

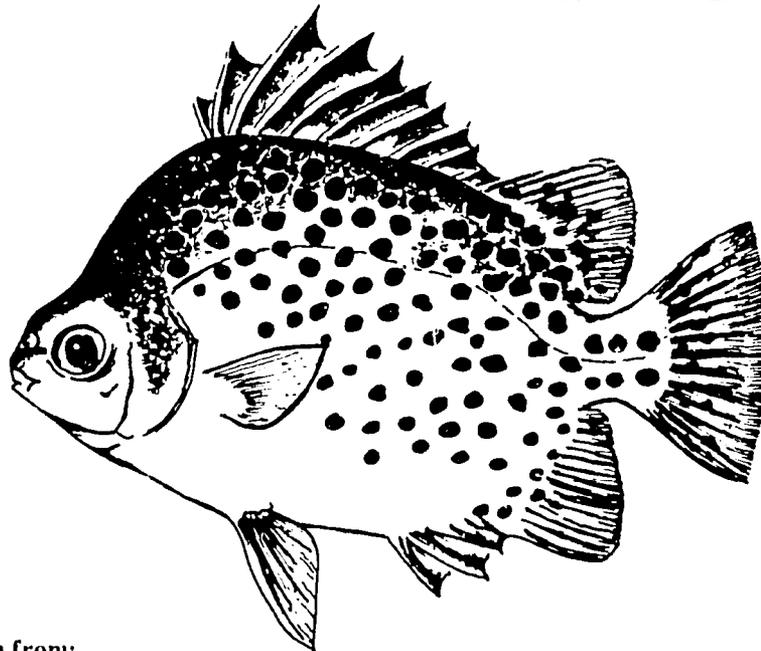
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**SPAWNING INDUCTION AND POND CULTURE OF THE SPOTTED SCAT
(Scatophagus argus Linnaeus) IN THE PHILIPPINES**

Arlo W. Fast, Editor
Hawaii Institute of Marine Biology

5.335



A Research Contribution from:

HAWAII INSTITUTE OF MARINE BIOLOGY
UNIVERSITY OF HAWAII AT MANOA
KANEOHE, HAWAII
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and

ILOILO STATE COLLEGE OF FISHERIES
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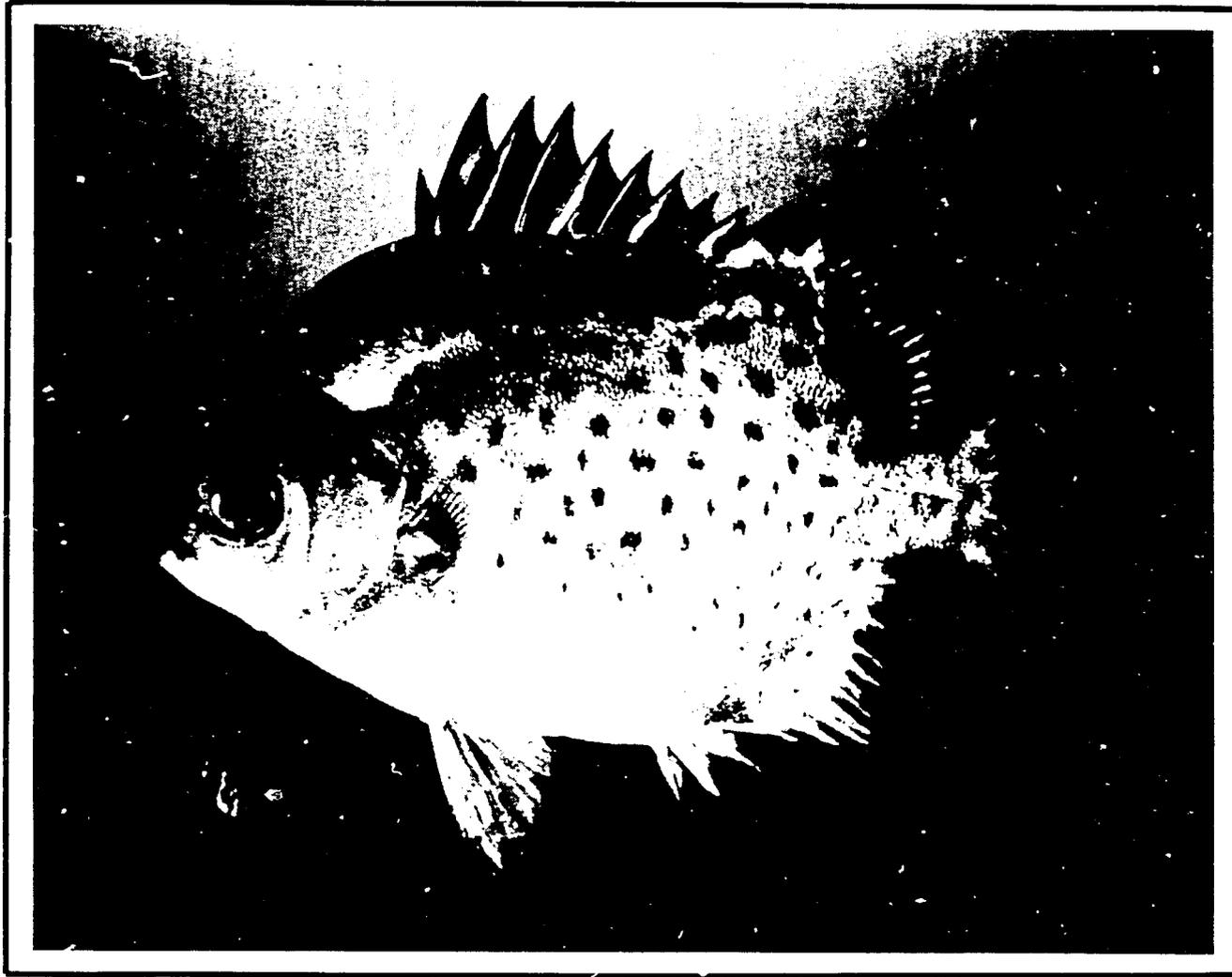
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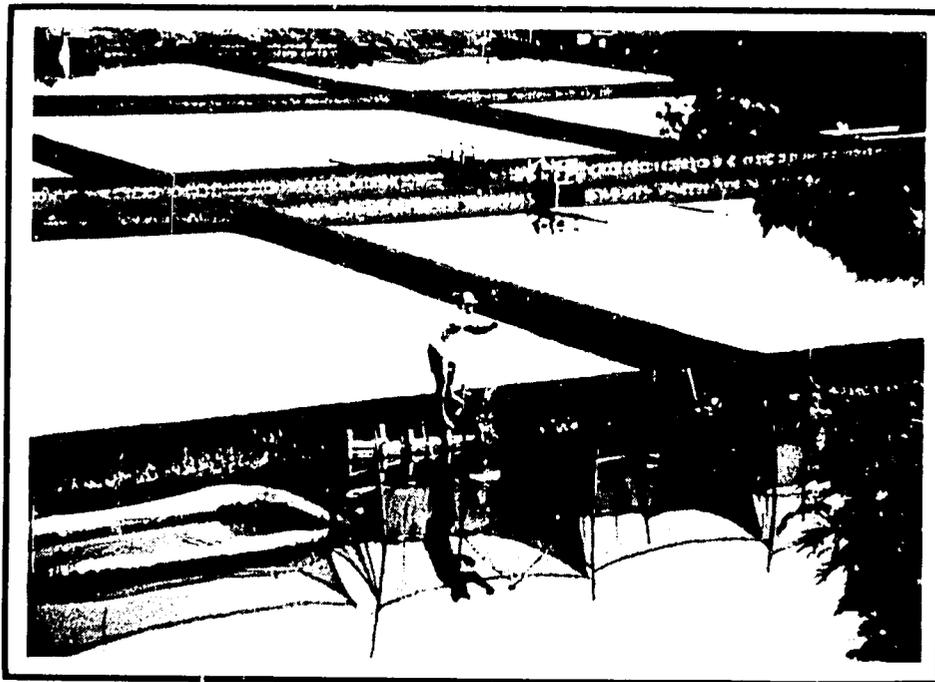
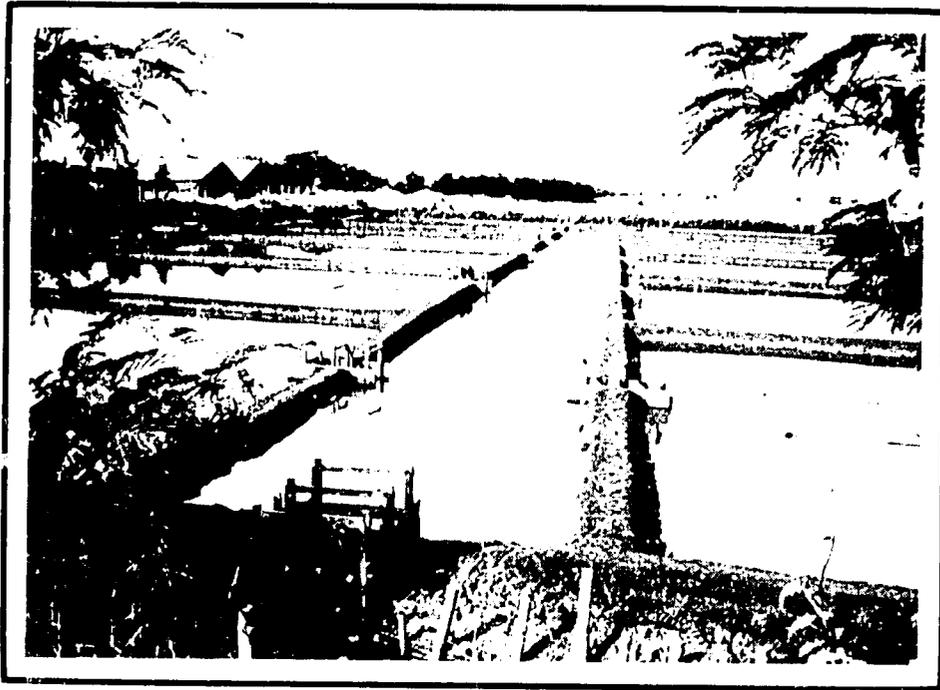
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A 400G ADULT SPOTTED SCAT (SCATOPHAGUS ARGUS) WITH A JUVENILE
(PHOTO BY KENT E. CARPENTER)



POND RESEARCH FACILITIES AT ILOILO STATE COLLEGE OF FISHERIES (ISCOF)

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Terence P. Barry and Arlo W. Fast

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PREFACE

The spotted scat is one of the highest priced food fishes in the Philippines. It is also a valued aquarium fish for the export and domestic market, with a far greater demand than supply. To date, there have been few attempts to culture the spotted scat through spawning induction, larviculture, and growout in tanks and ponds. Virtually all scat for the food market and aquaria trade are captured from the wild. None are grown to market size in ponds, except for incidental intrusions into milkfish or prawn ponds.

Dr. Jose Carreon, at that time the Dean of the College of Fisheries, University of the Philippines (UP), recognized the need to develop culture techniques for the spotted scat, and in 1984 proposed the present study to USAID in collaboration with the University of Hawaii, through USAID's PSTC grant program. The proposal was accepted, and research began during the spring of 1986 at the University of the Philippines in the Visayas (UPV) Brackishwater Aquaculture Center (BAC) at Leganes, Iloilo. As a result of facilities limitations at the BAC, the project was transferred to the Iloilo State College of Fisheries (ISCOF) in December 1986. Dr. Benigno P. Panistante and Mr. Henry Biona, Sr. became the Co-Principal Investigator and Co-Research Associate, respectively, as counterparts to myself and Mr. Terence P. Barry. Research continued at ISCOF through 1987. Concurrently, we continued collaboration with researchers at UPV, the Southeast Asian Fisheries Development Center (SEAFDEC), and the Bureau of Fisheries and Aquatic Resources (BFAR). The results of these combined research efforts and collaborations are reported here.

Although we did not meet all of our original goals, we are satisfied with the progress we made during our two year project period. We have clearly pointed out the direction where future research on the spotted scat should go, and feel that our contributions, reported here, will form the foundation on which to build additional progress towards developing practical and commercially viable methodologies for the spawning, larviculture, and growout of the spotted scat.

Arlo W. Fast
Editor

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We are very grateful to a number of institutions and individuals who contributed in many ways to the success of this project. The most important contributors were the individuals in the project personnel list which follows. The research assistants and aides were responsible for implementing most of the research work. We thank them for the long hours they devoted to the project, their willingness to take on additional assignments, and their steadfast dedication. We would like to single out Mr. Leopoldo Anas for the special contributions he made to the project. Special thanks also go to Amy Lynn Reyes Barry. Besides serving as the project accountant, she managed the data bases, performed all of the statistical analyses, and played an indispensable role in preparing the final project report.

We are also grateful for various assistances given by individuals not listed in the project personnel list. We thank Dr. Kent Carpenter for his numerous contributions; Ms. Josefa Tan-Fermin for preparing histological sections of the spotted scat ovary; Mr. Luis Ma. Garcia for his helpful ideas and discussions; Mr. Nestor Diasanta, for his artwork; Dr. Flor Lacanilao, Chief, SEAFDEC, AQD, for the use of SEAFDEC equipment; and USAID for providing the funds for our work.

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I. INTRODUCTION

Terence P. Barry and Arlo W. Fast

The spotted scat¹ (*Scatophagus argus*) is one of few teleost species of economic importance that could potentially thrive in tropical brackishwater fish ponds (Bardach et al., 1972). Scats are abundant in the nearshore waters of Southern and Southeast Asia where they inhabit estuaries, coastal mudflats, mangrove swamps, harbors, and upstream rivers. These habitats are characterized by extreme fluctuations in salinity, dissolved oxygen, temperature, tidal movements, river runoff, turbidity, and turbulence. The adaptations which allow the scat to live in this ever-changing environment endow it with many biological attributes highly desired in a cultured finfish.

Scats are economically important for two major reasons. First, they are an attractive species, and in body shape resemble the butterfly fishes, a group to which they are closely related. Scats are a popular aquarium species because of their appearance, hardiness, slow growth and "personable" behavior (Morgan, 1983). There is a continuous, high demand for scat in the tropical aquarium fish market, and they sell for a very good price². Secondly, in the Philippines, scats are considered to be one of the highest quality food fishes, and command an excellent price at the market (Table I.1). Scats as small as 50 gm are almost as highly priced per kilogram as larger specimens.

