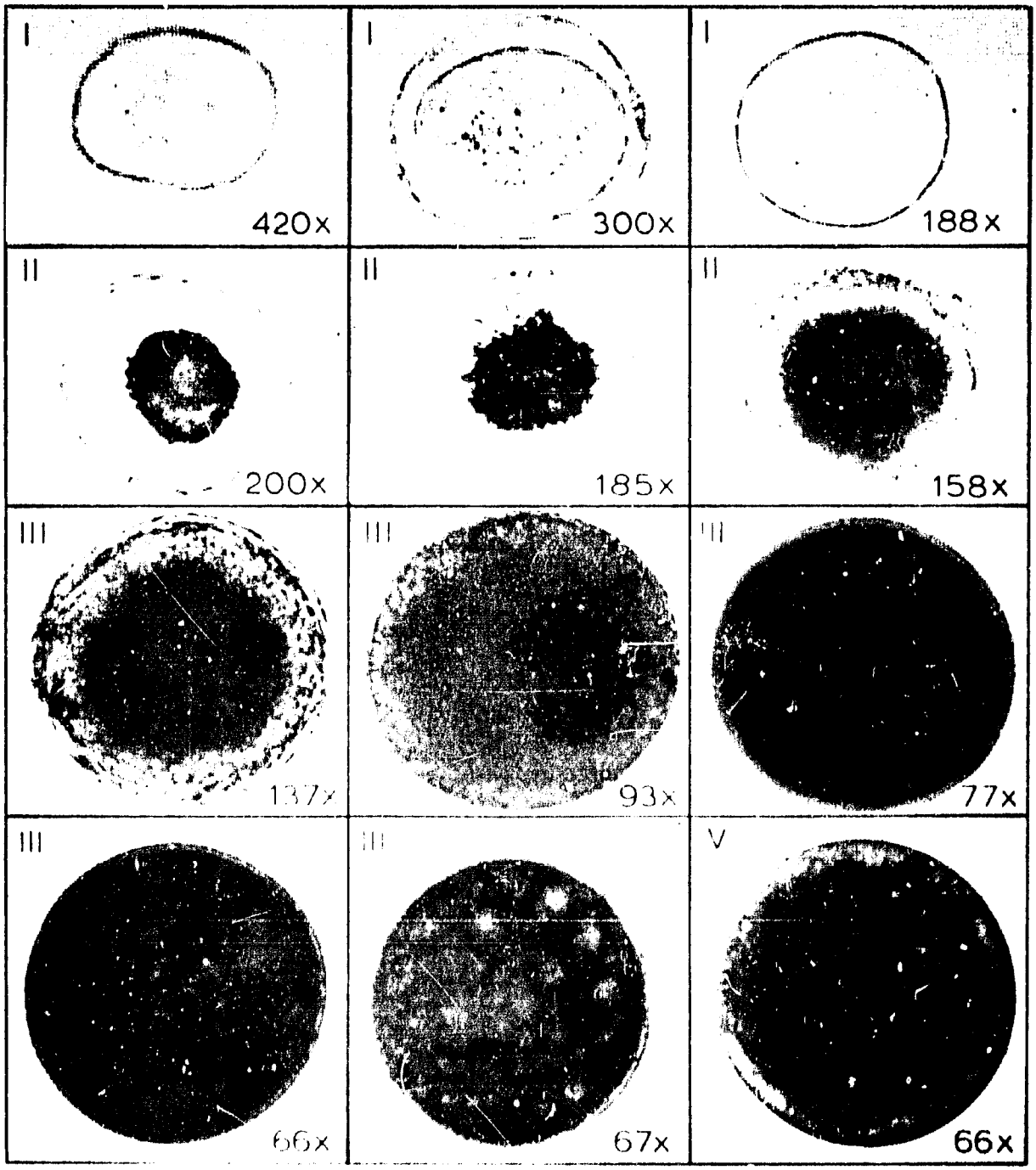


Fig. 5. Microscopic appearance of oocytes, stages I, II, III and V (atresia).

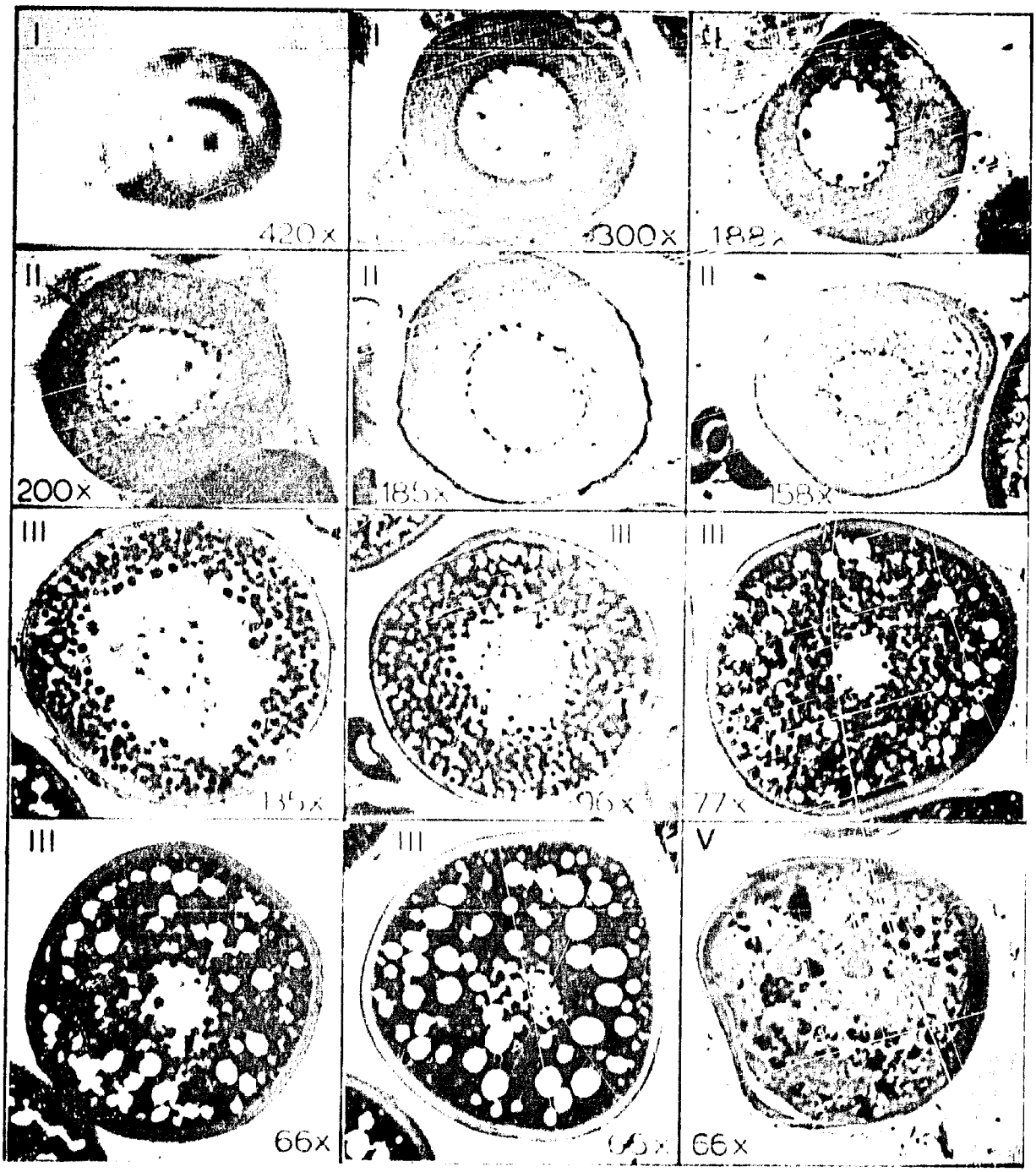


Fig. 6. Histological details of oocytes, stages I, II, III, and V (atresia)

The oocytes are removed from the cannula and washed and preserved in a solution of 1% formalin in 0.6% NaCl. They are then placed on a small plexiglass plate and measured with an ocular micrometer. Fine grooves cut in the plate align the oocytes and facilitate measurement. Egg diameters are measured along the horizontal axis and the measurements grouped into 50-μ class intervals. The sexual maturity of the fish is expressed in terms of mean egg diameter, calculated from the egg diameter frequency distribution.

The oocytes of *M. cephalus* develop in synchrony. Ovarian development therefore is determined accurately and quickly without sacrificing female fish. The method also provides a means to observe and record oocyte development in individual fish and thus precludes variation between females in the broodstock. Furthermore, it replaces the need for any histological processing and examination of oocytes.

INDUCED MATURATION IN SEASON

The annual cycle of ovarian development represented by the changes in gonadosomatic index (GSI) and the percentage of oocytes at each developmental stage are illustrated in Figure 7 for *M. cephalus* in Hawaii.

Oogenesis can readily be divided into five general stages by microscopic examination. The five stages, followed by microscopic and histologic appearance (Figures 5 and 6), are as follows:

Stage 1. Primary oocyte state (chromatin-nucleolus and peri-nucleolus stages): 12-170 μ in diameter

Primary oocytes are numerous and found in the ovaries throughout the year. At the commencement of the primary oocyte stage, the oocytes are small and spherical or oval in shape. They are transparent and vacuole-like nucleoli can be seen (Figure 5, I).

The spherical nucleus of each oocyte, through which one or more chromatin-nucleoli are distributed, occupies the greater part of the cell body within a thin layer of cytoplasm. With the growth of the oocytes, the cytoplasm increases considerably in relative volume and becomes more basophilic. The chromatin-nucleoli also increase in number and move toward the periphery of the nucleus. The oocytes become enclosed by a single thin layer of follicle cells.

As the primary oocyte stage progresses, the cytoplasm of the oocytes increases, becoming greater in volume than the nucleus and gradually losing its basophilic nature. The zonation of ooplasm is evident as the outer part of the ooplasm is distinct from the germinal vesicle. The chromatin-nucleoli lie close to the nuclear membrane. Oocytes in the later part of the primary stage are irregular and vary between spherical and tetragonal in shape.

Stage II. Yolk vesicle stage: 170-210 μ

At the beginning of the yolk vesicle stage, the appearance of each oocyte is characterized by the granular cytoplasm and darker zone surrounding the germinal vesicle. There are several outer layers of cells but the zona radiata is not distinct (Figure 5, II).

The general histological structure of the nucleus has not changed greatly from the late primary stage and chromatin-nucleoli are distributed close to the nuclear membrane in a single row. The nucleoli usually vary in shape from spherical to elliptical, but are at times very irregular. The oocyte is characterized by the appearance of yolk vesicles which, in the early development period of the stage, form a thin layer outside the nucleus. When stained with hematoxylin, these yolk vesicles are lighter in color than the ooplasm.

As the oocytes develop, the yolk vesicles increase in number and size, gradually filling the cytoplasm from the center of the oocyte toward the periphery. The zona radiata becomes apparent between the ooplasm and the follicular layer. At first it is narrow, compact and homogeneous but it thickens with the growth of the oocyte. The outer layer of follicle cells around the oocyte continues to thicken.

Stage III. Yolk globule stage (primary, secondary and tertiary yolk globule stages): 200-700 μ

In the early part of this stage, the yolk vesicle occupies the entire ooplasm and the appearance of each oocyte is characterized by a granular appearance (Figure 5, III). As each oocyte develops, the internal definition is slowly obscured from the center toward the periphery as the deposition of yolk material increases evenly. Finally, when the fusion of yolk globules is complete, the central portion of the oocyte appears to clear.

From histological examination, each oocyte at this stage is characterized by the appearance of minute spherical yolk globules which stain deep black with Heidenhain's hematoxylin. The yolk globules first appear in the outermost part of the ooplasm and increase in number and size as the oocyte grows, and the yolk layer continues to become thicker in proportion to the adjacent layer of cytoplasm. Finally the layer reaches the surface of the germinal vesicle.

The oocytes become nearly spherical or oval in shape. The nucleoli do not change in size and number but begin to lose their peripheral arrangement and become scattered randomly in the nucleus. The follicle layer becomes broader as the follicle cells increase in size and the zona

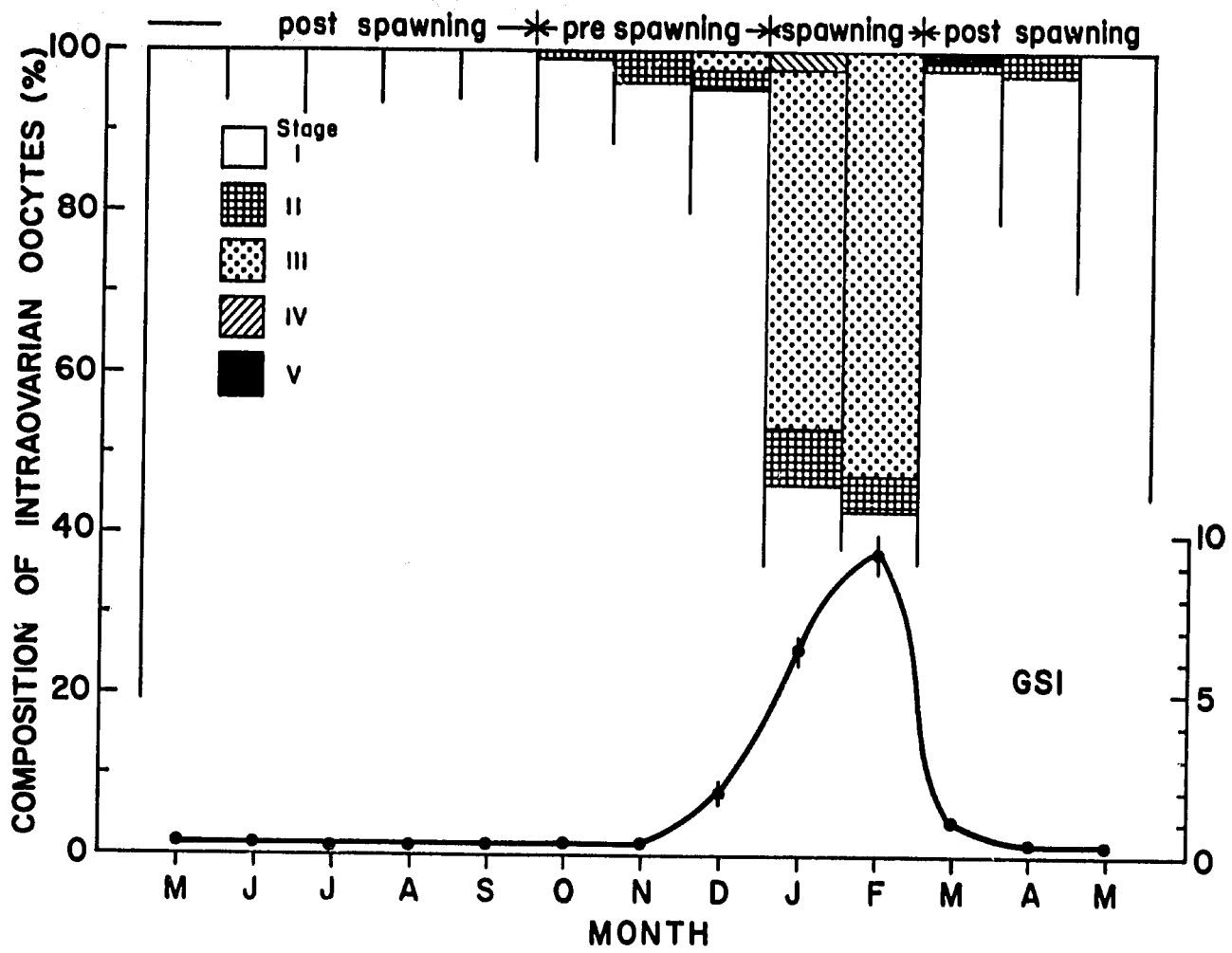


Fig. 7. Changes in gonadosomatic index (GSI) and stage composition of intraovarian oocytes of Hawaiian grey mullet (after Kuo and Nash 1976).

radiata thickens notably. The striation of the zona radiata is now clearly visible. As the oocyte grows, the yolk globules appear to accumulate very rapidly in the inner part of the ooplasm. The germinal vesicle does not change but the chromatin-nucleoli become more regular in shape.

In the final phase of this stage, the yolk globules accumulate throughout the ooplasm. Fusion of yolk globules and the numerous oil globules is apparent. The germinal vesicle becomes irregular in outline and consequently the nucleoli assume an irregular arrangement along the periphery. The nuclear membrane disappears, slowly followed by the nucleoli, which first become vacuolated and move toward the inner part of the germinal vesicle.

Stage IV. Ripe stage (migratory nucleus, maturation and ripe egg stages)

Ripe oocytes occur immediately before ovulation. The duration of this stage is short as the induced female is undergoing the final rapid development of oocytes before ovulation. The oocytes of this stage have been observed only from captive females after hypophysation. The oocytes are characterized by the migration of the nucleus to the animal pole, and fusion of the yolk globules and oil droplets. Finally, the yolk appears as an homogeneous mass filling the interior of the oocytes. The membrane enclosing the egg proper is comparatively thick. Many short villi are found distributed over the whole surface of the egg.

Stage V. Atresia

Visible degeneration of the oocytes occurs, mostly to stage III. The yolk globule contracts irregularly, beginning from the edge of the zona radiata and moving toward the center. The zona radiata begins to disintegrate and its outer surface becomes irregular. It eventually ruptures, invasion of the interior of the oocyte begins, and

the yolk is phagocytosed by granulosa cells which undergo hypertrophy (Figure 5, V).

In nature, vitellogenic oocytes in Hawaiian fish first appear in October. They are all in the yolk vesicle stage (stage II). The yolk-laden oocytes (III) are usually observed within ovaries sampled in December, when the percentage of oocytes at stages II and III are 47.2 and 52.9%, respectively. In January and February, the percentage of oocytes at stage III is between 83.3 and 92.4%. Atretic oocytes are first observed in oocyte samples taken in January. Peak spawning of grey mullet in Hawaii takes place during the months of January and February.

The spawning of *M. cephalus* has been induced successfully by injections of mullet pituitary homogenate (Tang 1964), carp pituitary homogenate (Yashouv 1969), and Pacific salmon or grey mullet pituitary homogenate with Synahorin (Liao 1969; Shehadeh and Ellis 1970; Liao et al. 1972). Spawning of captive mullet has been achieved since with the standardized salmon gonadotropin, SG-G100 (1 mg equivalent to 2,250 I.U. HCG) (Shehadeh et al. 1973a; Kuo et al. 1974 a), human chorionic gonadotropin, HCG (Kuo et al. 1973b) and most recently with combinations of SG-G100 with HCG or deoxycorticosterone acetate, DOCA (Kuo and Nash 1975), and with prostaglandins (Kuo and Watanabe 1978).

Despite prolonged usage, hypophysation practices are impeded by a lack of standardization among workers. The efficiency of any dose is related to the sexual maturity of both donor and recipient and their phylogenetic relationship, the gonadotropin potency of the injected preparation, and the physiological state of the recipient.

In order to standardize the hypophysation technique, the potency of a pituitary preparation or extract must be specified. An accurate expression of the stage of maturity of hypophysated females is also needed. Attempts to standardize the potency of the injected pituitary preparation have been ineffective to date, due to the variety of test animals being used, the bioassay technique and standard units employed, and lack of uniformity and consistency in gonadotropic potency of pituitary homogenates.

The use of mammalian gonadotropins and steroids as replacements for pituitary homogenates would compensate for the lack of uniformity in gonadotropic potency of pituitary homogenate. Attempts to test the possibility of using mammalian gonadotropins and steroids for induced spawning of teleosts have been made with limited success, but the dose rates and injection sequences of mammalian hormones have yet to be established. Tables 2 and 3 show the results of induced ovulation by *in vivo* and *in vitro* techniques, respectively.

HCG has been used effectively to induce spawning of the grey mullet (Kuo et al. 1973b). No relationship between spawning dose and maturity was found and the total dose required for spawning was found to be between 49 and 78 µg/g body weight. Estimation of the most suitable injection dose of HCG was very critical and precaution was needed to avoid an overdose which caused premature spawning characterized by the presence of multiple oil globules in each oocyte. Other gonadotropins and steroids, such as FSH, DOCA, estrone and estradiol, have been tested (Kuo and Nash 1975) for inducing ovulation *in vitro,* but a priming injection of SG-G100 was required.

In conclusion, salmon gonadotropin SG-G100 has proven to be the most potent ovulating agent and has produced reliable and predictable spawning. It is recommended above all others at this time for practical culture work on the grey mullet.

INDUCED MATURATION BY ENVIRONMENTAL CONTROL

Ovarian maturation was induced out of season by environmental manipulation, photoperiod/temperature in controlled experiments conducted by Kuo et al. (1974b). Female grey mullet adults were selected from established broodstock held captive for more than 2 yr. Only females were used which had been induced to spawn in the natural breeding season preceding the experimental period, or which were known to be suitable for induced spawning in the next natural breeding season (above 31 cm fork length). In order to be certain that the effects of the environmental parameters on ovarian oocyte development were not reflecting the natural reproductive cycle, all the experiments were initiated early in the refractory period or primary oocyte stage which occurs in Hawaii between March and October.

Full details of the experimental design on the photoperiod and temperature cycles were reported in Kuo et al. (1974b). The effect of photoperiod cycle on the vitellogenesis was examined by comparison of ovarian development for different females exposed to (1) a condensed natural daylight cycle, (2) a natural daylight cycle before a retarded light regime of 6L/18D was established, and (3) a natural daylight cycle acting as control. Constant temperatures between 17° and 26°C were maintained except for the control which reacted to ambient conditions.

In nature, vitellogenesis of ovarian oocytes begins shortly before the length of daylight is minimal (11 hr). The same response in ovarian development was not found from the condensed daylight cycles, but the results indicated that a constant photoperiod regime of 6L/18D was effective in stimulating vitellogenesis,

Table 2. Injection dose, schedule and reaction of grey mullet females to gonadotropins and steroids (after Kuo and Nash 1975).

Fish	Body weight (g)	Dose (units/g body weight) with oocyte diameter (μm) and injection sequence 0 h	12 h	24 h	48 h	72 h	96 h	120 h	Total dose/ g body weight	Hydration	Ovulation	Time of spawning (h)	Fertilization (%)	Result
A. Human chorionic gonadotropin (I.U.)														
1	634	27.6 607		50.0 630					77.6	+	+	21.0	45	Spawned
2	819	21.4 617		47.6 631					68.9	+	+	11.5	54	Spawned
3	872	16.1 617		32.9 630					50.0	+	+	16.0	60	Spawned
4	908	19.3 621		38.6 630					57.9	+	+	13.2	98	Spawned
5	759	19.6 645		30.4 651					50.0	+	+	15.0	84	Spawned
6	1092	14.1 645		42.2 659					56.3	+	+	13.8	92	Spawned
7	876	12.0 616		24.1 633	15.8 632				51.9	+	+	12.5	—	Partially spawned
8	1032	13.4 625	23.1 639	23.1 639					54.9	+	+	17.0	94	Spawned
9	795	13.3 641	20.3 649	16.4 654					50.0	+	+	18.3	—	Partially spawned
B. Partially purified salmon pituitary gonadotropin, SG-G100 (μg)														
1	968	3.1 702		10.3 713					13.4	+	+	13.0		Spawned
2	1093	9.1 670		4.6 672					13.7	+	+	12.5	53	Spawned
3	1010	5.0 665		9.9 676					14.9	+	+	10.0	93	Spawned
4	980	5.1 711		10.2 714					15.3	+	+	11.8	83	Spawned
5	838	6.0 723		6.0 723					12.0	+	+	14.7	93	Partially spawned
6	752	6.6 659		13.2 687					19.8	+	+	10.5	81	Spawned
7	862	5.8 613		13.9 637					19.7	+	+	10.5	87	Spawned
8	597	8.4 624		16.8 630					25.2	+	+	13.2	82	Spawned
9	847	5.9 689		11.8 716					17.7	+	+	14.2	85	Spawned
10	717	7.0 664		14.0 676					21.0	+	+	11.5	98	Spawned
11	1371	2.2 606		7.3 602	7.3 609	3.8 666			20.4	+	+	14.3	96	Spawned
12	596	0.8 630		0.8 629	0.8 637	1.6 648	0.8 649	0.8 649	21.0	+	+	14.2	60	Spawned
13	1060	4.7 657		0.0 677	9.4 694				14.1	+	+	13.1	93	Spawned
14	872	5.7 651		0.0 660	11.4 665				17.1	+	+	12.0	98	Spawned
15	1160	4.3 637		0.0 641	10.3 646				14.6	+	+	13.3	92	Spawned
16	714	7.0 623		0.0 622	14.0 626				21.0	+	+	12.3	92	Spawned
C. Purified salmon pituitary gonadotropin (μg): human chorionic gonadotropin (I.U.) (ratio 1 : 4)														
1	1097	11.6 645		34.8 660					46.6	+	+	13.2	93	Spawned
D. Deoxycorticosterone acetate (μg)														
1	491	10.2 611		10.2 616	10.2 636	10.2 654	50.9 648		91.7	+	—	—	—	Atresia
E. Pregnant mare's serum gonadotropin (I.U.)														
1	1305	1.2 698		1.2 723	1.2 683	1.2 675			3.6	—	—	—	—	Atresia
F. Deoxycorticosterone acetate (μg): hydrocortisone (μg) (ratio 5 : 1)														
1	1130	60.0 730		60.0 727	60.0 715				180.0	+	—	—	—	Atresia
2	605	60.0 736		60.0 726					120.0	—	—	—	—	Atresia
G. Purified salmon pituitary gonadotropin (μg): human chorionic gonadotrophin (I.U.): Deoxycorticosterone Acetate (μg) (ratio 1 : 1 : 0 then 0 : 0 : 1)														
1	450	22.0 659			50.0 653				72.0	+	+	18.5	0	Spawned

Table 3. Summary of grey mullet ovulation *in vitro* (24-hr incubation period) (after Kuo and Nash 1975).

Hormone	Dose (concn/ml; percentage of ovulation)				
Salmon pituitary gonadotropin (SG-G100)	25 µg	50 µg	100 µg	150 µg	
	16.8%	27.6%	26.9%	15.4%	
Follicle stimulating hormone (FSH)	1 I.U.	2 I.U.	3 I.U.	4 I.U.	
	5.6%	10.3%	15.0%	25.4%	
Luteinized hormone (NIH-LH-S11)	200 µg	200 µg	600 µg		
	19.7%	3.3%	6.8%		
Human chorionic gonadotropin	5 I.U.	10 I.U.	20 I.U.	40 I.U.	60 I.U.
	6.9%	11.9%	11.3%	21.9%	2.1%
Pregnant mare's serum gonadotropin (PMSG)	25 µg	50 µg	100 µg	150 µg	
	9.4%	4.2%	2.7%	7.5%	
Gonadal steroids	5 mg	10 mg	15 mg		
Estrone	1.8%	3.2%	2.6%		
Estradiol	2.0%	0%	6.7%		
Progesterone	7.1%	0%	0%		
Dehydroepiandrosterone (DHA)	0%	1.5%	0%		
Pregnenatone	0%	0%	0%		
Testosterone	0%	0%	0%		

irrespective of any preconditioning photoperiod adjustment within the temperature range examined. The experimental conditions stimulated vitellogenic oocytes being developed within 49-62 days at the photoperiod regime of 6L/18D. No vitellogenic oocytes were observed in fish in controlled conditions until 235 d, by which time the ovarian development seemed to reflect the normal breeding cycle.

For the temperature range examined, the progress of vitellogenesis was shown to be dependent upon temperature. The results (Figure 8) showed that only fish held at the lower temperature range (17-21°C) developed to functional maturity and were then induced to spawn by injections of purified salmon gonadotropin and larvae were raised through metamorphosis.

It is, therefore, possible to control ovarian development by proper combination of temperature and photoperiod regimes. The best results for grey mullet can be expected under a constant temperature of 21°C and photoperiod of 6L/18D. A higher dose of hormone is required to induce spawning of these fish than described for the standard procedure.

HYPOPHYSATION

In Hawaii and Taiwan, where induced spawning of *M. cephalus* is regularly performed, year class IV females weighing between 1 and 2 kg each are preferred for breeding. Hypophysation is initiated when intraovarian oocytes are in the tertiary yolk globule stage and the mean egg diameter is preferably larger than 650 µ but at least 600 µ. In the breeding season, the amount of gonadotropin required to induce spawning is inversely proportional to the initial egg diameter of the recipient female and varies between 12 and 21 µg/g body weight. The spawning dose is estimated from the regression illustrated in Figure 9, and is applied in two separate injections: one-third of the dose first, followed by the remaining two-thirds up to 48 hr later. A higher dose

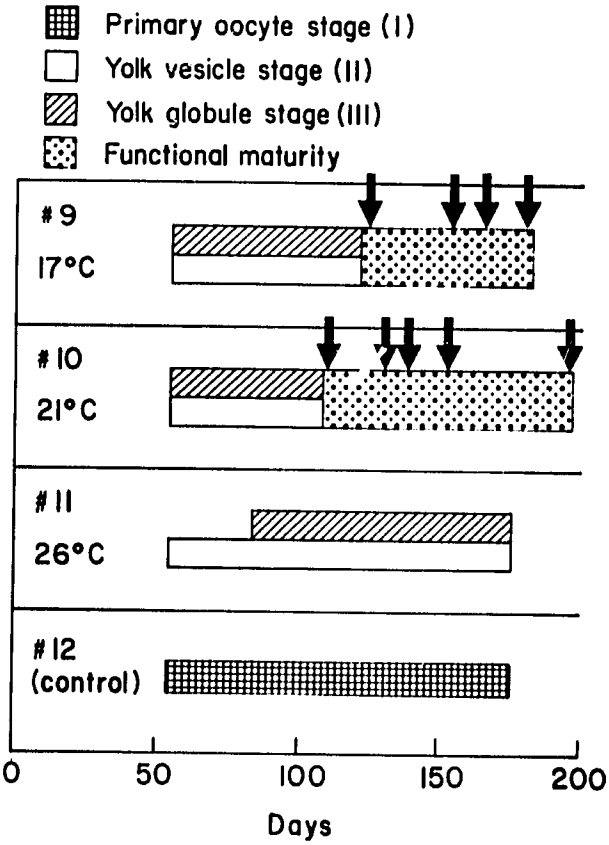


Fig. 8. Relationship of vitellogenesis to temperature in grey mullet. Arrows indicate successful induced spawnings of individual females (after Kuo et al. 1974b).

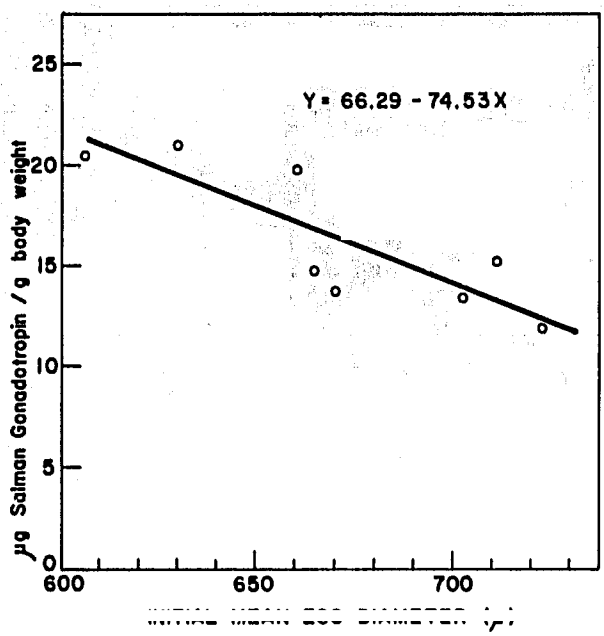


Fig. 9. Relationship between initial mean egg diameter of recipient grey mullet females and amount of gonadotropin required to induce spawning in the natural season (after Kuo and Nash 1975).

may be required for females induced to spawn out of season under environmental control. The sequence is critical to follow in order to avoid partial spawning. The priming injection initiates the final stages of oogenesis. No morphological or chemical changes in sampled oocytes are observed before 24 hr following the first injection, after which the central portion of the oocytes clears. The second injection, which is then given, induces hydration, indicated by a noticeable increase in body weight and by excretion of calcareous deposits. The increment of egg diameter related to injection doses at differing stages of ovarian maturity is illustrated in Figure 10. The quantity of gonadotropin corresponding

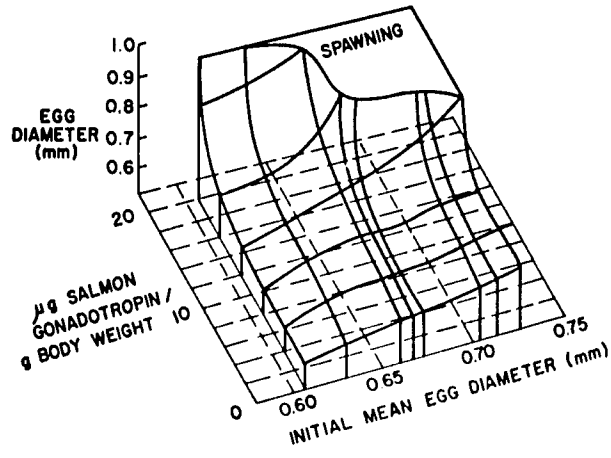


Fig. 10. Relationship of initial mean egg diameter, spawning dose, and egg development in grey mullet (after Kuo and Nash 1975).

to the area of spawning "platform" is required for successful spawning. A female will usually spawn some 12 hr after receiving the second and last injection.

ENVIRONMENTAL CONDITIONS FOR EGG INCUBATION

The vertical movements of the eggs of Mugilidae after release have been variously described. Sanzo (1936) observed that all fertile eggs of *M. chelo* sank soon after fertilization, while Yashouv (1939) reported sinking toward the end of incubation for eggs of *M. cephalus*. Kuo et al. (1973a) recorded that the majority of eggs of *M. cephalus* which sank within the first 12 hr were undeveloped or unfertilized (absence of perivitelline space). They noted that when eggs were prevented from settling by strong aeration in small vessels, survival was increased and the incubation period reduced by 2 hr at the same temperature. They concluded that water temperature and turbulence had a decided effect on incubation time, and this was supported by Tung (1973).

Tang (1964) noted that developing eggs of *M. cephalus* sank in standing water and so incubated them in suspension by circulating water. Yashouv and Berner-Samsonov (1970) described the development of *M. capito* eggs floating on the surface. Eggs of *M. cephalus* lost their buoyancy after 20 hr and sank but hatched successfully. Sebastian and Nair (1974) observed that eggs of *M. macrolepis* floated in shallow hatching trays, and Liao (1974) reported that the fertile eggs of *M. cephalus* were buoyant and floated near the surface. He maintained them in suspension during incubation by aeration as he noted that some did sink later. The rearing practices of Nash et al. (1974) attempted to maintain the fertilized eggs of *M. cephalus* in suspension by circulating water, particularly when incubating at a density of 250 eggs per liter.

Although incubation is apparently continued for eggs of *M. cephalus* which sink below the surface, the artificial environment of a container for hatching obviously introduces problems not normally encountered by eggs liberated in the sea. Density of eggs during incubation is a key factor influencing hatching rates and overcrowding produces problems of agglutination, bacterial contamination, oxygen depletion and increased metabolite production. Suspension of the eggs by water movement counters these problems, and few workers attempt to incubate fish eggs without the minimum of circulation or water movement through aeration, particularly when operating at high density.

Egg development and hatching time are both temperature dependent. Tang (1964) reported that hatching of *M. cephalus* took place in 59-64 hr at temperatures ranging from 20.0-24.5°C and salinity from 24.39-35.29‰. Fertilization was low (32%) and the rate of

hatching was below 10%. Yashouv and Berner-Samsonov (1970) noted that under laboratory conditions, the eggs of *M. cephalus* and *M. capito* developed and hatched within 36-44 hr at 22-32°C. Kuo et al. (1973a) stated that hatching of *M. cephalus* eggs was evident 36-38 hr after fertilization at 24°C, and 48-50 hr at 22°C. Total length of the newly hatched larvae was 2.65 ± 0.23 mm. Salinity was 32‰.

Liao (1974) stated that hatching of *M. cephalus* eggs took place in 34-38 hr at 23-24.5°C, and at 49-54 hr in 22.5-23.7°C, with salinities between 30.1 and 33.8‰. Tung (1973) described the relationships between mean water temperature (θ) and duration of incubation period (T) as $T_e^{0.35}\,\theta = 1{,}262$ in still water, and as $T_e^{0.037}\,\theta = 106$ in running water. Nash et al. (1974) described the temperature-incubation period relationship for *M. cephalus* in recirculating water (Figure 11). Finally, Sebastian and Nair (1974) recorded the incubation time of *M. macrolepis* as 23 hr between 26-29°C and 29-31‰ salinity.

Nash et al. (1974), Sylvester and Nash (1975), and Nash and Kuo (1975) report the survival of eggs of *M. cephalus* within broad ranges of temperature, salinity, and dissolved oxygen. Their results are summarized in Figure 12. Minimal mortalities of eggs occurred at 22°C for normal seawater (32‰), and an effective temperature range for incubation was 11-24°C. Houde et al. (1976) incubated *M. curema* between 26-27°C and salinities 35-38‰ satisfactorily.

Most workers prefer a working temperature range of 18-24°C for *M. cephalus*. Above 25°C the mortality is usually above 90% and often total. Optimal salinities

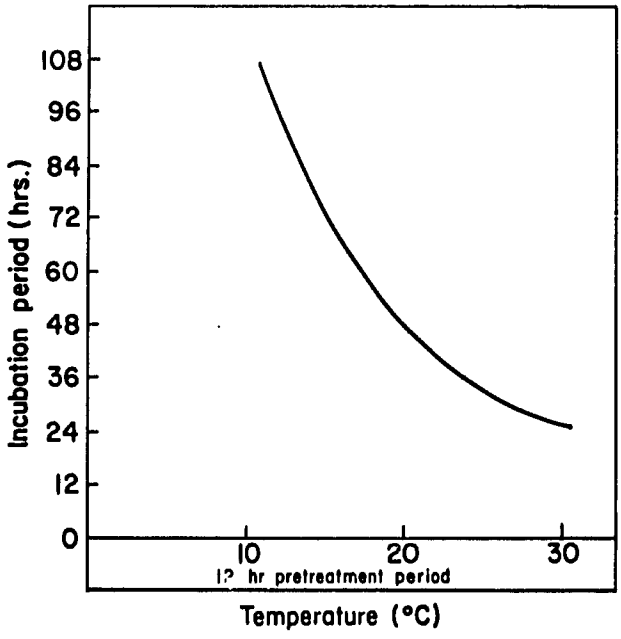


Fig. 11. Relationship between incubation period of grey mullet eggs and temperature. S = 32‰ (after Nash et al. 1974).

for incubation are 30-32‰ under ambient temperature conditions (19.5-20.5°C), and significant decreases in egg survival occur with eggs incubated in mean oxygen concentrations below 5.0 ppm.

Good temperature control during incubation is essential. Present work on the propagation of *M. cephalus* is conducted within the most desirable temperature range and mostly at the optimum level. Although individual daily temperature fluctuations may be responsible for some egg mortality, it is believed that temperature *per se* is not influencing the high mortalities experienced in the early stages of development. However, the working range of 18-24°C is suitable for rapid bacterial growth and therefore indirectly a causative factor of environmental instability during incubation.

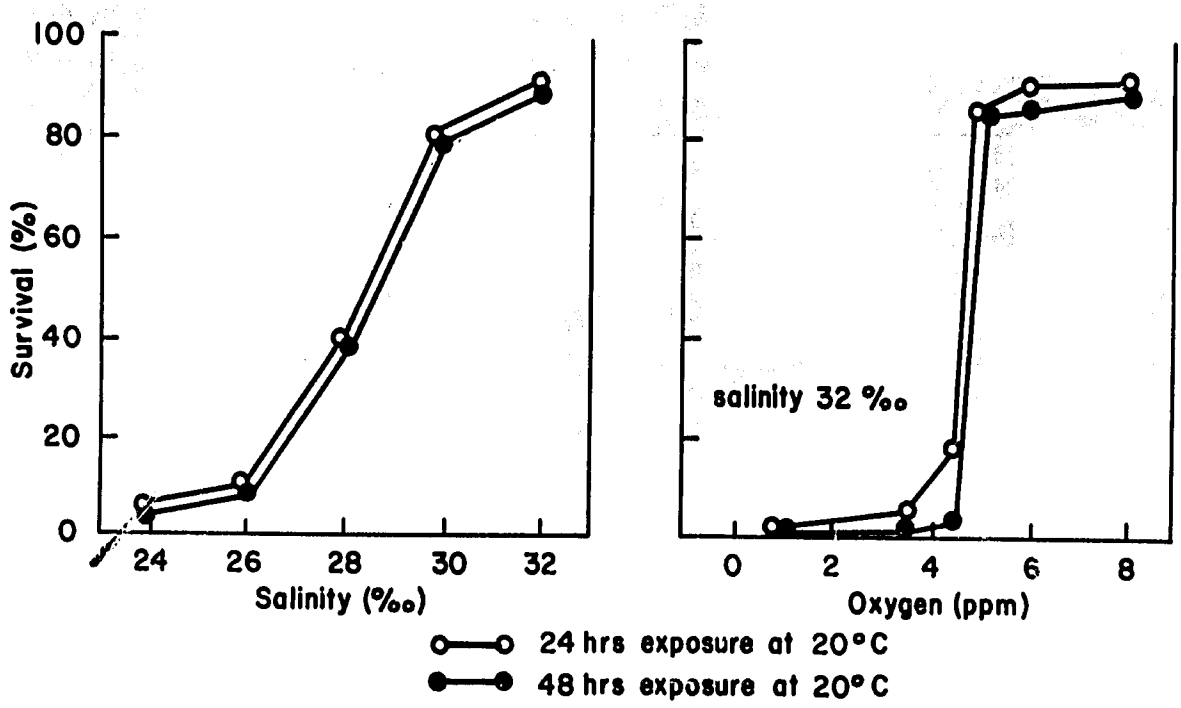
Kuo (unpublished) recorded the change in osmolarity of the eggs prior to spawning and that they were isotonic to seawater at the time of spawning. This fact, together with the migratory behavior of the adults at breeding time moving out into oceanic water, indicates that salinity level is most important for egg development, and that incubation should be conducted in full seawater.

INCUBATION FACILITIES

Liao (1974) reported that fertilized eggs of *M. cephalus* were incubated and hatched in two types of hatching equipment. The first was a flowing water type. It consisted of a fine-mesh net hanging in the water and provided with a continuous slow exchange of water. It was similar in design to that used for hatching eggs of Chinese carp. The second was a static system of simple containers, each with aerated seawater. After hatching, the larvae were transferred to other rearing tanks. The systems were later modified to avoid loss of larvae during transfer. All development stages were then completed in either plastic or large concrete tanks indoors with good environmental control.

Kuo et al. (1973a) incubated eggs in well-aerated static seawater (32‰) in 140-l fiberglass tanks. Incubation temperatures were between 22-24°C. They worked on a series of improvements and with Nash et al. (1974) developed a modified circular kreisel (Fig. 13) for incubating eggs of *M. cephalus*. The advantages of the kreisel were that the eggs could be pretreated with antibiotics to reduce bacterial growth and the density of eggs was high for the size of the apparatus. Also separation of the emergent larvae from empty shells and inviable eggs could be made during transfer of the larvae to the rearing containers. This reduced potential fouling in the rearing tanks.

The kreisel has proved to be an effective rearing apparatus and can be constructed with many dimensions. Aeration and circulation of the water are maintained by



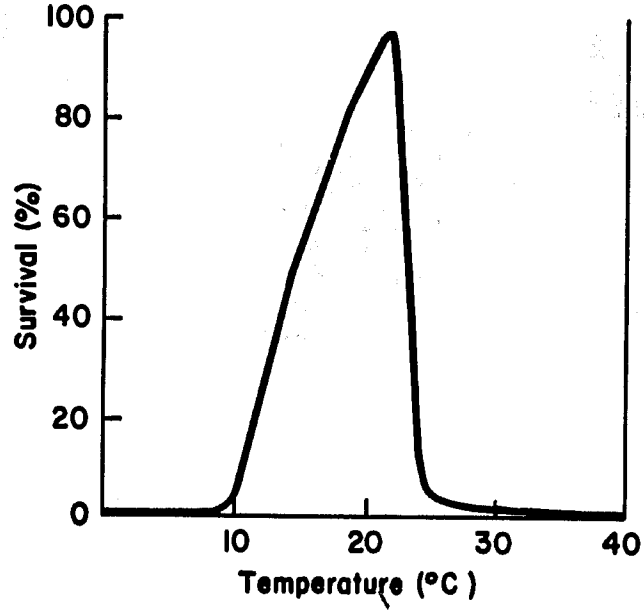


Fig. 12. Grey mullet egg survival (%) at varying incubation parameters (after Nash et al. 1974; Sylvester and Nash 1975; Nash and Kuo 1975).

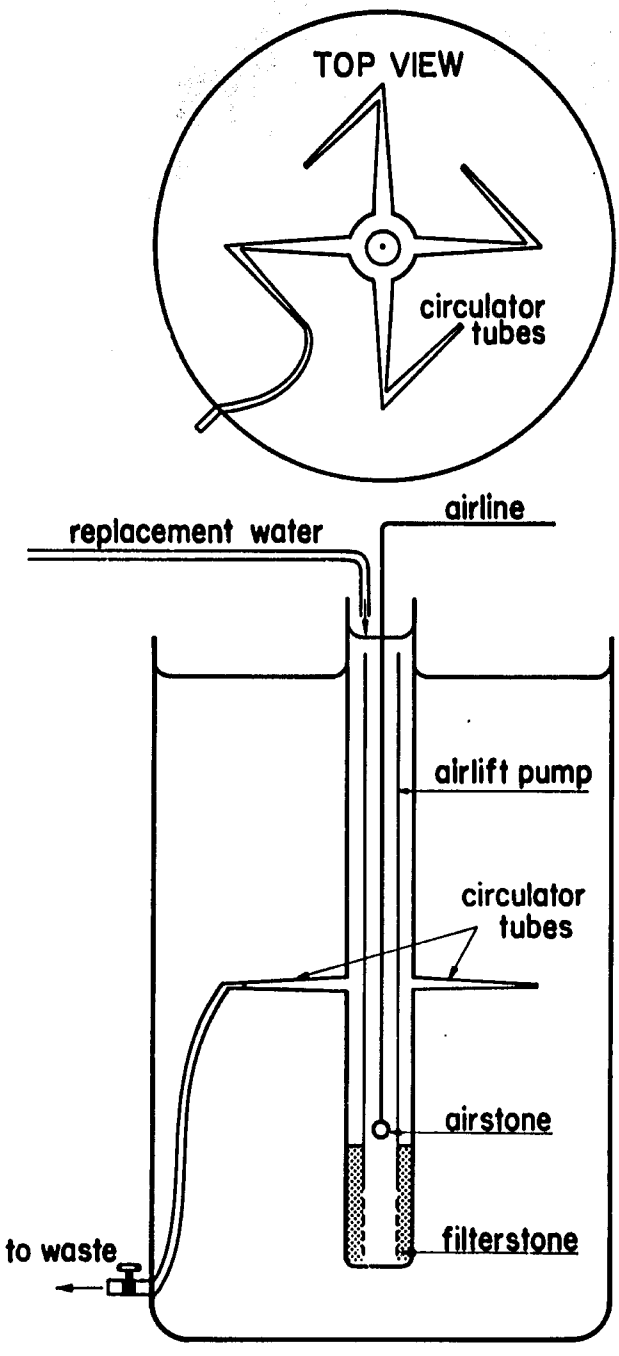


Fig. 13a. Rearing kreisel (after Nash et al. 1974).

an airlift pump located in the center column. The water is drawn by the airlift pump into the column through a large filter stone, aerated and passed back down the outside of the pump and redirected into the container. A connection on one of the discharge arms of the column directs a fraction of the water to waste, and that volume is replaced by an incoming supply located above the column.

Nash et al. (1974) used ambient seawater (32‰) in the kreisel. Before use, it was treated by filtering, irradiating, and treating with antibiotics. Both penicillin (19 IU/ml) and streptomycin (0.01 mg/ml) were added daily and effectively reduced marine bacterial growths. The eggs were suspended in seawater until after hatching and the larvae safely transferred by syphon into larger prepared rearing containers.

Yashouv (1969) used specially designed incubators to prevent the washing out of eggs with the changed seawater. Sebastian and Nair (1974) used shallow trays similar to those used for the incubation of salmonoid eggs.

Oppenheimer (1955) demonstrated bacterial growth on fish eggs shells by photomicrographs and found that the percentage hatch of a number of marine species increased by controlling marine bacteria with a mixture of penicillin and streptomycin. Shelbourne (1964) credited the successful culture of marine flatfish to the use of antibiotics. Using controlled amounts of the two, he demonstrated that the survival of young flatfish was significantly increased from 40% to 60% at metamorphosis.

The antibiotic treatment in incubation tanks reduces bacterial activity around the shell and prevents agglutination of the eggs. Physical damage to the egg shell, or the covering of the shell with bacterial slime or mucus, breaks down the osmotic balance between the eggs and the surrounding seawater. The osmotic regulation between the two is appearing to be a key factor in successful propagation of marine and brackishwater species, and needs a great deal of further attention and research.

Although the effectiveness of antibiotics can be replaced by ultraviolet sterilization and bacterial filters in the water system of a hatchery, the usefulness of antibiotic treatments cannot be underestimated (Struhsaker et al. 1973). Nash et al. (1974) utilized antibiotic washes and prolonged treatments during egg incubation. Buoyant viable eggs at the gastrula stage were removed from the spawning tank, washed in irradiated and filtered seawater, and then dipped for 1 min in a seawater bath containing potassium penicillin G (80 IU/ml) and streptomycin sulfate (0.05 mg/ml). They were then distributed into the kreisels at a density of about 250 eggs per liter and incubation was continued for the remainder of the period. Low levels of antibiotic, added daily, reduced all bacterial contamination.

Oppenheimer and Shelbourne theorized on the ways that bacterial activity affected and damaged eggs (Costlow 1969). Penicillin is effective against gram-positive organisms, and streptomycin against gram-negative organisms. The combination therefore sterilizes seawater if there are no resistant organisms present. Antibiotics have also proved effective in the culture of shellfish (Walne 1958). However, they are known to unbalance the mechanisms for calcium transfer in some species

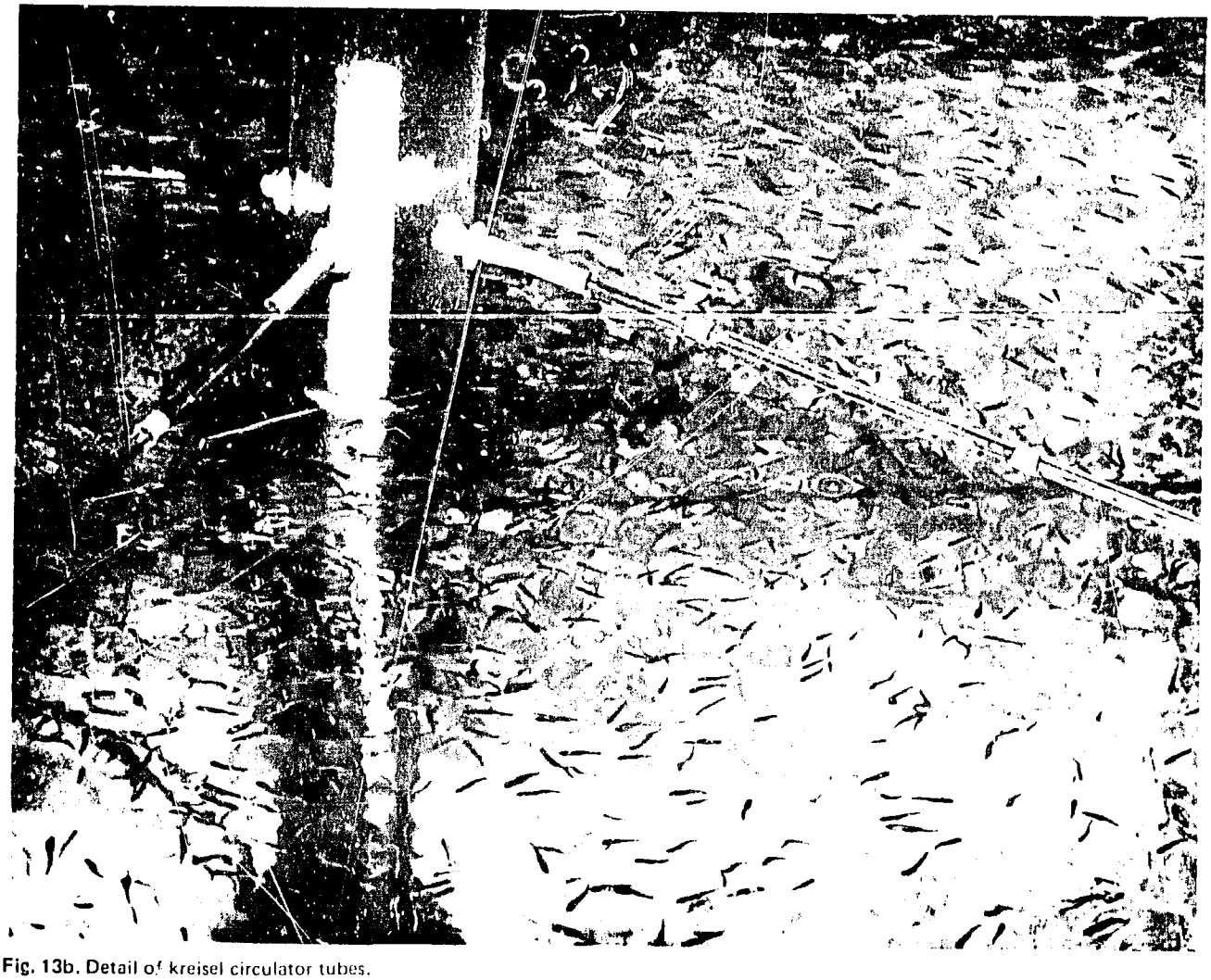


Fig. 13b. Detail of kreisel circulator tubes.

and could not be used for the culture of *Haliotis tuberculata* (Nash, unpublished data). Although the need for antibiotic treatment in the culture of *M. cephalus* has not been fully demonstrated, the need for bacterial control is vital for successful incubation of many marine and brackishwater species.

Little mention is made in the literature on construction materials used for incubation tanks. Shelbourne (1964) recommended the use of inert materials and the incubators used in the first flatfish hatchery were made of black polyethylene. The color was important to provide a nonreflective surface against which the larvae could clearly see living food particles. Other workers mentioned the use of fine-mesh net containers of terylene or nylon, but failed to specify the materials of the larger container in which the net bag was suspended. Both cement and fiberglass incubators have been used after prolonged leaching of metallic ions or resins but plasticizers have proved damaging. A great deal of information is lacking on the suitability of certain constructional materials. Until good data are available, most workers practice prolonged leaching of containers either in a heated atmosphere or submerged under water.

Larvae and Larval Culture

MORPHOLOGY

Hubbs (1943) defined the terminology for the young stages of fishes and separated prelarval, postlarval and juvenile stages on observed criteria. He considered that the postlarval stage began immediately on absorption of the yolk sac, and lasted as long as the structure and form were unlike that of the juvenile. The juvenile he considered to be the young stage similar to the adults in all essentials.

For most teleosts, the formation of the scales signifies the end of the postlarval stage. The Mugilidae, however, develop scales in the early postlarval stages (between 8-10 mm in length) and are soon well developed (12-14 mm in length). Hubbs' criteria do not therefore apply.

Roule (1917) divided the postlarval stage of Mugilidae

into two successive periods. The first had rudimentary scales as the diagnostic criterion, followed by the second with true scales. Anderson (1958) regarded the formation of the third spine of the anal fin as signifying completion of the postlarval stage, and classed individuals as juveniles if the third anal ray had fused into a spine. Tung (1973) described five stages of larval development and morphology.

Young mullet, which first appear in small schools along the coasts and in the estuaries, are fully scaled. They measure 18-28 mm in length. Extrapolated growth data for the species indicate that young of this size are 30-45 d old. The transition stage from postlarvae to juveniles used by Anderson (1958) does not occur until the young are 33-45 mm in length or between 45 and 60 d old. Thomson (1963) regarded the transition complete at about 50 mm when the third anal spine formed from the anterior ray and the adipose eyelid started to form.

For the purposes of this text, the artifical propagation of the mullet must include the culture of individuals to a stage of development when they can withstand relocation from hatchery to nursery pond. Heavy losses will occur if the young fish are mishandled or transferred too soon. This treatise considers young mullet up to 50 d old as the responsibility of the hatchery. They are technically larvae until that time. Young fish will be designated as juveniles when they are transferred from the hatchery to small ponds. They should be at least 50 d old. This arbitrary classification closely fits the morphological definition used by Anderson (1958). The use of the term "fry" for young fish is mostly avoided in the text. It is commonly used to describe all the resources of postlarvae and juveniles collected along the coastlines and transferred to nursery ponds.

Some of the first descriptions on the morphology of the larvae of Mugilidae were made by Sanzo. He observed and illustrated the early stages of *M. chelo* and *M. cephalus* (1936) and *M. labeo* (1937). He considered their prelarvae to be poorly developed. They measured only 2.2-2.5 mm in length. The mouths were closed and there was no trace of a branchial skeleton. Characteristic of the larvae were the voluminous yolk sac and large oil globule often accompanied by smaller oil droplets.

The first complete morphologic descriptions of larvae of Mugilidae were made by those workers involved in the induced spawning of the adults by hormone injection. Mostly they described the development of *M. cephalus*. Among them were Tang (1964), Yashouv (1969), Yashouv and Berner-Samsonov (1970), Liao et al. (1971), Kuo et al. (1973a), and Tung (1973). Sebastian and Nair (1974) described the development of *M. macrolepis*.

Newly hatched larvae of *M. cephalus* vary in length between 2.2 and 3.5 mm. The oil globule (and any additional small droplets) is situated in the posterior part of the yolk. Tung (1973) recorded 24 myotomes. He described the anterior half of the body bent on the yolk sac. The larvae had five or six pairs of cupulae on the body side from eye to tail, and several pairs on the front of the head. Feeding began 3-5 d after hatching. Yashouv and Berner-Samsonov (1970) gave full descriptions of the keys to the eggs and larvae of five mullet species: *M. cephalus, M. capito, M. saliens, M. chelo* and *M. auratus*.

Thomson (1963) reviewed work on *M. cephalus* and quoted in detail the embryonic development described by Sanzo (1936) and Anderson (1958). A full and accurate description of development and behavior of *M. cephalus* was made by Liao (1974) and reprinted in Table 4.

BEHAVIOR

Liao (1974) described the larvae of *M. cephalus* as having weak swimming activity with the posture of belly up and head down, sometimes moving with a jerky motion up and down. Kuo et al. (1973a) reported that newly hatched larvae were inactive and usually remained upside down suspended in the water column in an inclined position with the ventral side oriented toward the water surface. Occasionally each larva would go into a jerky motion and right its position. It would then dart upward rapidly, then sink passively back to its resting position (Figure 14). They noted that sustained larval activity increased after the second day.

The presence of the oil globule influences the activity of the larvae during early development. Kuo et al. (1973a) dealt at length with the vertical distribution of the larvae and changes in specific gravity. They noted that larvae gained sustained swimming powers between the 10th and 12th days after hatching and documented vertical distribution and measured specific gravity before that period. Their data are presented in Figure 15. They observed that during the first 2 d, the larvae were passively suspended in the water column and tended to become evenly distributed in depth as their specific gravity increased from 1.0263 g/cc at 12 hr to 1.0339 g/cc at 36 hr after hatching.

They described a vertical migration of the larvae between the second and third days. At the end of 60 hr, over 87% of the larvae came to rest on the bottom of the 80-cm experimental column. After 72 hr, the pattern was reversed with 85% of the larvae aggregated at the surface. During this migratory period, the larval specific gravity decreased from 1.0310 g/cc at 60 hr to 1.0264 g/cc by 96 hr at which time all the larvae were back at the surface. The larvae remained at the surface until the 6th or 7th day.

Table 4. Development of mullet larvae and behavior (from Liao 1974).

Days after hatching	Total length (mm)	Development and behavior
1	2.56~3.52	Newly-hatched larvae had large yolk and oil globule. Its front part of notochord being curved along the yolk sac and the curve degree was related with the duration of hatching. At low temperature, the duration was long and the curve was more distincted. Weak swimming activity with the posture of the belly up end head down, sometimes with jerky motion slightly up and down. Pigmentation dependent on individual. Colorless on eye. Mouth not developed. Digestive tube not well.developed.
2	2.64~3.28	Formation of organs was in progress. More pigmentation was found on eye and body. The total length was shorter than before. Mouth was under development. Bud of pectoral fin appeared. Nostrils were obviously seen.
3~4	3.11~3.53	Opening of mouth. Well-development of upper and lower jaws. Irregular peristalsis of stomach and intestines. Able to take food. Yolk was diminished being ¼ of original size. Oil globule was also reduced. It was the first critical period always accompanied by serious case of death. Gill clefts appeared. Being attracted and tend to concentrate at 600~1400 lux area. Distributed at upper level during nighttime.
5~7	3.06~3.40	Digestive tube was well developed. Moving up and down individually at both day and night. Feeding was easy to be observed but only limited at the daytime. Formation of abnormal thing in the bladder and swelling of hyoid could be inhibited by freshening of rearing water. Formation of stomach, intestine, gall bladder, pancreas, gas bladder and continued reduction of oil globule.
8	3.35~3.80	Complete disappearance of oil globule. Formation of gill filament. It was the flexing point of growth curve. The growth started to be accelerated.
10~13	3.45~5.10	Finfold moved backward. Gill filament well-developed. Body surface became dark in color. Strong phototaxis. Formation of hypural bone. It was the second critical period with very low survival rate.
14~15	3.85~5.70	Commence of swimming in schools. Formation of urostyle. 7~9 ray bases were found in each of anal fin and second dorsal fin. Gill lamella formed on gill filament.
16~19	5.40~6.60	17 soft rays in caudal fin. Black spots scattered on the whole body. Shinny silver white complexion appeared from gill cover along ventral part to anus. Schooling in upper level, sometimes into middle level.
20~21	6.00~7.65	Showing phototaxis during daytime while floating during nighttime. Appearance of brown color sometimes and silver green the other time. Higher variety was found in healthy larvae. 4 soft rays on the first dorsal fin.
22~24	8.25~10.9	Formation of complete 20 soft rays in caudal fin, 11 rays in anal fin, 6 rays in pelvic fin, 15 rays in pectoral fin. Fin membrane of dorsal fin and pelvic fin almost entirely degenerated. Appearance of scales and 1~3 circles of ridge on scale. The size of large scale reached 400x250 μ. Silver-white complexion. In daytime, larvae swam in upper level in schools and against aeration and stream. At night they floated and scattered on surface of water and liked to gather under the light.
25~28	8.80~15.0	All scales and fin ray were well-formed. Silver-green complexion. Appearance of teeth. Two nostrils separated.
29~32	16.6~20.7	Very sensitive. Gathering in small schools. In daytime, swam in middle and lower level. In nighttime continued to be floating but easy to be scared.
34~35	22.2~26.2	In daytime, larvae swam in large schools along the circle of rearing tank in the middle and lower levels. In nighttime, floating individually. Grass green in color, sometimes silver white on the dorsal part. Some diseased fish were found with "pop-eye" symptom.
37~40	23.1~29.3	Some changes in feeding behaviour. Feeding at late afternoon. Sensitive to light. No longer gathering under light.
45	27.5~32.8	Strongly resistant to environment. Suitable for stocking.

Between the 6th and 7th day after hatching, a second migration occurred. It was accompanied by a similar increase in the specific gravity of the larvae on the 7th day. All the larvae were on the bottom of the tank on the 8th day, with a complete reversal on the 9th day. Conditions at the time were full seawater (32‰) and ambient temperature (24°C).

Kuo et al. (1973a) concluded that the first sinking was probably related to the rapid absorption of the yolk sac and the resulting change in specific gravity (Figure 16). Planktonic larvae are normally able to distribute themselves through the surface waters. This ability may be more pronounced in Mugilidae because of the continuous references to the spawning of the adults over deep water and the rarity of eggs and larvae taken in plankton tows.

The second descent could not be fully explained. Although the larvae were not capable of sustained swimming at the time, they were capable of swimming to the surface of the water column. Kuo et al. (1973a) could not account for the sudden and transient increase in specific gravity of the larvae on the 7th day. Morpho-

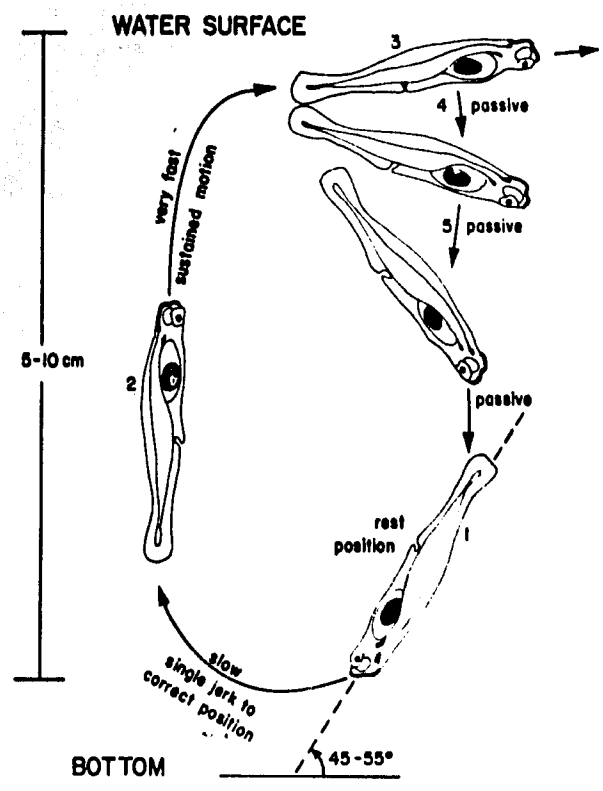


Fig. 14. Resting position and swimming movements of newly hatched grey mullet larva (after Kuo et al. 1973a).

logical observations of histologic sections of the larvae revealed that the pneumatic duct of the air bladder was occluded between the 6th and 7th days, but the relevance was not understood. The migration was known not to be a phototropic response.

During the mass propagation of the larvae, Kuo et al. (1973a) experienced heavy mortality at the critical stages associated with the two migratory periods. They attributed the mortality to mechanical and physical damage during prolonged contact with the bottom of the container as the larvae had no control over their ability to escape. The mortality was reduced slightly by using deeper containers (1.5 m) for rearing. Mortality was also associated with vertical migration by Houde et al. (1976) working with *M. curema*.

Nash et al. (1974) used a rearing kreisel and increased survival significantly during the first migration. The kreisel provided mechanical circulation but the larvae were protected from the damaginn effects of strong aeration by the simple central device (Fig. 13). Summarizing the migratory behavior, they believed that the larvae were responding first to changes in utilization of the yolk and sank to the bottom. After ascending the larvae had improved musculature control and lost the indiscriminate floating activity much earlier than previously stated.

The second vertical migration was associated again by Nash et al. (1974) with the changes in specific gravity of the larvae and suggested that two factors were concerned. The first was associated with the physiological and morphological changes; the second with nutrition. They observed that there were distinct differences between feeding and nonfeeding larvae as early as the 7th day after hatching. Established larvae were longer and more active, and were intensely pigmented. Unestablished or nonfeeding larvae remained in the surface water layers and used the increased surface tension along the sides of the container to support themselves. Development of the pigment was slow and little or no growth was evident. Such larvae died between day 7 and day 10 depending on the water temperature. Coincidental with the migra-

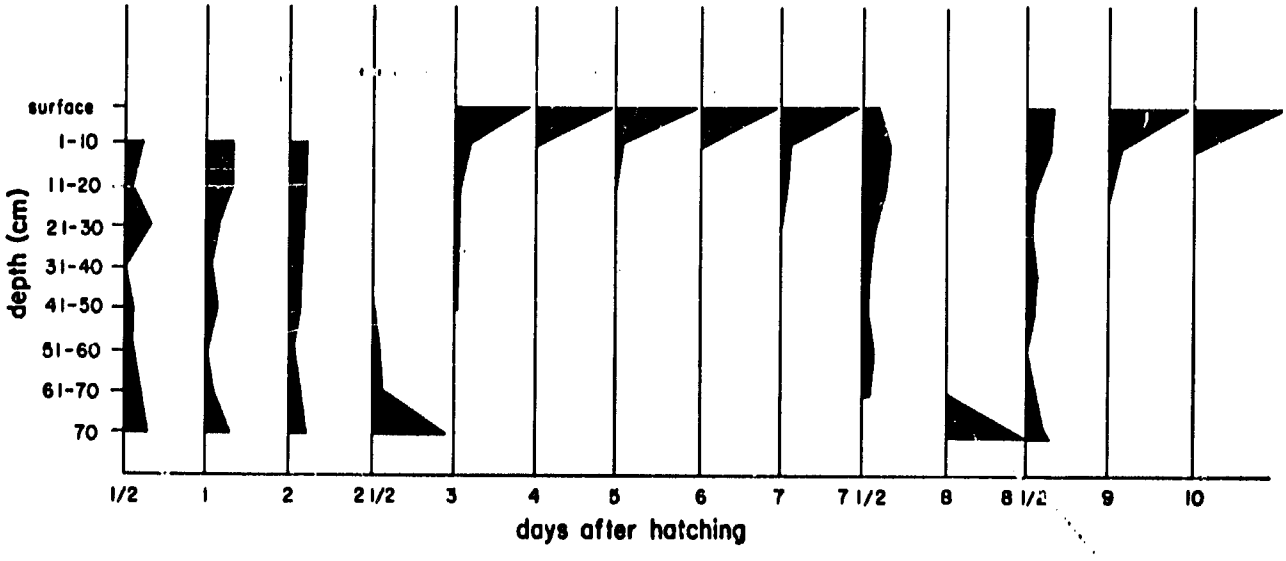


Fig. 15. Vertical distribution of grey mullet larvae during initial ten days of the larval period under conditions of full seawater (32‰) and ambient temperature, 24°C (after Kuo et al. 1973a).