Project Objectives. The primary objectives of our PSTC project were to determine the feasibility of culturing scat in brackishwater ponds, and to develop standardized techniques for its propagation. To achieve these objectives, we conducted experiments designed to 1) establish suitable pond growout strategies for scat, taking advantage, if possible, of the highly developed technology already widely used throughout the Philippines for culturing milkfish, and 2) develop reliable methodologies to induce gonadal maturation and spawning of captive male and female scat.

Research Priorities. Highest priority was given to the pond growout research. If scat are ever cultured commercially as food fish, their growout will most likely occur in earthen ponds. For this reason, we felt a need to determine the survival, growth and culturability of scat in ponds. Unpublished data obtained from researchers at the University of the Philippines in the Visayas (UPV), and the Philippine Bureau of Fisheries and Aquatic Resources (BFAR) suggested that scat grew very slowly. First, we needed to verify these reports, and then, if our results showed it was necessary, we planned to develop profitable, alternative growout strategies which took the scat's slow growth rate into consideration. We evaluated two growout strategies. First, we tested the effects of a

¹Spotted scat are also called scat, spadefish, spotted spadefish, butterfly, and spotted butterfly. In the Philippines, scats are commonly known as kitang or kikiro.

²According to Mr. Richard Bon Eng Ty, President of Aquascapes Philippines, a major Philippine tropical fish exporter, wild-caught scat cannot supply the current demand for this species in the world aquarium fish market. Aquascapes alone receives orders for more than 100,000 scat per month. Local suppliers receive from one-half to one peso per fish, depending on the size (2 to 5 cm). Current exchange rate is about 20 pesos/\$1.00 U.S.

In the U.S. aquarium trade, *S. argus* commands a high price. Fish with lengths of 5 to 10 cm (2 to 4 inches) typically wholesale for \$5 to \$10 each. Fish of this length range from 2 to 10 grams in weight, thus the price per pound is about \$450 wholesale. The retail cost may be approximately double.

TABLE I.1 A SURVEY OF THE PRICES OF FIRST CLASS FISH
IN THE ILOILO CITY CENTRAL MARKET

Species			Price/kg
Common Name	Local Name(s)	Scientific Name	₱
Spanish Mackerel	Tangigue	<u>Cybium commerson</u>	30-60
Red Snapper	Maya-maya	<u>Lutjanus malabaricus</u>	24-35
Grouper	Lapu-lapu	<u>Epinephelus sp.</u>	35-60
Sea Bass	Bulgan, Apahap	<u>Lates calcarifer</u>	30-60
Yellow Fin Tuna	Albacora	<u>Neothunnus macropterus</u>	30-35
Spotted Scat	Kitang, Kikiro	<u>Scatophagus argus</u>	60-80
Cavalla	Talakitok	<u>Caranx sp.</u>	20-40
Milkfish	Bangus	<u>Chanos chanos</u>	15-25

potent anabolic hormone, 17 α -methyltestosterone (MT) on the growth of scat in monoculture ponds at two densities. The MT was incorporated into prepared feed and administered to the fish with their daily rations. The other strategy evaluated the feasibility of culturing scat in polyculture with each of the two other widely cultured brackishwater species in the Philippines, namely milkfish (*Chanos chanos*), and the tiger prawn or "sugpo" (*Penaeus monodon*). We evaluated a growout strategy whereby two crops of milkfish or prawn would be produced for every one crop of the slower growing scat from the same pond.

High priority was also given to studies on induced maturation and spawning. For female scat, our experiments focused on the use of cholesterol-based, pelleted implants of synthetic LHRH (LHRHa). We sought to induce vitellogenesis, final oocyte maturation, and spawning in a predictable time. For males, we sought to find an effective method to stimulate spermiation on demand, using various hormones, and hormone combinations, administered in different ways.

We also investigated, in both aquaria and ponds, the dose-response effects of potential growth-promoting hormones in scat. We documented the occurrence of various diseases and parasites of the scat, and developed effective prophylactic and curative treatments against them. We also conducted experiments to determine the tolerance limits of scat fry to changes in salinity, temperature, and pH. In addition, we thoroughly analyzed our field broodstock and fry collection data, and obtained important information on the natural history and biology of the spotted scat.

II. NATURAL HISTORY OF THE SPOTTED SCAT (*Scatophagus argus*)

Terence P. Barry and Arlo W. Fast

II.1. Classification and Description of Spotted Scat

The spotted scat (*Scatophagus argus*, Linnaeus, Syst. Nat. ed. 12, 1766, p. 464), belongs to a small family of fishes, the Scatophagidae. There are only two genera in this family, with the following taxonomic classifications (Berg, 1940):

Phylum: Chordata
Subphylum: Vertebrata
Class: Osteichthyes
Subclass: Actinopterygii
Infraclass: Teleostei
Order: Periformes
Suborder: Percoidea
Family: Scatophagidae
Genera: *Scatophagus* spp
Selenotoca spp

The name *Scatophagus* is translated as "offal-eater", or "eater of feces". The name was derived from the habit of scat to gather in harbors and feed on offal and other wastes discharged from ships. Scat are also found around sewer outfalls, and in waters characterized by high organic waste inflows. Whether scat actually eat feces or offal, or whether they are instead feeding on attached algae associated with such discharges, is not known. We suspect the latter. Regardless of whether scat are true coprophages or not, many people refuse to eat this fish because of its reputation as such.

The word *argus*, translated as "thousand-eyed", clearly refers to the spots found on all juvenile and larger fish. The fry, by contrast have black coloration, and a more angelfish-like body form. The fry are also common in harbors, rivers, mangrove swamps and the like.

All members of the Scatophagidae have paired poison glands associated with each fin spine. Careless handling can result in a painful puncture, which may ache for many hours. The fish are not aggressive, however, and do not actively attempt to inflict wounds. They typically lie flat when handheld, but dart away when returned to the water. Cameron and Edean (1970) describe the poison glands.

Characteristics of the family Scatophagidae include: a deep, strongly compressed body; a small head and mouth, small jaw teeth in bands; small ctenoid scales; a distinct lateral line, running parallel with the back profile; a continuous dorsal fin; and 4 anal spines.

Several forms of *Scatophagus argus* have been reported. These are either distinct species, or merely color morphs of the same species. One of the more popular aquaria forms is the tiger or red scat (*S. argus* var. *rubrifrons*). There are also green spotted scat, and the common scat. We only observed one variety in the Iloilo area during our investigations. The juveniles had dark vertical bands with varying amounts and intensities of red markings (Fig. II.1B). These fish were very similar to the *rubrifrons* scats described in aquaria literature. Adults were characteristically brownish-green with black spots (Fig. II.1C). We observed the red-marked juveniles develop into the typical spotted adults. This suggests that the *rubrifrons* is not a distinct variety of scat, but merely a developmental stage of the common spotted scat.

II.2. Distribution of Spotted Scat

The spotted scat is widespread and common in many places throughout its range. We

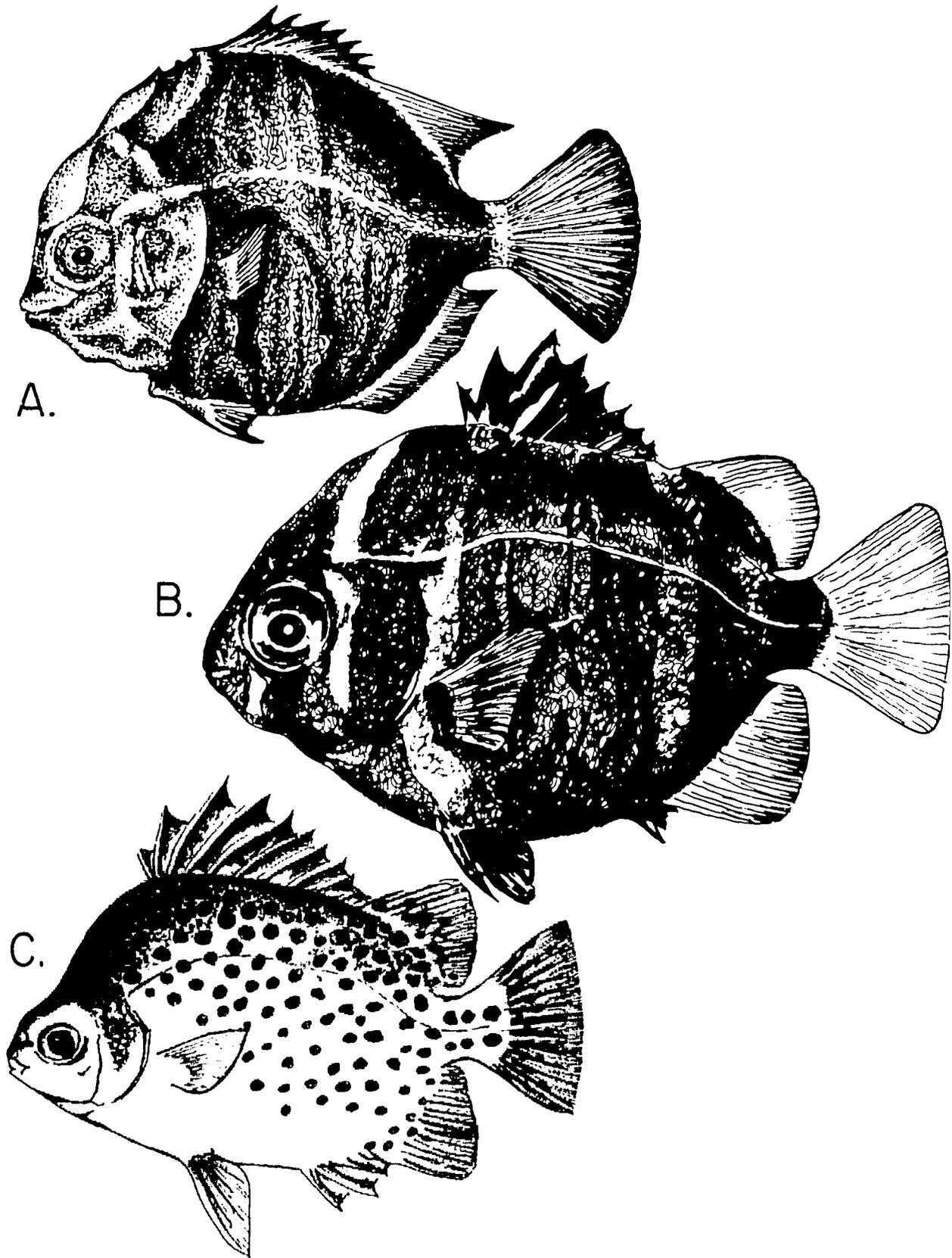


Fig. II.1. (A) *Tholichthys* larvae of the spotted scat (*Scatophagus argus*), (actual size, 0.60 to 1.2 cm, total length); (B) Juvenile of the spotted scat, (actual length, 1.2 to 4 cm); (C) Adult male spotted scat.

have personally seen *S. argus* in many locations in the Philippines, Taiwan, Thailand and Yap. Its reported range includes: freshwater, brackishwater and marine habitats of the Indo-Pacific, South and Southeast Asia, the Malay Archipelago, the Philippines, and Australia.

Spotted scat are known to have a very wide salinity tolerance range. They are found in waters ranging from fresh to greater than seawater (35 ppt). Aquarists have long recognized this euryhaline quality of the fish, but still generally recommend that the aquarium water be "hard" freshwater, or that salt be added. A typical recommendation is: "mix 3 or 4 teaspoons of salt per 2 gallons of freshwater, and increase the salinity as the scat grows."

II.3. Spotted Scat Field Collections and Fish Survivorship in Iloilo Studies

As part of our research efforts on spawning induction and pond growout, we collected large numbers of fish from the wild. In so doing, we learned much about the condition and habits of wild fish. This information is valuable for fish culture since it will establish breeding and stocking schedules based on wild-caught fish. At the same time, environmental and physiological data on wild-caught broodstock will provide clues on those conditions which trigger successful reproduction in nature. This information should allow us to develop more reliable and physiologically sound means to propagate the scat.

We observed stomach contents of wild-caught broodstock during our field collection, and observed feeding habits and preferences of scat held in aquaria and cages. Although a detailed study of the natural food preferences of the spotted scat was not made, routine inspection of the gut contents of fish which died soon following capture revealed that adult scats are primarily herbivorous. Their guts were typically packed with unidentified plant material. A green filamentous algae ("lumut") which grows floating or attached along the seashore in brackishwater, and a brown seaweed often found attached to the roots of mangrove trunks were commonly observed (Fig. II.2). Our principal fish collector told us that each year, he observes large spotted scat entering shallow water to feed on filamentous algae during March, April and May, before the rainy season begins. He said that this algae is abundant then, but disappears after the rains begin and the salinity drops.

We present our "field-generated" data here, not only because they relate to the natural history and biology of the spotted scat, but also because they set the stage for our "laboratory-oriented" studies which follow.

II.3.a. Scat Broodstock Field Collections. Broodstock were collected by one of two methods from the southern coastal waters of Panay Island, Philippines (Fig. II.3). With one method, known as the tambon method, nets were used to surround a floating aggregation device. The device was anchored at least two weeks beforehand in water 1.5 to 2 m deep at low tide, and typically within 0.5 km of the shore. The second method known as the barrier net technique, used a long net to enclose approximately three hectares of sand and mud flat immediately adjacent to a mangrove swamp at the mouth of a large river. The net was set after the fish entered the area at high tide. Fish were captured within the enclosure after the water receded and exposed the sand and mud bottom (Fig. II.4).

Captured scat were transported to the Iloilo State College of Fisheries (ISCOF), Barotac Nuevo, Iloilo, Philippines in plastic bags under oxygen. At ISCOF, all fish were acclimated to the appropriate salinity, treated for disease or injury as required, and on the day following capture, individually weighed (± 0.1 g), and measured (standard length, ± 0.1 cm) under anesthesia (0.3 ml/l 2-phenoxyethanol). Females longer than 14 cm and males longer than 12 cm (sizes when sexual maturity is attained) were individually tagged with colored beads sewn with nylon line through the dorsal musculature of the fish between the third and fourth dorsal spines. Oocyte samples were taken by cannulation from female fish using a 0.87 mm I.D., 1.27 mm O.D. polyethylene tube. Oocytes were preserved in a 0.1 M formalin solution with 0.60% NaCl, and their diameters measured within minutes to the



Fig. II.2. Mangrove area adjacent to the mouth of the Jalaud river, Zarraga, Iloilo, Philippines. Broodstock spotted scat captured in this area were often full of the unidentified seaweed shown in this photograph attached to mangroves.

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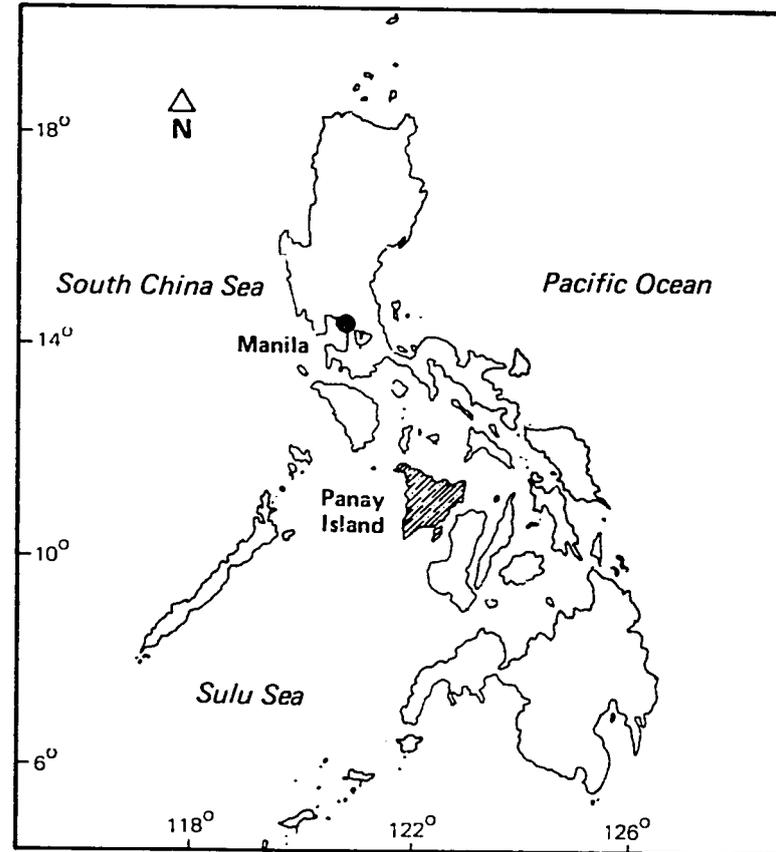
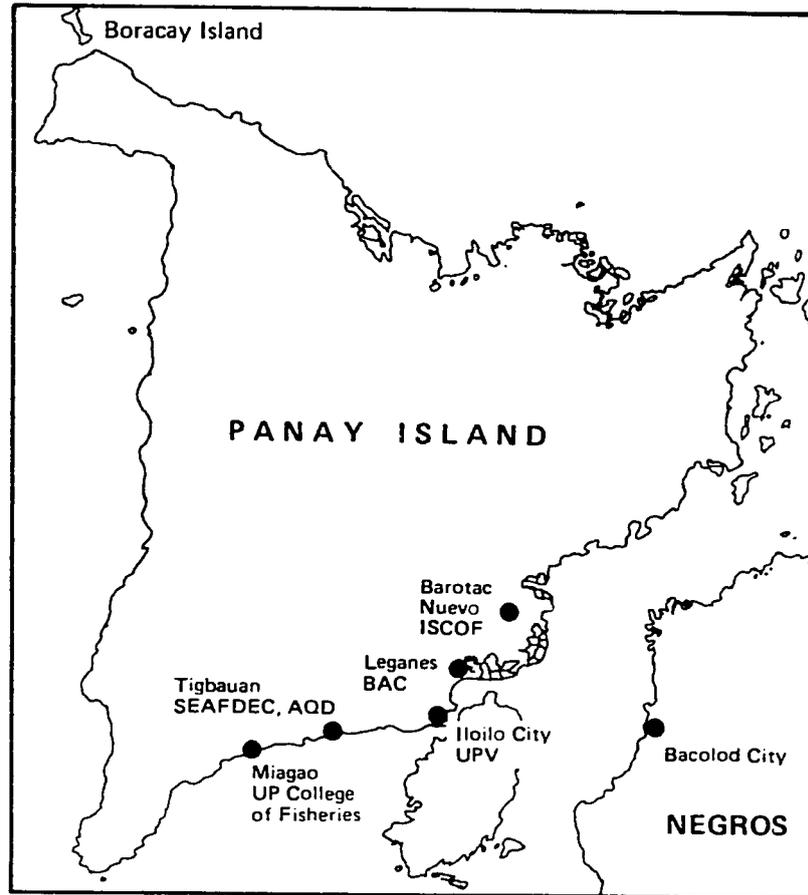


Fig. II.3. Maps showing the location of Panay Island within the Philippine archipelago, and the locations of the University of the Philippines College of Fisheries (UPVCF) in Miagao, the Southeast Asian Fisheries Development Center Aquaculture Department (SEAFDEC, AQD) in Tigbauan, the University of the Philippines in the Visayas (UPV) in Iloilo City, the University of the Philippines Brackishwater Aquaculture Center (UPV-BAC) in Leganes, and the Iloilo State College of Fisheries (ISCOF) in Barotac Nuevo. Most of the spotted scat used in the project were captured in the nearshore waters of Leganes and Barotac Nuevo.



Fig. II.4. The barrier net method of capturing spotted scat broodstock. The net was set at high tide when the fish came inshore to feed. The fish were captured within the net enclosure at low tide after the water had receded.

nearest 0.01 mm using an ocular micrometer. The position of the germinal vesicle was observed after fixing the oocytes in a clearing solution which made the opaque cytoplasm transparent. The clearing solution consisted of 85% ethanol, 10% acetic acid, and 5% formalin. Dimethyl sulfoxide (DMSO) was sometimes used to clear the oocytes. Male fish were sampled for running milt by gently squeezing their abdomens, and were classified according to the following scale: (0) = no milt; (+) = small volume of milt after repeated squeezes; (++) = large volume of milt after a single squeeze. The fish were stocked in hapa nets and floating cages in the ponds, or held in one-ton (1 m³) concrete, or nine-ton (9 m³) canvas tanks. Fish were fed commercial shrimp (35% protein) or milkfish (27% protein) pelleted feeds. Their diet was supplemented with filamentous and benthic algae (lab-lab).

II.3.b. Scat Fry Field Collections. Fry were usually collected by children using handheld, V-shaped, or circular nets, approximately one meter long, and made of bamboo and mosquito netting. The fry were caught along the seashore in the vicinity of the mouth of the Jalaud River, or along the banks of the river near its mouth. Most fry were caught at high tide. Fry were transported to ISCOF in plastic bags under oxygen. They were placed in 250-l plastic tanks, and fed artemia and filamentous algae for several days while acclimating slowly to the pond water salinity, before stocking into the experimental ponds, tanks, or aquaria. Bañada (1981) reports that spotted scat fry are only occasionally captured using traditional Filipino milkfish fry collecting techniques. This was also our finding.

II.3.c. Field Capture Data of Broodstock Scat. The length of spotted scat captured by the broodstock collection methods typically ranged between 7.5 and 25.5 cm (Table II.1). The smallest fish was less than 1.5 cm, and the largest was 28 cm. Monthly mean length varied between 4.3 and 16 cm.

During 16 months of collection, we captured a total of 1,162 scat by the broodstock collection methods. The total number of female scat captured each month always exceeded the total number of males. The overall ratio was 3.1 females captured for every 1.0 males (785/243), but the monthly female to male ratio ranged from 1.3 to 5.8 (Figs. II.5 and II.6). Arrunyasemsuke (1975) also reports that few males were captured during sampling for spotted scat in the Gulf of Thailand.

II.3.d. Broodstock Survival. Fig. II.7 shows the cumulative number of fish caught and maintained alive between April 28, 1987 and July 10, 1987. The differences between the number of fish in each line represents mortality following capture. Greater mortalities were encountered if the fish were captured using the tambon method. These fish were subjected to much more handling stress than the fish captured with the barrier net technique. The latter were in excellent condition when brought to ISCOF.

II.3.e. Fry Survival. Fig. II.8 shows the survivorship of all fry held in the hatchery before they were stocked into ponds. A high mortality rate of 38% occurred during the first three days following capture. Twenty percent of the fry died between the second and third day. Over the next seven-day period, however, the mortality rate declined significantly; an average of 1.43% of the remaining fish died daily. The initial high rate of mortality may be due to the stresses associated with capture, transport, and acclimation to a new food source. As discussed in the pond growout section, mortality after stocking in the pond for growout was low.

II.4. Food Preferences of the Spotted Scat

Literature aimed at the home aquarist indicated that spotted scat will accept a wide variety of food items, but that they have strong preferences for "vegetable matter". There is some feeling that plant material is even essential for long-term survival and growth.

TABLE II.1. STANDARD LENGTH-FREQUENCY DATA FOR ALL SCATOPHAGUS ARGUS CAPTURED BY THE "BROODSTOCK" COLLECTION METHODS BETWEEN JULY, 1986 AND OCTOBER, 1987

Class Limits Lower - Upper	Class Midlength	MONTH												
		JUL	SEP	OCT	NOV	APR	MAY	JUN	JUL	AUG	SEP	OCT		
0.5 - 1.49	1											1		
1.5 - 2.49	2													
2.5 - 3.49	3													
3.5 - 4.49	4													
4.5 - 5.49	5										1			
5.5 - 6.49	6													
6.5 - 7.49	7													
7.5 - 8.49	8				1	3	7	6	4	1	1		1	
8.5 - 9.49	9	1	1	1		10	13	12	20	8		1		
9.5 - 10.49	10	1	3	8		4	18	29	17		1			
10.5 - 11.49	11	5	5	9	1	20	19	25	49	23	1			2
11.5 - 12.49	12	9	16	14	1	16	17	22	43	13	5		3	
12.5 - 13.49	13	12	8	16	2	15	11	18	26	8	1		4	
13.5 - 14.49	14	6	12	10	3	10	4	5	12	11	3		8	
14.5 - 15.49	15	2	13	9		7	4	9	14	4	8		1	
15.5 - 16.49	16		6	6		9	2	4	9	3	6		4	
16.5 - 17.49	17	3	8	4		10	4	3	11	8	2		4	
17.5 - 18.49	18		8	5		11		41	7	5	5		2	
18.5 - 19.49	19		7	4		4	2	5	12	3	3			
19.5 - 20.49	20	4	8	9		1		3	10	1	1		3	
20.5 - 21.49	21	2	13	5		1	1	1	9	3	1			
21.5 - 22.49	22	2	4	6		1	1	1	3	1	1		1	
22.5 - 23.49	23		7	5		1	1		3	1	1			
23.5 - 24.49	24	1	4	2					3	1	1			1
24.5 - 25.49	25		2	0						1				
25.5 - 26.49	26		1	1										
26.5 - 27.49	27		1	0						1				
27.5 - 28.49	28		1	0										1
TOTAL		48	128	114	8	123	104	184	262	116	41		34	
MEAN LENGTH		14.3	16.0	14.4	4.3	13.6	11.2	12.3	13.2	12.8	15.4		15.8	

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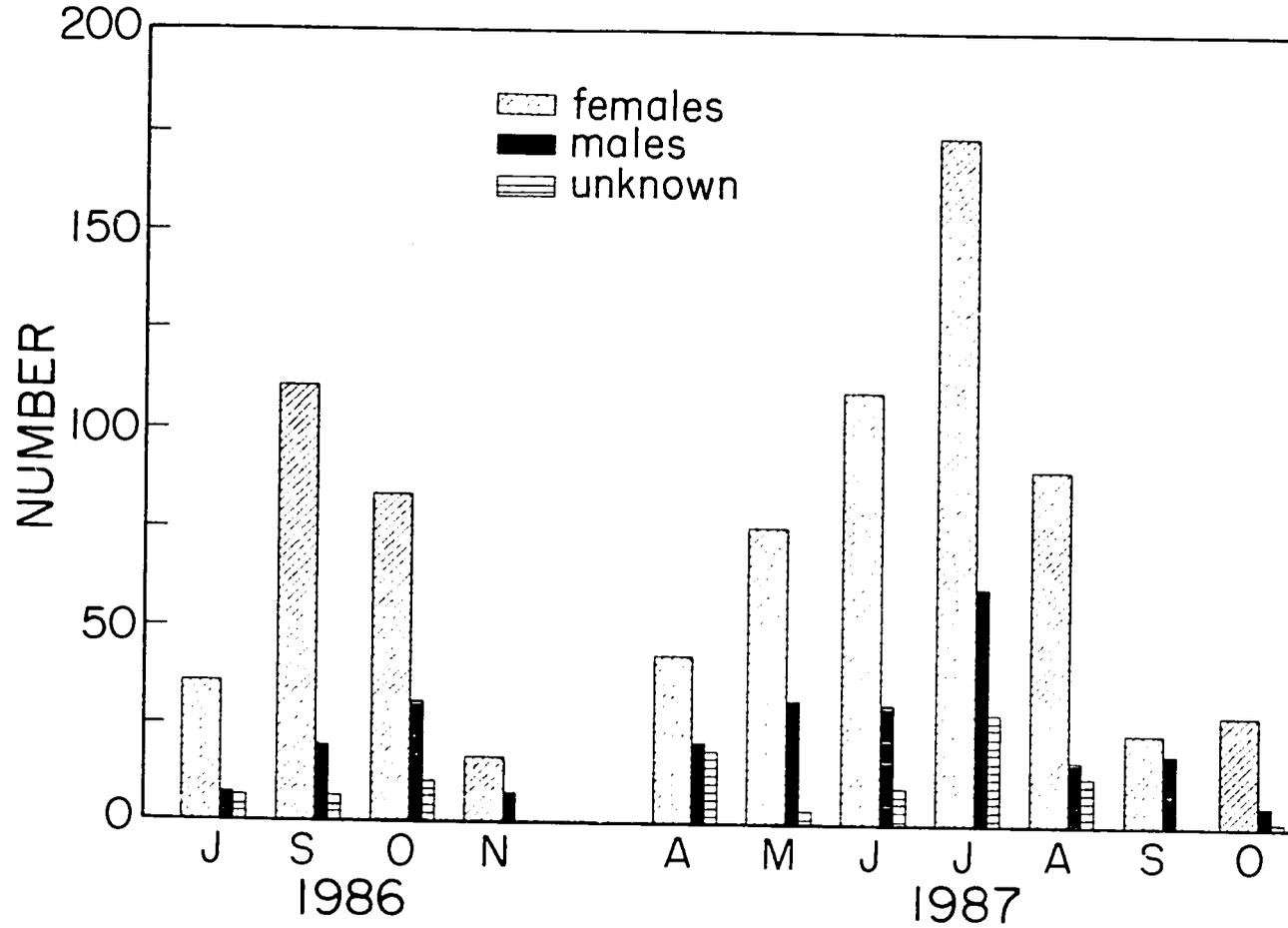


Fig. II.5. Total numbers of female, male and scat of unknown sex captured by the broodstock collection methods between July 1986 and October 1987.