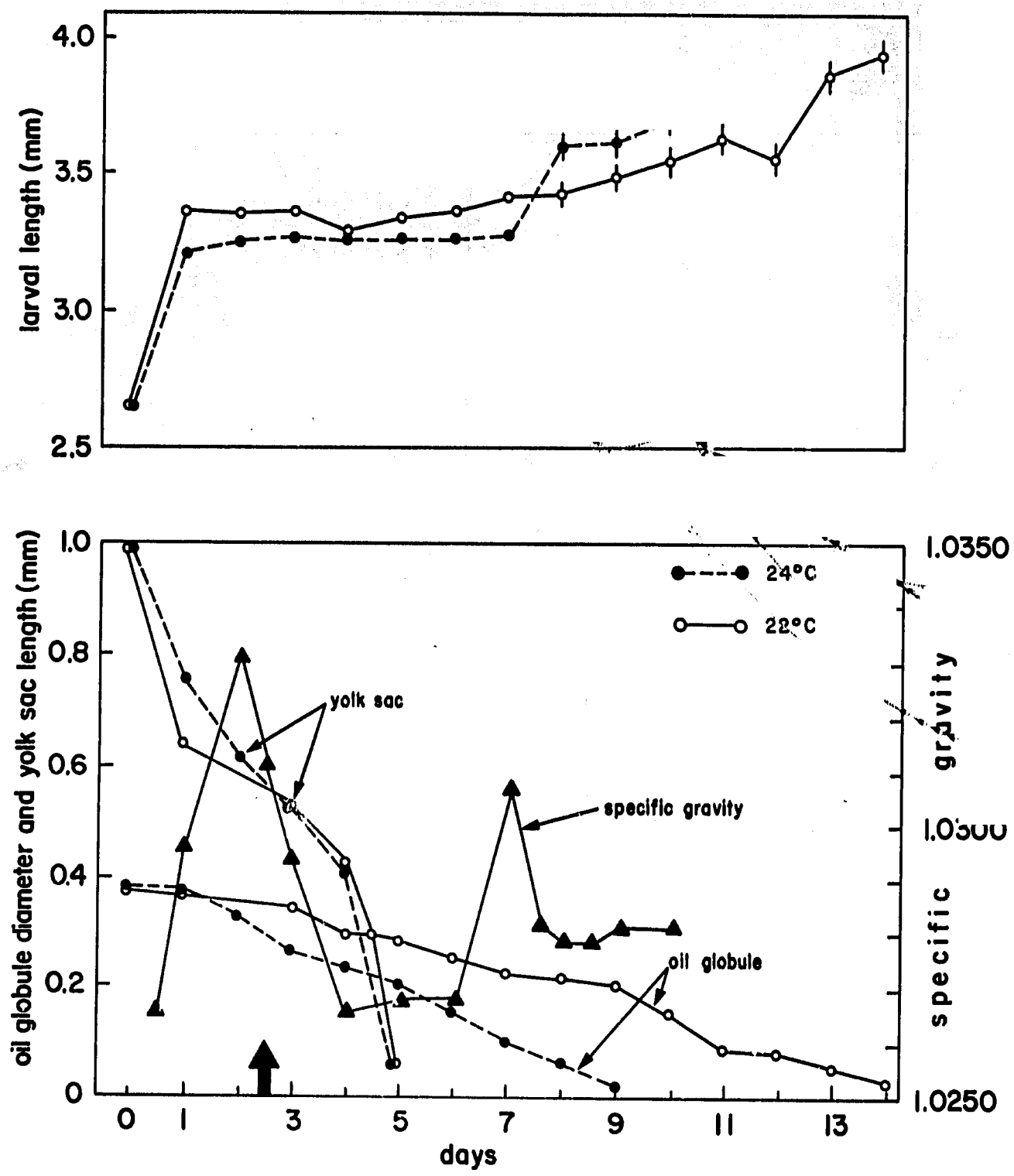


Fig. 16. Relationship between yolk sac and oil globule depletion, specific gravity, and larval growth in grey mullet at 22° and 24°C. Arrow indicates time of mouth opening (after Kuo et al. 1973a).

tory movement of the larvae to the bottom, many undeveloped individuals sank because they were unable to sustain themselves further and were moribund. Hence the earlier association of heavy mortality with the migratory period. Developed larvae also had to undergo the changes associated with the migration which must be critical to further development. As with other larval forms undergoing metamorphosis, the mullet larvae became inactive for a period and sank.

All workers now believe that those larvae which survive the second migration are capable of full development, providing that the conditions and food are suitable and if they are not mishandled. Established larvae after the second migration soon develop scales and then school together.

NUTRITION

The dearth of larval Mugilidae in plankton tows is responsible for the lack of information on their natural foods during this critical stage of development. This scarcity of knowledge during the first formative 50 d of life is to some extent compensated by the wealth of data on growth, food and feeding habits of juveniles and adults of Mugilidae from regions throughout the world, e.g., Hiatt (1944), Jacob and Krishnamurthi (1948), Thomson (1954), Sarojini (1957), Hickling (1970), Zismann et al. (1974), Cech and Wohlschlag (1975), Grant and Spain (1975), Chervinski (1976), and Marais and Erasmus (1977).

The data on acceptable foods for the larvae of Mugilidae have been produced by workers developing culture techniques, ususally following induced spawning practices. The variety of larval foods which have been tried and evaluated by fish culturists working with many marine and brackishwater species has been reviewed by May (1970). Aspects of food and nutrition of marine fish larvae important to the best culture practices have been developed by Houde (1972).

Following the format of May (1970), Table 5 lists the food organisms and prepared foods which have been used by culturists in their search for an adequate food for the larvae of the Mugilidae. The sources are predominantly the publications of workers in Taiwan, Israel, Hawaii and India, and in most cases were tried on the larvae of *M. cephalus.*

Emergent larvae of the grey mullet are believed to be entirely carnivorous during early development, becoming omnivorous and capable of digesting plant material sometime before metamorphosis. The most successful larval rearing results of Liao et al. (1971), Kuo et al. (1973a), Tung (1973), Nash et al. (1974), and Sebastian and Nair (1974), indicated the use of a mixed dietary regime during the first ten days of life. Some of these regimes are illustrated in Figure 17.

Table 5. Experimental foods for larvae of grey mullet species tried at one time or another (after many authors).

1. Wild plankton

2. Protista

Dunaliella sp.	*Prorocentrum micans*
Chlorella sp.	*Oxyrrhis* species
Gymnodinium splendens	*Coscinodiscus* species
Isochrysis galbana	*Chaetoceros* species
Monochrysis lutheril	*Biddulphia mobiliensis*
Skeletonema costatum	*Ditylum brightwelli*
Thalassiosira sp.	
Nitzschia sp.	and unidentified phytoplankton, diatoms and green water.
Platymonas subcordiformis	
Cryptomonas maculata	

3. Metazoa - planktonic forms

Brachionus plicatilis	*Arbacia* species
Artemia salina	
Crassostrea gigas	and unidentified Copepods

4. Prepared formulations, including at one time or another

Cod liver oil	*Artemia salina*
Powdered oil cake	Copepods
Bean cream	Oyster flesh
Fish ovaries	Fish meal
Egg albumin	Urea
Boiled egg yolk	Rice bran and flour
Liver juice and enzymes	Milk powder
Amino acids	Powdered oats
Marine vitamins	
Brewer's yeast	and commercially available
Freeze dried phytoplankton	tropical fish feeds
Freeze dried zooplankton	

Many workers include wild or natural zooplankton in their regimes. Shehadeh and Kuo (unpublished data) had some success with the nauplii of *Artemia salina* alone fed on the 7th day after hatching, but the results were later improved with a phytoplankton supplement (Kuo et al. 1973a). Sebastian and Nair (1974) noted that *M. macrolepis* fed exclusively on copepods. They found that the larvae grew best on copepods prepared in association with an algal bloom, particularly *Chlorella* species, as did Houde et al. (1976) with *M. curema.*

Many culturists rearing marine and brackishwater species of finfish and shellfish believe that the presence of phytoplankters in the rearing containers is beneficial to the technique, but not always of direct value as food. Although some phytoplankters are found in the guts of prelarvae, it is not thought generally that they are nutritionally sufficient. The indirect benefits of phytoplankters are probably the stabilization of the rearing environment through the removal of metabolites,

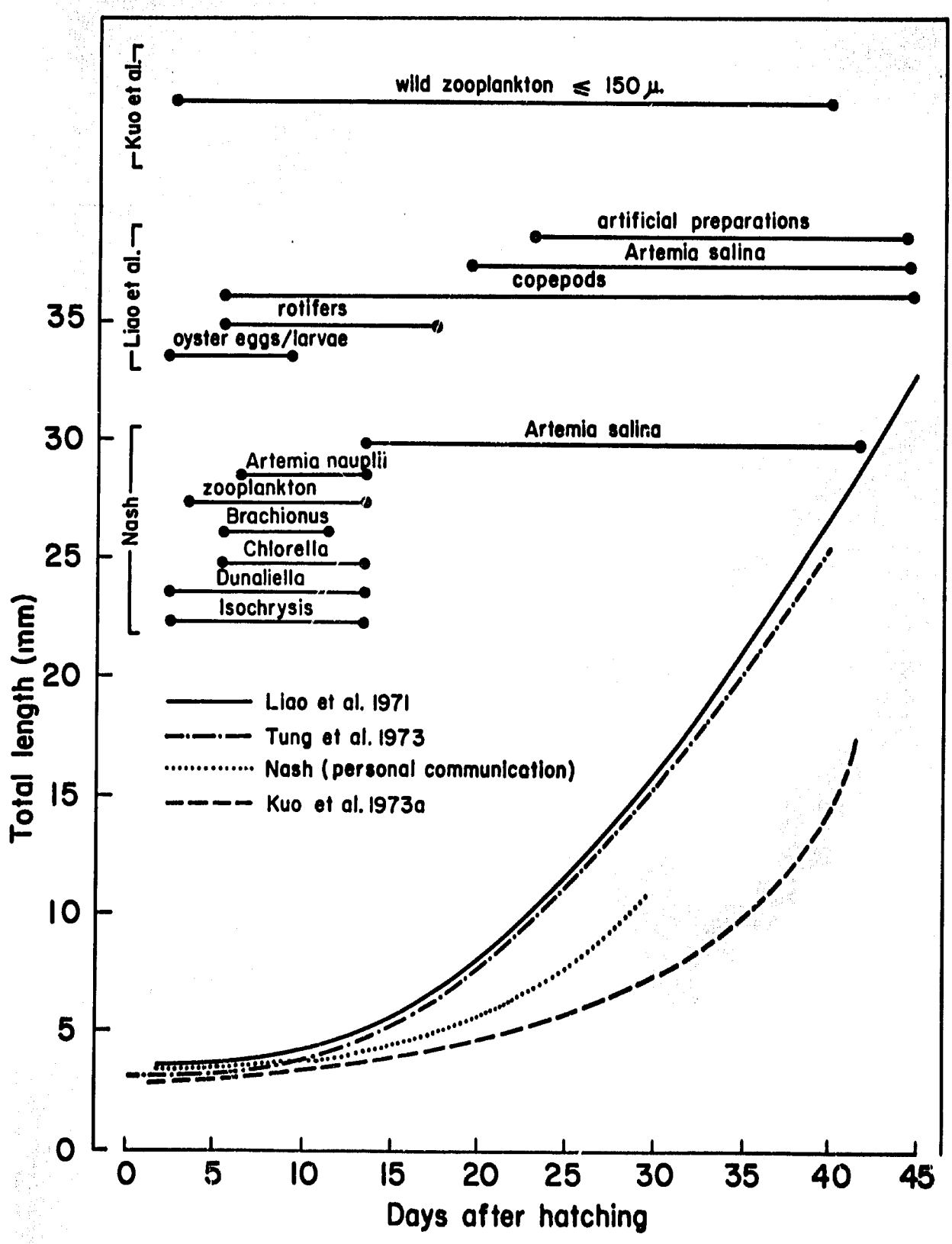


Fig. 17. Larval growth of grey mullet and dietary regimes used in trials (after several authors).

or for maintaining the nutritional level of the zooplankters before they themselves are consumed.

Several artificial diets compounded from both natural and synthesized materials have been fed to the larvae of mullet with limited success. Most have the disadvantage of increased fouling in the rearing containers. No data are available on artifical diets in association with naturai organisms. Against the poor larval survival data, which may be the result of other factors, the true nutritional value of many artifical diets cannot yet be estimated satisfactorily.

Nash and Kuo (1975) hypothesized that the larvae of the grey mullet were able to utilize at least one or several of the live or inert dietary organisms listed in Table 5. It was their belief that it was the preparation, feeding procedures, and practices which, among other things, were at fault and not the types of organisms tested. They stated several instances for the human influence to be an unintentional cause of larval mortality during larval feeding. For example, separation of the food organism from its own rearing medium was not always adequately performed, and the separation of the nauplii of *Artemia salina* from the cysts was often inefficient.

The selection of the food organism by the larvae is, in all probability, made on the simple criteria of movement and size in the first instance. The ability of larvae to feed is regulated by feeding behavior and vision. Blaxter (1969a) described the visual thresholds and spectral sensitivity of a number of marine species. As with many marine and brackishwater species, the eyes of the larvae of Mugilidae are large at hatching and become rapidly pigmented within three days. The eyes are only capable of coarse movement perception and, as with other fish species, are poorly equipped visually with a single type of visual cell. They have little ability to adapt to dark or light situations during the first hours after hatching. Most culturists follow the early techniques of Shelbourne (1964) and try to improve conditions for feeding by providing nonreflective dark surfaces for the rearing containers. The food organisms are clearly silhouetted as they move around.

Houde (1972) believed that food organisms within the size range 50-100 μ were preferred by fish larvae with relatively large mouths. The acceptable sizes of food increased rapidly as the larvae grew. The recent successes of a number of culturists using the rotifer *Brachionus plicatilis* (Theilacker and McMaster 1971), in association with a number of phytoplankters, infers that the size of about 100 μ is adequate for the first 2 d of feeding the larvae of the Mugilidae.

The ratio of food or organismic density to the larval density is another key factor to successful larval culture irrespective of species. The optimum food density must be maintained continuously through to the end of the larval period.

A superabundance of food can be as harmful as too little. A larva must not be intimidated by large or quick-moving food organisms or totally dominated by a number of them in one location; for example, organisms collect in a corner where the light intensity is often increased by the high light reflection of an interface. Conversely, the density of larvae can be too high. In addition to the problems of inhibitors produced by the larvae when overcrowded (Blaxter 1969b), the physical contact, the increased competition for space and food, and production of metabolites all lower the survival rate. Kuo (unpublished data) showed a higher survival of larvae of *M. cephalus* at 12/liter than either 8 or 16/liter. Shelbourne (1964) operated at higher densities for *Pleuronectes platessa;* a similar survival of about 70% was obtained from two populations of established feeding larvae at 28 and 56/liter. During development to metamorphosis, the survival decreased to 30% and the density to 12 and 24/l, respectively. May et al. (1974) successfully reared the larvae of *Siganus canaliculatus* at a density of 5/l. The data in Liao (1974) indicated effective rearing at a density of less than 10 larvae per liter. Each individual larval form must be provided with the opportunity to observe and attack live food particles. Many failures occur, particularly during the first crucial days of the learning process, and a larva cannot be without success for long.

Riley (1966) and Rosenthal and Hempel (1970) concluded that a higher food concentration was necessary at the time larvae initiated feeding than subsequently, probably because the younger stages were less capable of capturing food. Houde (1972) recommended a food level using wild zooplankton (copepod nauplii and copepodites) of 3.0/ml for the first 2 d of feeding larvae, but a level of only 1.5/ml on subsequent days. If rotifers were used, he suggested a higher concentration. If only nauplii of *Artemia salina* were fed, then he recommended a lower concentration.

Nash and Kuo (1975) specified an organismic density of 20-36/cc during the first feeding stages of *M. cephalus* which included all organisms. Liao et al. (1971) described the use of fertilized oyster eggs and cultured diatoms on the third day of development. Liao (1974) stated that the density of oyster eggs or trochophore larvae was maintained as high as 400-500 organisms/ml. Yeast and albumin were then supplemented on day 4 and rotifers and copepods on day 6.

Kuo (unpublished data) showed that feeding larvae of *M. cephalus* first on days 4 or 5 was better in terms of survival and management than that on either days 3 or 6. Furthermore, copepods smaller than 150 μ were not utilized until day 5. He also performed four series of experiments feeding emergent larvae with *Isochrysis,*

Brachionus, Chlorella, or natural zooplankton. Observations of stomach contents during development on days 5-11 were made. *Isochrysis* was taken first of all food organisms tested and ingested in quantity on day 5 after hatching. The food preference by mullet larvae for natural zooplankton or *Isochrysis* as an initial food was further examined. Again the food organisms were given singly and in combination, and the gut contents examined daily. No food preference was indicated, except that *Isochrysis* was once more consumed readily on day 5.

The logistics of aquaculture for the hatchery operation for marine and brackishwater fish are considerable. If certain species of fish require individual organisms over a long period of their development, it is possible that the larval food production system alone will be greater than that of the fish larvae themselves. Shelbourne (in Costlow 1960) quoted a daily requirement of 200 million nauplii for a flatfish hatchery producing 0.5 million juveniles. That is why the dehydrated cysts of *Artemia salina* are such an advantage at present as they can be conveniently stored and prepared with a minimum of delay and acclimation (Nash 1973). It also explains the strong interest in artificial foods.

The present methods of larval culture which are proving most successful are those using a mixed dietary regime, often by the creation and stabilization of an ecological system. The "green water" technique has been used successfully by Fujimura and Okamoto (1972) for the culture of *Macrobrachium rosenbergii,* and by several Japanese in the culture of penaeid prawns.

The use of the mixed dietary regime has several advantages. It requires culture of several species in one container before the release of the fish larvae. Following the preparation of the system with cured or conditioned seawater, the environment becomes stabilized. There are few rapid and diverse environmental changes which can upset the delicate biochemical balance of the larvae. The mixed regime is also economic in decreasing the numbers of individual organisms which have to be produced on a daily basis. The wide choice of organisms probably provides a better qualitative, as well as quantitative, diet for the larvae and also permits individual larvae to develop at their own rate by feeding on organisms relative to their size.

Pelagic copepods, according to Houde (1972) were probably ideal food for marine fish larvae. Liao (1974), Nash et al. (1974), Kuo et al. (1973a), and Tung (1973) all used natural zooplankton in their respective feeding regimes. It was the copepods which proved to be the most significant and beneficial organisms for the diet of the larvae of *M. cephalus.* The Japanese culturists regard copepods as the best larval fish food (Harada 1970) and consequently there have been many attempts to rear them intensively. In many cases large natural populations can be found in the filamentous green algae which grow in the enclosures retaining the broodstock or maturing juveniles.

ENVIRONMENTAL CONDITIONS FOR REARING

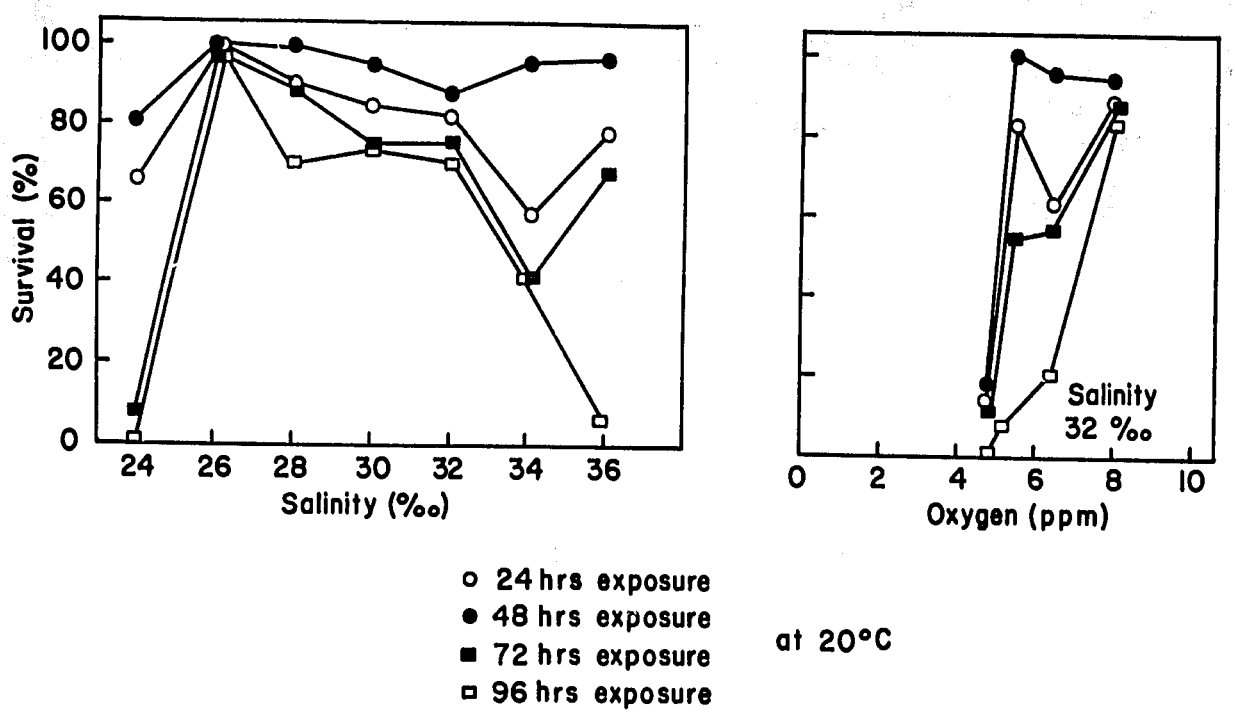
Salinity is probably the most unregulated and uncontrolled major parameter which influences the incubation and larval rearing of marine species. The majority of workers conclude that natural high-saline waters (32-35‰) are optimal. The eggs of most marine and brackishwater species are liberated into oceanic waters and, with the emergent larvae, are adapted to develop at high salt concentration. Holliday (1969) concluded that survival of embryos and larvae of many species could be increased at low salinities (10-16‰) because those levels were iso-osmotic with body fluids. Houde (1972) found that many species had high survival rates over a wide range of salinities. He did not consider salinity as critical as some other rearing tank conditions which affected growth and survival. Recent work by Alderdice and Forrester (1971) and Alderdice and Velsen (1971) indicated that salinity appeared to have an effect on the rate of yolk absorption.

In Israel, Taiwan and Hawaii, where the majority of work has been accomplished on the rearing of *M. cephalus,* offshore salinities and experimental conditions are all very similar, namely 32-35‰. Sebastian and Nair (1974) operated at slightly less (29-31‰) for the culture of *M. macrolepis.* However, the effect of salinity on larval survival and development is possibly more significant than that for incubation of the eggs.

Liao et al. (1971) reduced the salinity from 32 to 26‰ during larval development in three stages commencing on the sixth day after hatching. They concluded that there was an advantage rearing the larvae of *M. cephalus* in diluted or sweetened seawater. Sylvester and Nash (1975) provided tolerance levels of larvae of *M. cephalus* to varying salinities (Figure 18). They showed that the larvae could only withstand prolonged exposures to salinity between 25-34‰ at 20°C during the first week of development, with an optimum at 26-28‰ for 96-hr exposure.

The operational procedures for the culture of *M. cephalus* as outlined by Nash et al. (1974) in Hawaii did not include techniques for reducing the salinity of the water during the first 14 d. They used full saline conditions until completion of the second migration and then made dilutions. Better results were reported by Liao (1974) in Taiwan with the dilution technique.

Nash and Kuo (1975) theorized on the value of reducing salinity during early development and hypothesized that there was a link between the need to



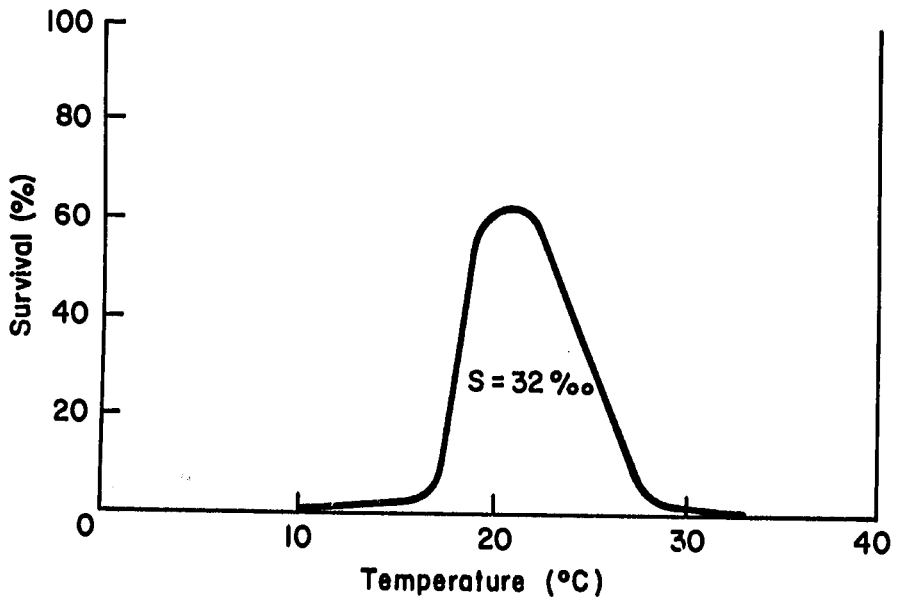


Fig. 18. Grey mullet larval survival at varying salinity, temperature, and oxygen levels (after Sylvester and Nash 1975; Nash and Kuo 1975).

change salinity and the second vertical migration of the larvae. They believed that the unexplained rise in specific gravity at the start of the second migration was unnatural and a result of osmotic imbalance. The larvae had not sufficient fresh water internally to maintain the balance and consequently sank. The larvae must therefore be cultured in seawater which is changed to suit the osmotic regulation. Such fine control was an external compensating reaction against the change in specific gravity. They also contended that the freshwater consumption of the larvae needed increasing and both cultured and artifical food preparations required greater consideration for freshwater content.

Larval development is temperature dependent (Blaxter 1969b). Shelbourne (1964) demonstrated the need for optimum temperature control for the culture of marine flatfish. He also noted differences between the survival of larvae in natural conditions and those in the intensive hatchery environment where bacterial activity was potentially more dangerous.

The reasoning of Shelbourne is particularly relevant to the culture of fish and shellfish in the tropical and subtropical latitudes. There the ambient temperatures are highly conducive to bacterial growth, and the optimum rates for yolk utilization by the larvae are probably narrowly defined and close to a critical level.

Strict temperature control for the incubation of the eggs of the Mugilidae is important. Emergent and developing larvae up to metamorphosis tolerate an ever-widening temperature range and their growth rate responds accordingly.

Liao (1974) reported the successful culture of the larvae of *M. cephalus* over a number of preceding years within the ambient temperature range of 19-24°C. Sylvester and Nash (1975) recorded minimum mortality of the larvae of *M. cephalus* between 18.9 and 25.3°C, although some larvae survived temperatures as low as 15.9°C and as high as 29.1°C. Sebastian and Nair (1974) operated within a higher range of 26-29°C for the culture of *M. macrolepis.*

It is believed by Nash and Kuo (1975) that, of the two development stages of egg incubation and larval growth, the former was more critical and required careful temperature regulation. Nash and Shelbourne (1967), working in the thermal discharges of coastal electrical generating plants, exposed the eggs and larvae of marine flatfish at various stages of development to the elevated temperatures. Emergent and developing larvae were able to withstand substantial thermal shocks and grew rapidly at the higher temperatures. The early start to growth produced significant savings in time taken to market size. However, there was no benefit in using elevated temperatures to increase the rate of egg development. Survival was in fact decreased.

A great deal of data on survival and temperature is available for juveniles of the Mugilidae, but is beyond the scope of this chapter dealing with the production of larvae up to 50 d.

Little information exists on the levels of dissolved oxygen suitable for the rearing of the larvae of the Mugilidae. Although the levels of dissolved oxygen are measured regularly as part of many culture operations, the data are often excluded from reports. Sylvester and Nash (1975) determined that the survival of the larvae of *M. cephalus* significantly changed at mean oxygen concentrations below 5.4 ppm (Figure 12).

The size of the culture system does not necessarily influence the success of a mass propagation effort. Many workers operating in carefully monitored small units, and with high-density feeding but low larval density, have produced exceptional survival figures. However, in terms of mass propagation, the larger units are more appropriate for rearing the numbers of larvae which are necessary for a culture practice to become economic.

The successful culture system of Fujinaga (1963) with *Penaeus japonicus* encouraged both Liao et al. (1971) and Nash et al. (1974) to use larger containers for the culture of *M. cephalus.* Survival of the larvae in them was definitely increased but the reasons were obscured. At present the usefulness of the larger container may be in the lowering of larval density and increasing spacial freedom. It would also decrease the influence of any specific inhibitors released by the larvae, and prevent a size hierarchy. However, they stocked the containers with eggs or larvae at densities of 50-250/l initially, with a final density of between 5 and 50/l on day 21.

The disadvantage of the larger container is the great demand on available larval food, particularly as food density is a key factor for survival. The logistics to supply the larval food on a single daily supply basis would be tremendous for a farm of any size. Shelbourne (in Costlow 1969) operated an intensive *Artemia salina* nauplii system capable of producing over 200 million nauplii per day. However, in the context of a mixed feeding regime, populations of differing organisms can make up the organismic content to the required density in the simplest way.

The advantage of the large container, as theorized by Nash and Kuo (1975), is the stabilizing of the rearing environment. Although good tank hygiene is necessary and water has to be replenished and salinity reduced, the effects of the exchanges or the additions of food are buffered by the size of the system. The larvae are therefore protected from sudden shocks or exposure (albeit for a short period) to adverse conditions.

Little data are available on the suitability of materials for containers for rearing the larvae. A study of the effects of materials on several small marine organisms

was made by Bernhard and Zaffera (1970). Plastic, polyethylene, fiberglass, concrete, vinyl and wood have all been used at some time for Mugilidae with little comment on their value or suitability. Most workers leach potentially toxic chemicals and plasticizers from molded or fiberglass units.

The color of the rearing units can be important. Nonreflective black polyethylene tanks to prevent areas of high light intensity have been widely adopted.

An even light intensity over the tanks prevents localized gathering of the larval food and the larvae. Exposure of larvae to direct sunlight has to be avoided and all rearing operations are preferably conducted indoors. Liao (1974) noted that the larvae were sensitive to light. Four-day-old larvae exhibited phototaxis and 6-day-old larvae migrated up and down according to the time of day, but fed only in the day. Larvae avoided strong illumination but were attracted by dim light intensity of about 600-1400 lux.

POSTLARVAL DEVELOPMENT AND SURVIVAL

Between the present critical stage of early development (day 12-14) and the juvenile stage there appear to be few problems in the propagation of *M. cephalus*. The larvae which survived beyond day 14 were usually hardy, fed well, and grew rapidly. By the end of the third week the scales appeared and the postlarvae began to school.

Growth was rapid. The postlarvae fed voraciously on nauplii of *Artemia salina*, photoplankters, diatoms, copepods and artificial dry preparations (Figure 17). Waterflow through the rearing tanks was greatly increased accompanied by strong aeration. The most damaging effect was physical handling. Many postlarvae underwent a handling trauma and became inactive and died after violent quivering. Handling was not advised until they became fully scaled juveniles and ready for transfer, which was about 50 d old. Mass propagation was therefore considered to be a 50-d process.

Survival at the 50-d limit has improved gradually over the last 5 yr. In Taiwan production of *M. cephalus* was not always related to individual survival, as the method of propagation relied on the collection and spawning of a large number of migrating adult broodstock. All female fish collected were injected with a homogenate of mullet pituitaries and Synahorin. About 65% of the females completed hypophysation, and the fertilization rate was also about 65%. All the eggs were incubated and the available larvae reared until ready for transfer to the farms. About 25,000-50,000 juveniles or more were produced each year, from an unestimated number of emergent larvae. Liao (1975) reported a survival of 19.35%.

In Hawaii, the individual adult *M. cephalus* females were spawned with the injection of salmon gonadotropin. Hypophysation was assured and the fertilization rate was over 90%. After incubation, the emergent larvae were stocked at a known density in rearing tanks, and counted at the end of the trial. An accurate survival figure for 50-d-old juveniles was therefore known. In a recent season (Nash et al. 1977), final survival at 50 d for the best test production units was 33.5 and 16.9%. Only the outbreak of a ciliate infection in one tank reduced survival from 29.9% to 16.9% during the last five days (Figure 19).

In similar rearing trials with *M. curema*, Houde et al. (1976) reported survival at 20 d of 1.2-23.0%. Little mortality occurred after day 12. These survival figures from large laboratory-scale trials are more than adequate for the construction of pilot-scale hatcheries, and the practice of mass propagation of grey mullet.

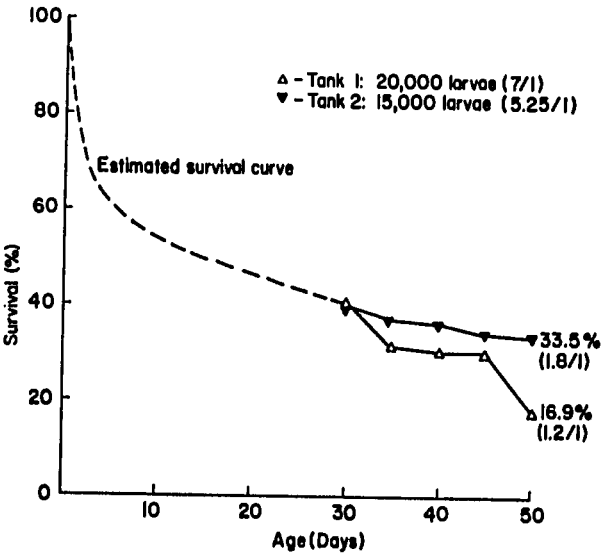


Fig. 19. Survival of grey mullet larvae, 1977 season in Hawaii.

INTENTIONALLY
LEFT
BLANK

Chapter 4. Hatchery Techniques

This chapter is a guide for hatchery managers and operators to raise mullet. It incorporates the most recent techniques used at the Oceanic Institute. In certain areas the methods outlined supersede those of earlier publications. For example, several simplifications have been effected in food production and larval rearing; larval stocking densities have been increased while adequate levels of food organisms were maintained.

Some important shortcomings still exist. (1) The most reliable hormone, salmon gonadotropin, SG-G100, remains expensive and difficult to obtain. The use of other less expensive hormones and induced spawning techniques remain experimental. (2) A food gap exists for postlarvae between the ages of 25 and 45 d, during which time they are too large to utilize brine shrimp nauplii efficiently but are not yet ready to eat food off the bottom of the tank. (3) More experience is necessary working with photoperiod and temperature controls on the process of maturation. (4) Although larval survival through day 50 has now reached an acceptable level, too much variation still exists between fish and within fish from tank to tank.