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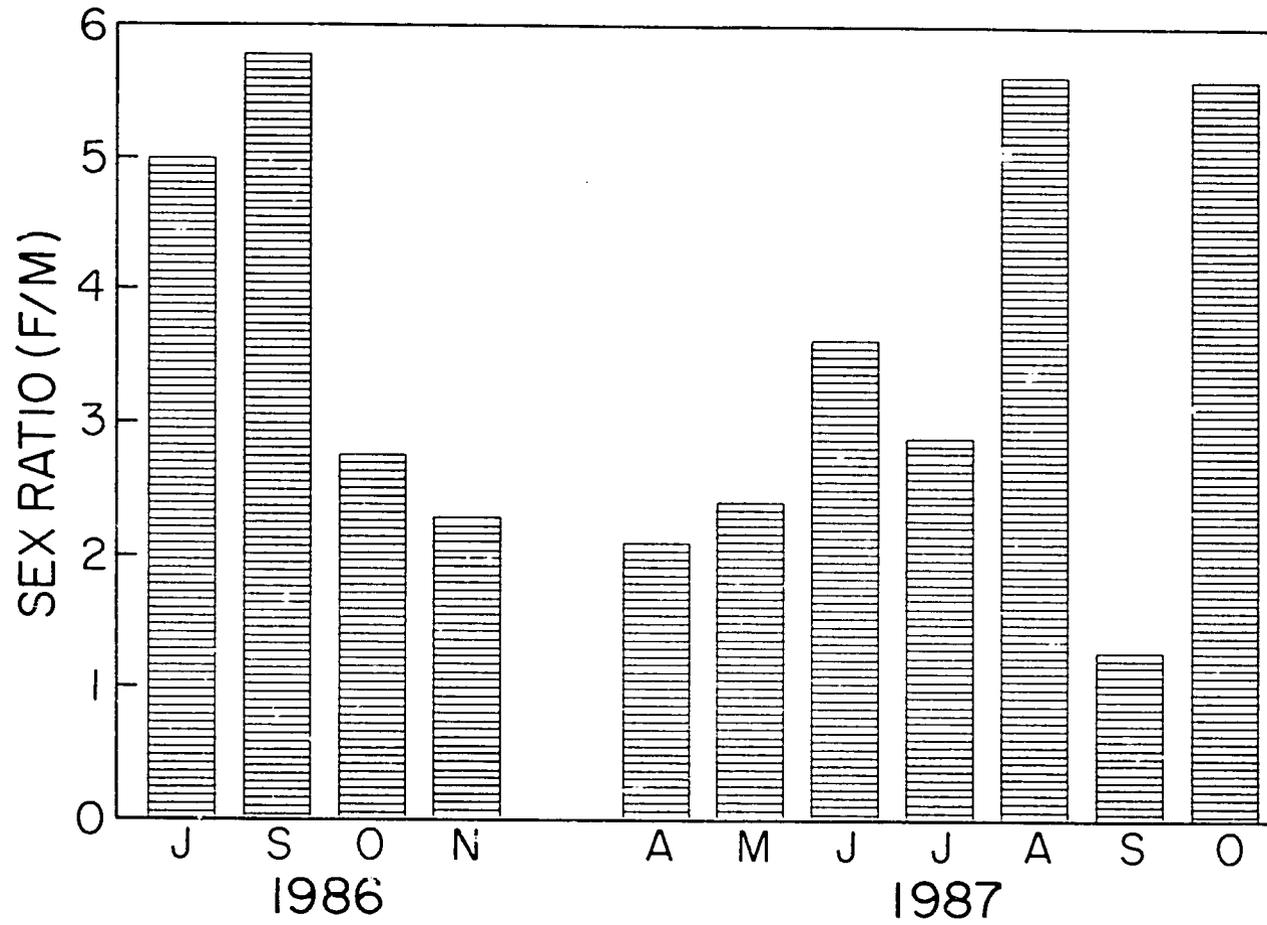


Fig. II.6. Female to male sex ratio of spotted scat captured by the broodstock collection methods between July 1986 and October 1987.

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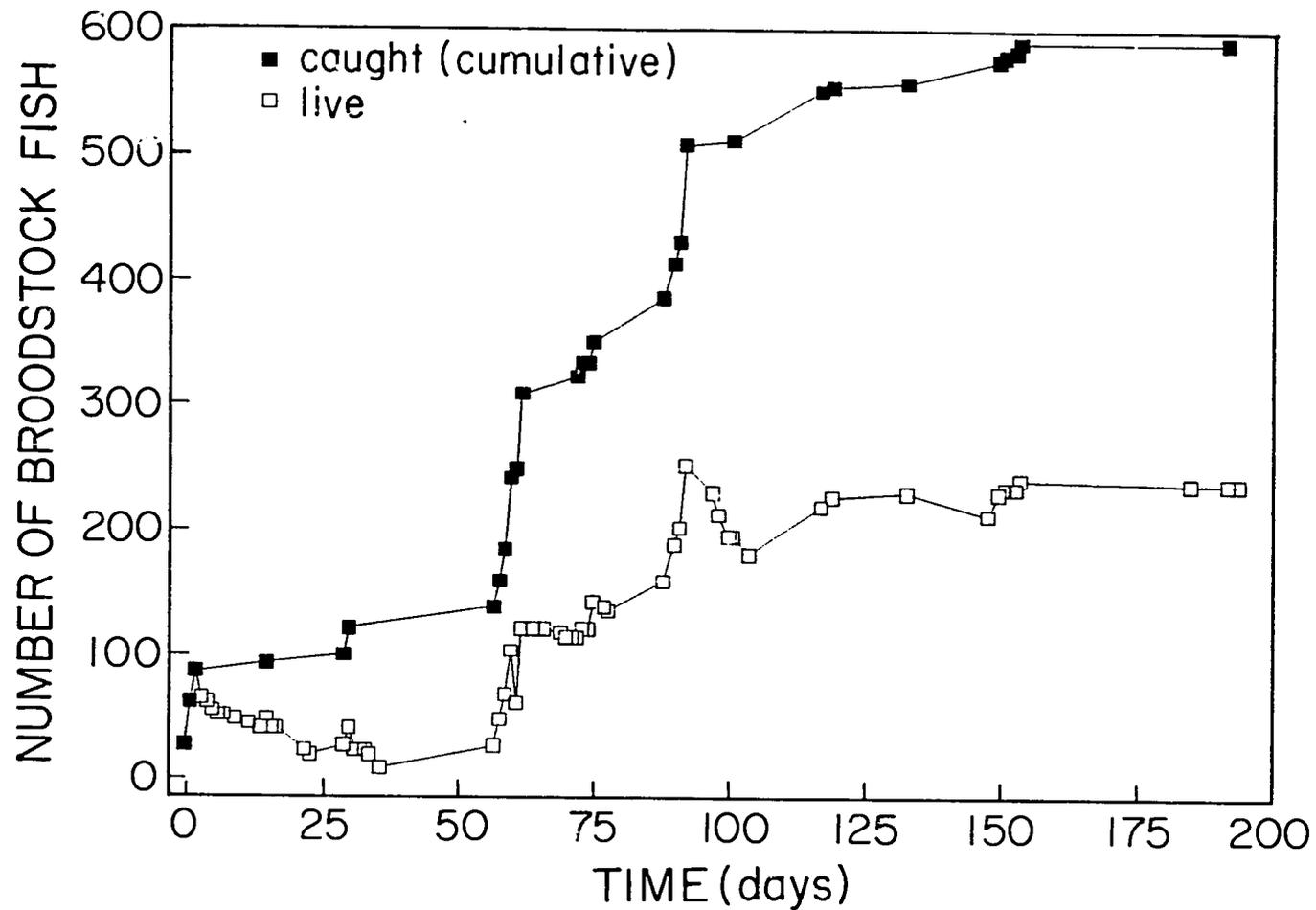


Fig. II.7. The cumulative number of spotted scat broodstock caught between April 28, 1987 and July 10, 1987, and the number of living broodstock held at ISCOF during the same period. The differences between the two lines are the result of post-capture mortalities.

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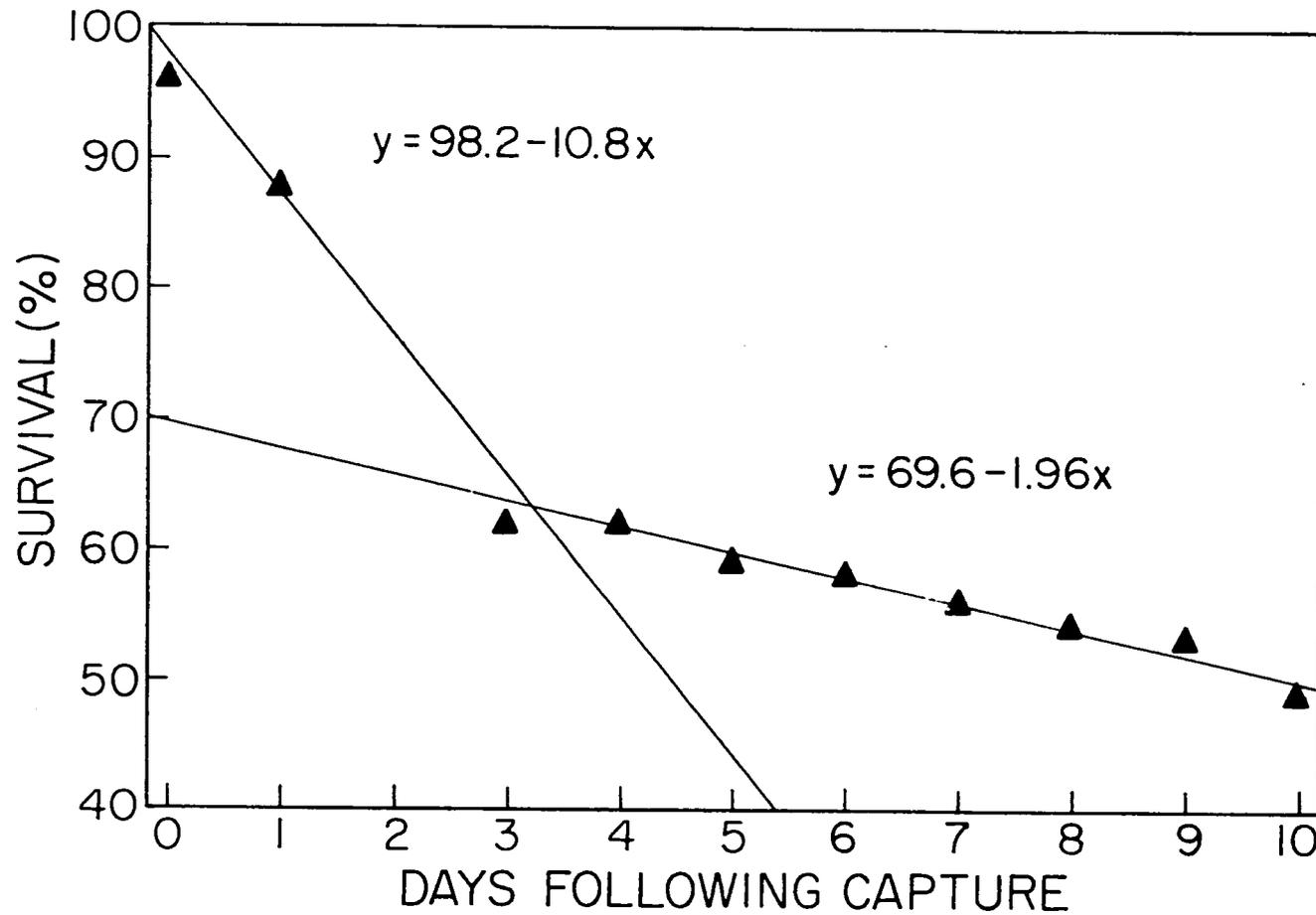


Fig. II.8. Survival of spotted scat fry held in tanks at ISCOF during the first 10 days following capture from the wild. A total of 19,019 fish were captured and evaluated between June and October 1987. Regression lines were fitted through the points representing the percent survival from days 0 to 3, and from days 3 to 10. The slopes of these lines indicate the mortality rates of captured fry over these two time periods.

Food recommended for aquarium fish include: boiled lettuce or spinach, steeped oatmeal, and common aquaria plants such as *Nitella* or *Riccia*. Scat will also take water fleas (*Daphnia spp.*), worms, insects, and dried feeds.

Winfree and Sultzman (unpublished report) conducted feeding trials with aquaria-held *S. argus*. They found that "scat rarely refused feed when squid, krill or filamentous algae *Enteromorpha* were included in the diet." These diets, however, did not support rapid growth. They found that "fish fillet, beef liver and chicken fillet were superior to the former ingredients in supporting growth." Their scat also preferred a soft or firm pellet with a 60% moisture content, rather than hard pellets. On their better diet formulation, scat grew from 2 g to 15.1 g in 6 months.

As noted earlier, many authors refer to the offal and fecal feeding habits of the scat. Their acceptance, and/or preference for these items has not been confirmed.

Structural adaptations to the scat's herbivorous diet are: 1) its long, coiled intestine, which in adult scats was found to be approximately 3.5 times the body length (Fig. II.9), and 2) small, sharp teeth used for scraping and shredding plant material.

Captive scat of all sizes readily ate filamentous algae. After approximately one week in captivity, scats began to accept pelleted commercial feeds, and eventually accepted them readily. They always preferred algae, however, if both foods were offered together. Fish kept in floating cages in the pond were observed nibbling on the algae which grew on the sides of the cages. Postmortem examination of cage-confined, pellet-fed fish revealed that their guts were often full of algae eaten from the sides of their cage. These fish generally thrived, probably because of the nutritional benefit they received from this additional food source. Captive scats would also feed on the benthic flora and fauna common in milkfish ponds ("lab-lab"). Although the fish appeared to prefer filamentous algae over "lab-lab", they nevertheless accepted it readily, and as the pond growout studies indicated, their growth rate and survivorship were good when "lab-lab" was their main food source (Biona et al., VII, this volume). Captive fry and juvenile scat were also observed to feed readily on zooplankton, artemia, and mosquito larvae.

II.5. Growth and Length-Weight Relationships of Spotted Scat

II.5.a. Length-Weight Relationship. The length-weight relationships for male and female spotted scat are shown in Fig. II.10. The relationships, which differed between the sexes, were derived using individual length and weight measurements of 252 male and 797 female scat. The graphs indicate that once males are more than approximately 15 cm in length, they tend to be heavier than females of equal length. Males longer than 15 cm have reached sexual maturity, and the weight differences between the sexes may be due to the anabolic actions of endogenous androgens in males (see Cruz and Barry, X, this volume).