As an introduction to this chapter, a flowchart (Figure 20) illustrates hatchery operations. In a diagrammatic way, it relates the major hatchery functions to each other. It shows the progression of procedures from catching the wild adults through the various hatchery stages, including spawning, incubation, and larval rearing. The flowchart illustrates an open life cycle, implying that existing broodstock is composed of adults collected from the wild. For genetic improvement, large numbers of progeny should be retained separately each year for future breeding programs. In time all broodstock should be hatchery progeny.

Broodstock Collection

The resources of broodstock can be either wild fish on a migratory spawning run or resident populations from fishponds. Wild fish on migration are more likely to be well advanced in egg development than captive fish in brackishwater ponds, and probably close to spawning. Pond fish, therefore, need collecting well in advance of the natural spawning season.

Gill nets or surround nets are used for catching wild fish so that the chance of injury is lessened. Surround nets are preferred, as gill nets often cause damage to the eyes and gills. The nets are set and examined at least every 30 min so that captured fish are not left struggling in the nets for long periods. The most suitable mesh for the net is 6-7 cm (stretched measure) nylon monofilament. Pond fish are usually caught in the trap at the gate of the pond or by netting with a suitable available net.

The fish are transported as quickly and safely as possible back to the hatchery holding ponds. Anaesthetics are not necessary if the fish are shipped in a live-bait well or an aerated transport container. Only good individuals are taken back to the hatchery. All should be sexually mature or approaching maturity. Fish about 4 yr of age are preferred; they should appear healthy and strong and be free from external parasites or

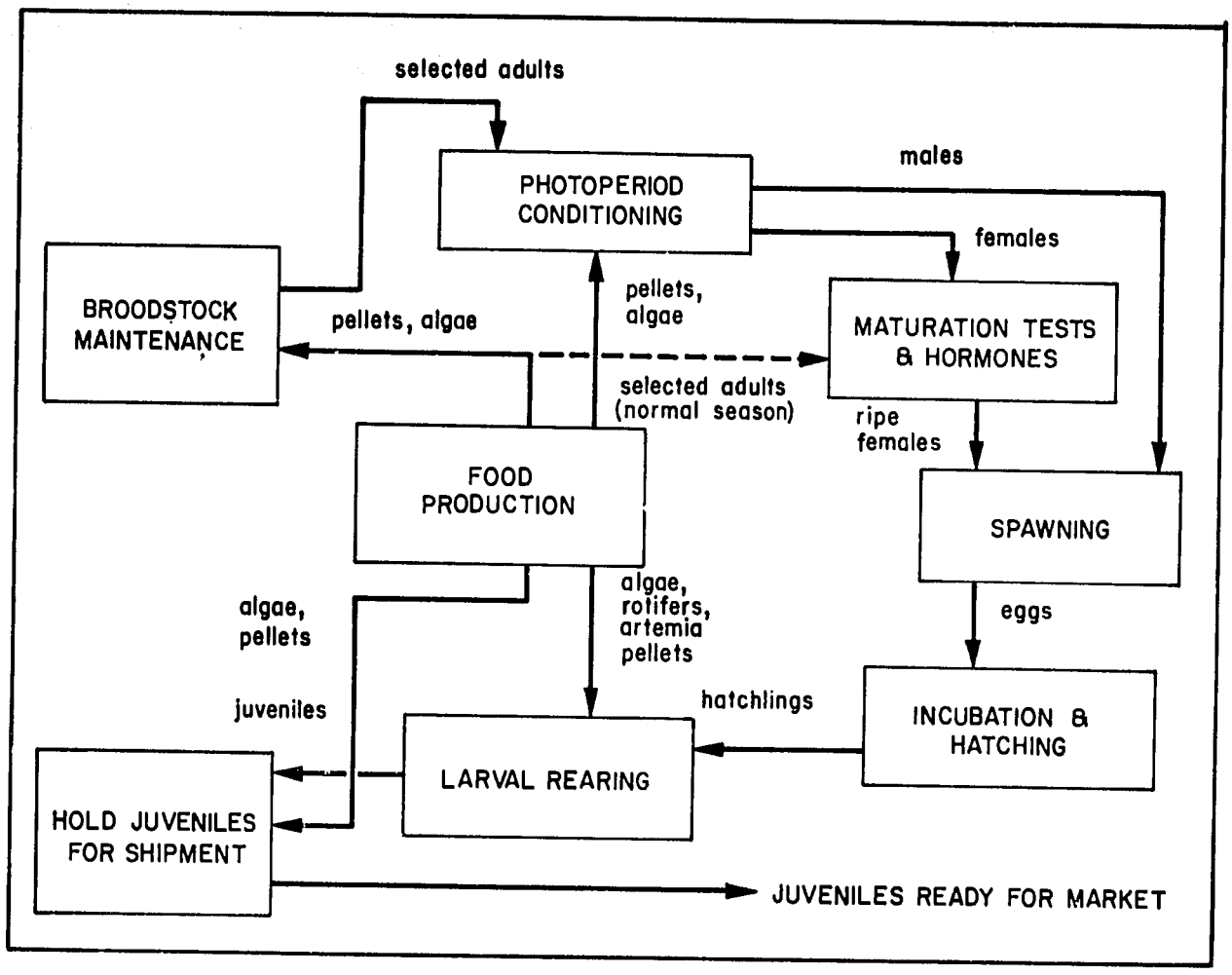


Fig. 20. Flowchart of hatchery operations.

obvious disease.

A circular transport tank is used if available and of dimensions to suit the available space on a fishing boat and/or the transport truck. A convenient size tank to fit a small boat or a 1-t flatbed truck is 1.5 m. diameter and 1.0 m deep. The tank can be made of fiberglass, resined plywood, plastic or metal, as long as it is maintained in good repair. Before use, the tank is washed with fresh water for 24 hr, drained, and allowed to dry in the sun for a day.

During collection, the tank is kept filled with seawater which is well aerated. At sea this is achieved by connecting a hose to the ship's pump and exchanging water continuously, or by aerating the water using a portable air pump.

The transport tank is fitted with lifting brackets to transfer it directly to the transport vehicle, or the fish are transferred to a second transport tank on the vehicle. Again, during transit, aeration is maintained by either a portable aeration unit or by compressed air or oxygen cylinders. If oxygen is used, the regulator, tubing and airstones are properly assembled to provide a slow, continuous delivery.

A cover over the top of the transport tank is necessary at all times. Fine-mesh net is adequate as it prevents the fish from becoming trapped in folds, but a solid cover is preferred. An adequate holding volume per adult fish during transport is 25 l of aerated seawater.

All captured fish are anticipated to receive some external damage or injury. A general treatment antibiotic, such as nifurpirinol (trade names: Furacin or Furanace), is added to the tank. As the antibiotic is rapidly absorbed across the gills and the skin of the fish, a concentration of 1 ppm (1 mg/l) is recommended.

The fish are examined in more detail on arrival at the hatchery and before release among other resident individuals. The new fish are first held in temporary tanks indoors, again under treatment with the antibiotic bath. The fish are examined, treated for any injury or obvious disease, weighed and measured, sexed and numbered with tags or by fin clipping and kept indoors for a further period of observation.

The mullet are a hardy group of fish, thriving well in either salt or freshwater. However, as with all fish, they are always handled with care to avoid future problems with disease or accident. The handling equipment is maintained in good condition and cleaned regularly to avoid contamination by disease which would spread from pond to pond.

Broodstock Maintenance

HOLDING PONDS

Holding ponds for the broodstock are of dimensions to allow 1,000 l of water per fish. They are either dirt ponds, lined with 1 mm thick butyl rubber, or clay-bottom ponds. Clay-bottom ponds of fine particle size 50-60 μ are preferred as the fish need a mixed diet of living and detrital organic matter, together with a proportion of fine sand to grind the diatoms and other food.

Pond size and configuration depend on the size of the hatchery and its location. Water depth is at least 1 m. Very small ponds are avoided because (1) construction cost per m^3 is higher, (2) natural solar heating causes large fluctuations in water temperature and (3) algal growth is more difficult to control. If the holding pond is too large, identification and selection of individual spawners before catching becomes a problem.

Each pond has both a fresh and seawater inlet, with regulators, has its own independent large drainage system, and is able to empty fully in 2 hr or less. A surface overflow should be located at one end, according to the direction of prevailing wind, and fitted with a skimmer trap.

A natural growth of algae and diatoms is established in the ponds before introducing the broodstock. This is accomplished by filling the ponds with seawater, adding available organic and inorganic fertilizer, and maintaining static water conditions for about 2 wk. If the ponds are lined, additional substrate can be provided to increase the surface area for grazing. Vertical plastic surfaces, or 0.003 inch translucent polyethylene sheet, can be fabricated by heat-sealing the sheet over a lead-core line and then subdividing it along its width at 15 cm intervals. This produces separate vertical surfaces, anchored to the same lead-core line. The line is stretched across the bottom of the pond and weighed down. Other innovations may be devised and used to increase the surface area within the pond, as long as circulation in the pond is not impaired.

The optimum salinity for growth of broodstock mullet in the ponds is brackish water of 10-25‰. Although mullet tolerate fresh water for extended periods, they do not grow as rapidly. The gonads only reach full maturity at 30‰ and above, and broodstock must be preconditioned in full saline water immediately before and during the spawning season. Preferred values for some of the most important pond parameters are presented in Table 6 below.

If the organic biomass of the pond is above and beyond the control of the fish, the pond must be drained and cleaned. For a clay-bottom pond, the clay is first allowed to dry. It is then plowed thoroughly (using lime if available) and allowed to dry again before refilling. A lined pond is hosed down with fresh water and allowed to dry. If organic buildup on the plastic strip is heavy, the strip is also hosed down and cleaned, or replaced. The plastic deteriorates with time and needs replacing every 6 mo.

Table 6. Parameters of seawater in ponds to precondition broodstock prior to induced spawning.

Temperature	15-25°C	
Salinity	32-35%	
pH	7.50-9.00	
Dissolved oxygen	7.8-8.3 ppm	Should not go beyond 8.3; avoid high fluctuations in DO levels.
Water flow-rate	0.5-1 change/day	

HUSBANDRY

The broodstock are observed and examined regularly for infections and parasites. The chances of parasitic infestations are increased during high-temperature conditions in the summer months, and during low flow conditions or when water quality is poor. Infestation by marine ectoparasites is treated by bathing the fish in freshwater for 30 min or by bathing in 40% formalin solution for no more than 1 hr at 200 ppm (1:5000 in seawater), or both. The formalin treatment is hard on the fish and should be accompanied by strong aeration. The fish are observed continually and the treatment stopped if signs of disorientation or respiratory distress appear.

If the parasite infestation is epidemic, infested ponds should be flushed continuously with fresh water for 3-5 d and allowed to dry thoroughly before reusing.

Because parasite and bacterial infestations and treatment differ from place to place, it is important that all available information on local parasites and bacteria and their treatment is acquired. Further pathology includes autopsies of dead fish, identification of new parasites and bacteria and control treatments, and locating sources of infestation and preventing infection.

For each anticipated spawn, seven females and at least three males of broodstock are required. The females

should be mature adults, capable of producing stage III eggs during the spawning season. The broodstock are all tagged, preferably when first captured, and are held separately by sex in the outdoor ponds.

A variety of supplemental feeds have been prepared and used successfully. The foods are high in protein and have vitamin additives. The feeds are offered at a rate of 1% of body weight per day for fish in outdoor ponds with natural growth, and 5% of body weight per day for fish in indoor tanks. Females which have been selected as spawners, and are being induced to spawn by injection, are not fed.

The quantity and composition of supplemental rations depend to some degree on the level of natural food in the outdoor ponds. The formulations in Table 7 have been used at some time and proved adequate. The ingredients are milled and sieved to 0.5 mm size, mixed and stored dry at ambient temperature. Fresh water is added just before feeding, sufficient to make the dry feed mix stick together. Because preparation of the mix is time consuming, a large supply can be made at one time if it is correctly stored in a dry atmosphere.

Table 7. Feed formulations for mullet.

1. Commercial catfish chow (crumbs)	
2. Wheat middlings	55%
Cottonseed meal	14%
Soybean meal	14%
Tunafish meal	14%
Propylene glycol	1.4%
Visorbin (vit. B complex)	1.4%
Vitamin mix	0.2%
3. Commercial catfish chow (crumbs) or wheat middlings	4 parts
Fish meal	1 part
Soybean meal	1 part
4. Wheat middlings	4 parts
Soybean meal	1 part
Fish meal	1 part
Dried ulva	1 part

These formulations are used for both adults and juveniles and can also be used for postlarvae if milled and sieved to a finer texture. However, knowledge of the nutritional requirements of brackishwater fish is not well advanced and acceptable feeds can probably be formulated from a variety of animal protein and cereal wastes. A general rule might be to prepare a feed with about 45% animal protein for broodstock fish, and the balance in cereal or plant wastes. A vitamin additive is desirable but not essential if the broodstock have natural feeds available.

Spawning and Fertilization

SELECTION OF SPAWNERS

For induced spawning in season, the first selection of spawners from the broodstock takes place before the beginning of the natural spawning season. The natural spawning season can differ slightly from year to year because of climatological and meterological changes. Captive broodstock usually mature about one month later than adults in the wild. Depending on the desired number of spawnings, the selection process is repeated several times during the season. Figure 21 illustrates the selection process.

Collection of the broodstock from the holding ponds is performed early in the day when temperatures are low. The water level is dropped to assist in catching the fish. They are herded by a net into one corner, or they are attracted into a feeding box. Once the fish are concentrated, they are kept separate so that unwanted fish are readily returned to the pond.

For individual examination, the fish are lifted out with a dipnet and placed in a small holding tank containing seawater with 2-methylquinoline (trade name: Quinaldine) (5-10 ppm) or MS-222 (30 ppm). The fish are anaesthetized safely for short periods at these levels and at normal temperatures (20-25°C). It is important that the fish are handled properly and do not damage themselves during recovery. If ripe males are found, they are transferred at once to separate tanks or to the indoor facilities containing seawater at 30-35‰. An egg sample is taken from each female, identified, and microscopically examined and measured. Until the individual egg diameter is determined for each female, the fish are held in separate recovery tanks. Selected females are then transferred indoors for prespawning holding. The rest are returned to the pond.

For every anticipated spawn, seven females and three males should be available, from which two females are selected to prepare for final induced breeding with the three males.

Each male must be healthy and vigorous, and yield milt when gentle pressure is applied along the abdomen toward the cloaca. Selected males, which are already marked, are transferred indoors but kept separate from the females. They are fed a prepared diet at a rate of 5% of body weight per day. The males can be used for more than one spawning.

Selected females must show egg maturation well into stage III, with a mean egg diameter of at least 500 μ. If the egg diameter is greater than 600 μ, the female is ready for hormone injection at once but is allowed to acclimate for 24 hr indoors before the injection treatment is started. If the eggs are between 500 and 600

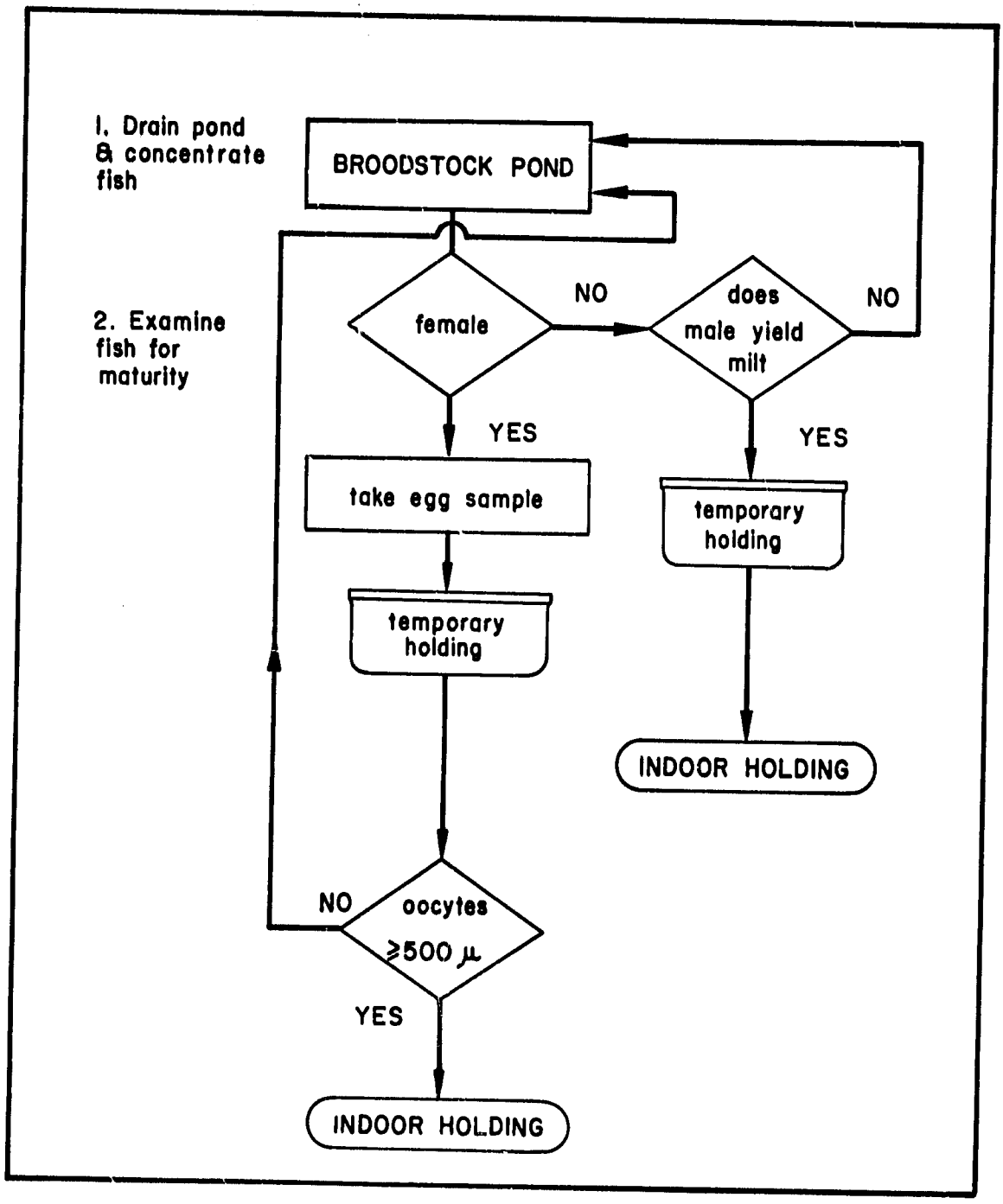


Fig. 21. Spawner selection procedure.

µ, the female is held indoors and the eggs allowed to maturate further. Egg maturation is benefited in the late stages by controlling the water temperature to about 20°C.

The procedure for determination of the egg maturation stage *in vivo* is discussed previously. Each egg sample is identified and stored in a 1% solution of formalin in seawater. This concentration does not produce any shrinkage of egg diameter.

It is important that the females are identified as individuals. All fish are tagged when first captured. If, for some reason, a fish has to be marked again, tagging is not recommended at this point as it induces stress. The individual females are held separately and the tanks identified according to the egg development stage of the fish. A female is not fed if she is about to undergo the

final hormone injections to induce breeding.

THE INDOOR ENVIRONMENT

The indoor holding tanks are relatively small to preserve individual fish identity and facilitate handling. Each unit allows a volume of 100 l/fish with a maximum of 10 fish. An ideal depth is 0.75 m. A hard, smooth, interior finish is needed to facilitate cleaning, and each tank has a bottom sloping to a center drain. It also has an overflow. A tank cover made of netting or framed screen is required to keep the fish from jumping out.

Each tank has a water system to deliver seawater at 32‰ salinity capable of a turnover rate once per hour, and freshwater for mixing or cleaning purposes. Seawater temperature (within the range of 18-24°C) is maintained by cooling units or air temperature control, and the oxygen level is maintained by aeration. Air temperature is maintained also at 18-24°C.

A clear incandescent lamp (300 W) or fluorescent lamp (north white light) is suspended over the center of each holding tank and adjusted vertically to provide 800 lux (75 ft-c) at the water surface. Activity of each lamp is controlled by separate timers and dimmers, and each operates automatically.

Each unit is cleaned daily and uneaten food and debris removed. When the tank is not in use, it is cleaned more thoroughly by brushing the interior surfaces with dilute hydrochloric acid, followed by intensive rinsing with fresh water.

The spawning tank has a minimum volume of 200 l and a minimum width of 40 cm, with glass sides for observation, and overflow standpipe. The tank is filled with seawater at 32‰ salinity flowing rapidly through the tank at a rate of once every 30 min. Both water temperature and air temperature are controlled to 20-22°C. Heavy aeration is required to maintain oxygen levels at saturation point. Light is subdued, giving 110-260 lux (10-25 ft-c) at the water surface.

A few moments before spawning, the seawater flow is shut off to prevent loss of eggs, but aeration is continued. It is important that disturbances in the laboratory are kept to a minimum during spawning.

MATURATION AND INDUCED BREEDING

For a female with eggs between 500 and 600 μ, several weeks may elapse before she is ready for hormone injection. At optimum water temperature (20°C), this time can be reduced. Continual egg sampling stresses the fish and it should be kept to a minimum during this maturation period. As maturation proceeds, sampling should be performed no more frequently than once every 3 d. It is discontinued after the eggs reach 600-650 μ and the fish is ready for induced breeding, except for the single sample taken 24 hr after the first hormone injection in order to estimate the proper time for the second injection.

Ovarian maturity of the female mullet is most accurately measured by the following method. The eggs are removed from the female through a polyethylene cannula 0.85 mm in diameter (Fig. 22). The fish is either anaesthetized or inverted gently with a black hood over the head. The cannula is inserted into the oviduct for a distance of 6-7 cm from the cloaca, and eggs sucked orally into the tube by the operator as the cannula is withdrawn. Eggs sampled from the mid-portion of the ovary are the most representative, and sampling error is minimized by avoiding the extremities. The eggs are removed from the cannula and washed and preserved in a solution of 1% formalin in 0.6% NaCl. They are then placed on a small plexiglass plate and measured with an ocular micrometer. Fine grooves cut in the plate align the eggs and facilitate measurement. Egg diameters are measured along the horizontal axis and the measurements grouped into 50 μ class intervals. The sexual maturity of the fish is expressed in terms of mean egg diameter, calculated from the egg frequency distribution.

When the eggs reach the tertiary yolk globule stage and have a mean diameter above 600 μ (and preferably 650 μ), the female is ready for hormone injection. Before hypophysation is initated, the female is removed from the holding tank and transferred to the spawning tank, where the injections are given. The black hood over the head and eyes again minimizes stress during the hormone treatment.

The hormone to induce spawning and which produces the most reliable results is partially purified salmon gonadotropin, SG-G100, standardized at 1 mg equivalent to 2,150 IU of HCG. In the breeding season, the amount of gonadotropin required to induce spawning is inversely proportional to initial mean egg diameter of the female, and varies between 12 and 21 μg/g body weight. The spawning dose is estimated from the regression (see also Figure 9):

$$Y = 66.29 - 74.53\,X$$

where Y = total hormone dose

X = initial mean egg diameter

The dose is applied in two separate injections: one-third of the dose first, followed by the remaining two-thirds up to 48 hr later. This sequence is critical to avoid partial spawning.

The hormone is injected in the dorsal muscles near the base of the dorsal fin in a constant volume of 0.50 cc of 0.6% NaCl. During the injection procedure, the female is held gently with the black hood over the head and eyes (Fig. 23).

Fig. 22. Top to bottom: Holding female mullet for egg sampling or hormone injection; inserting cannula for egg sampling; withdrawing cannula while sampling.

The first injection initates the final stages of egg development. In females with initial mean egg diameter less than 650 μ, the first injection induces a shift in egg diameter frequency distribution and an increase in mean egg diameter toward the critical range of 650-700 μ. These changes do not occur in females with initial mean egg diameter equal to or greater than 650 μ.

Timing of the second injection depends on observed changes in the eggs. A final egg sample is taken 24 hr after the first injection. Between 24 and 48 hr after the first injection, the central portion of the eggs clears. Then the second injection is given. Time to spawning after injection of the second dose varies from 10 to 14 hr.

SPAWNING AND FERTILIZATION

Three males, selected for running milt, are placed in the spawning tank with the female about 2 hr after the second injection. Rapid abdominal distention and

Fig. 23. Hormone injection procedure.

protrusion of the cloaca of the female is observed about 8 hr after the second hormone injection as a result of hydration of the eggs. The cloaca changes from a horizontal (related to the baseline of the anal fin) to a vertical position. As hydration progresses, indicated by continuous distention of the belly and frequent excretion of calcium, the males become more active and remain in close contact with the female. Occasionally the males will spin around the female or nudge the cloaca. The prelude to spawning is a violent quivering by the males which are then lying parallel to and facing the same way as the female, and touching. The first release of a small number of ripe eggs stimulates the males to liberate spermatozoa. The female will then respond with an explosive and continuous release of eggs (Figure 24).

Fecundity ranges from 650-850 eggs/g body weight and consequently between 500,000 and 1 million eggs are released. It is important that fecundity is estimated before the fertilized eggs are transferred from the spawning tank. This estimate is the base upon which all subsequent density levels are calculated. A complete record is kept of fecundity, fertilization rate and hatch rate, with reference to the individual fish and the method of hypophysation, e.g., hormone type and dosage. Accordingly, eggs are sampled and counted before transfer using a statistical procedure to determine fecundity.

A few moments after spawning, all the adults are removed from the tank. The eggs remain in the spawning tank until fertilization is estimated. Aeration is intensified to maintain the eggs in constant motion and assure fertilization. The first cleavage of the eggs is observed about one hour after spawning. The percentage fertilization is then determined by microscopic examination of the eggs according to the following method.

An hour after spawning, a small sample of about 100 eggs is taken from the spawning tank, drained, and placed on a grooved microscope slide. The percentage fertilization is equal to the number of eggs which show cell division divided by the number examined.

A low fertilization rate may indicate poor egg quality, and subsequently produces a poor hatch and poor quality larvae. Moreover, the unfertilized eggs provide an ideal substrate for rapid growth of bacteria during incubation and during the early days of larval life if they are not separated. Only a minimum fertilization rate of 85% is acceptable by hatchery management.

PHOTOPERIOD/TEMPERATURE CONTROL

Experiments with photoperiod and temperature control have indicated that functional ovarian maturity of female mullet can be achieved out of season. Early in the refractory period, it is desirable to select from the female broodstock a group of fish to accelerate gonad maturation, and another group to retard. The remainder are allowed to mature naturally. In this way it is possible to have females available for spawning before, during and after the natural breeding season. Figure 25 outlines the photoperiod/temperature control procedure.

The selection of females for photoperiod/temperature manipulation is made in the refractory period when the oocytes are in the primary stage of development. The fish may be those which have been induced to spawn in the previous natural breeding season, or those females suitable for induced spawning in the next natural breeding season. Selected females can be held under environmental regulation and control for up to 2 yr and produce viable eggs, if the artificial food diet can be supplemented by natural growth of algae and diatoms in the indoor holding tanks.

The regimes for accelerating or decelerating egg development in the females are indicated in the flowchart (Figure 26). Timing of each regime depends on the desired number of early and/or late spawnings. Total conditioning period in 6L/18D at 21°C is about 120 d before the desired spawning time. Late spawners can be maintained outdoors under natural photoperiod and temperature until the end of the refractory period. Then they must be moved indoors and maintained under conditions of natural summer photoperiod; 18L/6D, and temperature to delay egg development. They are then exposed to the 120-d regime prior to induced spawning time. The eggs of the female are sampled monthly to determine the development stage. The first signs of egg development to stage II are observed after about 60 d at 6L/18D and 21°C. Eggs in tertiary yolk globule stage III are anticipated after about 120 d, and any time thereafter the fish is ready for hormone injection. Throughout photoperiod regulation and until hormone treatment is started, the females are fed the prepared diet at 5% of body weight per day.

Males for spawning out of season are also conditioned together with the females in the same tank. To assist spermiation out of season, the males are injected with 17-alpha methyltestosterone (2.5 mg/100 g body weight in 0.6% NaCl) or HCG (0.1 mg/100 g body weight). SG-G100 can also be used. During photoperiod control the males are fed similarly to the females.

The hypophysation technique for spawning out of season is the same as described earlier. Higher doses of hormone may be required and more frequent injections needed to induce spawning. Total doses of between 21.5 and 41.5 µg/g body weight of salmon gonadotropin, SG-G100, administered in two to four injections are usually adequate.

Photoperiod and temperature regulation are conducted indoors separate from the other indoor holding facilities. Photoperiod, water and air temperature controls, and

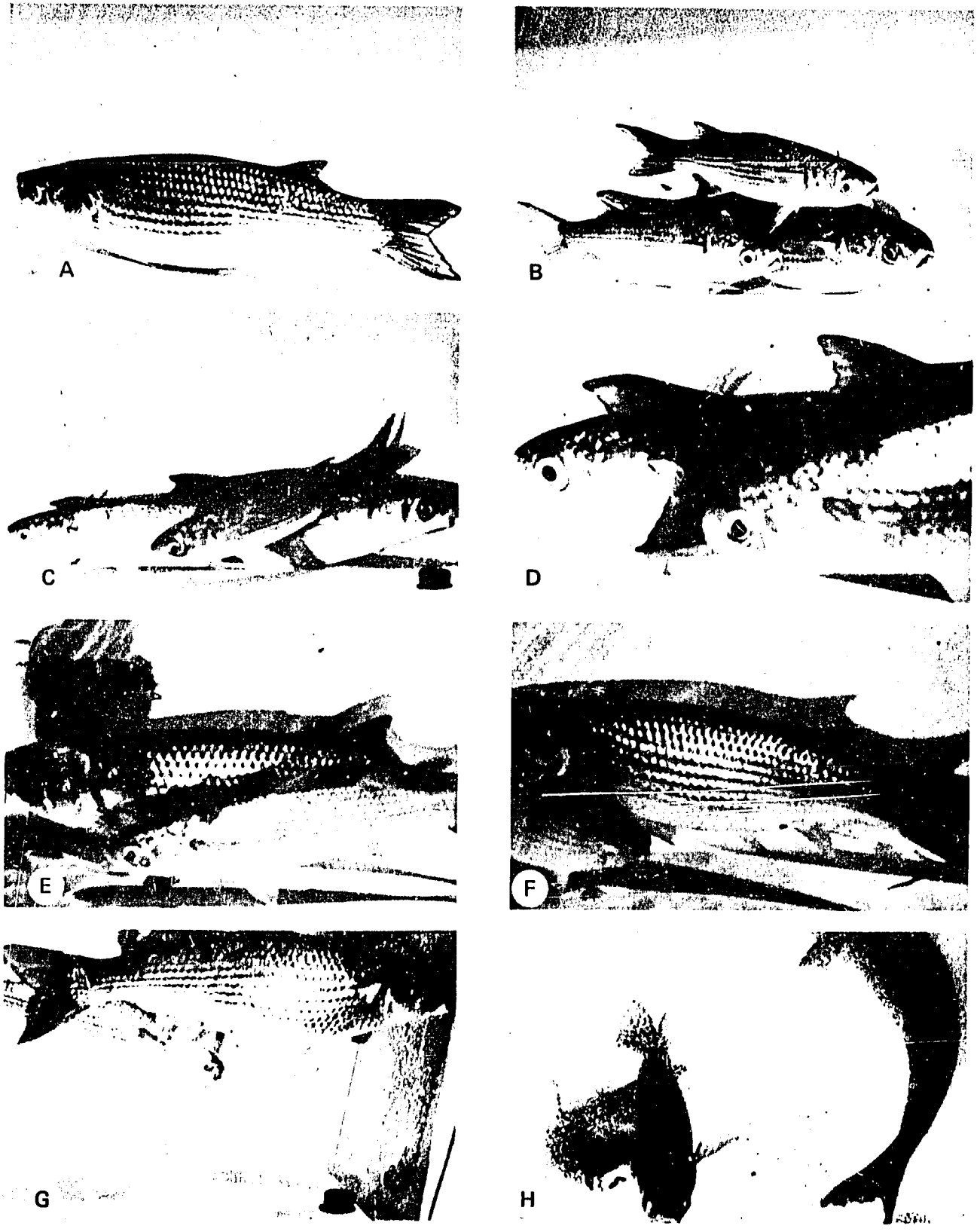


Fig. 24. Mullet spawning sequence. A. Female mullet a few hours after the "effective" gonadotropin dose. Note distention of abdomen. B. Males in a typical courtship position with the female. C. Males turned caudally. D. Males approaching female caudal fin. E. & F. Males turning to touch female's cloaca. G. Female mullet in the act of spawning. Male is approaching the egg mass. H. Two males (left) in the act of fertilization. Note the quick disparsal of the eggs by the action of male caudal fins.

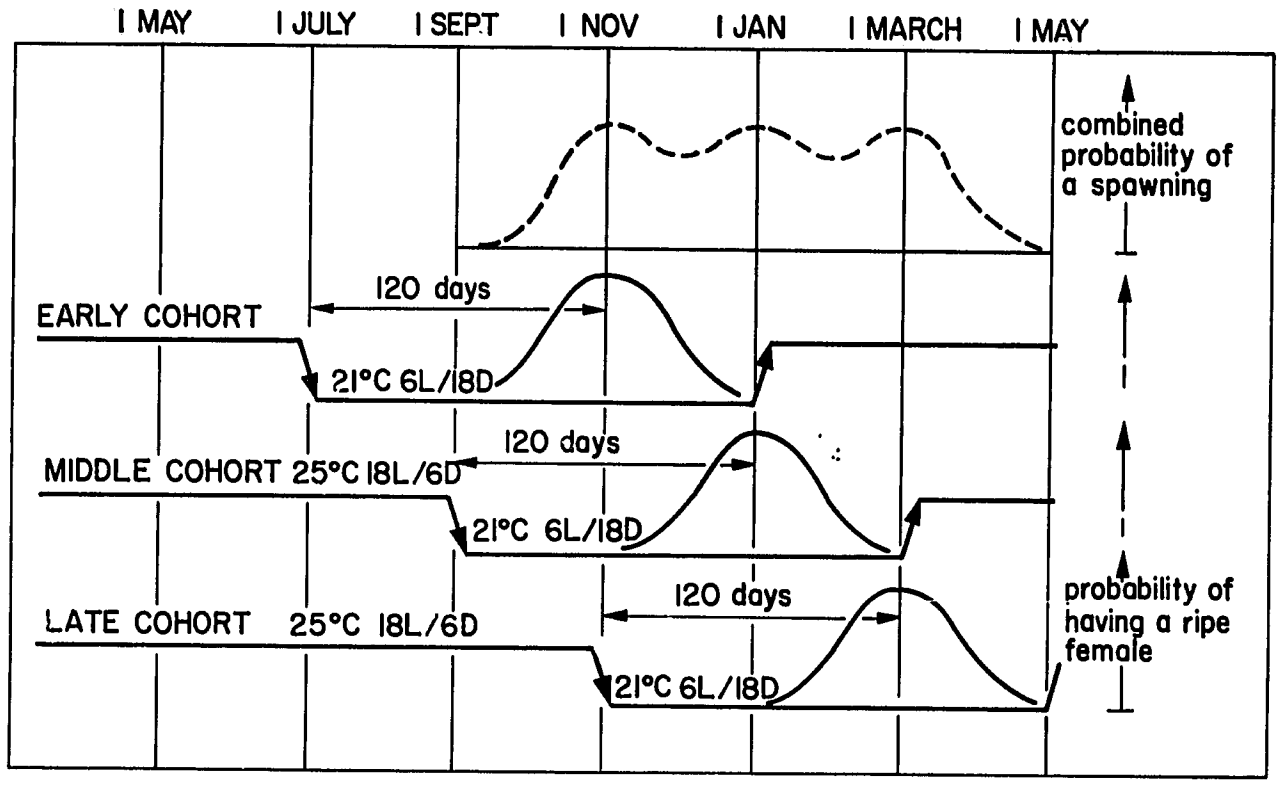


Fig. 25. Photoperiod/temperature control and spawning time (Hawaii).

light cycles can all be regulated completely. The facilities are designed in such a way that the different regimes of 6L/18D at 21°C and 18L/6D at simulated summer temperatures can be run concurrently.