The biggest fish captured was a female 28 cm long and weighing 1.20 kg. The largest male captured was 27 cm long and weighed 1.05 kg. Khan (1979) reported a spotted scat 33.4 cm in total length, weighing 1.2 kg. The equations to estimate the average weights of male and female scats, given their standard length, are as follows:

$$\text{Males: Weight} = 0.07058 \times (\text{Length}^{2.881})$$

$$\text{Females: Weight} = 0.18976 \times (\text{Length}^{2.526})$$

$$\text{Both sexes: Weight} = 0.17587 \times (\text{Length}^{2.552}).$$

The exponents of these equations are all less than 3, which was expected considering the laterally compressed body form of the scat.

II.5.b. Age and Growth Predictions. The von Bertalanffy growth equation:



Fig. II.9. The coiled gut tract of an adult spotted scat 22 cm in standard length. The specimen was a female; note the ovary (o), and the head shape.

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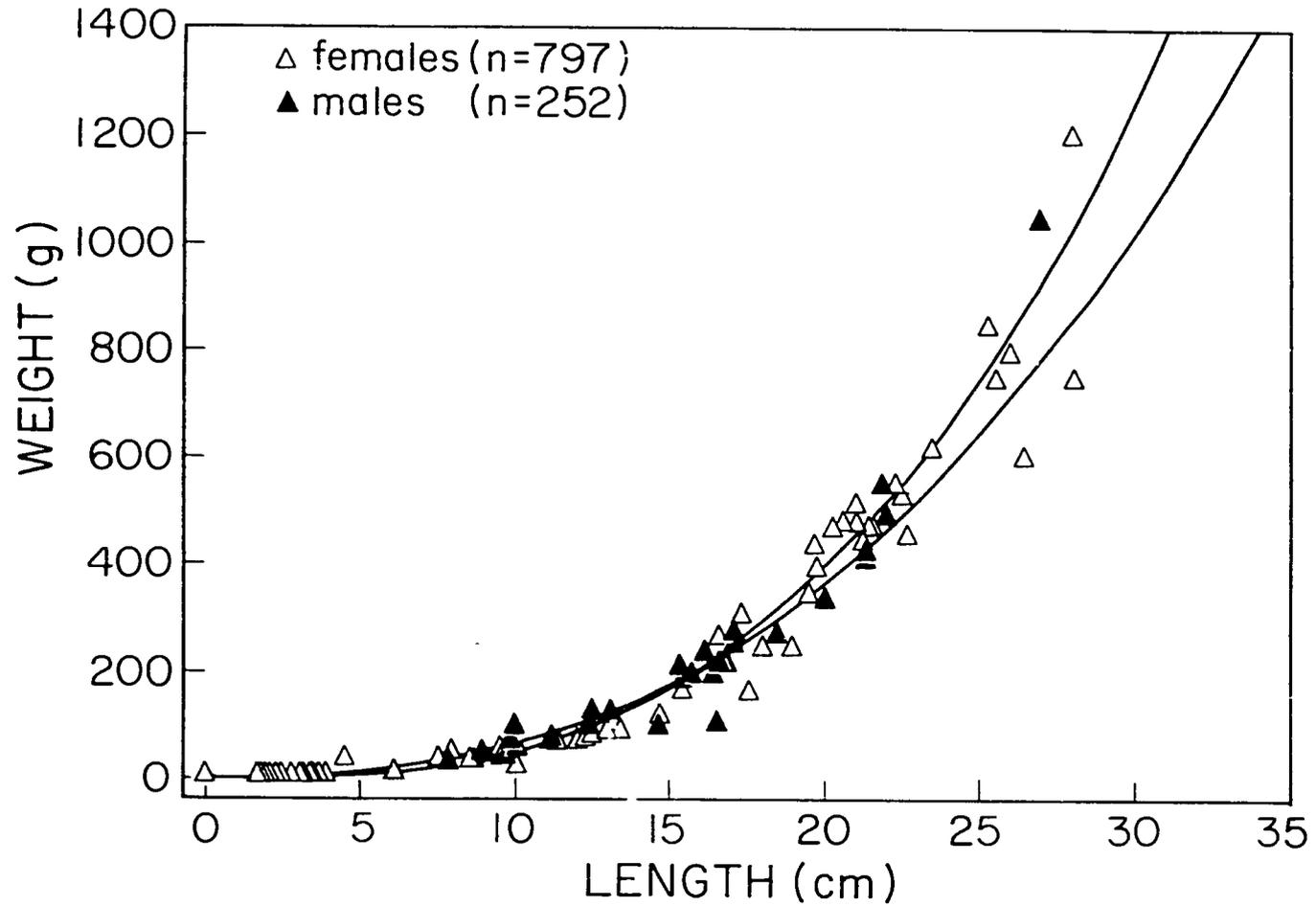


Fig. II.10. Length vs. weight relationship for male (upper line, solid triangles) and female (lower line, open triangles) spotted scat. To generate these curves, length and weight measurements of 797 females and 252 males were taken. Only representative points are shown.

$$L_t = L \times (1 - e^{-K(t-t_0)});$$

where L_t is the mean length at time t , L is the average maximum length attainable, K is a growth constant which describes the rate at which L_t approaches L , and t_0 is the age of the fish when its length was zero, is used to describe the indeterminate growth of fishes. The parameters L and K for the spotted scat have been estimated from previously published length-frequency data (Ziegler, 1979; in Ingles and Pauly, 1984) using the ELEFAN I program (Ingles and Pauly, 1984). The estimates are $L = 25.0$ cm and $K = 1.20$. Fig. II.11 shows the von Bertalanffy growth curve generated using these parameters. Another curve was also generated setting $L = 30$ cm. The value of t_0 can not be estimated from the length-frequency data, therefore, the graphs do not give the ages of fish that are a given size, but only give a relative age.

II.6. Size at First Reproduction of Spotted Scat Captured in Iloilo

The size when females become sexually mature was determined by plotting their gonad weight as a function of standard length (Fig. II.12). The rise in gonad weights for fish longer than 14 cm (heavier than approximately 150 g) indicates that this is the size at which females first reach sexual maturity.

Plotting testes weight as a function of length did not reveal a pattern useful in determining the size of first reproduction in males. Testes weight did not differ significantly between small and large males (Fig. II.12). The smallest male found with running milt (++) was 11.5 cm long and weighed 83.5 grams; this may be a reasonable estimate of the size at first reproduction in male scat.

II.7. Sexual Dimorphism and Sex Determination in Spotted Scat

The sexes can be differentiated by head shape (Fig. II.13). The head profile of the female ascends at a relatively constant slope, whereas in the male, there is an obvious curvature of the head above the eye which is absent in females. This difference is more prominent in larger animals, but is noticeable in fish as small as 100 g. Other external features which aid in differentiating between the sexes are: 1) males tend to be heavier than females of equal length at lengths greater than 20 cm; and 2) females are often a lighter, olive-green color compared to males, which tend to have a darker color. The sex of an animal greater than 14 cm could be verified by attempting a gonadal biopsy using a polyethylene cannula. A cannula could always be easily inserted into a female's oviduct. It was not possible to enter the sperm ducts of males.

II.8. Spawning Season of Spotted Scat in the Iloilo Area

Based on the clues provided by our broodstock and fry capture data, and from weather observations, we have tried to determine the natural spawning season of the spotted scat.

Broodstock scat were collected during July, September, and October 1986, but not during August 1986, nor from November 1986 through March 1987. Broodstock were again collected during April through October 1987. Analysis of oocyte diameters indicated that over 40% of the females with standard lengths greater than 14 cm (reproductive size) had mature oocytes (greater than 0.4 mm dia.) in July 1986; only 12% had mature oocytes by October 1986. During 1987, 30% of the females 14 cm or larger had mature oocytes in April. This decreased to less than 10% in August. The percentage of mature females ranged between 7% and 44% for the remaining months in 1987 (Fig. II.14).

Fry were collected from the Jalaud River primarily by one fisherman's family who lived on the river. The family was in intimate contact with the river, and due to their continuous observations, were aware of when fry were abundant. When they noticed scat fry present, they made a major effort to collect fry to sell to our project. Fry collection data indicates that scat fry were abundant between August and October 1986, and from June

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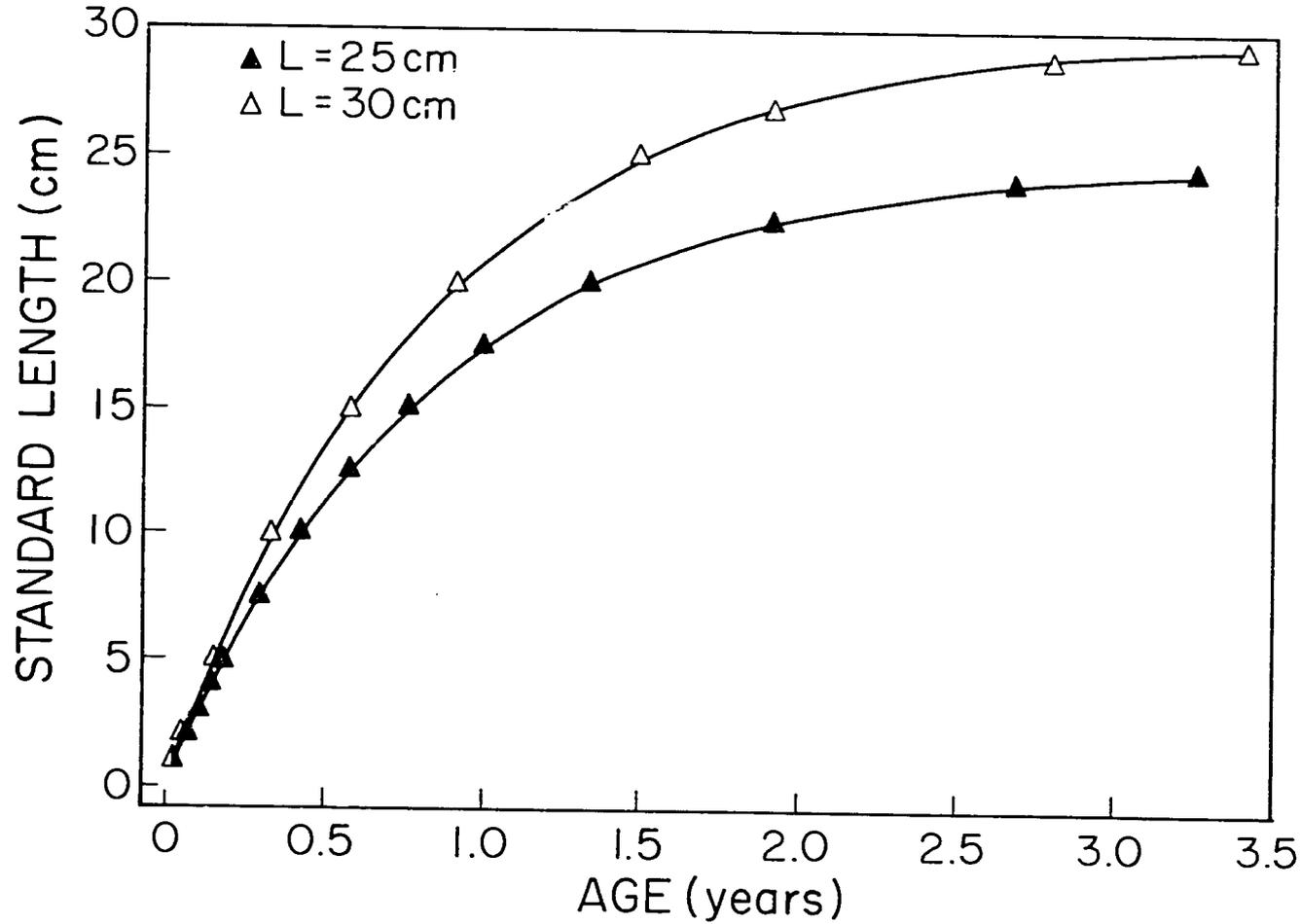


Fig. II.11. Growth curves generated for spotted scat using the Von Bertalanffy growth equation with assumed average maximum lengths (L) of 25 and 30 cm, and a growth constant of K = 1.2 (Ingles and Pauly, 1984.).

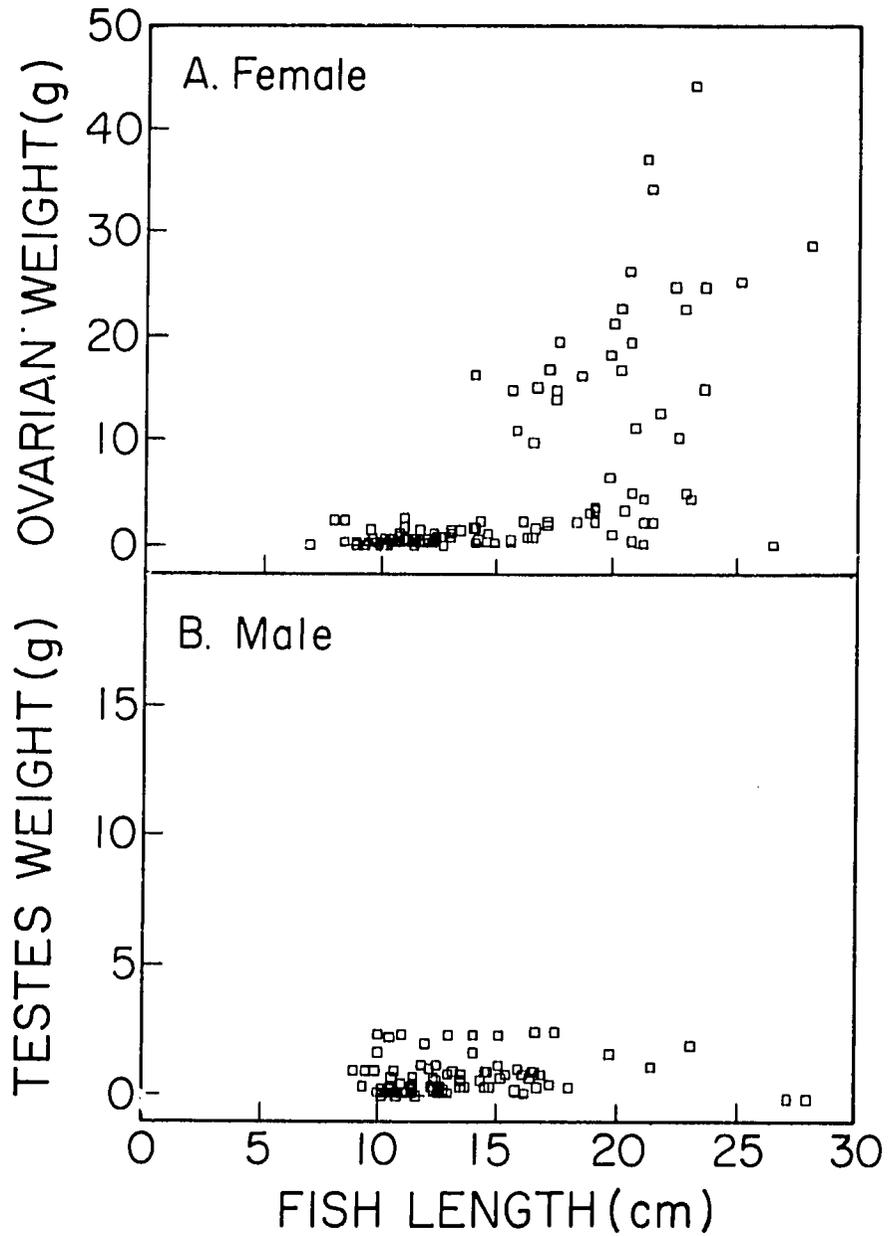


Fig. II.12. Relationship between ovarian weight and standard fish length of female spotted scat (A), and testicular weight of male spotted scat (B). Fish were captured between July 1, 1986 and October 30, 1987. Standard lengths and gonad weights of 140 females and 90 males were taken.



Fig. 11.13. The head profiles of a female (upper) and male (lower) spotted scat showing the sexual dimorphism which can be used to differentiate between the sexes.

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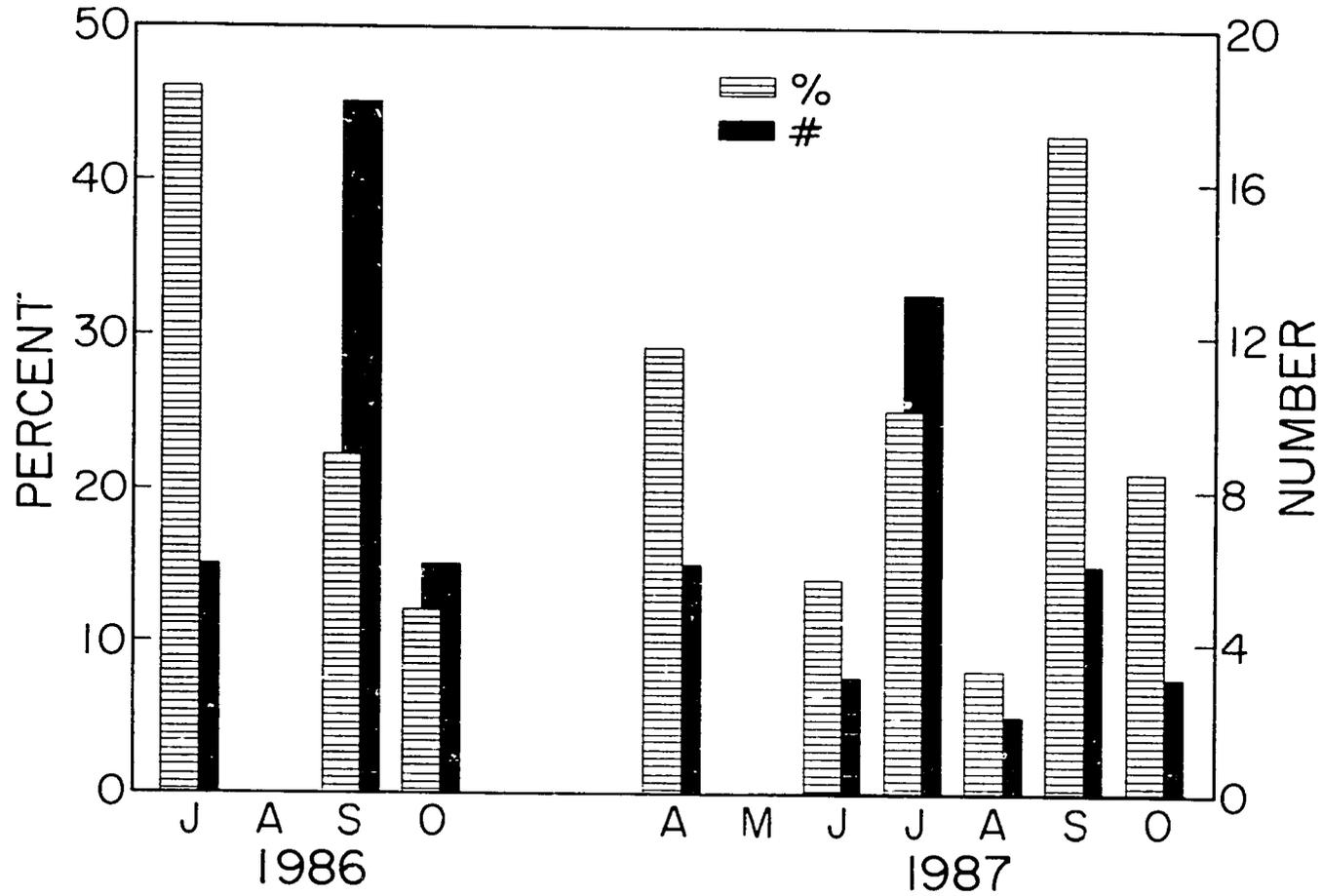


Fig. II.14. Number and percent of female spotted scat captured during 1986 and 1987, with standard length greater than 14 cm (size at first reproduction) and oocyte diameters of 0.40 mm or greater.

through September 1987 (Fig. II.15). The greatest single monthly collection was in August 1987 when over 18,000 scat fry were collected. The fry collection data for 1987 are most telling since they show no fry captured before June, with a sharp increase and decrease through the summer months. Our data for 1986 were less complete.

Weather data for 1986 and 1987 clearly indicate the presence of wet and dry seasons, and related temperature and wind conditions. During 1986, the rainy season began in June and continued through late fall (Fig. II.16). Monthly rainfall from January through May 1986 was less than 100 mm, but increased to more than 300 mm in June, and to 900 mm in August. From January through May 1987, monthly rainfall was less than 50 mm, but increased to more than 150 mm in June and 450 mm in July 1987. Average monthly air temperatures were lowest during the winter months of December through March, and typically reached monthly maxima during April and May, just before the rains began (Fig. II.16). There was a sharp drop in air temperature in June, concurrent with the rainy season. The average wind directions by month also reflect the rainy season pattern. From December through May, wind direction is from WNW to NNE. During May and June, the wind direction shifts 180° as the SW monsoons begin, bringing rain (data from Philippine Atmospheric, Geophysical and Astronomical Services Administration, PAGASA).

From the above data, we conclude that December through March is a refractory period for the scat. The fish probably live offshore in deeper waters and have immature oocytes. We believe that oocyte maturation begins in March and April, and is stimulated by increasing water temperature, and perhaps day length. In April, a high percentage of the female scat of reproductive size have mature oocytes. At this time, the fish move inshore in preparation for spawning; however, as evidenced by the fry collection data, they probably do not spawn in any number. Spawning probably begins in June, and reaches a peak in July. This conclusion is supported by the fry collection data, and the broodstock collection data which shows a decline in the percentage of females of reproductive size with mature oocytes from July to August in both 1986 and 1987. In both years, the start of the rainy season correlates with the peak of the spawning season, suggesting that this environmental clue may be an important stimulus for spawning in the spotted scat. A high percentage of mature females were caught in September and October, suggesting that the spotted scat may undergo rematuration and spawn more than once in a single breeding season.

The SW monsoonal winds undoubtedly contribute to fry survival, and may help trigger spawning. Near Iloilo, the WNW through NNE winds tend to blow offshore, and to move plankton-rich waters away from the shore. The SW monsoons not only bring rain and runoff, but also blow plankton-rich water onshore. Plankton blooms are promoted by the rains which wash nutrients from the land into the sea. Thus, larval fish developing nearshore at the beginning of the rainy season should have an abundant supply of plankton-rich water.

In conclusion, in the spotted scat, oocyte maturation may be controlled by temperature, and perhaps photoperiod. Spawning may be triggered by rainfall, associated river outflows, and perhaps wind direction.

II.9. Fecundity of Spotted Scat Captured in Iloilo

The number of mature oocytes, 0.50 mm diameter or larger, in a pre-spawned female scat, is proportional to the weight of the fish. The highest number of eggs we found were from a fish that weighed 947 g and produced 807,000 eggs (Fig. II.17). The samples came from five fish whose eggs were counted following spawning and seven other fish which were accidentally killed, and whose oocytes were counted following removal of their ovaries. The best fit regression line for the data set is:

$$\text{Number of eggs} = -66,940 + (982.9 \times \text{Weight of the fish in grams})$$

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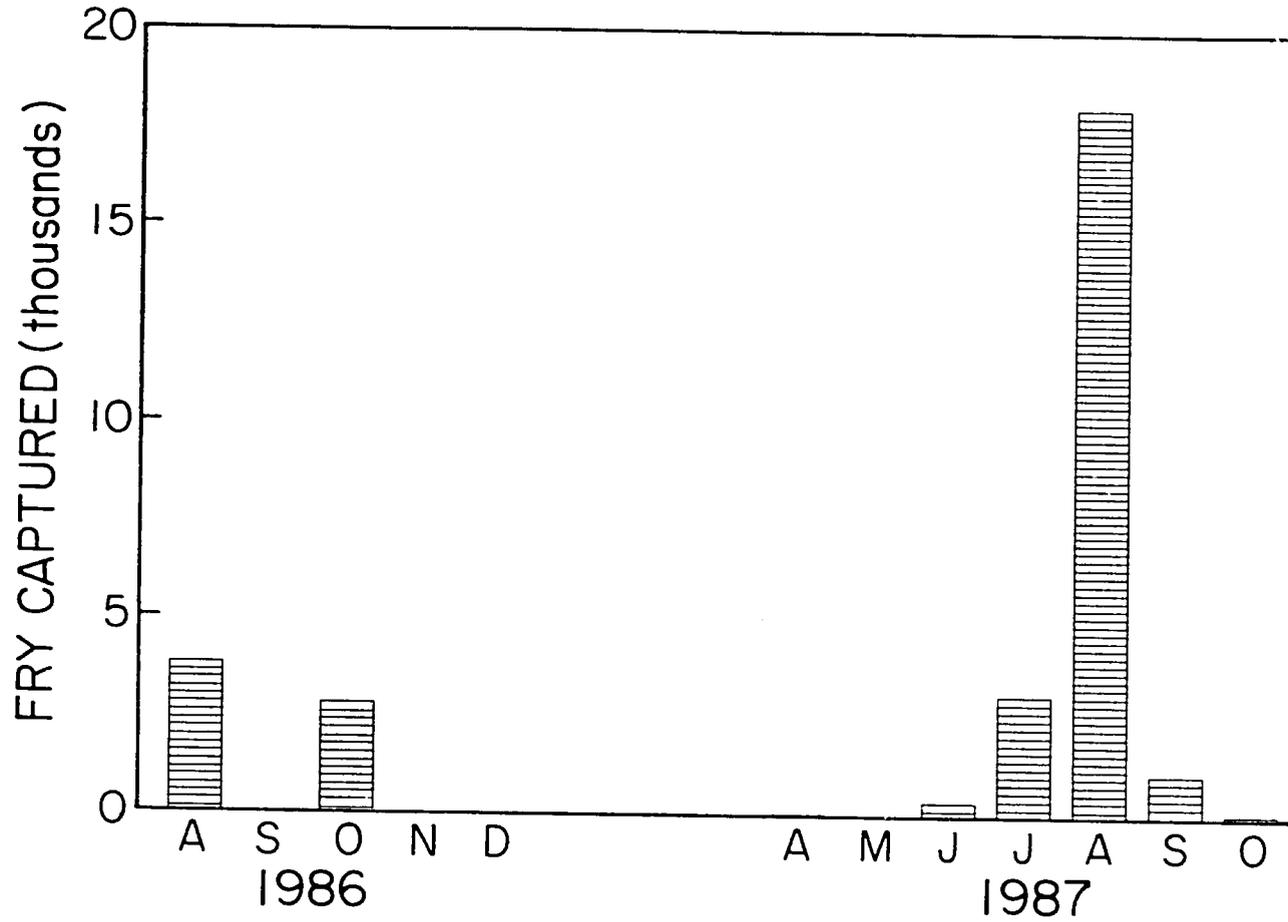


Fig. II.15. Total number of fry collected from the southern coastal waters of Panay Island, Philippines from August 1986 through October 1987.