The water system supplies prechilled and preheated seawater of 32‰ salinity; the desired water temperature is maintained by adjusting the flowrate of either the prechilled or preheated seawater. Air temperature in each section is maintained by air conditioning regulated by a separate thermostat. Air temperature is identical to water temperature in the holding tanks. Lamps in each section of the photoperiod holding facility should be controlled by separate electrical dimmers and timers, and operate automatically.

Except for the items specifically related to temperature and photoperiod control, the requirements for operating and maintaining the photoperiod holding facilities (and the fish) are the same as those in the other indoor holding facilities.

Incubation and Hatching

INCUBATION

After the fertilization rate is determined, the eggs are transferred to the incubators. Aeration in the spawning tank is stopped and the fertile buoyant eggs rise to the surface. These are transferred by syphon directly into the incubators. An alternate method is to remove the viable eggs with a soft fine-mesh handnet. The eggs are then washed gently under running irradiated and filtered seawater, and dipped for 1 min in a seawater bath containing potassium penicillin G (80 IU/ml) and streptomycin sulfate (0.05 mg/ml). Care must be taken to transfer only the buoyant and fertile eggs. It is important that the transfer is as clinical as possible, and all the equipment is washed and sterilized before use.

The preferred incubation density is 75-100 eggs per liter. Although higher densities can be used, it is found that the incubation rate is reduced. The egg number is estimated by volume transfer from the spawning tank into the incubators. For example, from a 200-l spawning tank, and knowing the estimated fecundity of the female fish, an aliquot volume is calculated and transferred which contains the number of eggs to stock an incubator of known volume to a density of 75-100 eggs per liter.

The incubation unit meets the following specifications:

1. Minimizes chances of contamination and fouling.
2. Subdues light levels.
3. Prevents agglutination and crowding of eggs, and minimizes contact of eggs with surfaces.
4. Cleans easily.
5. Allows maximum dispersion of oxygen throughout the water column.

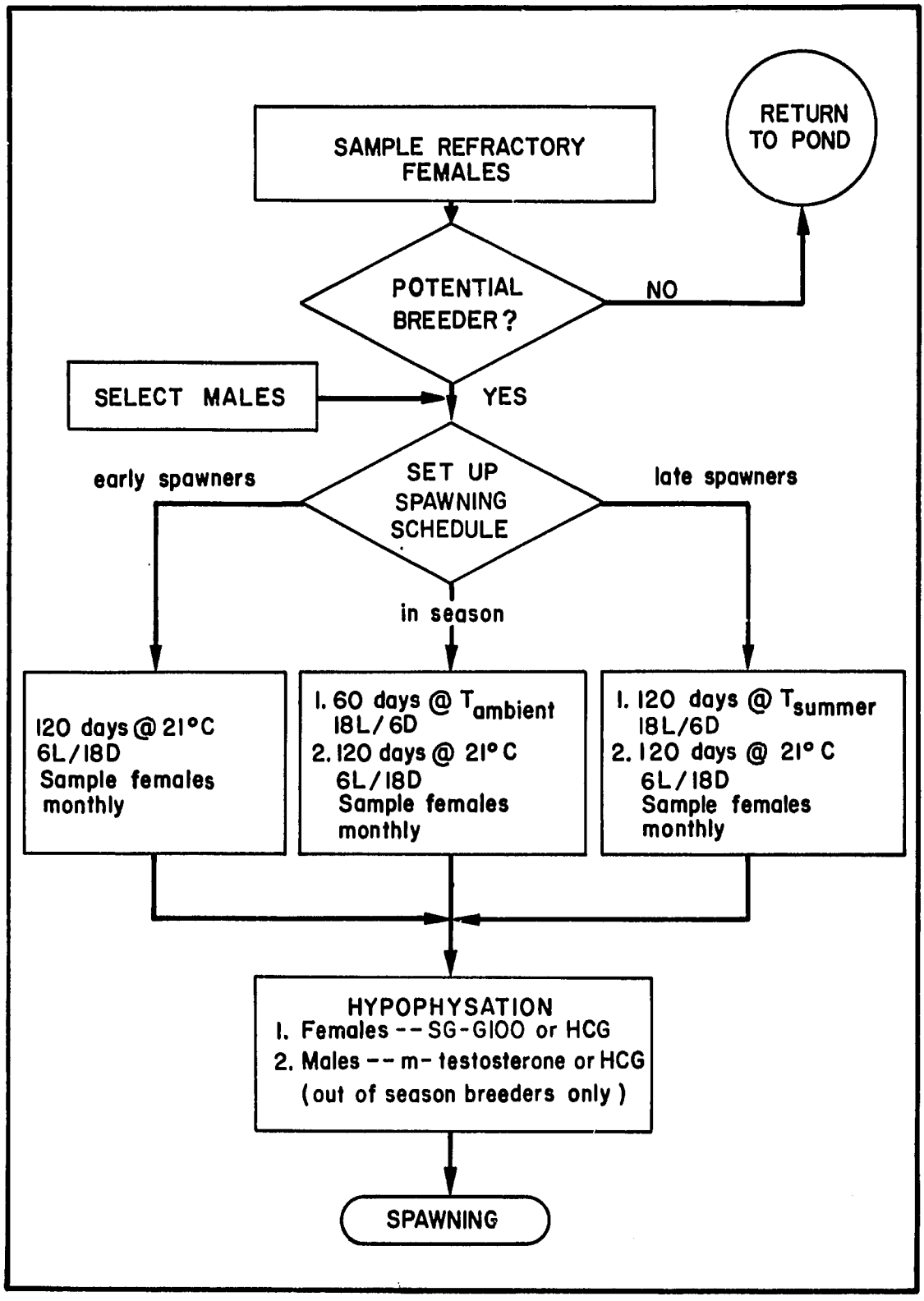


Fig. 26. Photoperiod/temperature control procedure.

A cylindrical incubator with a cone-shaped bottom meets all these requirements. It has a minimal depth of 1.5 m to provide a long water column. A diameter of 0.75-1 m allows maximum circulation from airstones placed in the base of the cone and of a size which produce small bubbles to prevent damage to the eggs. The cone-shaped base enhances concentration and collection of dead eggs. Plastic, polyethylene, fiberglass, concrete, vinyl and wood are all materials acceptable for an incubator. Toxic chemicals and plasticizers from newly molded plastic and fiberglass units must be leached in a heated atmosphere for a day or submerged under water for a week before use. The interior finish of each incubator is smooth with rounded edges, and matte black in color.

Cleaning of the incubator before use is extremely important. The interior is scrubbed with antibacterial solution of potassium permanganate, followed by a thorough rinsing with fresh water. It is then filled with seawater (30-35‰) which is treated first by an ultraviolet sterilizer and passed through a bacterial filter. No further water exchange is made.

Water and air temperature are regulated within a range of 18-24°C, with an optimum at 20-22°C. Dissolved oxygen levels are maintained at saturation point. Aeration is vigorous and well dispersed through the water column. The eggs are maintained in suspension. Incubation is conducted in reduced light. If desired, an antibiotic treatment of 10 IU/ml penicillin and 0.01 mg/ml streptomycin can be added daily to prevent the chances of marine bacterial infections occurring.

Egg incubation is conducted in separate units. It requires strict control of conditions with at least twice daily checks of water temperature, aeration, and dissolved oxygen levels. Incubation time is inversely proportional to temperature. Mortality increases at temperatures higher or lower than the effective range. At higher temperatures the chance of bacterial contamination increases. At 22°C the total incubation time from fertilization is about 48 hr. For each degree below or above the optimum, 6 hr are added or subtracted to get incubation time. For example:

Temperature (°C)	Time (hr)
20	60
21	54
22	48
23	42
24	36

HATCHING

At the optimum temperature range of 20-22°C, incubation and hatching take 48-60 hr. Just before hatching, the oxygen demand of the eggs increases sharply and it is essential that oxygen levels are maintained near saturation at this time. Scum and foam on the water surface of the incubator generally indicate a low hatch rate. Although higher rates are possible, a hatch rate of at least 50% is expected consistently. Hatchery management will determine a minimum tolerable level below which it is not economical in terms of hatchery operations to continue with that particular batch.

Before transferring the emergent larvae to the larval rearing containers, the number of larvae in the incubator is estimated for density and hatch rate determinations. When the contents of the incubator are evenly distributed, a sample is counted. Ten such samples are removed, counted, and averaged. Extrapolated to the total volume of seawater in the incubator, this aliquot density provides a good estimate of the number of larvae in the incubator which hatched.

Hatch rate is estimated by

$$\text{Hatch rate} = \frac{\Sigma \text{ hatched larvae}}{\Sigma \text{ eggs}} / \text{ fertilization rate}$$

The number of larvae for each rearing unit is then calculated by the transfer of known volumes from the incubator. At a projected survival at day 50 of 25% of the larvae at a maximum density of five juveniles per liter, 20 larvae per liter are stocked in each of the larval rearing containers. If lower survival is anticipated, higher initial stocking densities to about 50 larvae per liter are used.

After estimating the larval count, the air is shut off and aeration hoses are disconnected and removed. It is advisable not to dredge the bottom of the incubator because empty egg shells and unhatched eggs sink and present a potential substrate for marine bacteria if transferred with the larvae. The larvae are transferred by syphoning slowly the calculated volume of water from the incubator to give the desired larval density in the rearing unit.

Larval Rearing

REARING UNITS

The rearing unit is fabricated from plastic, polyethylene, fiberglass, concrete, vinyl sheet, or wood. A smooth interior surface is essential and this is achieved by coating the interior with fiberglass, epoxy resin, or lead-free paint. The unit is thoroughly leached before use to remove any plasticizers or toxic chemicals by prolonged air drying in the sun, or submersion for several days in running water. The sides of the unit are

painted with a matte or nonreflective black paint or dyed resin, and the base white.

Each unit is fitted with a surface overflow, which is screened, and a drainage outlet at the center of the inward sloping base (1 in 20). A removable screened standpipe fits into the outlet.

The volume of a typical unit for indoor work is 4,800 l, with dimensions 2.5 m diameter and 1 m deep. The outdoor unit can be larger with a volume of 14,000 l, with dimensions 3.5 cm diameter and 1.5 m deep. The outdoor unit needs a protective cover fabricated from wood and fine screening.

Before use the tanks are scrubbed clean and hosed down with fresh water. No chemicals are used. The unit is then filled with seawater which is treated by passing through an ultraviolet sterilizer and through a bacterial filter.

THE REARING ENVIRONMENT

Good quality sterilized seawater at 32-35‰ is required for larval rearing. A source of fresh water also has to be available for mixing with the seawater during the rearing process. Water temperature control is necessary between the range 20-26°C. At the top of the range the larvae grow at a faster rate, but the conditions are more conducive to bacterial growth. A controlled temperature of 20-22° C is preferred. This is maintained for an indoor unit by controlling the air temperature.

The water in the rearing unit is not exchanged continuously but is circulated in the tank by the apparatus called the kreisel (Figure 13). About 20% of the water volume in the unit is exchanged each day for the first 25 d of the 50-d rearing cycle. A slow reduction in salinity is begun on day 10, aimed at reducing the salinity to about 24‰ by day 25. After day 25 a continuous exchange of water is begun, completely replacing the tank volume once every 24 hr. Later the flow rate is increased depending on the density and growth of the survivors. By day 50 the salinity can be as low as 15‰ and the exchange rate two or three volumes per day.

Above the rearing unit is a fluorescent or incandescent lamp fitted with a diffuser to provide a light intensity of no more than 1400 lux at the water surface. As the young larvae and postlarvae are sensitive to light shock, it is advisable to maintain constant light conditions or avoid sudden changes. Natural daylight is acceptable for any outdoor rearing unit.

The rearing tank is cleaned daily. The tank surface and bottom are skimmed with a suction device to remove dead larvae and uneaten food. Daily levels of temperature, salinity, pH and dissolved oxygen are recorded. If equipment is available, periodic determinations of nitrite, nitrate, ammonia, sulfide and phosphate are useful to monitor metabolite buildup.

LARVAL FEEDING

Larval feeds used most successfully are indicated in Table 8.

The phytoplankton culture is introduced into the larval rearing unit to the required density before the rotifers are added. As the rotifers graze down the existing algae, more culture is added daily to maintain the required density of 10^3 cells/ml. An indication of the correct density is a visible green color to the water, but more precise density is measured with a cell counter. Counts are made after the culture is added and distributed. It is recommended that new culture be added each day even though the density does not appear to have changed.

The rotifer population is maintained at 5-10/ml until the larvae metamorphose and are large enough to prefer bigger organisms. At this point the addition of the algal culture is stopped. Rotifer density is estimated daily, by counting a minimum of three samples. More rotifers are added as needed to maintain the correct density. Copepods, if available, are added to the larval diet at an initial density of 2-3/ml on day 9. They are fed until day 40. Smaller copepods are selected first by the larvae, followed by larger copepods later on. Amphipods are also readily eaten by postlarvae. Day-old nauplii of the brine shrimp are fed to postlarvae starting on day 14/15 at an initial density of 2-3/ml. As the postlarvae grow older this density is increased or larger nauplii are used. The quantity is estimated by consumption and demand.

The daily ration of artifical feed for older postlarvae is not yet determined. The feed formulae used are identical to those prepared for adult mullet, but the dry particle size is smaller (100 μ). Before feeding, the crumbs are mixed to dough by adding fresh water. This dough mix is then hand fed and broadcast so that the particles remain suspended for a time. For younger larvae (30-40 d old), the feed is taken in the water column. Later on, the older larvae pick feed particles from the bottom of the tank. As it is added in sufficient quantities to permit all the larvae to feed on demand, the artifical food presents a fouling problem. The uneaten food is therefore removed daily by flushing or cleaning the tank bottom with a suction device.

Although there are several operations which cause physical disturbances in the rearing unit, the postlarvae younger than 45 d are not themselves handled or moved. Almost all the young fish react traumatically to handling and die. This is suggested to be a nutritional deficiency but as yet is not confirmed. In consequence, the hatching and rearing activity is considered to be a 50-d operation. After 45 d the young fish school together and can be

Table 8. Type and size of larval feeds.

Organism	Density/ amount	Size of particle	Time of feeding
Phytoplankters (*Chlorella* and *Navicula* sp.)	10^3 cells/ml		Day 3/4-day 14/18
Rotifers (*Brachionus plicatilis*)	3-5/ml	50-175 μ	Day 4/5-day 14/18
Copepods (*Euterpina acutifrons*)	2-3/ml initial density (or as available)	250-1000 μ	Start at day 9/10, and feed increasingly larger sizes to day 50
Brine shrimp nauplii (*Artemia salina*) (day old)	2-3/ml initial density	250 μ then above	Day 14/15, then increase density as needed to day 50
Amphipods (*Corophium insidiosum*)	1-2/ml (or as available)	500-4000 μ	Day 30 and after
Artificial preparations	On demand	⩽ 100 μ	At the earliest on day 30, preferably day 40

safely handled, and transferred after 50 d.

Phytoplankton Culture

CULTURE ROOM

Except for the large-scale batch culture, all stages of the phytoplankton culture procedure are conducted successively indoors under strictly controlled conditions. A culture room is therefore an essential component of the hatchery.

The culture room provides the following utilities and services:

- Fresh and seawater, both delivered sterilized by ultraviolet light and filtered through 5 μ cartridge filters. If possible, the seawater is heated to 26-27°C. Water demand is intermittent, mainly for cleaning and filling purposes.
- A compressed-air distribution system with filters and water traps. The plastic airlines are 3.0 mm i.d. and the tubing system has to be totally reliable.
- Air conditioning, to keep the room temperature at 23°C; this system also has to be totally reliable.
- Fluorescent lighting, 50:50 agrolamps and cool-white (40 W), with automatic controls to give a photoperiod of 18L/6D.
- Steam cleaning equipment.
- An autoclave large enough to hold 50% of the culture carboys and capable of 15 lb/in^2 pressure at 250°F.
- Sufficient space to hold basic culture test tubes, flasks, carboys, and cylinders in racks.
- Bench space and large sink with fresh and saltwater.
- Refrigerator, microscope, and cell counter.

CULTURE PROCEDURE

A culture flowchart is shown in Fig. 27. Although a number of phytoplankters can be used, only the inocula of the organisms *Chlorella salina* and *Navicula marina* are cultured. These inocula are maintained individually year round, with a subculture started each month, and are used to start the larger culture as required. To develop a culture, the required ratio of inoculum to flask culture is two tubes of each organism per liter of flask culture.

The intermediate flask culture is recommended as a backup to the larger cultures in glass carboys. A few flasks are maintained year round. During the larval rearing season, small volumes of the carboy cultures are used as inocula for new carboy cultures.

The flasks (1.5-2.5-l capacity and made of Pyrex glass) are cleaned by rinsing with fresh water and sterilized in an autoclave after they are steam cleaned. They are then filled with sterile seawater at 26-27°C and a nutrient mix added at 6.35 ml/l. They are then inoculated using sterile techniques. A subculture is started every 2 wk. The flasks are shaken daily. The volume ratio of the flask to carboy culture system is 1:16.

Some carboys are maintained year round to provide batch culture inoculant for supporting the culture of rotifers. Small volumes of the carboy cultures are used

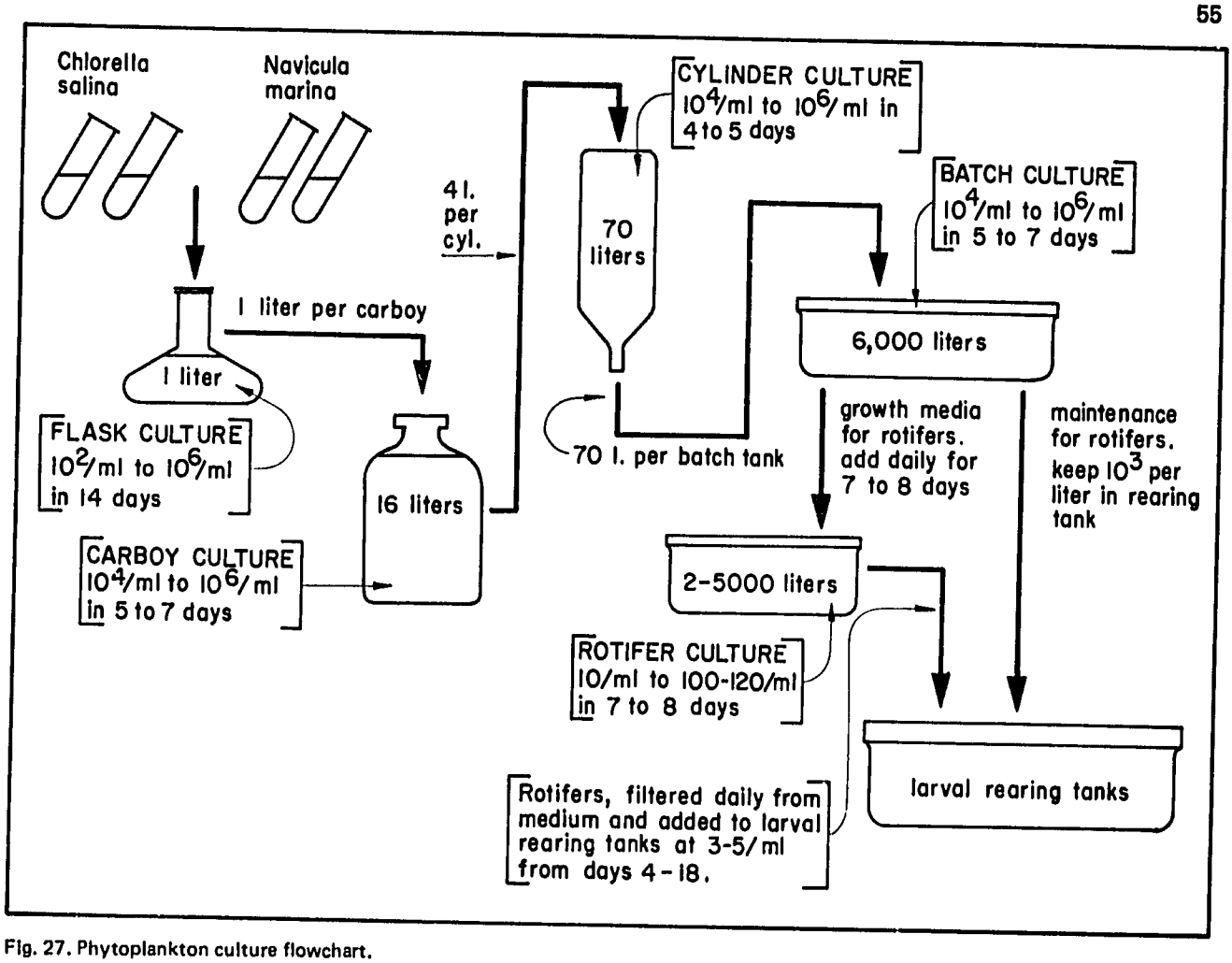


Fig. 27. Phytoplankton culture flowchart.

to inoculate other carboys.

The carboys (16-l capacity and made of Pyrex glass) are cleaned by rinsing with fresh water and autoclaving after steam cleaning. No soap or detergent is used. They are filled with sterile water at 26-27°C and nutrient mix at 6.35 ml/l is added. They are inoculated with cultures from other carboys or flasks to an initial density of 10^4 cells/ml. The culture cycle takes 5-7 d, yielding a final density of 10^{6+} cells/ml. Vigorous aeration in the carboys is required throughout the culture period. The volume ratio of the carboy to cylinder culture system is 1:17.

The large cylinder culture system is maintained during the larval rearing season only. It is inoculated with samples from the carboy cultures to the required density. The cylinders (capacity 70 l) are made of resined fiberglass. The sides are translucent with a smooth interior finish. The cone-shaped bottom has a bottom outlet to empty the culture.

The cylinders are steamcleaned at least every 3-4 cycles. They are filled with sterile seawater at 26-27°C, and nutrient mix at 6.35 ml/l is added. They are inoculated with small volumes of culture from the carboys to an initial density of 10^4 cells/ml. The culture cycles every 5 d with a final density of 10^{6+} cells/ml. Vigorous aeration is required throughout the culture cycle. The volume ratio of the cylinder to batch culture system is 1:85.

The batch culture system is maintained during the larval rearing season only. The tank size is 2.7 m diameter and 1.5 m deep with a capacity of 6,000 l. The sides are translucent and the bottom slopes to a center outlet for emptying. The tanks are drained at the end of each cycle and cleaned according to the same procedure used for the cylinders. Fertilizer is added to the sterilized water at 25 g per 1,000 l. They are then inoculated to an initial density of 10^4 cells/ml with cultures from the cylinders or carboys. The culture cycles every 5-7 d with a final density of 10^{6+} cells/ml.

The outdoor tanks are exposed to ambient air temperature, but the cultures prefer temperatures above 23°C. The tanks are protected under a translucent roof. Vigorous aeration is necessary. The photoplankton batch culture can be conducted anywhere as long as exposure to direct sunlight is avoided, and the usual biological requirements of the organisms are met. It is preferable to

batch culture organisms in a hothouse or glasshouse.

A batch culture is harvested when at peak density. This is usually maintained for 1-2 d. It is used to give the required 10^3 cells/ml density of phytoplankton in each larval rearing unit for the rotifers and to support the rotifer production tanks. An addition to the larval rearing unit is made daily in order to maintain the required rotifer density of 3-5/ml. Algal density in each larval rearing unit is monitored before and after addition of phytoplankton culture. The volume ratio of the batch culture to the larval rearing system volume is 1:6. This includes the phytoplankton culture needed for rotifer production. The ratio to the rotifer rearing system volume is about 0.5:1.

NUTRIENT MIX AND FERTILIZER

A nutrient mix is added to the flask, carboy and cylinder culture before inoculation. It is used at a rate of 6.35 ml/l of seawater. The ingredients are separately dissolved in distilled water in small portions of 1 l. After autoclaving, they are stored separately in the culture room. The solutions will keep for about 6 mo. The formula of the nutrient mix is:

Ingredient of nutrient mix	Solution (ml/l)	Quantity (parts)
KNO_3	1.0	2.5
$FeCl_3$	1.0	0.5
K_2HPO_4	0.1	2.5
Burkholder's solution		0.85

Burkholder's solution is prepared separately in the laboratory. The ingredients (listed below) are dissolved separately in distilled water in 1-l flasks to make 7 1. Burkholder's solution is also stored in the culture room.

Ingredients of Burkholder's solution	Weight g/l	Weight g/7 l
H_3BO_3	0.57	3.99
$MnCl_2.H_2O$	0.36	2.52
$CuCl_2.2H_2O$	0.268	1.876
$Na_2MoO_4.2H_2O$	0.252	1.764
$Fe_2(SO_4)_3$	0.912	6.388
$ZnSO_4$	1.25	8.75
NaEDTA		1.8

A fertilizer mix is added to the large batch cultures and used at a rate of 25 g dry fertilizer mix/1,000 l of seawater. The formula of the fertilizer mix is:

Ingredient	Weight g/1,000 l
Urea	1.39
$(NH_4)_2SO_4$	19.44
$CaHPO_4$	4.17

Rotifer Culture

The rotifer production system (Fig. 27) is necessary to supply rotifers at a density of 3-5/ml to each larval rearing unit from day 4-5 to day 14-18, until the post-larvae prefer larger organisms. Rotifers are reared in circular tanks (capacity 6,000 l) 2.7 m diameter x 1.5 m deep, made of resined fiberglass. The interior is smooth and each tank is covered with a polyethylene sheet.

Before use the tanks are steamcleaned and filled with 1,200 l of phytoplankton culture at 10^{6+} cells/ml density. It is then inoculated with rotifers from another culture to a density of 10 rotifers/ml. As the rotifers graze down the algae, between 600-1,200 l phytoplankton culture at 10^{6+} cells/ml is added daily. The rotifer density peaks at about 100-120/ml in about 7-8 d. By that time the total culture volume is about 6,000 l. The rotifers are harvested using an airlift pump and filtering through a 48 µ mesh Nitex bag. The rotifers are then transferred to the larval rearing units in clean buckets, and stocked at a density of 3-5 rotifers/ml.

The appearance of protein-rich foam on the surface of the culture tanks after about 1 wk is indicative of increasing ammonia levels and imminent culture decline. Harvesting is conducted immediately. The culture is discarded when the rotifer counts decline or there is obvious metabolite contamination. Rotifer density, oxygen levels, and ammonia levels should be monitored in the tanks daily if possible. The volume ratio of rotifer culture to the larval rearing system is 0.25:1.

Rotifers prefer a temperature range of 23-27°C and salinity of 32-35‰. The cultures need some artificial light or indirect and subdued daylight. It is recommended that surplus rotifer cultures be maintained during the season to ensure against unexpected losses. It is also useful to keep a minimal system operating in the off season. Auxiliary cultures are maintained in small tanks. The culture techniques are identical except that a tank is filled to capacity at the start of the culture cycle and not by daily addition over a period.

Copepod and Amphipod Production

Large-scale controlled production of copepods and

amphipods has not been achieved as yet due to their low fecundity. However, all hatcheries generate a large volume of nutrient-rich effluent water. If this effluent is discarded into a waste-water pond, which provides a valuable food resource, it is recycled back into the system in the form of these invertebrate organisms.

A separator system is used successfully to harvest phototaxic, free-swimming invertebrate organisms from such a waste-water pond. These copepods and amphipods are readily consumed by mullet larvae, and are believed to be nutritious. So far no preferred feeding schedule is recommended, but copepods and amphipods are given to the postlarvae if sufficient quantities are available. The separator system is described in detail in Chapter 5.

Brine Shrimp Nauplii Production

An automated production system for the nauplii of the brine shrimp gives reliable daily yields. It is also capable of immediate conversion to manual operation in the event of a failure of automatic controls. It is described in full detail in Chapter 5. The system operates on a 48-hr cycle using two incubators with a common separator. The time for incubation is 36 hr leaving the remaining 12 hr for cleaning and recharging. The cysts of the brine shrimp are stocked in the incubator units at a density no higher than 0.75 g/l.

In an attempt to conserve on *Artemia* cysts, an intensive culture system is being tried out at present to grow brine shrimp nauplii to 4-5 d for feeding to older postlarvae (20 d and over). The nauplii are fed dried *Spirulina* and grow at densities of 3-5/ml until they are large enough to substitute for a greater number of newly-hatched nauplii. The system is still experimental, but it is estimated that overall cyst demand may be cut by 50-60% using this method to supply nauplii to older larvae.

Staffing

The most important qualities for a competent hatchery staff are conscientious attention to details, rather than a high level of academic training. The distinction between hatchery production operations and controlled experimentation is often not clear. Usually the hatchery production operations suffer. Therefore, it is recommended that the hatchery operations staff and research staff, and their roles, are clearly defined and separate.

Listed below are the staff and skills necessary to operate a small hatchery, such as the one shown in Figure 28 (300,000 annual production of juveniles). For a larger hatchery (three to five million annual output), the main staff additions would be at the unskilled and semiskilled levels further assisting the manager and his technicians.

MANAGER

The duties of the manager are to plan operations, supervise people, control funds, prepare reports, order or authorize the ordering of supplies and equipment and arrange for transportation and sale of the hatchery's product. He should therefore have a strong business background, experience in dealing with people in a supervisory capacity, and a strong background in some aspect of biology. In addition, if the hatchery is supporting experimental or extension training projects, he must define the extent of teaching responsibilities for himself and his staff, and the facilities to be used. The manager's main responsibility is production, not extension.

FISHERIES BIOLOGIST

The duties of the fisheries biologist are to supervise broodstock collection and maintenance, maturation testing and hormone treatments, photoperiod conditioning and spawning. A Ph.D. or experience in reproductive physiology of fishes is not required. A B.S. in fisheries biology is desirable. Prior experience in handling hatchery animals is more important.

HATCHERY TECHNICIAN FOR LARVAL REARING

This technician is responsible for incubation, larval rearing, and juvenile holding. He must be an experienced hatchery operator and a background in fisheries biology would be useful but not essential. He must be a good fish handler, understand fish husbandry practices, and be observant.

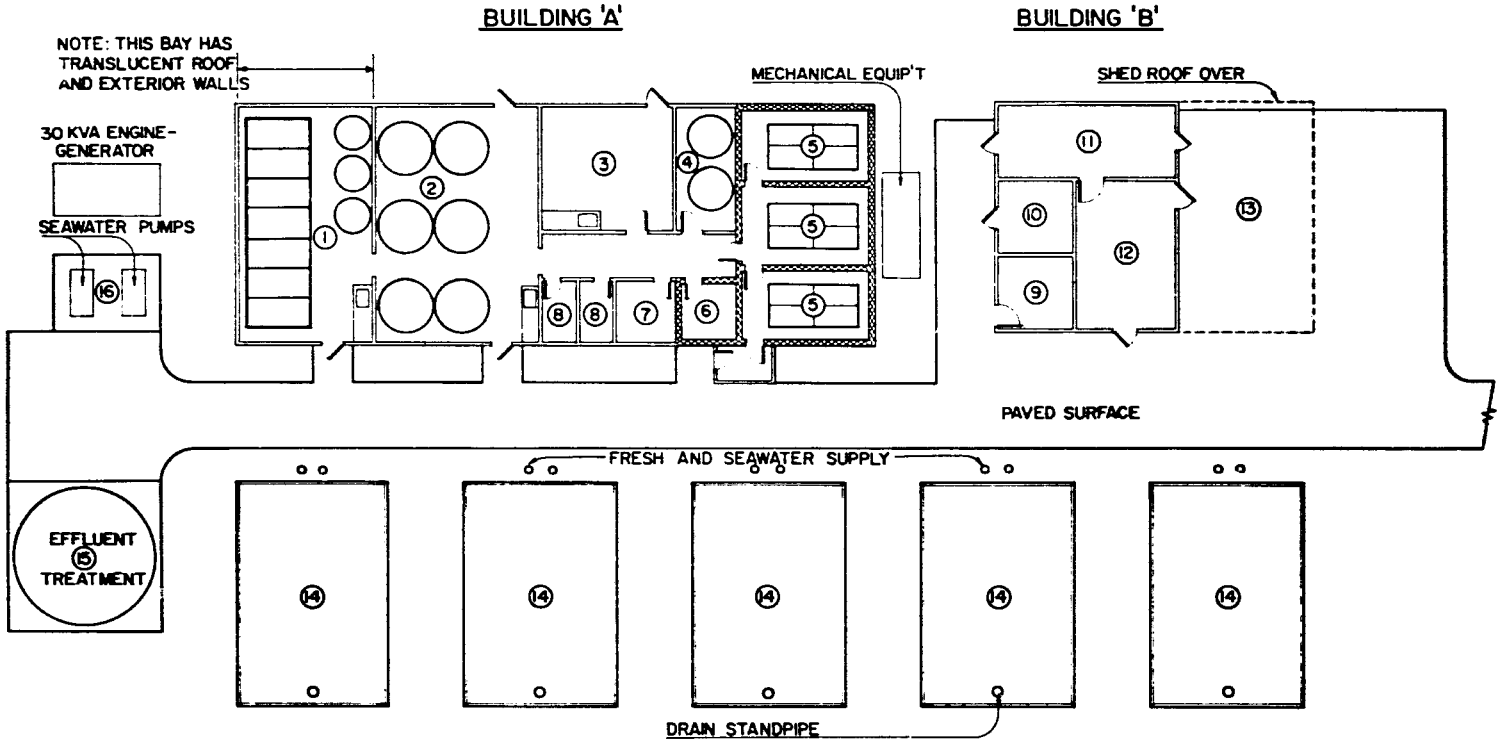
FOOD PRODUCTION TECHNICIAN

The duties of the food production technician include all aspects of the food production system, i.e., phytoplankton culture, rotifer culture, brine shrimp production, copepods and other invertebrate production, and artificial food. He must also maintain the productivity of the outdoor holding ponds. A background in marine biology with experience in phyto- and zooplankton culture is essential.

MAINTENANCE HANDYMAN

The responsibilities of the maintenance handyman include routine maintenance and minor repairs of the hatchery. He should be skilled in carpentry, electrical wiring and plumbing for general maintenance, but it is

FACILITY	VOLUME	CAPACITY	PRODUCTION		
OUTDOOR PONDS	43 m^3	43 ADULTS		HATCHERY OUTPUT	300,000 JUVENILES
ALGAE/ROTIFER TANKS	17,000 L.			SALTWATER USAGE	156,000 L/DAY
LARVAL REARING	20,000 L.	4.0×10^5 LARVAE	100,000/CYCLE		
BRINE SHRIMP INCUBATOR	2,700 L.		4.0×10^8/DAY		
ΞPHOTOPERIOD TANKS	4,440 L.	24 ADULTS			



PLACE	USE	DIMENSIONS	AREA	PLACE	USE	DIMENSIONS	AREA
1	ALGAE/ROTIFER PRODUCTION	5.50 x 9.75	54	9	OFFICE	3.00 x 3.00	9.0
2	LARVAL REARING	6.75 x 9.75	66	10	DRY FOOD PREP	3.00 x 3.00	9.0
3	SPAWNING & INCUBATION	5.50 x 4.90	27	11	WAREHOUSE	3.00 x 7.30	21.9
4	BRINE SHRIMP ROOM	2.50 x 5.00	12.5	12	MAINTENANCE SHOP	4.25 x 6.00	25.5
5	PHOTOPERIOD ROOMS (3)	3.20 x 5.37	17.2	13	VEHICLE/BOAT SHED	5.50 x 9.00	49.5
6	ALGAL CULTURE	2.50 x 2.50	6.25	14	BROODSTOCK POND (5)	6.00 x 9.00	54.0
7	LABORATORY	2.70 x 2.70	7.3	15	SEAWATER DISCHARGE	—	—
8	RESTROOMS (2)	1.80 x 2.70	4.9	16	SEAWATER SUPPLY	—	—

Figure 28
EXPERIMENTAL FINFISH HATCHERY

recommended that motors, pumps, sterilizers, refrigeration equipment and all laboratory instruments are repaired outside the hatchery workshop.

TEMPORARY LABOR

Temporary unskilled labor, about two additional persons, may be required intermittently during the season. The need is for reliable observant workers who can carry out instructions.

OUTSIDE SERVICES

The following outside services are required by the hatchery:

1. Bookkeeping
2. Pathology
3. Fishing for broodstock
4. Major mechanical and electrical repairs
5. Technical advice on phyto- and zooplankton culture, artificial feed mixtures, and hormone selection and use.

The above staffing arrangement assumes that the hatchery is located near a center from which the various listed outside skills and services may be obtained. In keeping with the principle that the hatchery employees are there primarily to produce fish, they should not be expected to have a variety of skills which are only used occasionally. If, on the other hand, the hatchery is built in a remote location, the staff must be augmented with many of these service specialists.

INTENTIONALLY
LEFT
BLANK

Chapter 5. Hatchery Design

Determining the Hatchery's Mission

The first basic question to ask in designing the fish hatchery is: "What will the facility be required to do, once it is built?" For each of the three alternative answers, a somewhat different facility is indicated, not only in capacity, but also in the way it is arranged and equipped.

1. It may be purely an experimental facility, with a number of experimental goals, for example, development of new spawning and rearing techniques; testing of existing techniques on new species, or in a new area; selective breeding for genetic improvement of existing stock; production of large numbers of standardized larvae needed for testing of pond farming methods or for some other purpose; or some combination of the above.

2. Alternatively, the hatchery may support pilot operations, for example, explore the economics of mass propagation of mullet, develop production techniques to the highest efficiency, or provide significant numbers of juveniles for field tests leading to establishment of a grow-out industry.

3. Finally, it may be predetermined that an economic climate already exists and that mass-propagation of juveniles is sensible using hatchery methods at their present stage of development, in which case the hatchery may be a production unit at the foundation of a food-production system.

The second basic question to ask is: "How will the hatchery be managed?" Again there are alternatives.

1. It may exist as an independent enterprise, entirely self-sufficient in facilities, or forming part of a larger complex from which it can draw services and personnel on demand, such as pathologists, laboratory analysis, transportation and maintenance, or perhaps an existing seawater system.

2. Alternatively, the hatchery may be integrated into a broader program of aquaculture development, or may be a pioneering effort.

The importance of recognizing these distinctions lies in the possibility of taking advantage of existing facilities, thereby not having to build new ones. It also relates closely to site selection.

Sizing the Hatchery

Before serious effort is put into site selection and hatchery design, it is essential to decide how big the hatchery needs to be. Therefore:

1. What is the predicted demand for the hatchery's product?
2. Is selling price an important factor in this demand?
3. Is the hatchery experimental or commercial?
4. Is the hatchery to be built in increments to deal economically with increasing demand, or are new facilities to be added as demanded by the market?
5. Is the hatchery to have additional duties, such as the production of other species, or the support of extension or educational services?

These questions are more or less sensitive to differences in locality, in-country programs for aquaculture development, and external technical and financial

assistance programs. Hence, the size calculations which follow are based on the activities directly related to the production of juvenile mullet, and develop figures normalized *per million juveniles.*

ASSUMPTIONS

The calculations presented below are based on assumptions which are valid for production techniques and practices in Hawaii. Whereas in general they will be good for tropical coastal situations, caution should be exercised in cases where climatic conditions or mullet species are known to be markedly different from those in Hawaii.

1. By controlling the temperature and photoperiod regime in which adult mullet are kept during the months preceding the spawning season, it is possible to create a cohort of early spawners and a cohort of late spawners, in addition to those fish ready to spawn during the normal season. See Figure 25.
2. Routine 25% survival or larvae through 50 d is projected; hence the hatchery is sized for this survival figure.
3. Since the safe transfer of larvae between tanks has not been demonstrated for larvae between the ages of 15 and 45 d, the rearing unit in which emergent larvae are initially stocked is committed for the full 50-d rearing period, after which it can be cleaned and restocked immediately.
4. Safe stocking levels and water exchange rates are shown in Table 9.
5. Holding of large numbers of adult mullet and juveniles 50 d or older is in outdoor ponds or raceways.
6. Conditioning of selected spawners, spawning and larval rearing, algae and rotifer production are all accomplished indoors under a greater or lesser degree of environmental control.
7. Extensive pretreatment and/or recycling of fresh or saltwater is not required or desirable.

LARVAL REARING VOLUME

In Hawaii and Taiwan, individual high larval survival rates have been achieved (30%) but it is still not possible to reproduce these survival rates consistently. For planning purposes, a mean survival of 25% appears realistic. Under the current rearing practice, the density of 50-d-old juveniles should not exceed 5/l. Thus, the initial density should be

$$\frac{5(\text{density/l})}{25\ (\%\ \text{survival})} \times 100 = 20$$

That is, 20 larvae are transferred from the incubators for each liter of rearing unit volume. If a lower survival is expected, higher initial stocking densities are used, up to about 50/liter.

Three uses of each rearing unit per season are assumed. Therefore, for a projected total of one million juveniles, the required rearing volume is

$$\frac{106}{5(\text{density/l}) \times 3(\text{uses/tank})} = 66{,}667\ \text{l}\ (67{,}000\ \text{l})$$

BROODSTOCK REQUIRED

Current practice depends heavily on adult mullet taken from the wild. As the condition of these animals is variable and in general not optimum for gonad maturation and successful spawning, it is prudent to have on hand an excess of males and females. These fish are kept in outdoor holding ponds, from which promising animals are selected for photoperiod conditioning, to retard or accelerate gonad development, or to hold indoors for spawning during the natural season. The quality of broodstock is expected to improve in the future with increased domestication. In the meantime, an acceptable number of broodstock on hand is seven females and three to five males for each intended spawning. From this number, two females are finally selected as candidates for each spawning, using as criteria their general physiological condition and state of maturation. In this way, a substitute is available in case either female proves difficult to induce.

The yield of emergent larvae from the eggs produced per spawning averages 300,000. Thus the number of successful spawnings required per million juveniles is

$$\frac{106}{300{,}000\ (\text{yield}) \times 25\ (\%\ \text{survival})} \times 100 = 13$$

The total number of broodstock to be kept on hand per million juveniles is therefore

$$13\ (\text{spawnings}) \times [7\ (\text{females/spawning}) + 4\ (\text{males/spawning})] = 143\ \text{adult fish}$$

An acceptable figure for spatial volume required to maintain an adult in good condition is 1 m^3 per individual. Supplemental feeding is provided, and water turnover rate is no greater than once per day. Hence pond volume requirement for the broodstock per million juveniles is 143^3, which is 4 ponds 6 m × 6m × 1 m deep.

PHOTOPERIOD FACILITIES

Experience to date shows that at least 80% of sexually mature females, under photoperiod control during their refractory period and injected, spawn successfully the following season. Allowing that at least half of all those females under photoperiod control exhibit normal

Table 9. Projected safe stocking densities for each life stage.

Stage	Facilities	Stocking density	Water volume exchanges/day	Remarks
Adult	Outdoor broodstock holding	1 adult/m^3	1 ±	Lined pond, supplementary feeding, no active aeration.
Adult	Indoor broodstock holding (e.g., photoperiod)	10 adults/m^3	12-24	1.5 m^3 tanks, all artificial feed, temperature control, active aeration.
Adult	Spawning tanks	1 female and 3 males/200 l tank	12-24	200 l aquarium, vigorous aeration, no feeding, waterflow cut off just before spawning.
Eggs	Incubators	75-100 eggs/l	Static	1,200 l incubators, covered to exclude light; moderately active aeration; temperature control.
Larvae	Larval rearing tanks, day 1-25	20-50/l	20%	Moderate aeration, use of kreisel recommended. Feed is live rotifers and brine shrimp nauplii, with algae provided for grazing; temperature control.
Postlarvae and juveniles	Larval rearing tanks, day 25-60		< 1	Programmed salinity reduction via introduction of fresh water.

gonad development and become eligible for injection, the number of individuals placed under photoperiod control per million juveniles will be

$$\frac{13\ \text{(spawning)} \times 2\ \text{(females/spawning)}}{50\%\ \text{(expected to mature)}} = 52\ \text{females}$$

$$\frac{13\ \text{(spawning)} \times 3\ \text{(males/spawning)}}{2\ \text{(spawnings/male)}} = \frac{20\ \text{males}}{72\ \text{adult fish}}$$

Adult mullet are kept indefinitely in small well-aerated indoor tanks with rapid seawater exchange (once per hour) at a density of 1 fish/200 l in either accelerated, retarded or ambient photoperiod conditions.

Indoor holding volume for photoperiod fish per million juveniles therefore equals

$$72\ \text{(fish)} \times 200\ \text{(l/fish)} = 14{,}400\ \text{l}.$$

FOOD PRODUCTION

Algal production volume per million juveniles, based on current production technique, is

60% × larval rearing volume = 0.6 × 67,000 l = 40,000 l capacity

Rofiter production volume is

25% × larval rearing volume = 0.25 × 67,000 l = 16,700 l capacity

Brine shrimp nauplii production capacity is based on demand by the growing larvae. When the larvae begin feeding on nauplii on about day 14, 2 nauplii/ml of rearing volume per day is sufficient. For 30-d-old larvae, this increases to 20/ml. For three rearing cycles per season, nauplii production is required at any one time for only one cycle, i.e., one-third of the season's larvae.

An automatic tandem nauplii incubator/separator with two sets of incubators, each holding 4,500 l, produces 1.34×10^9 nauplii daily. This is sufficient to feed over a third of a million 30-d-old larvae. It requires 4.5 kg (dry weight) of brine shrimp eggs per day.

Artificial feeds for mullet larvae aged 30 d and older are formulated but experimental. However, for reference, provision is made for the preparation and

storage of at least 15 kg of artificial feed per day.

SEAWATER REQUIREMENTS

Table 10 gives the seawater budget per million juveniles based on the volumes and flow rates described earlier in this chapter.

Table 10. Seawater budget.

Facilities	Volume (l/d)	Maximum delivery (l/min)
Outdoor holding ponds	143,000	200
Photoperiod facility	360,000	300
Larval rearing	30,000	300
Food production	10,000	300
Total	543,000 (or 143,000 gpd)	

APPLICATIONS

For illustrative purposes, three hatchery sizes are discussed: (1) an experimental hatchery to produce about 300,000 juveniles per season; (2) a pilot-scale hatchery for a million juveniles; and (3) a production hatchery able to produce three million juveniles or more. Actual plant layouts, floor space requirements, and detailed component size summaries are included for the experimental and production hatcheries, based on the sizing calculations presented above.

The experimental hatchery (300,000 juveniles, Figure 28) is adequate to support studies in reproductive physiology, genetics, artificial feeds, and intensive culture experiments using raceways.

The pilot-scale hatchery (one million juveniles) can, in addition to the studies listed above, support technicians' training, some extension work, studies in the development of inexpensive hormones, and refinement of the hatchery techniques themselves. It can also support tests requiring significant quantities of juveniles such as bait trials for the tuna industry or controlled pond culture.

The production hatchery (three million, Figure 29) is offered as a projection into large-scale mass propagation, as might be required to support a farming operation of 500 to 1,000 ha.

FLOOR SPACE REQUIREMENTS– EXPERIMENTAL HATCHERY

This is a small-scale hatchery which, in addition to production, can support a small training program. Photoperiod control is very much in evidence. Additional space is required for toilet and shower rooms, feed storage, and general warehouse.

Table 11. Requirements of the experimental hatchery.

Facility	Dimensions (m)	Area (m^2)
Manager's office	3.0 × 3.0	9.0
Staff offices	–	–
Algal culture room	2.5 × 2.5	6.25
Wet laboratory	In spawning room	
Histology laboratory	2.7 × 2.7	7.3
Pathology laboratory	In histology lab	
Chemistry laboratory	In histology lab	
Maintenance shop	4.25 × 6.0	25.5
Spawning & incubation room	5.5 × 4.9	27
Feed preparation room	3.0 × 3.0	9.0
Algal preparation room	–	–
Advanced photoperiod laboratory	3.2 × 5.4	17.2
Retarded photoperiod laboratory	3.2 × 5.4	17.2
Ambient photoperiod laboratory	3.2 × 5.4	17.2
Larval production room	6.75 × 9.75	66
Algal production room	5.50 × 9.75	54

FLOOR SPACE REQUIREMENTS– PRODUCTION HATCHERY

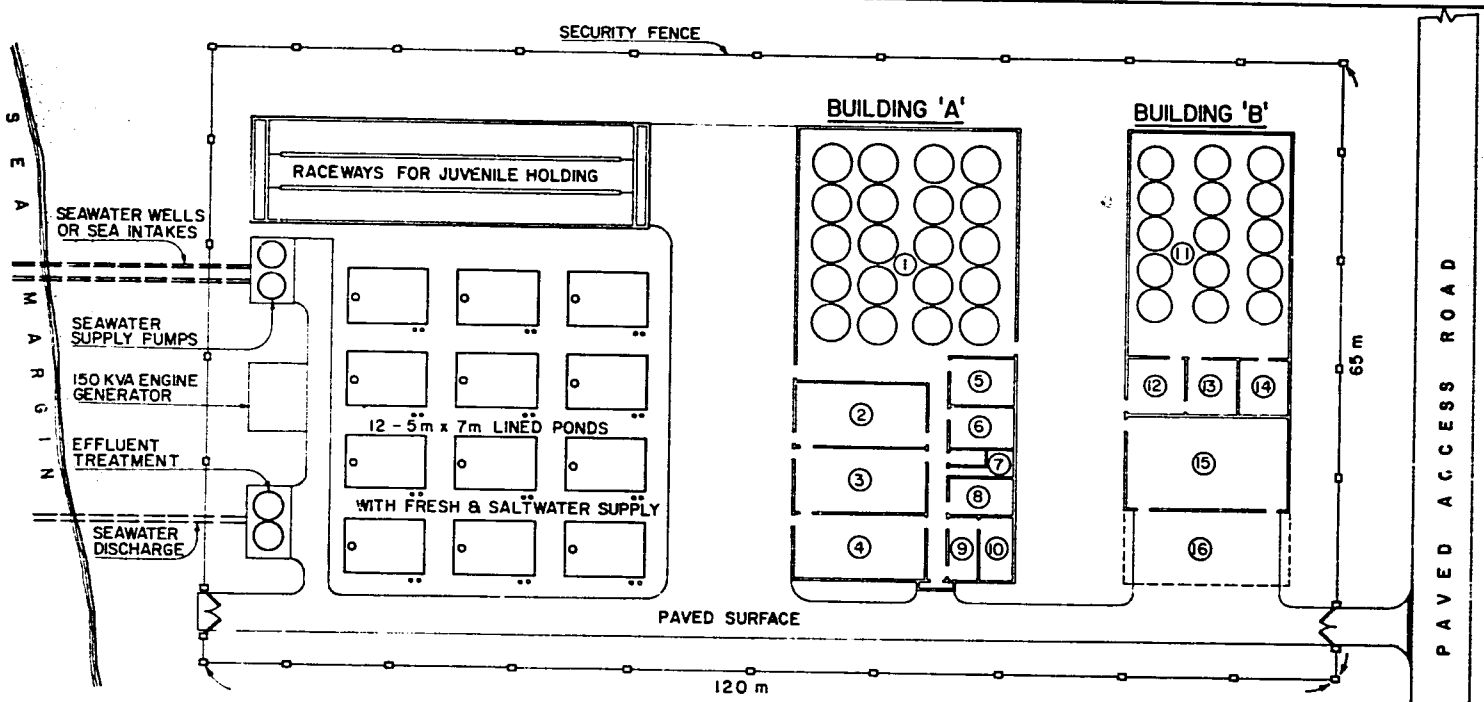
The figures below are projected for a production hatchery with capacity for three to five million juveniles per 6-mo operating season. Additional space is required for toilet and shower rooms, feed storage, and general warehouse.

Table 12. Requirements of the production hatchery.

Facility	Dimensions (m)	Area (m^2)
Manager's office	3.0 × 4.0	12.0
Staff offices	3.0 × 4.0	12.0
Algal culture room	5.0 × 4.0	20.0
Wet laboratory	6.0 × 5.0	30.0
Dry laboratory	5.0 × 4.0	20.0
Maintenance shop	10.0 × 15.0	150.0
Spawning room	6.0 × 5.0	30.0
Feed preparation room	5.0 × 5.0	25.0
Advanced photoperiod laboratory	6.0 × 12.0	72.0
Retarded photoperiod laboratory	6.0 × 12.0	72.0
Ambient photoperiod laboratory	6.0 × 12.0	72.0
Larval production room	20.0 × 20.0	400.0
Algal production room	15.0 × 20.0	300.0

FACILITY	VOLUME	CAPACITY	PRODUCTION
OUTDOOR PONDS	143 m^3	500 ADULTS	—
ALGAE/ROTIFER PRODUCTION	170,000 L		
LARVAL REARING	200,000 L	4×10^6 LARVAE	10^6 JUVENILES/CYCLE
BRINE SHRIMP INCUBATOR	27,000 L		1.3×10^9 NAUPLII/DAY
RACEWAYS	150 m^3	10^6 JUVENILES	—
Σ PHOTOPERIOD TANKS			

HATCHERY OUTPUT	3,000,000 JUVENILES
SALTWATER USAGE	1,500,000 L/DAY



BUILDING A - HATCHERY			
SPACE	USE	DIMENSIONS	AREA
1	LARVAL REARING	20 x 20	400
2	PHOTOPERIOD-RETARD	6 x 12	72
3	PHOTOPERIOD-NORMAL	6 x 12	72
4	PHOTOPERIOD-ACCEL.	6 x 12	72
5	BRINE SHRIMP ROOM	6 x 6	36
6	SPAWNING	6 x 5	30
7	REST ROOMS	2.5 x 5	12.5
8	LABORATORY	6 x 5	30
9	STAFF OFFICE	3 x 4	12
10	MANAGER'S OFFICE	3 x 4	12

BUILDING B - FOOD PRODUCTION			
SPACE	USE	DIMENSIONS	AREA
11	ALGAE/ROTIFER PROD.	15 x 20	300
12	DRY FOOD PREPARATION	5 x 5	25
13	OFFICE/LABORATORY	5 x 4	20
14	CULTURE ROOM	5 x 4	20
15	SHOP & WAREHOUSE	10 x 15	150
16	VENICLE/BOAT SHED	8 x 15	120

0 5 10 15
scale, METERS

Figure 29
PRODUCTION-SCALE FINFISH HATCHERY

SUMMARY OF HATCHERY COMPONENT SIZES

Component sizes for both the experimental and production hatcheries are tabulated below. The numbers are for output per hatchery season.

Table 13. Hatchery component sizes.

Component	Experimental hatchery (per season)	Production hatchery (per season)
Hatchery output	300,000 juveniles	3 million juveniles
Larval rearing volume	20,000 l	200,000 l
Initial stocking density	20/l	20/l
50-d larval density	5/l	5/l
Spawnings/season	4[1]	40
Σ Broodstock population	43[1] adults	430 adults
Outdoor pond volume	43[1] m^3	430 m^3
Σ Animals in photoperiod	24[1] adults	220 adults
Photoperiod volume	4,440 l[1]	44,000 l[2]
Algal production volume	12,000 l[1]	120,000 l
Rotifer production volume	5,000 l	50,000 l
Brime shrimp nauplii capacity	4×10^8/d	1.3×10^9/d
Brine shrimp eggs required (maximum)	1.66 kg/d	15 kg/d
Brine shrimp incubator volume	2,700 l	27,000 l

[1]Minimum level
[2]Maximum level

Table 14. Average and peak seawater delivery rates.

Facility	Experimental hatchery	Production hatchery
Outdoor holding ponds, av.	43,000 l/d	430,000 l/d
Outdoor holding ponds, peak	200 l/min	200 l/min
Photoperiod facility, av.	100,000 l/d	10^6 l/d
Photoperiod facility, peak	300 l/min	300 l/min
Larval rearing, av.	10,000 l/d	100,000 l/d
Larval rearing, peak	300 l/min	300 l/min
Food production, av.	3,000 l/d	30,000 l/d
Food production, peak	300 l/min	300 l/min

Site Selection

Much has been written about site selection for fish farms of various kinds, and many lists are available in the literature. A less frequently seen list for a hatchery site is included in Appendix 1.

In the last analysis, only a few items from these lists are critical. The most critical of all is water supply. Almost any construction problem can be overcome on a site with an abundant supply of high-quality seawater or fresh water. A site which yields only poor-quality water or water requiring extensive pretreatment or recycling must be considered worthless.

Although mullet are coastal and estuarine dwellers, tolerant of large and rapid variations in water composition and temperature, they are oceanic breeders whose eggs and larvae spend their first weeks of life in the purity and stability of an oceanic environment. Every effort should therefore be made to select a location for a hatchery where abundant seawater with almost oceanic properties can be obtained. Little data appear in the literature on the limits of water quality suitable for rearing mullet larvae.

The second critical item for hatchery or fish farm selection is accessibility. The site should be near a town of sufficient size to provide dwelling accommodations for the staff, supplies and maintenance items, and fabrication and repair capabilities for electrical and mechanical components. If possible, the hatchery should not have to generate its own electricity except during rare power outages. The site should also be within reach of any special services from related insitutions, if available. Finally, it should be close to the end users of the juveniles it will produce, or to central distribution points. Though all of these advantages may not be found at any one site, the combined effect of distance and difficulty of transportation can be a very important burden to effective hatchery management.

The third critical item is the topography of the site for building. If the site is steeply sloped, provides poor foundation qualities or otherwise presents construction difficulties, these can probably be overcome but at a cost in capital, maintenance, and convenience of operation which are subsequently excessive. The ideal site is spacious, on flat to gently sloping ground, well drained but out of the way of floods, storm waves and high tides, on competent soil, and laying between the sea and a paved road.

The fourth critical item is land acquisition. A site such as that described above may find keen competition from other kinds of developers because of its general desirability. It is well to pay attention to land values early in the site selection procedure so as to ensure that the site is in fact available, and at a price consistent with the project budget.

Seawater System

DEVELOPMENT OF A SOURCE

As a hatchery site for mullet is close to the seashore or on a saltwater estuary, the sources of seawater are wells drilled or pits dug into porous strata near the water's edge, or intake pipes laid on the bottom and

extending seaward from the hatchery shoreline. Local circumstances determine which one is best. A careful investigation of the water quality of the site is essential and a futuristic review of the site for developments which might cause the quality to change with time.

INFILTRATION PIT

If the shoreline contains sand or gravel layers, it is feasible to dig a pit a few meters below sea level, install cribbing to stabilize the sides, and fit vertical-shaft centrifugal pumps on a platform over the pit. The size of the pit is determined by soil porosity and water flow (factor Q). A pumping test is conducted first to determine soil porosity and infiltration rate. Water samples are taken to test for salinity, chemistry, and the presence of micro-organisms. Plenty of depth is left below the pump intake for the collection and settling of sediment.

The advantages of this system are that it is inexpensive to install, does not require elaborate construction machinery, is easy to maintain, and the soil acts as a natural filter. The disadvantage is the possible contamination by freshwater if a groundwater lens exists. The infiltration pit is useful only for shorelines of highly porous material, such as old beaches or sand dunes.

WELLS

Underlying strata which contain useful amounts of saltwater are not always obvious. Local water authorities, well drillers, and farmers can give opinions on the probability of developing seawater wells. A test well is drilled first which, if successful, has a bore size adequate to put a well into service. The drawdown at various delivery rates is then measured and water samples tested for salinity, chemistry and the presence of micro-organisms. The temperature of delivered water is also measured as it can be markedly different from water surface temperatures. In the past it was customary to fit a long-shaft centrifugal turbine pump to the well head. Today, a wide variety of submersible pumps are available and are preferred. Every well is cased with a 15 cm minimum casing, and a 30+ cm casing for a major installation. At least two wells are sunk so that the total demand of the hatchery is met at all times even if one well is closed for any reason.

The advantage of the well is that, if aquifers exist, it is a highly reliable source of water at moderate cost. The disadvantage is that the water properties, while probably consistent, are often different from the nearby seawater.

SEAWATER INTAKES

A direct intake system is a good alternative if wells or infiltration pits are infeasible or provide poor-quality seawater. The design and installation of reliable seawater intake lines are special problems, requiring considerable knowledge of the nearshore environment. First, the tidal rise and fall and longshore current patterns are studied in relation to bottom water quality to determine the minimum distance from shore where good seawater is obtained at all times. This distance is also beyond the range of storm runoff from the land or other sources of coastal pollution during any state of the tide. Next, an alignment for the intake lines is chosen which optimizes line length, the amount of protection or armor required to prevent damage to the lines during storms, the pumphouse location, the intake strainers and protective cribbing, the anchors or burial points for the lines, and protection for the lines in a surf zone. At least two lines are installed with intake structures separate so that one line can be backflushed and primed without contaminating the other. A coastal engineer familiar with local conditions is best consulted.

The advantage of this system is that it is the best and, in many localities, the only source of high-quality seawater. The disadvantages are that the intake lines can be long and costly, and are continually vulnerable to storm damage. Pump specifications and pumphouse location are chosen with care, and the size of the intake lines is generous to avoid any destructive cavitation in the pumps due to the effects of the lengthy suction path. The total suction head, including friction, gravity and minor losses, is limited to 7.5 m. Figure 30 indicates the water flow (factor Q) and pump horsepower which can be expected for a variety of intake line diameters and lengths.

MATERIALS

In general, metals in the seawater system are avoided to forestall exposing any fish in the hatchery to toxic metallic ions, and also to reduce corrosion. Suitable materials are rigid polyvinyl chloride (PVC), polyethylene, and glass fiber-reinforced polyester resin or fiberglass, all of which are widely available. Any plastic piping is specified opaque (e.g., grey PVC, not white), and rough piping (e.g., fiberglass) is coated with an opaque paint to prevent fouling by algae internally. Other suitable materials are vitrified clay pipe, Portland cement concrete pipe, and Portland cement/asbestos pipe. Cheaper cast iron and wrought iron pipes are used for drain lines, but their life in saltwater is short. Pumps, valves, and fittings with neoprene and plastic parts are preferred, if available, to those with corrosion-resistant metal parts, such as bronze, Monel or No. 316 stainless steel. As these metals corrode by galvanizing reaction, isolation of dissimilar metals is essential. Metal hardware

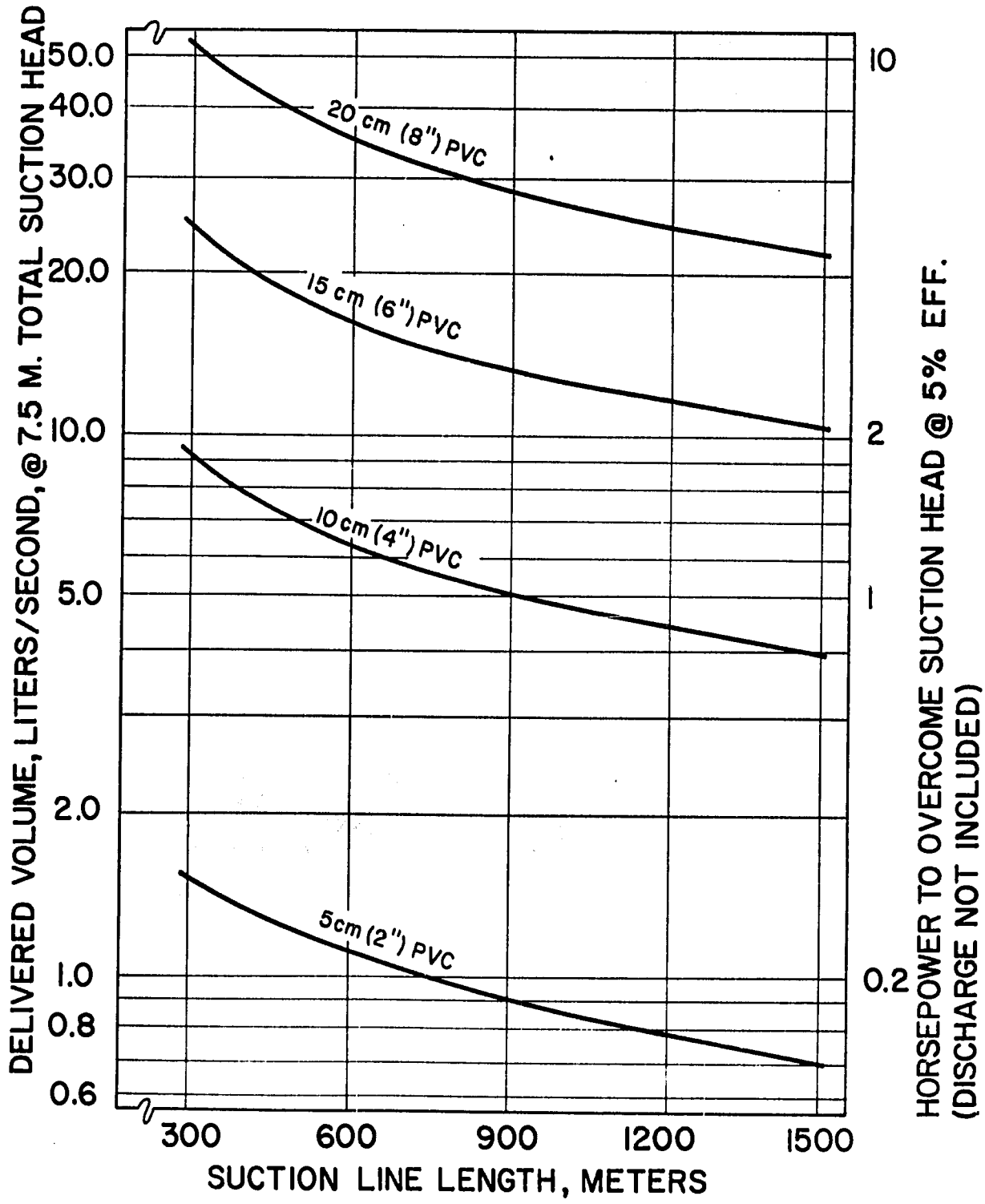


Fig. 30. Waterflow (Q) and pump h.p. vs. size and length of intake line.

is also certified by the manufacturer for seawater service.

DISTRIBUTION AND DELIVERY

For the experimental hatchery, where the water requirement is relatively small and variable, the delivery system works on demand. Distribution is from a central interconnected double reservoir constructed at a high point above the hatchery. The pump and auxiliary pump are controlled by a high level/low level automatic/manually operated float switch in one section of the reservoir. Water fills and flows into both sections and can then be shut off or allowed to overflow continuously. Pumping capacity is equal to the rate of water use in the hatchery peak demand. The receiving section of the reservoir acts as a sump or filter. The system is simple and accommodates a great variation in water demand.

For the large hatchery complex, where water use is more predictable, pumping capacity is greater than the average demand. One pump runs continuously (with another in reserve) pressurizing the system directly, but a reservoir is linked into the system in case delivery is surplus to demand. The reservoir is fitted with a float switch that shuts the pump off to prevent continuous overflow.

All reservoirs are lined and covered to reduce fouling by marine organisms.

If the seawater quality is good, it is not necessary to filter the water going to the outdoor ponds or the indoor holding tanks. For the algal and rotifer production systems, the larval rearing tanks, and the spawning and incubation tanks, the water is passed through one or two 5 μ cartridge type filters and an ultraviolet sterilizer unit permanently installed in each delivery line. This combination of treatments is essential for efficient production in each operation. For temporary delivery lines, a portable rig comprising a 5 μ cartridge filter in series with a small sterilizer, with a hose for attaching to any seawater outlet, is adequate.

OPERATION AND MAINTENANCE

Marine algae and other fouling organisms are a constant trouble to any seawater system. Some control is possible by reducing exposure to ambient light, using nontoxic antifouling paints, or by periodically (about four times a year) filling part of the system with fresh water and allowing it to stand for 24 hr. However, the system is designed with adequate access points for cleaning rods.

Pumping seawater is expensive. Leaks are always corrected promptly, and good operational procedures specify that outlets are turned off when not in use. An alarm system is installed if possible to warn of pump failure, linked to the pump itself or the float switch in the reservoir.

An inventory of mechanical spares, including pumps, motors, and valves, and the tools to install and change parts is always kept on hand in the workshop.

Materials and Construction

BUILDING CONSTRUCTION

Conventional materials and construction methods are selected which suit conditions in the local building industry, with some important points for caution. Metal structural elements and builder's hardware, such as steel joists, pipe columns, beams and rigid frames, are first treated with a corrosion-resistant paint. Interior surfaces are finished in smooth continuous fabric and are painted in light colors with durable, high-quality paints. In all enclosed spaces containing open tanks, especially those with strong aeration or continuous water supply, humidity is high and in time a moist film of microscopic salt crystals forms on all surfaces. In refrigerated or cooled hatchery rooms, e.g., the photoperiod, spawning and culture rooms, good insulation is provided.

All floors are concrete, finished with a nonslip surface, sloped to floor drains and wide gutters. All gutters, which slope to a central drainage point in the hatchery system, are fitted with removable wooden covers, inset flush with the surrounding floor. Finish hardware is top commercial quality and corrosion resistant.