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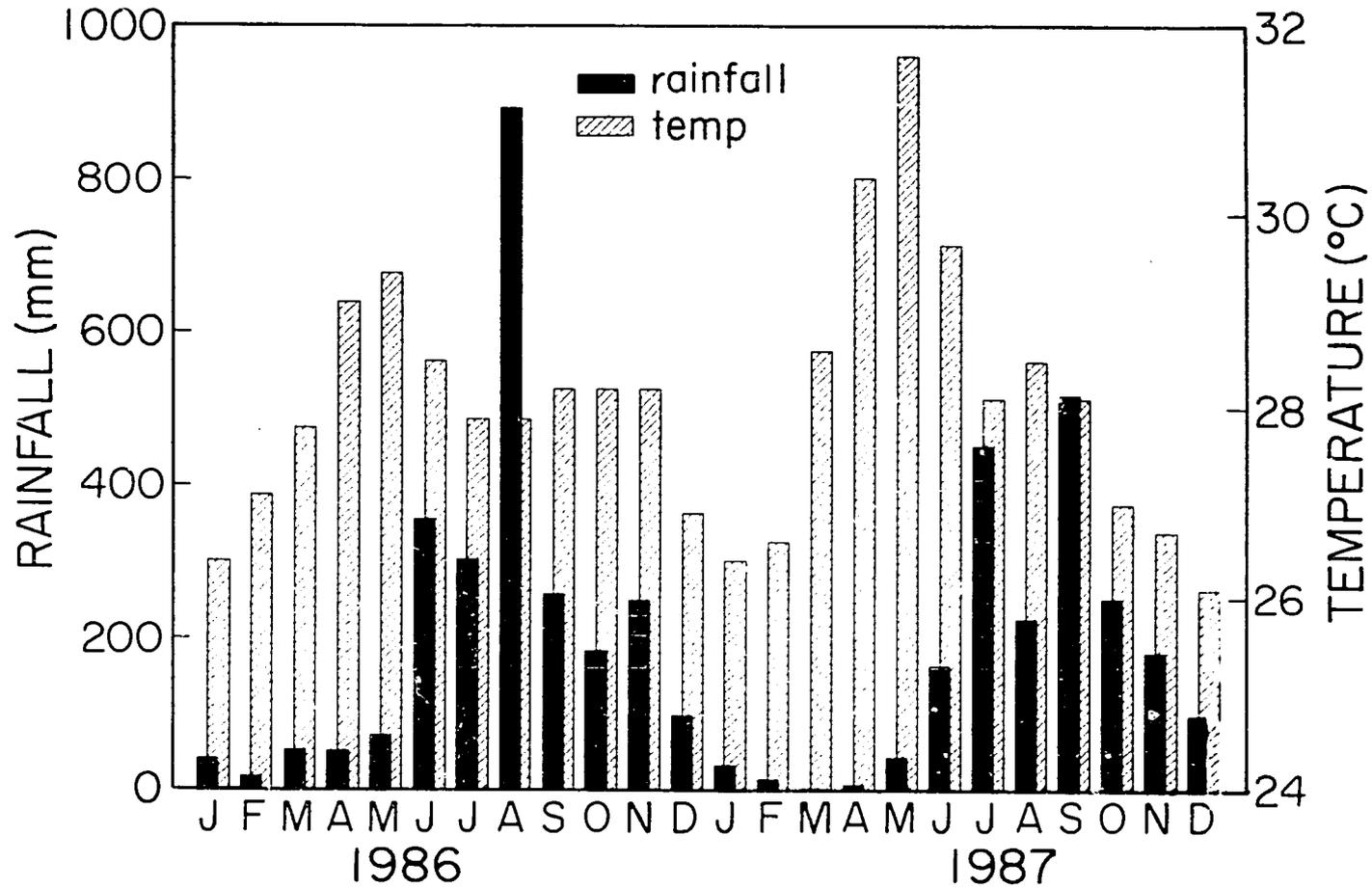


Fig. II.16. Total monthly rainfall and average monthly air temperatures at Iloilo during 1986 and 1987. Data is from the Philippine Atmospheric, Geophysical and Astronomical Services Administration (PAGASA) in Iloilo City, Philippines.

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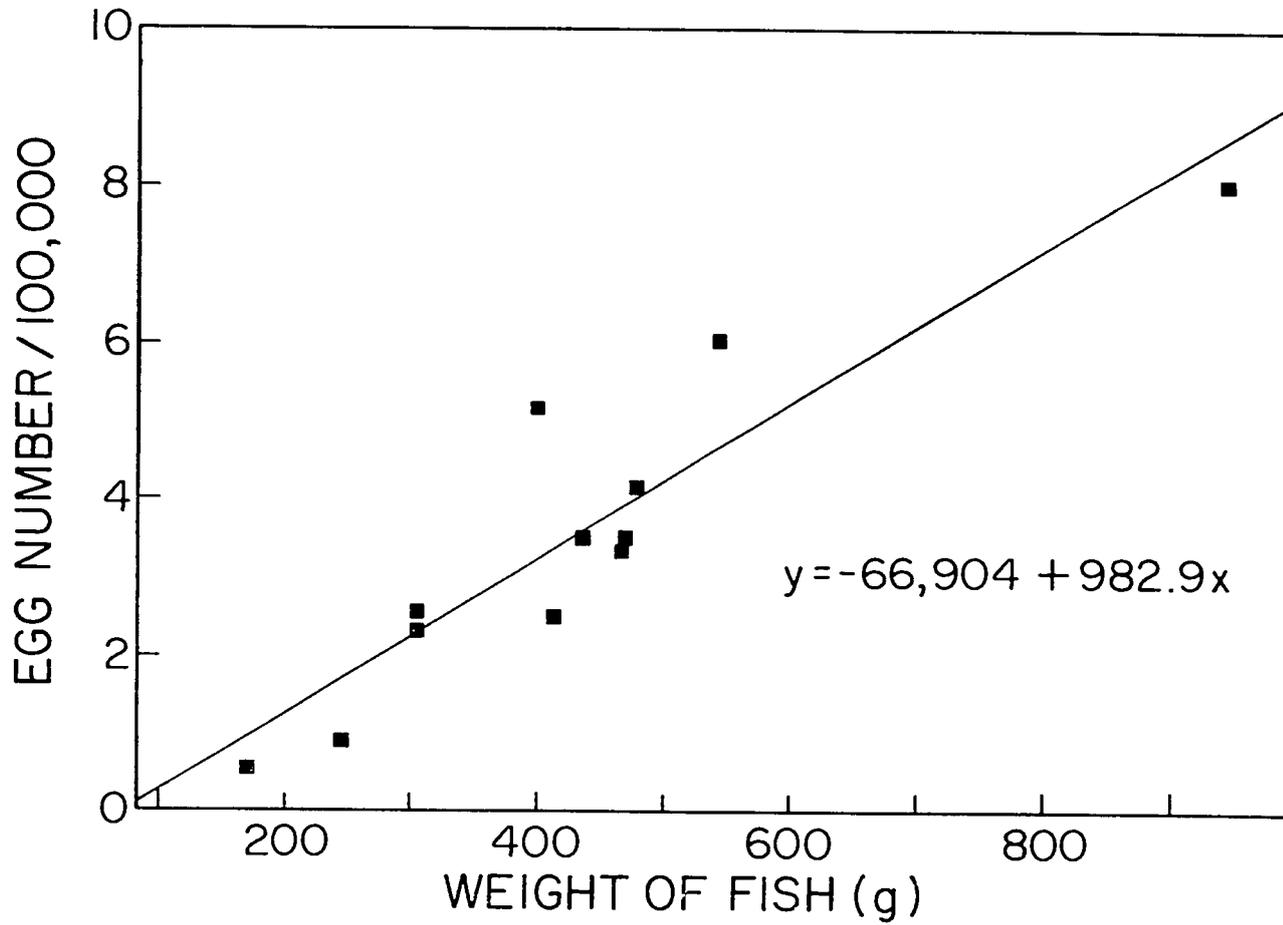


Fig. II.17. Fecundity of prespawned female spotted scat with oocyte diameters greater than 0.50 mm. A regression line, which can be used to predict the number of oocytes (y) which will be spawned by a female of a given weight (x), was fitted to the points.

II.10. Stages of Oocyte Development.

Classifications and descriptions of oocyte development in teleost fish can be found in Nash and Shehadeh (1980), and Nagahama (1983). The classification of the stages of oogenesis which follows was designed to serve as a practical guide for those interested in inducing final oocyte maturation and spawning in this species.

i) Primary Oocytes. Diameter: 0.04 to 0.10 mm (Fig. II.18A). The oocytes are white or transparent in color. The nucleus is visible and occupies most of the central area of the oocyte. Cannulated oocyte samples often included attached pieces of white ovarian tissue, and small bright orange granules of unknown function interspersed among the oocytes. Immature oocytes were abundant, and present year-round.

ii) Immature Oocytes. Diameter: 0.10 to 0.35 mm (Figs. II.18B-D). The oocytes have a uniform, granular cytoplasm. They are white or light yellow in color. Their surfaces are slightly textured. The follicle layer is not obvious when viewed with transmitted light. The germinal vesicle is visible after clearing, and located in the center of the cell. Oocytes in this stage may be in the yolk vesicle, and oil drop stages described by Yamamoto et al. (1965) (in Nagahama, 1983). Oocytes at this stage did not respond to LHRHa treatment.

iii) Maturing. Diameter: 0.35 to 0.60 (Fig. II.18E,F). The oocytes at this stage are uniformly yellow in color, and perfectly round. Yolk globules become incorporated into the oocyte and completely fill the cytoplasm. These small, membrane-bound globules give the cytoplasm a textured appearance (Fig. II.18E). When the oocytes are approximately 0.50 mm in diameter, oil droplet coalescence begins in their center. Initially, the process is only visible in oocytes treated with clearing solution. The germinal vesicle is centrally located, but begins to migrate (GVM) to the periphery with the start of oil droplet fusion. The yolk globules also begin to fuse at this time, and the globules filling the center of the oocyte become larger than those at the periphery. The follicle layer is clearly visible when viewed with transmitted light, and is approximately 0.01 mm thick. Short, twisted, cord-like structures of unknown function fill the area between the follicle and oocyte membranes (Fig. II.18F). Often a space can be observed between the two membranes, which is presumably an indication that ovulation is imminent. The center of the oocyte clears further as yolk globule fusion, and oil droplet coalescence progresses. The germinal vesicle continues its migration and approaches the oocyte periphery. At this stage, the smaller yolk globules at the periphery of the cell often appear translucent, and white, compared to the oocyte interior now dominated by several large yellow oil droplets (Fig. II.18F). Ovulation can occur in oocytes with diameters as small as 0.50 mm, as evidenced by the presence of post-ovulatory follicles.

iv) Final Maturation. Diameter: 0.60 to 0.75 mm (Fig. II.18G,H). The germinal vesicle reaches the cell periphery and breaks down (GVBD). Following ovulation, the oocyte, now an egg, undergoes a rapid increase in size as the result of hydration. The coalescence of yolk globules and lipid droplets continues from the center outward as the egg grows. The cytoplasm eventually becomes colorless and transparent prior to spawning. A single, large, yellow oil droplet floats to the oocyte surface after the yolk globules are completely fused (Fig. II.18I).

v) Atresia. Atresia is a process in which hypertrophied granulosa cells phagocytize the yolk, and the oocytes are resorbed. Atresia often occurs in fish subjected to stress (cf. Nagahama, 1983). Samples of atretic scat oocytes were characterized by the presence of broken cell membranes; freed, membrane-bound oil droplets; white, flocculent cellular "debris"; and a yellowish, oily fluid that is probably oil from broken droplets.

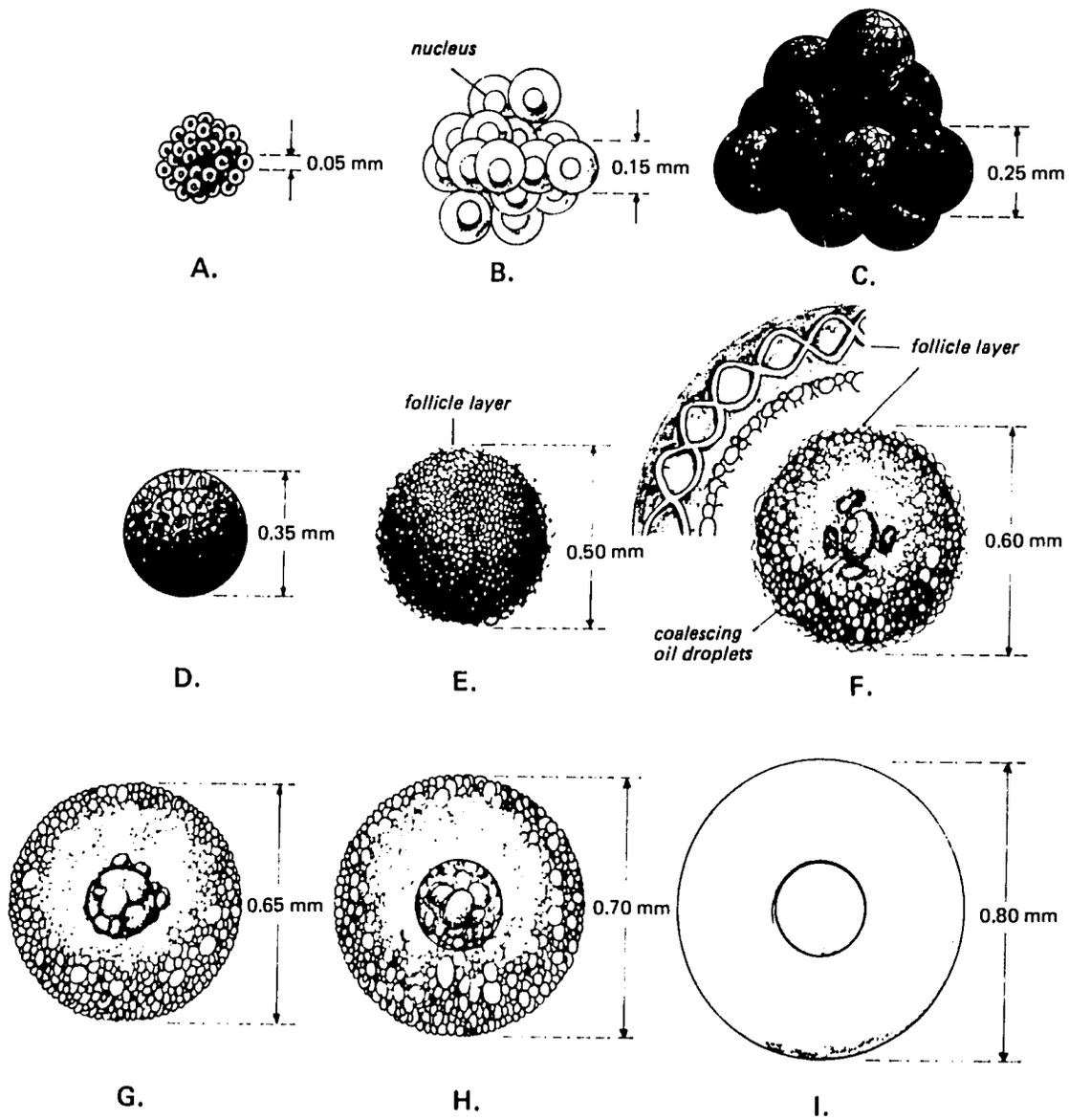


Fig. II.18. The developmental stages of spotted scat oocytes. See text for description.

II.11. Pairing and Mating Behavior of Captive Spotted Scat

We observed the pairing and mating behavior of spotted scat confined to tanks during September 1986. Two males, with running milt, (weights 267 and 256 g) and one female with oocyte diameters of 0.52 mm (weight 265 g) were placed together in a 200-l tank on September 15, 1986. The female was implanted with LHRHa. The salinity of the water was 25 ppt. Pairing behavior was immediately observed between both males and the female. The two males followed the female as she swam around the tank, and occasionally nudged her abdomen from below with their snouts. Antagonistic behavior was seen between the males, although this was not intense. The males avoided each other by staying on opposite sides of the female. At times, one of the males would make a sudden attack on the other. When this occurred, the attacked male tilted his head downwards and held his dorsal spines erect. The aggressive male attempted to swim around the attacked fish to nip its flank. The attacked male oriented himself quickly to avoid being struck by the aggressor. We never observed the aggressor actually bite the other male, although wounds were visible on the latter's flank. After chasing the other male for a short time (~1 min), the aggressive male returned his attention to the female.