Hatchery spaces not temperature controlled are ventilated, if possible, using operable vents low down on the exterior walls and continuous monitors on the roof ridge. All ventilation openings are fitted externally with heavy-duty screening for insects, birds and rodents. For ventilation, the site layout and building orientation takes advantage of prevailing winds.

In all areas except the enclosed photoperiod control rooms, fixed skylights are used. They are fitted with obscure material transmitting no more than 8% of incident light. Fiberglass sheet is recommended. In the algal production room, the maximum amount of sunlight is utilized. The roof and walls are covered with single-weight translucent corrugated fiberglass sheet, attached with appropriate fasteners over corrugated weatherstripping. This material transmits about 20% of incident sunlight initially, but in time it is affected by photo-oxidation. Its projected life of 3 yr in the tropics can be doubled if it is painted every two years with diluted catalyzed finishing resin.

The hatchery buildings are designed with column-free interiors where possible. The convenience of the open workspace more than offsets the added cost of wood

trusses, laminated beams, or steel rigid frames used to replace interior columns. The utility services (air, electricity, fresh and saltwater) are carried and distributed by suspended lightweight frames fitted about 2.5 m above floor height.

HEATING AND COOLING

Space heating inside the hatchery interior is not required, with the possible exception of some laboratory and office heating in winter or at night. However, some incubators and production tanks may need individual heating during cold weather. Localized heaters or heat-transfer jackets can be used, utilizing solar collectors or a fuel-fired water heater.

Good temperature control is maintained in the insulated photoperiod rooms and the algal culture room. The culture room generates little heat and is cooled effectively with a window air conditioner. The photoperiod rooms, with their large dehumidifying load, require the incoming seawater to be chilled first. The large hatchery complex requires a central refrigerator-chilled water unit with heat exchangers and fan-coil units on separate thermostats for the three photoperiod/temperature regimes. It is essential that the heat exchangers are certified for saltwater use.

ELECTRICAL SYSTEM

The electrical power service entrance, metering, distribution, and fusing are conventional. All switches are specified for outdoor use, and all are grounded or earthed. All motors, solenoids, actuators, and controls are at least of drip-proof classification, and located clear of all saltwater. Electrical detailing in all rooms containing open tanks is such that the entire room can be safely hosed down with freshwater.

Auxiliary power generators are necessary. Depending on the reliability of the local power service, one or two emergency generators, each capable of meeting hatchery demands, are on site. Not all loads are connected during outage of normal power, but the seawater pumps, certain lights, and the air system are essential to be permanently connected. If the hatchery is ever unmanned for long periods, for example, overnight, then automatic switchgear for the generators is necessary. This switchgear, upon detecting a power fault, in sequence disconnects from the local power service, starts both generators, and selects one to take the emergency bus, shutting the other down. Resetting the gear when the local power service returns is manual. If the hatchery is manned continuously, no automatic switchgear is necessary. A power-out alarm, operating on the engine-generator starting battery, is sufficient, and portable battery-operated emergency lights are used at critical locations. As for the automatic system, a manual reset of the gear is sufficient. An inventory of spares for the generating plant is essential in the workshop.

COMPRESSED AIR

All aeration operations in the hatchery use compressed air. The central system consists of air mains fed by a large receiver operating at 5.3-8.8 kg/cm.^{2} This is charged by two low-speed compressors each fitted with filters, and water and lubricant traps. The pump and trap assemblies are certified as suitable for producing air of medical quality. The air distribution mains are liberally fitted with tees, valves and unions so that subsystems are isolated and reconfigured easily. A low-pressure alarm is fitted on the receiver.

In each subsystem, there are pressure regulators to reduce pressure to about 0.35 kg/cm^2 for distribution through the small-diameter plastic tubing to individual tanks. Water traps and particle filters are fitted near the pressure regulators.

In addition to the central system, a number of small, portable, electrically driven air compressors are available in storage. These are the diaphragm type, which do not use lubricants in the airways of the compressor, and have the capacity to meet small emergencies.

Equipment

TANKS

The hatchery uses a large number of tanks of various sizes, but all have similar characteristics. The fabrication material is anything from plastered brick to gel-coated fiberglass, but the interior finish is hard, smooth and durable, with filleted corners and rounded edges. The bottoms slope gradually (1:20) to a central outlet in the bottom. Surface color is important, both for cleaning and for enhancing visibility of the live organisms under culture. Matte black interior sidewalls and white bases are recommended. All drain fittings are sized to accept a standpipe overflow assembly (Figure 31). If forms or molds are used in tank fabrication (e.g., concrete or fiberglass), substantial economies are gained by standardizing on a few tank sizes. If possible the forms or molds are stored for future use.

All tank outlets are fitted with appropriately sized screens. Monofilament woven screen material is available in a wide range of sizes, from 50 μ to several mm mesh, in nylon or polyester. The polyester screen is stronger and less water-absorbent than nylon. Screening material is fastened to plastic or wood frames with epoxy cement. In finer sizes it can be sewn into any shape of a filter

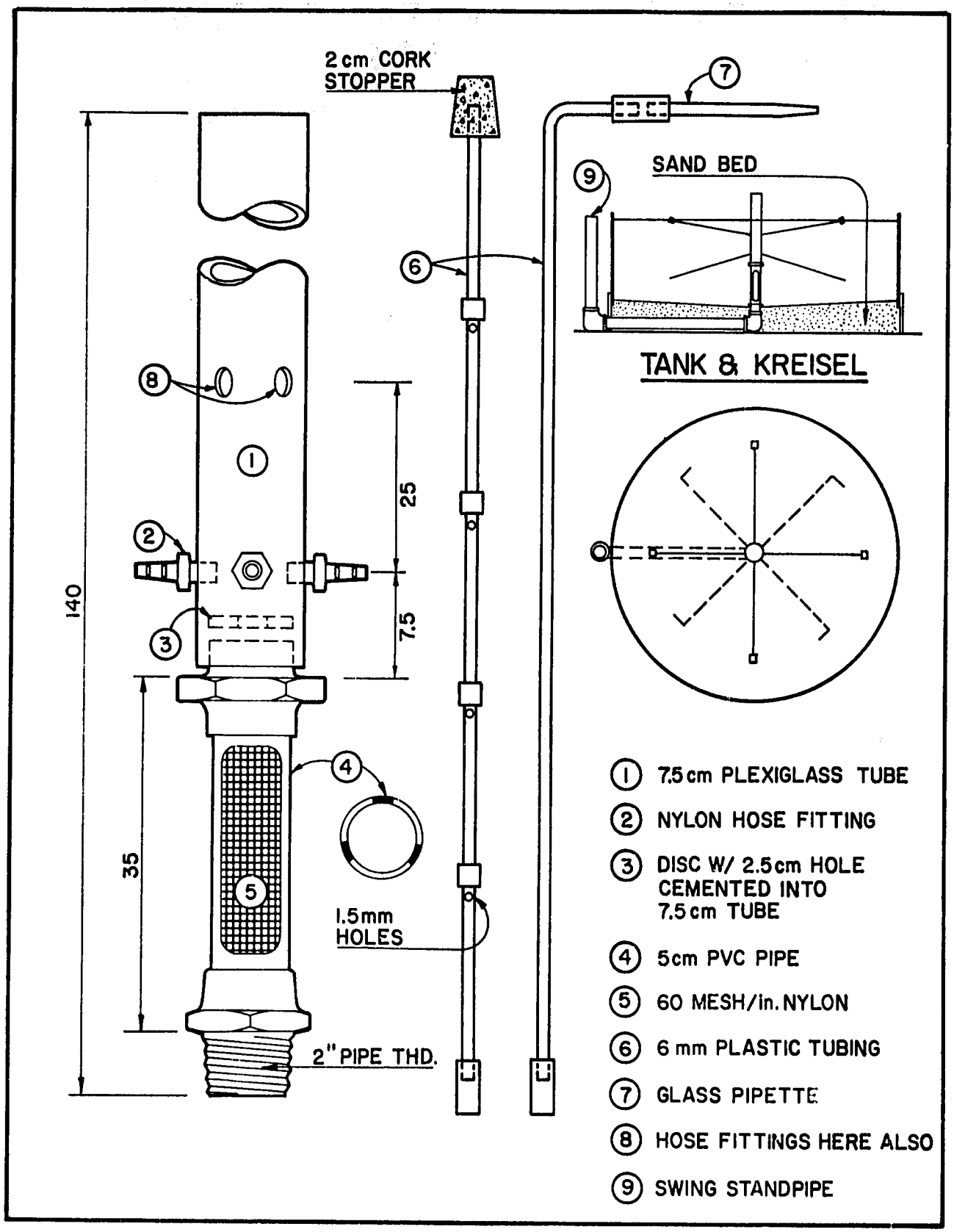


Fig. 31. Rearing tank and kreisel. This standard design is easily modified to suit tanks of different sizes, and can be made from other materials than those shown. It is driven by an air stone which rests on the disc marked 3 in the drawing.

sock or bag. The surface area of screen used is generous to keep the velocity of water passing through the protective screen about 0.5 cm/sec.

Screen covers are required for tanks holding adult fish to prevent them from jumping out. A nylon mosquito screen on a wood frame similar to a screen sash is sufficient, but the frame is weighted or clamped to the edge of the tank.

MECHANICAL EQUIPMENT

Some general comments about specifying and using mechanical equipment are in order. First, one is always wary of manufacturers' claims about the ability of their product to withstand prolonged saltwater exposure. The only acceptable proof is demonstrated performance in similar installations together with a warranty. Equipment suitable for this type of service is not cheap. It is better to pay the price for dependable performance than to stand the inevitable losses caused later by frequent equipment failure.

Second, it is well to verify before making an important purchase that spare parts and repair service are available locally. This is extremely important.

Third, it is necessary to have a program of preventive maintenance for mechanical equipment. This program has two parts: (1) regular inspection, service and repair; and (2) maintenance of accurate records of repairs, failures and replacements. For reliable hatchery production performance, there is no substitute for taking good care of all mechanical equipment.

SPECIAL ITEMS

Most components of a saltwater hatchery are conventional. Presented here are descriptions of four specialized hatchery components which are used in the hatchery.

Mullet egg incubator (Figure 32). The incubator is a fiberglass tank 1.25 m diameter and 1 m deep, with a 90° cone-shaped bottom. The interior sidewalls are gel-coated black; the bottom is gel-coated white. The incubator has a close-fitting cover. It is mounted on a wood frame with its top 2.5 m above the floor. A removable standpipe with a screened outlet is fitted into the bottom drain. A shut-off valve is also provided below the incubator. Aeration is supplied by a fine airstone connected by small-diameter tygon tubing.

The incubator is operated in static conditions of water flow. A portable screened box allows water to be siphoned out of the incubator if the larvae are concentrated before removing them.

The volume of the standard incubator is 1,200 l, but this can be doubled or tripled using the same proportions for increased sizing. The standard size incubates about 200,000 mullet eggs.

Rearing tank and kreisel (Figure 31). The standard 6,000-l rearing tank is made of fiberglass, 3 m in diameter and 1 m deep. It has black gel-coated sides and white gel-coated bottom with a slope (1:20) to a central drain. The drain holds a standpipe 10 cm o.d. which constitutes the main column of the rearing kreisel. Beneath the drain fitting are a 90° elbow and a length of pipe extending beyond the edge of the tank. This is fitted with a swing-type overflow pipe.

The kreisel has two purposes: (1) to circulate water evenly in tanks throughout the tank volume, and (2) to provide protected aeration. The kreisel is capable of operating continuously through a rearing cycle, but is usually replaced by a screened overflow for more rapid water exchange during the second half of the 50-d rearing cycle.

The circulation effected by the kreisel is gentle. Experiments show that larval growth in a tank fitted with a kreisel is superior to that in a tank with only continuous or intermittent airstone aeration. This is probably because the kreisel is less disruptive on the feeding behavior of the larvae.

Brine shrimp nauplii incubator (Figure 33). The system is composed of two 900-l incubators with a common separator and collector box. The incubators are made of black polyethylene with the bottom sloping (1:20) to a central main drain. The incubators are operated in tandem, each being loaded with 1 g of brine shrimp cysts per liter of incubator volume on alternate days. Incubation time in 35‰ seawater at 24°C is 36 hr. It is conducted in total darkness and with strong aeration.

On hatching, the nauplii are strongly photopositive. The unhatched and discarded cysts tend to sink to the bottom or float to the surface, respectively, while the nauplii remain in the water column. After the aeration is stopped, the nauplii are attracted to light from a lamp located above a drainhole which is located in the incubator sidewall below mid-depth. After 30 min, the drainhole valve is opened, taking the nauplii from the central water column into the separator box if separation is performed in the box. The technique uses the combined effect of slow lateral current flow through the side screens and further photopositive attraction to a second lamp at the end of the box. The nauplii are drained off into a collector box. Except for the manual operation of the main drain valve, the dual incubator/separator arrangement is operated automatically by means of time clocks, relays and solenoids. The construction and operation of the incubator is fully described in Nash (1973).

Copepod/Amphipod separator (Figure 34). Although the importance of copepods and amphipods to the

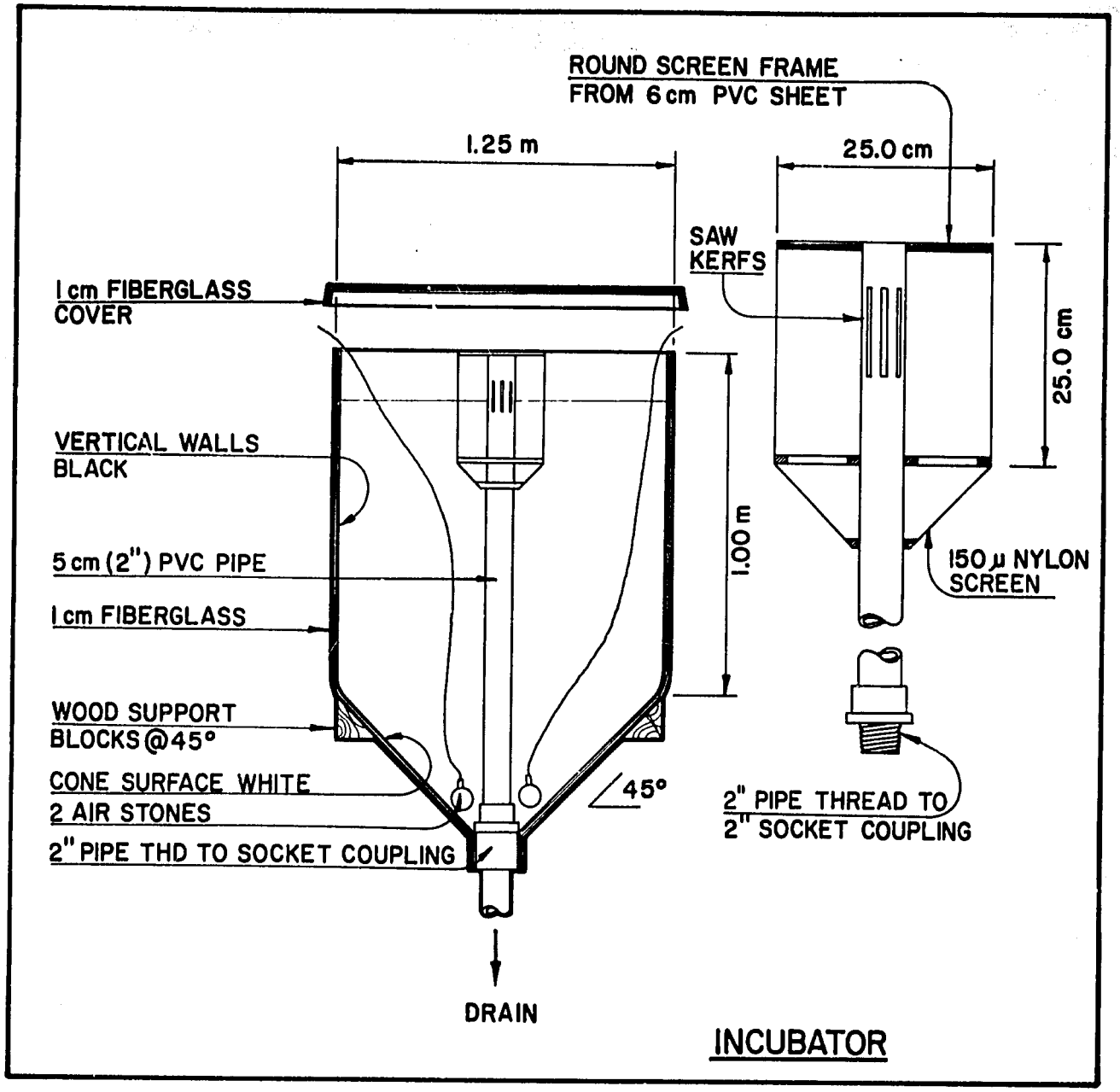


Fig. 32. Mullet larval incubator. This standard design may be operated either with or without continuous water flow. If static operation is desired, the standpipe may be removed and a shut-off valve substituted below the tank.

growth and survival of mullet larvae is not confirmed, they are thought to be beneficial. Mullet larvae eat them readily if presented in the proper size range. A separator is used to harvest the naturally occurring populations of copepods and amphipods which exist in any waste-water ponds. The system is in effect utilizing the nutrients that are washed down the drains of a hatchery, by recycling them as invertebrate food organisms for mullet larvae.

The collection apparatus consists of three floating enclosures, each surrounded by a predator screen of 4.8 mm mesh. The enclosures are made of 2.8 mm mesh screen. Each contains a 12 V lamp in a watertight case to attract the organisms, and a submersible centrifugal pump (11 l/min) transfers the organisms to the separator box on shore.

The separator box has five serial compartments separated by nylon screens of 1,600 µ, 355 µ, 183 µ, and 73 µ mesh size. A light at one end of the box attracts the organisms through successively finer screens and, together with the current flow in the separator, operates to grade the organisms by size. The invertebrates are drawn off

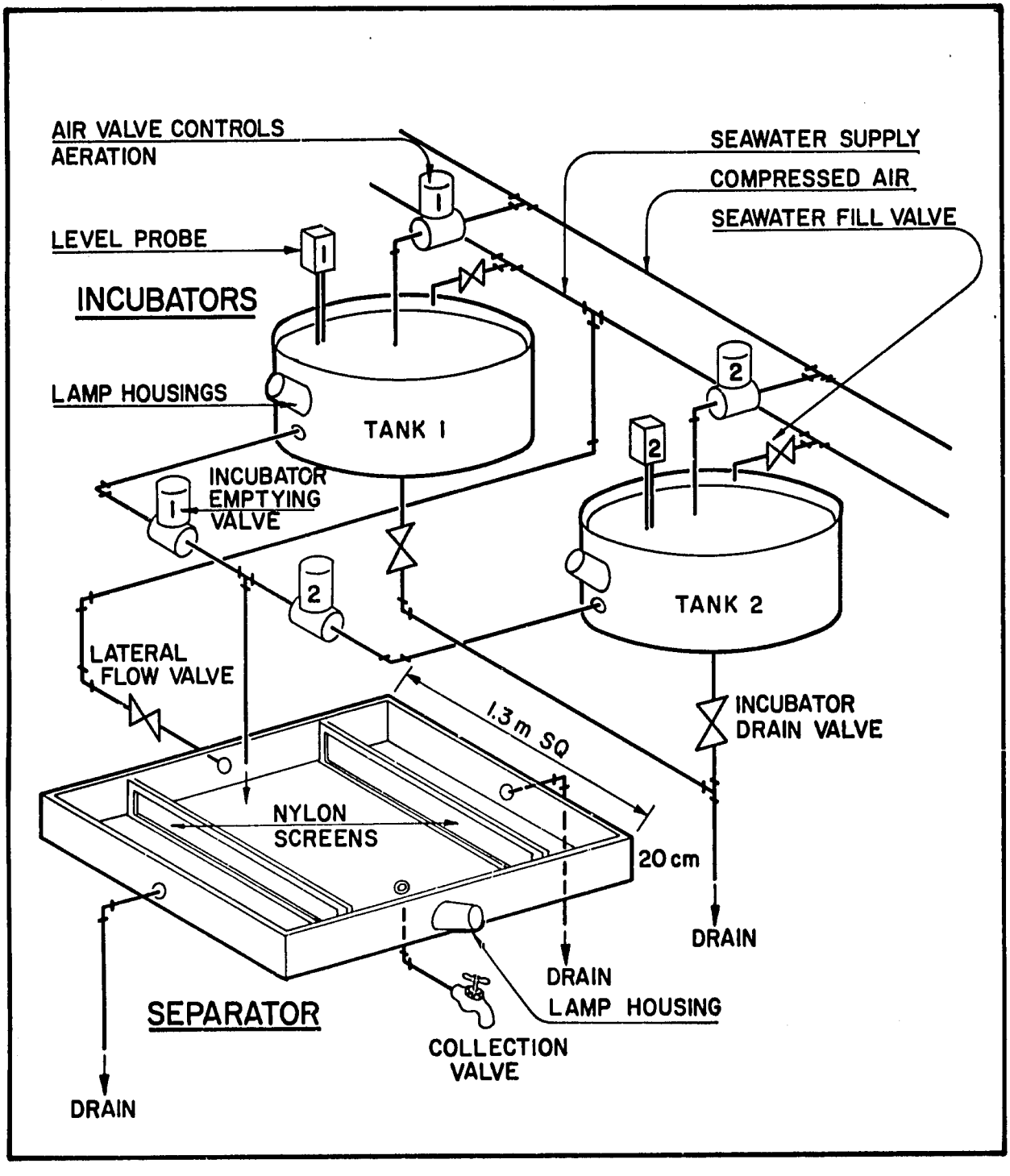


Fig. 33. Artemia nauplii incubator. For proper operation, this equipment should be enclosed in a darkened room. Neither the incubators nor the separator need to be covered.

from each compartment in fine-mesh bags and used to feed the mullet larvae according to their age and demands for organisms of a specific size.

The use of copepods and amphipods as mullet food is still experimental. So far no feeding schedule is recommended, and no effort has been made to optimize the growth of these organisms. The separator and its operating technique are fully described in Paulsen (1977).

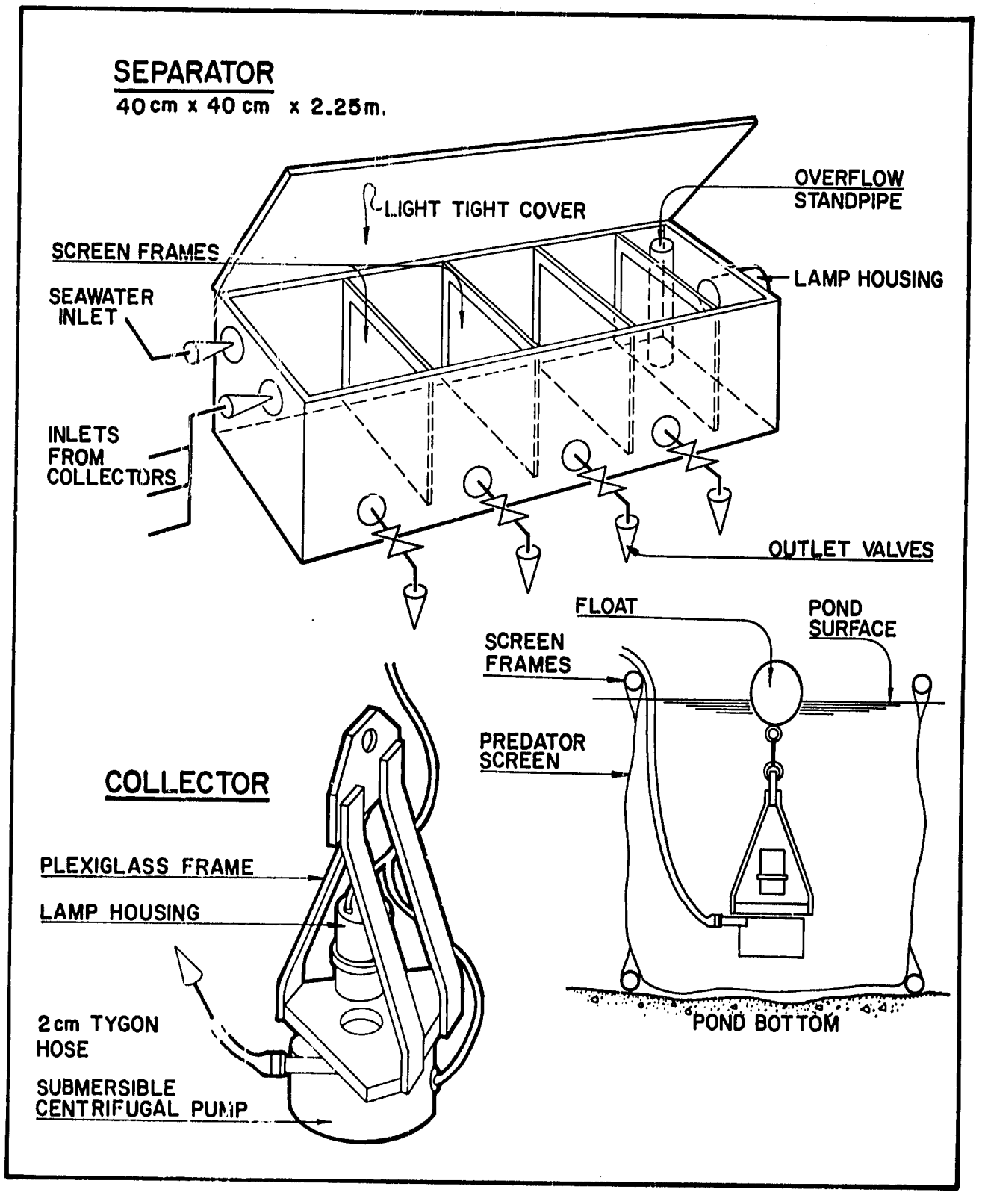


Fig. 34. Copepod-amphipod harvester. Sorted organisms are usually collected by tying a fine mesh bag over the outlet and flowing water through it continuously from each compartment.

INTENTIONALLY
LEFT
BLANK

Chapter 6. Speculation for the Future

The Mugilidae probably have the brightest future of all the marine and brackishwater finfish in the developing technology of aquaculture. The majority are desirable fish with good flesh texture and taste, particularly when captured or taken from water of high salinity and have a high percentage of body fat. They are distributed widely geographically and have a capacity for tolerating extreme conditions of temperature, salinity and dissolved oxygen. They are naturally hardy animals which thrive on good husbandry but are also capable of withstanding poor farming practices.

The many species of Mugilidae give them genetic potential paralleled by few other aquatic species. It will be the manipulation of the genes and the selection of strains and breeding lines which will make them the aquatic equivalent of the first fatstock land animal.

Sufficient knowledge on the artificial propagation of *M. cephalus* has been produced to justify an investment in a pilot-scale hatchery operation. The purpose of the hatchery would be to improve the existing techniques by operating at the increased scale. After 6 yr of development with hatchery production, a basis for the commercial production of flatfish is now very much refined. Survival and larval rearing density has been increased considerably through management experience and consequently has made a significant saving in capital construction requirement.

For the projected production-scale mullet hatchery, if the final rearing density is increased by increasing larval survival, the same facilities can be used to produce many more juveniles during the hatchery season. Either the facilities and capital cost could be reduced substantially for the original production of three million larvae, or the same facilities could produce many more per year. Tang (1974) estimated that the aquaculture fishery of 2,000 mt of grey mullets in Taiwan is based on the collection of 10 million juveniles annually.

In addition to the advantages of genetics for increased growth and production in ponds, genetic engineering and cross-breeding within the Mugilidae may result in the production of larvae with hybrid vigor sufficient to increase survival for rearing at increased density. The benefits of genetic engineering were described by Purdom (1972). He said that gynogenesis promised to be a rapid way of producing inbred lines, and the production of artificial triploids already showed greater growth rates than the diploids. Triploids also had the advantage of being sterile, which has always been a significant factor in fatstock production.

Liao et al. (1972) reported the problem of obtaining sexually mature males throughout the breeding season. He described the ease with which they could be caught and used early in the season, but by the end they were spent, leaving several female fish unfertilized. Considerably larger populations of males are necessary to support a hatchery production system so that this does not occur. Environmental control should also be used for males retained indoors. The shortage demonstrates the need for more work on the cryogenic preservation of sperm first developed for fish by Blaxter (1953) and followed by Hwang et al. (1972) and Chao et al. (1974) for the males of *M. cephalus*. Unpublished work by

Watters in Hawaii demonstrated the differences in activity of sperm in a number of media. The motility and viability of the sperm suspended in ambient seawater (32‰) lasted for about 1 hr and much longer than that in other media. However, there were differences between sperm of different males.

These factors demonstrate once more the need to define the quality of eggs and sperm from mature fish, and to establish that the quality is maintained year by year. Fish tend to get treated as part of a population, used and returned to the group. The first process of marking or isolating good breeding fish can commence now without waiting for the biochemistry of egg quality to be established. Much fundamental work is possible now which will lay the foundation for improvement by genetics and stock selection.

The induced breeding and mass-propagation technique is technical in its approach. Although experience and improvements in the technique will, in the future, lessen what presently appears to be a complicated procedure, the fecundity of the Mugilidae and the high survival will make the cost: benefit ratio of the hatchery favorable. One large hatchery complex, with a few specialized and trained technicians, will be able to provide annually, many millions of juveniles to many fish farms over an extensive geographic area. For example, a hatchery producing 10 million juveniles per year would be able to meet the demands of 1,000 ha of productive ponds.

Ironically, the pressure points of the hatchery system will not be directly concerned with the Mugilidae themselves. The first is the inconsistent availability of the purified salmon gonadotropin, SG-G100. Prepared from the pituitaries of salmon, this purified and standardized hormone is expensive and in short supply. Alternatives, such as human chorionic gonadotropin, are available but as expensive. The need is either to replace these gonadotropins with synthetic organic compounds which also stimulate maturation and oogenesis or to work on the schooling behavior and nutrition of the Mugilidae to induce them to spawn in captivity without hormone treatment.

The second pressure point will be the shortage of cysts of the brine shrimp, *Artemia salina*. The nauplii of the brine shrimp play a significant role in the hatchery production system for mullet, and in many other fish and crustacean culture techniques. The cysts are presently in short supply and expensive. The cysts are packaged in a convenient form for storage and use in hatchery operations and it is therefore more desirable to find artificial ways of producing cysts (using industrial effluents or desalination plants) than to discover and culture other live food organisms. Another alternative is to feed the larvae earlier with powdered artificial feeds, but as yet no suitable form ,ators have been found.

The Mugilidae have the greatest potential for becoming the most important supplier of aquatic animal protein for mankind. However, this potential can only be released by the successful artifical propagation of juveniles from hatcheries. The wealth of information that is available on the culture of the mullet is more than enough to justify the establishing of a coastal hatchery for the pilot-scale production of juveniles. Many enterprises have succeeded with much less basic knowledge and have not had as much at stake.

Acknowledgments

ICLARM acknowledges with thanks the generosity of the Elsevier Scientific Publishing Company and the Fisheries Society of the British Isles for permission to reproduce material previously published in *Aquaculture* and the *Journal of Fish Biology*.

Many of the technical procedures on hatchery systems for the grey mullet are based on the research work of Drs. Ziad H. Shehadeh, Ching-Ming Kuo and Colin E. Nash, together with the members of the multidisciplinary staff of the Oceanic Institute aquaculture team between 1971 and 1977. Also included are the significant technical contributions made by Dr. I-Chiu Liao and his colleagues of the Tungkang Marine Laboratory, Taiwan Fisheries Research Institute.

References

Abraham, M., Blanc, N. & Yashouv, A., 1966. Oogenesis in five species of grey mullets (Teleostei, Mugilidae) from natural and land locked habitats. Isr. J. Zool, 15 (3-4): 155-172.

Abraham, M., Blanc, N. & Yashouv, A., 1968. Persistent yolk nuclei in the oocytes of *Mugil cephalus* when confined to fresh water environment. Ann. Embriol. Morphol., 1:169-178.

Adams, W., Montalban, H.R. & Martin, C., 1931. Cultivation of bangos in the Philippines. Philip. J. Sci., 47:1-38.

Ahsan, S.N., 1966. The control of cyclical changes in the testicular activity of the lake chub *Couesius plumbeus* (Agassiz). Can. J. Zool, 44-149-159.

Alderdice, D.F. & Forrester, C.R., 1971. Effects of salinity and temperature on embryonic development of the petrale sole *(Eopsetta jordani).* J. Fish. Res. Bd. Can., 28-727-744.

Alderdice, D.F. & Velsen, F.P.J., 1971. Some effects of salinity and temperature on embryonic development of Pacific herring *(Clupea pallasi).* J. Fish Res. Bd. Can., 20:1545-1562.

Anderson, W.W., 1957. Early development, spawning, growth and occurrence of the silver mullet *(Mugil curema)* along the South Atlantic coast of the United States. Fish. Bull. U.S. Fish. Wildl. Serv., 57(119):397-414.

Anderson, W.W., 1958. Larval development, growth and spawning of striped mullet *(Mugil cephalus)* along the South Atlantic coast of the United States. Fish. Bull. U.S. Fish. Wildl. Serv., 58(144):501-519.

Angelos, H.G., 1971. A preliminary report on the observation and possibilities of induced spawnings in mullet and milkfish. Occas. Pap. Indo-Pac. Fish. Coun.. 71(8):1-12.

Anonymous, 1940. Mullet experiment successful. Fish. Gazet., 57(8):21.

Apyekin, V.S. & Tronina, T.M., 1972. Experiments on stimulation of maturing and spawning in mullet (tentative results). Gidrobiol. Zh., 8(1):82-89.

Arnold, E.L. & Thompson, J.R., 1958. Offshore spawning of the striped mullet (Mugil cephalus) in the Gulf of Mexico. Copeia, (2):130-132.

Avanesov, E.M., 1972. Present spawning conditions of mullets (genus Mugil) in the Caspian Sea. J. Ichthyol. (Engl. Transl. Vopr. Ikhtiol.), 12(3):419-426.

Babaian, K.E., 1958. Caspian mullet. Zool. Zh., 36:1505-1513. In Russian, English summary.

Babaian, K.E., 1960. Vyrashichivanie kefali v karpobykh prudakh (Rearing mullet in carp ponds). Rybovod Rybolov, 6:23. In Russian.

Babaian, K.E. & Zaitsev. Y.P., 1964. New data on the biology of the grey mullet and prospects for increasing grey mullet rearing in the U.S.S.R. Zool. Zh., 43(9):1342-1345. In Russian, English summary.

Baggerman, B., 1957. An experimental study on the timing of breeding and migration in the three-spined stickleback *(Gasterosteus aculeatus L.)* Arch. neerl. Zool., 12:105-317.

Basu, S.P., 1946. Possibilities of mullet culture in India. Indian Farm., 7(11):517-522.

Belloc, G., 1938. Biologie et peche de l'etang de Biguglia. Rapp. P. -v. Reun. Comm. int. Explor. scient. Mer Mediterr., 11:405-431.

Bernard, M. & Zaffera, A., 1970. The importance of avoiding chemical contamination for a successful cultivation of marine organisms. Helgol. Wiss. Meeresunters., 20:655-675.

Blanco, G.J. & Acosta, P.A., 1958. The propagation of the grey mullet in northern Luzon brackish water fishponds. Philip. J. Fish., 6(1):1-5.

Blaxter, J.H.S., 1953. Sperm storage and cross-fertilization of spring and autumn spawning herring. Nature, 172:1172-1190.

Blaxter, J.H.S., 1969a. Visual thresholds and spectral sensitivity of flatfish larvae. J. Exp. Biol., 51:221-230.

Blaxter, J.H.S., 1969b. Development: eggs and larvae. In, Fish physiology, Vol. 3, edited by W.S. Hoar & D.J. Randall, Academic Press, New York, pp. 177-252.

Breder, C.M., 1940. The spawning of *Mugil cephalus* on the Florida West Coast. Copeia, (2):138-139.

Broadhead, G.C., 1953. Investigations of the black mullet, *(Mugil cephalus* L.) in northwest Florida. Fla. Board Conserv. Mar. Res. Lab. Tech. Ser., (7):1-33.

Bromhall, J.D., 1954. A note on the reproduction of the grey mullet (*Mugil cephalus* Linnaeus), Hong Kong Univ. Fish. J., 1:19-34.

Bullough, W.S., 1940. The effect of the reduction of light in spring on the breeding season of the minnow *(Phoxinus laevis* L.). Proc. Zool. Soc. Lond. (A), 100-149-157.

Campbell, A.Y.G., 1921. Madras Fisheries Administration Report for the Year 1919-1920. Madras Fish. Bull., (13):1-34.

Cech, J.J. & Wohlschlag, D.E., 1975. Summer growth depression in the striped mullet (*Mugil cephalus* L.) Mar. Sci., 19:

Cervigón, F. & Padrón, M., 1974. Hypersaline lagoons as an ideal environment for mullet culture. Paper presented at IBP/PM International Symposium on the Grey Mullets and their Culture, Haifa, Israel.

Chen, F.Y., Chow, M. & Sim, B.K., 1969. Induced spawning of the three major Chinese carps in Malacca, Malaysia. Malay. Agric. J., 47:211-238.

Chepurnov, V.S. & Dmitriyev, Y.I., 1962. Results of an investigation on the rearing of mullet in the estuaries of the Odessa Region and measures recommended to increase productivity. Uch. Zap. Kishinve G.s Univ., 62(1).

Chervinski, J., 1976. Growth of the golden grey mullet (*Liza aurata* [Risso]) in saltwater ponds during 1974. Aquaculture 7(1):51.

Chow, I., 1958. Growth characteristics of four species of pond fish in Hong Kong. Hong Kong Univ. Fish J., 2:29-36.

Costlow, J.D., 1969. Marine Biology, Volume V. Gordon and Breach, Science Publishers, New York, 606 pp.

D'Ancona, U., 1955. Les lagunes mediterraneennes et leur exploitation (Mediterranean lagoons and their utilization) Proc. Tech. Pap. Gen. fish. Counc. Mediterr., 3:145-149.

De Angelis, R., 1969. Mediterranean brackish water lagoons and their exploitation. Stud. Rev. Gen. Fish. Coun. Mediterr., 12-41 pp.

Dekhnik, T.V., 1953. Reproduction of *Mugil cephalus* in the Black Sea. Dokl. Akad. Nauk. SSSR, 93:201-204. In Russian, English summary.

Dekhnik, T.V., 1954. Reproduction of the anchovy and mullet in the Black Sea. Tr. Vses. Nauchnolssled. Inst. Morsk. Rybn. Khoz. Okeanogr., 28:34-48. In Russian.

Demir, N. 1971. On the occurrence of grey mullet postlarvae off Plymouth. J. Mar. Biol. Assoc. U.K., 51:235-246.

Devanesan, D.W. & Chacko, P.I., 1943. On the possibility of culture of certain marine mullets in fresh water tanks. Proc. Natl. Inst. Sci. India, 9:249-250.

Donaldson, E.M., Yamazaki, F., Dye, N.M. & Philleo, W.W., 1972. Preparation of gonadotropin from Salmon *(Oncho-rhynchus tshawytscha)* pituitary glands. Gen. Comp. Endocr., 18: 460-481.

El-Zarka, S. El-D., 1968. Rehabilitation of the fisheries of an inland saline lake in the United Arab Republic. Stud. Rev. Gen. Fish. Coun. Mediterr., (35):21-44.

Erman, F., 1958. About the artificial feeding of grey mullets in Israel. Balik Balikcilik, 6(3):9-12.

Erman, F., 1961. On the biology of the thick-lipped grey mullet *(M. chelo Cuv.).* Rapp. P. -v. Reun. Comm. int. Ezplor. scient. Mer Mediterr., 16:276-285.

Faouzi, H., 1936. Successful stocking of Lake Qarun with mullets (*Mugil cephalus* Linn. and *Mugil capito* Cuv. and Val.) from the Mediterranean. Int. Rev. gesamten Hydrobiol. Hydrogr., 33(5/6):434-439.

Fitch, J.E., 1972. A case for striped mullet *(Mugil cephalus)* spawning at sea. Calif. Fish. Game, 58(3):246-248.

Fujimura, T., & Okamoto, H., 1972. Notes on progress made in developing a mass culturing technique for *Macrobrachium rosenbergii* in Hawaii. In: Coastal Aquaculture in the Indo-Pacific Region, T.V.R. Pillay editor, Fishing News (Books) Ltd., London, 497 pp.

Fujinaga, M., 1963. Culture of Kuruma shrimp *(Penaeus japonicus).* Indo-Pac. Fish. Counc. Curr. Aff. Bull., 36:10-11.

Ganapati, S.V. & Alikunhi, K.H., 1949. Experiments on the acclimatization of salt water fish seed to fresh water. Proc. Indian Sci. Congr., 36(3):161.

Gandolfi, G. & Orsini, P., 1970. Some observations on the reproductive biology of *Mugil saliens* from the Venice Lagoon. Boll. Pesca Piscic. Idrobiol., 25(1)85-93. In Italian.

Grant, C.J. & Spain, A.V., 1975. Reproduction, growth and size allometry of *Mugil cephalus* L. from North Queensland Inshore Waters, Aust. J. Zool, 23:181-201.

Gravelry, F.H., 1929. Madras Fisheries Administration Report for the Year 1928-1929. Madras Fish. Bull., 23(1):1-86.

Hamis, A.H.H., 1972. On young mullet from the Black Sea and the Southeast Mediterranean Sea. Tr. Vses. Nauchno-Issled. Inst. Morsk. Rybn. Khoz. Okeanogr., 90:142-147.

Harada, T., 1970. The present status of marine fish cultivation research in Japan. Helgol. Wiss. Meeresunt., 20:594-601.

Harrington, Jr., R.W., 1959. Photoperiodism in fishes in relation to the annual sexual cycle. In: Photoperiodism and Related Phenomena in Plants and Animals, Volume 55. American Association for the Advancement of Science Publication, Washington, D.C., 651-667.

Heldt, H., 1948. Contribution a l'etude de la biologie des muges des lacs Tunisiens. Bull. Stn. oceanogr. Salammbo, (41), 50 pp.

Helfrich, P. & Allen, P.M., 1975. Observations on the spawning of mullet, *Crenimugil crenilabis* (Forskal), at Enewetak, Marshall Islands. Micronesia, 11(2):

Henderson, N.E., 1963. Influence of light and temperature on the reproductive cycle of the eastern brook trout, *Salvelinus fontinalis* (Mitchill). J. Fish. Res. Bd. Can., 20:859-897.

Hiatt, R.W., 1944. Food chains and food cycle in Hawaiian fish ponds. I. The food and feeding habits of mullets *(Mugil cephalus),* milkfish *(Chanos chanos)* and the ten-pounder *(Elops machnata).* Trans. Am. Fish. Soc., 74:250-261.

Hickling, C.F., 1970. A contribution to the natural history of the English grey mullets (Pisces, Mugilidae). J. Mar. Biol. Ass. U.K., 50(3):609-633.

Hines, R. & Yashouv, A., 1971. Some environmental factors influencing the activity of spermatozoa of *Mugil capito* Cuvier, a grey mullet. J. Fish. Biol., 3:123-127.

Hoar, W.S., 1959. Endocrine factors on the ecological adaptation of fishes. In: Comparative Endocrinology, A. Gorbman, editor. Wiley, New York, 1-23.

Holliday, F.G.T., 1969. The effects of salinity on the eggs and larvae of teleosts. In: Fish Physiology, Vol, 1, W.S. Hoar and D.J. Randall, editors. Academic Press, New York, 293-311.

Hora, S.L. & Nair, K.K., 1944. Suggestion for the development of salt water fisheries or Bhasahada fisheries in the Sunderbans. Fishery Development Pamphlet, Department of Fisheries Bengal, (1):12 pp.

Hornell, J., 1922. The Madras Fisheries Administration Report for the Year 1920-1921. Madras Fish. Bull., 15(1):44 pp.

Hotta, H., 1955. On the mature mugilid fish from Kabashima, Nagasaki Prefecture, Japan, with additional notes on the intestinal convolution of Mugilidae. Jap. J. Ichthyol., 4:162-169. In Japanese, English summary.

Houde, E.D., 1972. Some recent advances and unsolved problems in the culture of marine fish larvae. Proceedings of the Third Annual Workshop of the World Mariculture Society, St. Petersburg, Florida.

Houde, E.D., Berkeley, S.A., Klinovsky, J.J. & Schekter, R.C., 1976. Culture of larvae of the white mullet *(Mugil curema* Valenciennes) Aquaculture, 8(4):365.

Hubbs, C.L., 1943. Terminology of early stages of fishes. Copeia, (4):260.

Hwang, S.W., Chen, H.P., Lee, D.L. & Liao, I.C., 1972. Preliminary results in the cryogenic preservation of grey mullet *(Mugil cephalus)* sperm. J. Fish. Soc. Taiwan. 1(2):1-7.

Jacob, P.K. & Krishnamurthi, B., 1948. Breeding and feeding habits of mullets *(Mugil)* in Ennore Creek. J. Bombay Nat. Hist. Soc., 47:663-668.

Jhingran, V.G. and Natarajan, A.V., 1970. A study of the fisheries and fish populations of the Chilka Lake during the period 1957-65. J. Inland Fish. Soc. India. 1:49-126.

Job, T.J. & Chacko, P.I., 1947. Rearing of salt water fish in fresh waters of Madras. Indian Ecol., 2(1):1-9.

Johnson, M.C., 1954. Preliminary experiments on fish culture in brackish water ponds. Progr. Fish. Cult., 16(3):131-133.

Kennedy, M., Fitzmaurice, P., 1969. Age and growth of thick-lipped grey mullet, *Crenimugil labrosus* (Risso) in Irish waters. J. Mar. Biol. Ass. U.K., 46:683-699.

Kesteven, G.L., 1953. Further results of tagging sea mullet (*Mugil cephalus* Linnaeus) on the Eastern Australian coast. Aust. J. Mar. Freshwater Res., 4(2):251-306.

Kuo, C.M. & Nash, C.E., 1975. Recent progress on the control of ovarian development and induced spawning of the grey mullet (*Mugil cephalus* L.). Aquaculture, 5:19-29.

Kuo, C.M., Shehadeh, Z.H. & Milisen, K.K., 1973a. A preliminary report on the development, growth and survival of laboratory reared larvae of the grey mullet, (*Mugil cephalus* L.) J. Fish. Biol., 5:459-470.

Kuo, C.M., Shehadeh, Z.H. & Nash, C.E., 1973b. Induced spawning of captive grey mullet (*Mugil cephalus* L.) females by injection of human chorionic gonadotropin. Aquaculture, 1:429-432.

Kuo, C.M., Nash, C.E. & Shehadeh, Z.H., 1974a. A procedural guide to induce spawning in grey mullet (*Mugil cephalus* L.). females by injection of human chorionic gonadotropin. Aquaculture, 3:1-14.

Kuo, C.M., Nash, C.E. & Shehadeh, Z.H., 1974b. The effects of temperature and photoperiod on ovarian development in captive grey mullet (*Mugil cephalus* L.). Aquaculture 3:25-43.

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

Kurian, C.V. 1960. Spawning of *Mugil cephalus*. Indian Farm. 15(12):10-11.

Kurian, C.V., 1974. Mullets and mullet fisheries in India. Paper presented at IBP/PM International Symposium on the Grey Mullets and their Culture, Haifa, Israel.

Le Dantec, J., 1955. Quelques observations sur la biologie des mugos des reservoirs de Certes a Audenge. Rev. Trav. Off. (Sci. Tech.) Peches Marit., 19(1):93 97.

Liao, I.C., 1969. Artificial propagation of grey mullet (*Mugil cephalus* Linnaeus). Fish. Ser. Chin.-Am. Jt. Comm. Rur. Reconstr., 8:10-20.

Liao, I.C., 1974. The experiments on the induced breeding of the grey mullet in Taiwan from 1963-1973. Paper presented at IBP/PM International Symposium on the Grey Mullets and their Culture, Haifa, Israel. Also 1975. Aquaculture, 6(1):31.

Liao, I.C. 1977. On completing a generation cycle of the grey mullet *(Mugil cephalus)* in captivity. J. Fish. Soc. Taiwan 5(2):121-130.

Liao, I.C., Lu, Y.J., Huang, T.L. & Lin, M.C., 1971. Experiments on induced breeding of the grey mullet (*Mugil cephalus* Linnaeus). Fish. Ser. Chin.-Am. Jt. Comm. Rur. Reconstr., (11):1-29.

Liao, I.C., Cheng, C.S., Tseng, L.C., Lim, M.Y., Hsieh, L.S. & Chen, H.P., 1972. Preliminary report on the mass propagation of grey mullets (*Mugil cephalus* Linnaeus). Fish. Ser. Chin.-Am. Jt. Comm. Rur. Reconstr., (12):1-14.

Liao, I.C.,Lee, D.L., Lim, M.Y., & Lo, M.C., 1971. Preliminary report on induced breeding of pond-reared mullet (*Mugil cephalus* Linnaeus). Fish. Ser. Chin.-Am. Jt. Comm. Rur. Reconstr., (11):30-35.

Lin, S.Y., 1940. Fish culture in ponds in the new territories of Hong Kong. J. Hong Kong Fish. Res. Stn., 1(2):161-193.

Linder, D.R., Strawn, K. & Luebke, R.W., 1974. The culture of striped mullet (*Mugil cephalus* Linnaeus) in ponds receiving heated effluent from a power plant. Paper presented at IBP/PM International Symposium on the Grey Mullets and their Culture, Haifa, Israel.

Lunz, G.R., 1951. A saltwater fish pond. contribution from the Bears Bluff Laboratories, No. 12, Bears Bluff Laboratories, Wadmalaw Island, South Carolina.

Marais, J.F.K. & Erasmus, T., 1977. Body composition of *Mugil cephalus, Liza dumerili, Liza richardsoni* and *Liza tricuspidens* caught in the Swartkops Estuary. Aquaculture, 10(1):75.

May, R.C., 1970. Feeding larval marine fishes in the laboratory: a review. Rep. Calif. Coop. Oceanic Fish Invest., 14:76-83.

May, R.C., Popper, D. & McVey, J.P., 1974. Rearing and larval development of *Siganus canaliculatus* (Park). Micronesia, 10(2):285-298.

Medlen, A.B., 1951. Preliminary observation on the effect of temperature and light upon the reproduction of *Gambusia affinis.* Copeia, 148-153.

Merriman, D. & Schedl, H.P., 1941. The effects of light and temperature on gametogenesis in the four-spined stickleback, *Apeltes quadracus* (Mitchell). J. Exp. Zool., 88:413-449.

Mitson, R.B., 1963. Marine Fish Culture in Britain. V. An electronic device for counting the nauplii of *Artemia salina* L. J. Cons. Perma. Int. Explor. Mer., 28(2):262-269.

Mohanty, S.K., 1973. Temperature tolerance of grey mullet. FAO Aquaculture Bull., 5(2):9.

Mookerjee, H.K., Ganguly, D.N. & Sircar, A., 1946. On the composition of food of the Indian mullet (*Mugil parsia* Ham.) with suggestions to culture them in fresh water ponds of Bengal. Proc. Indian Sc. Congr., 33(3):132.

Nash, C.E., 1973. Automated mass-production of *Artemia salina* nauplii for hatcheries. Aquaculture, 2:289-298.

Nash, C.E. & Kuo, C.M., 1975. Hypotheses for problems impeding the mass propagetion of grey mullet and other finfish. Aquaculture, 5:119-133.

Nash, C.E. & Shelbourne, J.E., 1967. Power station effluent as an environment for flatfish culture. I.C.E.S. Fisheries Improvement Committee, CME:10.

Nash, C.E., Kuo, C.M. & McConnell, S.C., 1974. Operational procedures for rearing larvae of the grey mullet (*Mugil cephalus* L.). Aquaculture, 3:15-24.

Nash, C.E., Kuo, C.M., Madden, W.D. & Paulsen, C.L. 1977. Swim bladder inflation and survival of *Mugil cephalus* to 50 days. Aquaculture, 12:89-94.

Nikolskii, G., 1954. Special Ichthyology. Sovietskay nauka. Moscow. 2nd Edition translated from Russian, Jerusalem, 1961, 538 pp.

Oppenheimer, C.H., 1955. The effect of marine bacteria in the development and hatching of pelagic fish eggs, and the control of such bacteria by antibiotics. Copeia, (1):43-49.

Oren, O.H., 1971. International biological programme: coor-

dinated studies on grey mullets. Mar. Biol., 10(1):30-33.
Paget, G., 1923. The determination of the rate of growth of Bouri *(Mugil cephalus)* in Lake Mariyut. Rep. Fish. Serv. Egypt, 1922-43-49.
Panikkar, N.K., 1951. Problems of marine and estuarine fisheries. In: Indian Fish., Ministry of Agriculture, 79-87.
Patnaik, D.A. 1966. On the biology of *M. cephalus* of the Chilka Lake. 1962. Proc. 2nd All India Congr. Zool, Part 2: 454-464.
Paulsen, C.L., 1977. Apparatus for collection and separation of invertebrate organisms from a pond, for use as fish food. Progr. Fish. Cult. 39(2):101-102.
Perceva-Ostroumova, T.A., 1951. Reproduction and development of grey mullets introduced in the Caspian Sea. Tr. Vses. Nauchno-Issled. Inst. Morsk. Rybn. Khoz. Okeanogr., 18:123-134.
Perlmutter, A., Bograd, L. & Pruginin, J., 1957. Use of the estuarine and sea fish of the family Mugilidae (grey mullets) for pond culture in Israel. Proc. Tech. Pap. Gen. Fish. Coun. Mediterr., 4:289-304.
Pillay, T.V.R., 1947. Possibilities of mullet culture in India. Indian Farming, 8(11):544-549.
Pruginin, Y. & Kitai, H., 1957. Cultivating mullets as a secondary fish in the carp ponds. Bamidgeh, 9(4):70-75.
Purdom, C.E., 1972. Genetics and fish farming. Laboratory leaflet (New Series) No. 25, Ministry of Agriculture, Fisheries and Food, Fisheries Laboratory, Lowestoft, Suffolk, U.K.
Riley, J.K., 1966. Marine fish culture in Britain. VII. Plaice *(Pleuronectes platessa)* post-larval feeding on *Artemia salina* L. nauplii and the effects of varying feeding levels. J. Cons. Int. Explor. Mer., 30:204-221.
Rosenthal, H. & Hempel, G., 1970. Experimental studies in feeding and food requirements of herring larvae *(Clupea harengus* L.). In: Marine Food Chains, J.H. Steele, editor, University of California Press, Berkeley, 552 pp.
Roughley, T.C., 1916. Fishes of Australia and their technology. Tech. Educ. Ser. Publ. Instr. Dep. N.S. Wales, (21):296 pp.
Roule, L., 1917. Sur le developpement larvaire et post-larvaire des poissons du genre *Mugil.* C.R. Hebd. Seances Acad. Sci., Paris, 164(5):194-196.
Sanzo, L., 1930. Uova e larve di *Mugil cephalus* Cuv. ottenute per fecondazione artificiale. Mem. R. Com. Talassogr. Ital., 179:3.
Sanzo, L., 1931. Uova e larve di mugilidi. Atti. Soc. Ital. Prog. Sci., 20:324-325.
Sanzo, L., 1936. Contirbuti alla conoscenza dello sviluppo embrionario e post-embrionairo nei Mugilidi. I. *Mugili cephalus* Cuv. II. *M. chelo* Cuv. Mem. R. Com. Talassogr. Ital., 230:1-11.
Sanzo, L., 1937. Uova e larve di *Mugil labeo* Cuv. Boll. Pesca Piscio. Hidrobiol., 13(5):506-510.
Sarojini, K.K., 1957. Biology and fisheries of the grey mullets of Bengal. 1. Biology of *Mugil parsia* Hamilton with notes on its fishery in Bengal. Indian J. Fish., 4(1):160-207.
Sarojini, K.K., 1958. Biology and fisheries of the grey mullets of Bengal. 2. Biology of *Mugil cunnesius* Valenciennes. Indian J. Fish., 5(1):56-76.
Sebastian, M.J. & Nair, V.A., 1973. The induced spawning of the grey mullets. Seafood Exp. J., 5(1):1-4.
Sebastian, M.J. & Nair, V.A., 1974. The induced spawning of the grey mullet, *Mugil macrolepis* (Aguas) Smith and the large scale rearing of its larvae. Paper presented at IBP/PM International Symposium on the Grey Mullets and their Culture, Haifa, Israel.
Sharpe, B., 1945. 5000 Pounds of fish a year per acre. Country Gentleman, 115(12):26-28.
Shehadeh, Z.H. & Ellis, J.N., 1970. Induced spawning of the striped mullet (*Mugil cephalus* L). J. Fish. Biol., 2:355-360.
Shehadeh, Z.H., Madden, W.D. & Dohl, T.P., 1972. The effect of exogenous hormone treatment on spermiation and vitellogenesis in the grey mullet (*Mugil cephalus* L.). J. Fish. Biol. 5:479-487.
Shehadeh, Z.H., Kuo, C.M. & Milisen, K.K., 1973a. Validation of an *in vivo* method for monitoring ovarian development in the grey mullet (*Mugil cephalus* L.). J. Fish. Biol., 5:489-496.
Shehadeh, Z.H., Kuo, C.M. & Milisen, K.K., 1973b. Induced spawning of grey mullet *Mugil cephalus* L. with fractionated salmon pituitary extract. J. Fish. Biol., 5:471-478.
Shehadeh, Z.H., Kuo, C.M. & Nash, C.E., 1973c. Establishing brood stocks of grey mullet (*Mugil cephalus* L.) in small ponds. Aquaculture, 2:379-384.
Shelbourne, J.E., 1964. The artificial propagation of marine fish. Adv. Mar. Biol., 2:1-83.
Shelbourne, J.E., 1969. In: Marine Biology, Volume V. John D. Costlow, editor, Gordon and Breach, Science Publishers, New York, 606 pp.
Shelbourne, J.E. & Nash, C.E., 1966. Sea fish culture in Britain. Proc. Nutr. Soc., 25(2):133.
Shireman, J.V., 1974. Preliminary experiments on striped mullet culture in fresh water catfish ponds. Progr. Fish Cult., 36(2): 113.
Smith, J.L.B., 1935. The fishes of the family Mugilidae in South Africa. Ann. S. Afr. Mus., 30(19):587-644.
Stenger, A.H., 1959. A study of the structure and development of certain reproductive tissues of *Mugil cephalus* Linnaeus. Zoologica, N.Y., 44(2):53-68.
Struhsaker, J.W., Hashimoto, D.Y., Girard, S.M., Prior, F.T. & Conney, T.D., 1973. Effects of antibiotics on survival of carangid fish larvae *(Caranx mate),* reared in the laboratory. Aquaculture, 2:53-88.
Sundararaj, B.I. & Goswami, S.W., 1969. Techniques of hypophysation of catfishes. FAO/UNDP Regional Seminar on Induced Breeding of Cultivated Fishes. FRi/IBCF/15, 1-23.
Sylvester, J.R. & Nash, C.E., 1975. Thermal tolerance of eggs and larvae of Hawaiian striped mullet (*Mugil cephalus* L.). Trans. Am. Fish. Soc., 104(1):144-147.
Sylvester, J.R., Nash, C.E. & Emberson, C.R., 1975. Salinity and oxygen tolerances of eggs and larvae of Hawaiian striped mullet (*Mugil cephalus* L.). J. Fish. Biol. 7:621-629.
Tamaru, T., 1970. Marine Aquaculture. Translation from the Japanese of the revised and enlarged, second edition, 1968, by Mary I. Watanabe. National Science Foundation, Washington, D.C., PB 194 051T, Part 1.
Tang, Y.A., 1964. Induced spawning of striped mullet by hormone injection. Jap. J. Ichthyol., 12(1/2):23-28.
Tang, Y.A., 1974. Collection, handling and distribution of grey mullet fingerlings in Taiwan. Paper presented at IBP/PM International Symposium on the Grey Mullets and their Culture, Haifa, Israel.
Theilacker, G.H. & McMaster, M.G., 1971. Mass culture of the rotifer *(Brachionus plicatilis)* and its evaluation as a food for larval anchovies. Mar. Biol., 10:183-188.
Thomson, J.M., 1954. The organs of feeding and the food of some Australian mullet. Aust. J. Mar. Fresh Water Res., 5(3):469-485.
Thomson, J.M., 1955. The movements and migrations of mullet (*Mugil cephalus* L.). Aust. J. Mar. Fresh Water Res., 6(3):328-347.
Thomson, J.M., 1963. Synopsis of biological data on the grey mullet (*Mugil cephalus* Linnaeus) 1758. Fish. Synop. Div. Fish. Oceanogr. C.S.I.R.O. Australia, (1).

Thomson, J.M., 1966. The grey mullets. Oceanogr. Mar. Biol. Ann. Rev., 4:301-335.

Thong, L.H., 1969. Contributions a l'etude de la biologie des Mugilides (poissons teleosteens) des cotes du massif Armoricain. Trav. Fac. Sci. Univ. Rennes. (Ser. Oceanogr. Biol.), (2):55-182.

Tung, I.H., 1973. On the egg development and larval stages of the grey mullet (*Mugil cephalus* Linnaeus). Rep. Inst. Fish. Biol. Min. Econ. Aff. Natl. Taiwan Univ., 3(1):187-215.

Vodyanitskii, V.A. & Kazanova, I.I., 1954. The identification of pelagic eggs and larvae of Black Sea fishes. Tr. Vses. Nauchno-Issled. Inst. Morsk. Rybn. Khoz. Okeanogr., 28:240-325.

Wallace, J.H., 1974. Aspects of the biology of *Mugil cephalus* in a hypersaline estuarine lake on the east coast of South Africa. Paper presented at IBP/PM International Symposium on the Grey Mullets and their Culture, Haifa, Israel.

Walne, P.R., 1958. The importance of bacteria in laboratory experiments on rearing the larvae of *Ostrea edulis*. 1. Feeding experiments. J. Mar. Biol. ass. U.K., 43:767-784.

Wimpenny, R.S. & Faouzi, H., 1935. The breeding of a grey mullet (*Mugil capito* Cuv.) in lake Qarun, Egypt. Nature, London, 135(3425):1041.

Yang, W.T. & Kim, U.B., 1962. A preliminary report on the artificial culture of grey mullet in Korea. Proc. Indo-Pac. Fish. Coun., 9(2/3):62-70.

Yashouv, A., 1966. Breeding and growth of grey mullet (*Mugil cephalus* L.) reared in captivity in fresh water ponds. Bamidgeh, 21(1):19-24.

Yashouv, A. & Ben-Shachar, A., 1967. Breeding and growth of Mugilidae. 2. Feeding experiments under laboratory conditions with *Mugil cephalus* L. and *M. capito* Cuv. Bamidgeh, 19(2/3):50-66.

Yashouv, A. & Berner-Samsonov, E., 1970. Contribution to the knowledge of aggs and early larval stages of mullets (Mugilidae) along the Israel coast. Bamidgeh, 22(3):72-89.

Yoshioka, H., 1962. On the effects of environmental factors upon the reproduction of fishes. 1. The effects of day-length on tha reproduction of the Japanese killifish *(Oryzias latipas)*. Bull. Fac. Fish. Hokkaido University, (13):123-136.

Zismann, Lyka, Vivane Berdugo & Kimor, B., 1974. The food and feeding of early stages of grey mullets in the Haifa Bay region. Paper presented at IBP/PM International Symposium on the Grey Mullets and their Culture, Haifa, Israel.

Zismann, L. & Ben-Tuvia, A., 1975. Distribution of juvenile mugilids in the hypersaline Bardawil Lagoon January 1973 - January 1974. Aquaculture, 6(2)143.

Zviagina, O.A., 1961. The distribution of eggs of mackerel *Pneumatophorus japonicus* (Houttuyn) and mullet (*Mugil so-iuy* Basilewsky) in Peter the Great Bay. Tr. Inst. Okeanol., 43:328-336.

INTENTIONALLY
LEFT
BLANK

Appendix 1

This Appendix is presented as a checklist for the most important hatchery supplies and equipment. It is by no means comprehensive. Water supply, air temperature control, and compressed air supply systems, and specifications for production and culture tanks are not included as these are covered fully in Chapters 4 and 5 of the main report.

BROODSTOCK COLLECTION

- transport holding tanks with covers
- small boat with space for holding tanks, or a boat with built-in live bait well
- boat trailer
- flatbed truck with sufficient loading capacity to hold filled tank
- gill nets/surround nets, nylon monofilament 6·7 cm mesh (stretched measure)
- dipnets, about 50 cm in diameter
- portable aeration unit, or compressed oxygen/air with regulator, tubing and airstones
- scuba gear, desirable when surround nets are used
- antibiotic (e.g., nifurpirinol)

BROODSTOCK MAINTENANCE

Holding Ponds:

- organic or inorganic fertilizer
- clay bottom ponds: hand/motorized plow, and lime
- rubberlined dirt pond: brushes, hoses and hose nozzles, 3-mm translucent polyethylene sheeting, lead-core line
- instrument(s) to measure temperature, salinity, pH and dissolved oxygen levels
- water flow measuring device
- feeding box, if desired

Husbandry:

- Pathology supplies: refrigerator/freezer combination
  binocular dissecting microscope
  compound microscope with oil immersion
  microtome
  laboratory table
  wet table with drain
  warming tray
  small autoclave/oven
  centrifuge
  reagents, chemicals and antibiotics (e.g., formaldehyde and nifurpirinol)
  distilled water unit
  embedding wax

miscellaneous laboratory glassware

- measuring board
- weighing scale (e.g., Ohaus Triple Beam balance)
- buckets
- dipnets, about 50 cm in diameter
- tags and applicators
- feeding:
  - food mix ingredients
  - weighing scale
  - grind mill, with 0.2 - 2 mm screens
  - assorted sieves
  - large storage containers with lids

SPAWNING AND FERTILIZATION

Selection of Spawners:
- surround net
- dipnet, about 50 cm diameter
- small holding tanks
- anaesthetic (2-methylquinoline, MS-222)

Indoor Holding:
- aeration equipment (tubing and airstones)
- incandescent or fluorescent lamps
- tank cleaning: brushes, hydrochloric acid, hoses and hose nozzles
- spawning tanks with covers
- aeration equipment

Maturation Testing & Hormone Treatment:
- anaesthetic (as before)
- polyethylene canula (0.85 mm in diameter)
- holding box and black plastic hood
- formladehyde and NaCl 0.6% solution
- distilled water
- hormones
- injection needles and syringes
- microscope
- ocular micrometer
- microscope slides, with and without grooves
- 8 key cell counter
- calculator
- refrigerator/freezer combination
- work bench and large sink
- miscellaneous lab glassware (e.g., reagent bottles, eye droppers, micropipets, dispersion beakers)

Incubation & Hatching:
- incubators
- aeration equipment
- cleaning: potassium permanganate, brushes, scouring pads (no soap), hoses and hose nozzles
- clear vinyl tubing for transfer, 2.5 cm diameter
- fine mesh handnet
- antibiotics (potassium penicillin G and streptomycin sulfate)
- buckets
- instrument(s) to measure temperature, dissolved oxygen

LARVAL REARING
- larval rearing tanks
- rearing kreisels
- fluorescent or incandescent lamps
- suction cleaning device
- instrument(s) to measure temperature, salinity, pH and dissolved oxygen
- hand counters

CULTURE OF FOOD ORGANISMS

Phytoplankton Culture
- aeration equipment (1/8 inch clear plastic tubing)
- fluorescent, agro lamps and cool white lamps
- steam cleaning equipment
- autoclave
- algal cultures (e.g., *Chlorella salina* and *Navicula marina)*
- test tubes
- Pyrex flasks, 1-5-2.5 l.
- Pyrex carboys, 16 l.
- cylinders, 16 l.
- holding racks for culture glassware and cylinders
- work bench and large sink
- refrigerator/freezer combination
- microscope
- cell counter
- culture transfer lines, clear plastic tubing
- nutrient mix ingredients
- Burkholder's solution
- glassware for mixing and storage of nutrient mix and Burkholder's solution
- fertilizer mix ingredients
- sealed containers for fertilizer storage
- phytoplankton production tanks
- aeration equipment for production tanks (1/2 inch clear plastic tubing)
- hand cleaning: scouring pads (no soap), brushes, hoses and hose nozzles, chlorine solution
- distilled water unit

Rotifer Culture
- production tanks with covers
- small tanks
- airlift pump

- Nitex bag, 48 μ mesh, collector
- buckets
- instrument(s) to measure salinity, dissolved oxygen, temperature, and ammonia levels

Copepod/Amphipod Production

- floating enclosures, 2,830 μ mesh screen, with 12 V lights
- predator screens, 4.8 mm mesh
- submersible, centrifugal pumps (11 l/min)
- clear plastic tubing, 2.5 cm diameter
- separator chamber
- Nitex screen of selected mesh sizes
- timers for pumps and lights

Brine Shrimp Production

- 2 incubators, with common separator and collector box
- Nitex screen, 75 μ
- brine shrimp eggs (grade A quality)
- aeration equipment: lines and airstones
- immersion heaters or water heating unit, if required
- 7 W lights in waterproof plexiglass caps
- assorted valves and plumbing
- production control unit
- cleaning: chlorine solution, scouring pads (no soap), brushes, hoses and hose nozzles

Appendix 2

The Oceanic Institute in Hawaii has prepared a *technical training film* on the induced breeding and rearing of the grey mullet. The film illustrates the culture techniques and procedures described and used as the basis of this study on hatchery production systems.

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

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

All information relevant to the techniques is covered visually or by soundtrack. The film was awarded a Certificate of Merit at the International Festival of Aquaculture Films at the FAO Technical Conference on Aquaculture, Kyoto, Japan, May - June, 1976.

The copyrighted 23-minute 16mm color film, with soundtrack, was produced by Ahuimanu Productions. It can be purchased from the Oceanic Institute, Waimanalo, Hawaii 96795, U.S.A. The film is distributed with a copy of the text in English.