On September 16, the dominant male and the female were seen hovering at mid-depth, facing each other. They both undulated their bodies in a rhythmic motion. This behavior lasted from 30 seconds to several minutes; the frequency of the undulations increased with time. At this point, we observed the male bite and hold the upper lip of the female for several seconds. Her upper lip was scraped open and bloody as a result of this behavior. The dominant male resumed following the female around the tank and placed his snout to her urogenital opening with greater frequency than was seen the previous day. The beginning of abdominal distension (perhaps because of egg hydration) was observed in the female at this time.

On the morning of September 17, the male-male antagonism had markedly increased and the dominant male spent much of his time trying to attack the other male. Interestingly, the female moved to position herself between the two males. The aggressive male made repeated attempts to go around the female to get at the submissive male, which held its dorsal spines erect most of the time. After these attacks, the aggressive male immediately returned his attention to the female, nudging her abdomen from below with greater force and frequency. The dominant male, but not the submissive male, was observed with a wounded, bloody upper lip like the female's. The submissive male had more wounds along its flank. On the morning of September 18, the submissive male was found dead on the tank bottom. The female had spawned. Actual spawning behavior was not observed. It was subsequently found that all male and female scat mated for spawning had wounded upper lips.

II.12. Spawned Eggs and Larvae

II.12.a. Spawned Eggs. The spawned eggs of the spotted scat varied in size from 0.68 to 0.75 mm in diameter. The eggs are transparent and spherical, and contain a single yellow oil droplet 0.30 mm in diameter. We observed that unfertilized eggs turned opaque within one hour. Unfertilized eggs are easily suspended by aerating the water, but sink if the aeration is turned off.

According to Winfree (1983, unpublished report) "fertile eggs could be distinguished within 1 to 2 hours by gradual changes in internal structure. Oil droplets also continued to coalesce in the fertile eggs but not in the infertile ones. Viable eggs were buoyant in seawater."

II.12.b. Early Larval Development. According to Winfree (op. cit.), 12 hrs after fertilization, embryonic differentiation was obvious. Larvae hatched 17 to 18 hrs after fertilization. About 3 hours after hatching, the larvae oriented head down and began to

sink. At this stage, the eyes and mouth were forming. Fin buds formed within 24 hrs. The eyes, mouth, and gut became functional 2 to 3 days after hatching. Winfree estimated the optimal size of food at first feeding to be 50 ± 25 μm diameter, based on a maximal gape of just less than 100 μm . "Feeding behavior was apparent when larvae collected at the surface, along the sides and corners of the tank, 3 days after hatching. Mortalities increased in frequency a day later, probably because of starvation. By 9 days, the surviving larvae had grown large enough that rotifers (125 μm) were suitable as food. *Artemia nauplii* were added 8 days later."

II.12.c. Tholichthys Larvae. The larvae of the spotted scat pass through a developmental stage known as the tholichthys (Fig. II.1A and II.19). This stage is unique to a few genera of teleosts, including the butterflyfishes (Chaetodontidae), and scats (Scatophagidae) (Bloom, personal communication). The tholichthys larvae of the spotted scat are deep-bodied and laterally compressed. They are usually very dark, have rough, scaleless skin, and a well-developed lateral line. They range in size from approximately 0.60 to 1.2 cm. The most distinctive feature of tholichthys larvae are the bony plates which completely encase the head in a thick protective sheath. In the spotted scat, one of these plates dorsal to the eye has posteriorly-oriented projections which form spiny horns on either side of the head. These plates are slowly absorbed as the tholichthys larva develops into the juvenile form.

The duration of the planktonic period of the spotted scat is not known, nor is it known where the nursery grounds are for this species. Based on the seasonal abundance of mature females and larvae, we think that the newly hatched egg may develop to the fry stage within one month. The majority of fry obtained by collectors are actually tholichthys larvae, caught along the shore in brackishwater estuarine habitats.

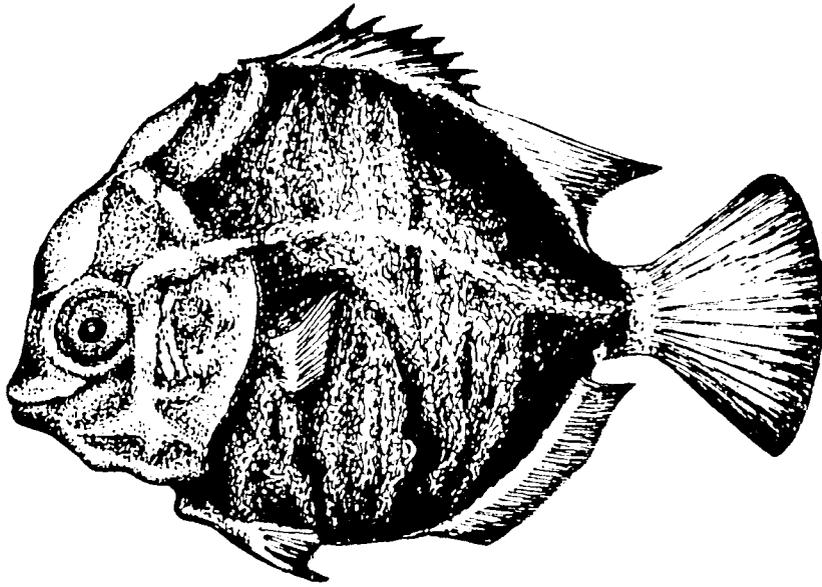


Fig. 11.19. The tholichthys larval stage of the spotted scat. Actual size is 1.2 cm in total length.

III. GONADAL MATURATION AND SPAWNING INDUCTION IN FEMALE SPOTTED SCAT (*Scatophagus argus*)

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and Maria Paz Soccoro C. Macahilig

INTRODUCTION

A culture industry for the spotted scat could be sustained if the supply of wild-caught fry is sufficient to meet the needs of the industry. Most likely, however, this will not be the case since the current supply of wild-caught scat fry is insufficient to meet even the needs of the aquarium trade. Development of a growout industry of scat for foodfish would put additional demands on the fry supply from the wild.

The alternative to wild fry is to produce fry from captive broodstock through induced maturation, spawning and larviculture. With this in mind, we conducted experiments on induced gonadal maturation and spawning of female spotted scat. Our investigations focused on the use of the synthetic luteinizing hormone-releasing hormone analogue, des-Gly¹⁰, [D-Ala⁶] LHRH (LHRHa).

LHRHa is a highly effective agent for inducing final oocyte maturation, ovulation, and spawning in female teleosts. LHRHa has important advantages over other substances commonly used to achieve these purposes, such as pituitary extracts, or exogenous piscine and mammalian gonadotropins (for review, see Donaldson and Hunter, 1983; Harvey and Hoar, 1979). LHRHa is a synthetic molecule that is at least 35 to 50 times more potent than native LHRH, and hundreds of times more potent than gonadotropins on a per weight basis (Donaldson and Hunter, 1983). Consequently, LHRHa dosages are easily standardized, and because so little of the hormone is needed, it is more economical to use than gonadotropins. In addition, LHRHa can be administered in chronic-releasing, cholesterol-based pellets which can deliver the hormone for as long as weeks at a time, and thus maintain elevated hormone levels in the fish without the handling and stress associated with constant hormone injections (Almendras et al., 1987; Crim et al., 1983; Crim, 1985; Lee et al., 1986; Nacario and Sherwood, 1986; Sherwood et al., 1987).

In the present study, wild-caught female scat, at different stages of oocyte maturity, were implanted with cholesterol-based pellets containing various doses of LHRHa, and sampled at regular intervals by ovarian biopsy to monitor the progress of oocyte development. Our goals were to 1) determine if LHRHa could induce gonadal maturation and spawning in female spotted scat, and, if successful, 2) determine the minimum effective doses of LHRHa required to induce maturation and spawning, and 3) find the approximate time to spawning following LHRHa administration.

MATERIALS AND METHODS

Making Hormone Implants. The synthetic luteinizing hormone-releasing hormone analogue, des-Gly¹⁰, [D-Ala⁶] LHRH (LHRHa), and cholesterol were purchased from the Sigma Chemical Co., St. Louis, MO, USA. Cholesterol-based pellets of LHRHa were made according to the method of Lee et al. (1985) with several modifications. A 1 mg/ml stock solution of LHRHa was prepared using 95% ethanol. To make a single pellet, an appropriate amount of LHRHa stock solution was mixed with 12.16 mg of cholesterol to form a paste. The ethanol was allowed to evaporate at room temperature. The dried powder was mixed with 0.64 mg of melted cocoa butter, and formed into pellets which had the following characteristics: weight \approx 8.1 mg, length = 4.0 mm, and diameter = 1.5 mm.

Hormone Administration. Fish were implanted by making a deep, narrow incision in the dorsal musculature into which the pellets were placed using a device made from two sections of a collapsible radio antenna. Fish were usually implanted in the early afternoon within 24 hours following capture, after being tagged, weighed, and measured. In initial experiments, the pellets contained either 5, 10, 25, or 50 ug of LHRHa. The hormone dose on a per weight basis, therefore, varied with the weight of each fish. Later, the amount of LHRHa per pellet was adjusted for the individual weights of each fish to deliver a specific dose.

Experimental Design

We conducted two sets of experiments: (A) Maturation experiments (25 fish); and (B) Spawning experiments (34 fish).

A. Maturation

Three maturation experiments were conducted to test the ability of LHRHa to stimulate the development of immature oocytes. We used small sample sizes in each experiment due to a limitation in the number of available broodstock.

Experiment 1 ran from Aug. 28 to Sept. 23, 1986. Nine fish received control pellets and nine were implanted with 50 ug LHRHa. Fish were at different stages of gonadal development. Those with oocytes of comparable size were divided equally between the control and experimental groups. The fish were stocked in 0.25 m³ cages floating in a 200 m² pond, and sampled weekly.

Experiment 2 ran from Sept. 15 to Oct. 1, 1986. Six fish were implanted with 50 ug pellets and six served as controls. Fish had different initial oocyte diameters. The experiment was conducted in 50-l glass aquaria; fish were stocked at one fish per tank, and sampled weekly.

Experiment 3 ran from March 3 to April 28, 1987. Ten fish were stocked directly into a 250 m² pond. Three fish each received a 5, or 25 ug LHRHa pellet; two fish each received 50 ug LHRHa pellets or served as controls. The fish were sampled every one to three weeks.

B. Spawning

In addition to the above broodstock experiments, all female scats with oocyte diameters greater than 0.35 mm were implanted on the day of their capture, or the following day, with pellets containing various doses of LHRHa. Females left to spawn naturally were placed into 200-l polyethylene, or 1-ton concrete tanks, and mated with from one to five males. Fish which were to be stripped were held in tanks or cages floating in the pond, and were not mated. All fish were sampled at regular intervals to monitor the progress of final maturation. For fish with oocyte diameters of less than 0.50 mm, oocyte samples were taken once every two or three days. Samples were taken daily once the oocyte diameters exceeded 0.50 mm. To closely monitor and record the progress of final oocyte maturation, several fish were sampled at eight-hour intervals. A total of 34 broodstock-sized female scat were used in the spawning experiments.

RESULTS

The developmental stages through which scat oocytes pass could be easily differentiated by observing characteristic cytoplasmic and nuclear changes through a

dissecting microscope. These developmental stages were well correlated with changes in oocyte diameter (Barry and Fast, II, this volume).

As used here, successful maturation of female fish refers to conditions where oocyte diameter increases following initial observation. Spawning success refers to the completion of final maturation of the eggs, and their release from the female, either through natural spawning, or manual stripping.

Maturation Experiment Results. No oocyte maturation was observed in any of the caged or aquaria-held fish from experiments 1 and 2. There was no response to LHRHa in these fish, regardless of the dose of LHRHa administered; their eggs all became atretic. Two of the 10 fish from experiment 3, however, successfully matured and spawned. These 2 fish had initial oocyte diameters of 0.01 and 0.15 mm, and received LHRHa doses of 175 ug/kg and 100 ug/kg, respectively (Fig. III.1).

In the pond maturation experiment, i.e., experiment 3, oocytes of the control fish grew slowly from the first to the fourth week. The growth rate increased from week 4 to week 6, accelerated between weeks 6 and 7, and then declined (Fig. III.2). All experimental fish received LHRHa pellets containing 5, 25 or 50 ug on weeks 1, 4, and 7. The rates of oocyte growth were slow from weeks 1 to 4 in all groups, as in the controls. On week 4, however, following administration of their second LHRHa pellet, the rates of oocyte development were much greater for the fish in the 25 and 50 ug groups compared to those in the control and 5 ug groups. Oocytes of the fish receiving 50 ug pellets continued to develop rapidly. By week 6, the 2 fish in this group had spawned. In the 25 ug group, the rate of oocyte growth slowed between weeks 5 and 7. Following reimplantation of another LHRHa pellet on week 7, however, the rate of oocyte growth accelerated. In the 5 ug group, the oocytes developed steadily until the seventh week. By the following week, atresia had set in. On week 7, the oocytes of all fish which did not spawn had likewise undergone atresia, perhaps because of stress caused by the high temperatures (32 C) and salinities (greater than 55 ppt) in the pond at that time.

Spawning Experiment Results. Female scat tested in the spawning experiments exhibited one of three general response patterns: 1) the fish showed no response to LHRHa, and the oocytes eventually became atretic; 2) the fish responded to LHRHa by increased oocyte size and development, but they did not ovulate and spawn, and their oocytes eventually became atretic; and 3) the fish ovulated and spawned. These responses are shown by three fish implanted with a control pellet and two dose levels of LHRHa on July 26, 1987 (Fig. III.3).

LHRHa was highly effective in stimulating spawning in female *Scatophagus argus*. Thirty-four fish were implanted with pellets; 6 of these served as controls. Of the 28 test fish, LHRHa stimulated maturation and spawning in 14 females, and maturation only in the remaining 14 (Fig. III.4; Table III.1). No oocyte development or spawning occurred in any female which received a control pellet. In every case, the oocytes of control fish underwent atresia, regardless of how well-developed they were.

Minimum Effective Dose. The minimum dose of LHRHa needed to induce final maturation and spawning was estimated by fitting regression lines through the lowest doses of LHRHa, at a given initial oocyte diameter, that 1) had a positive effect in increasing oocyte growth, but failed to induce spawning (Fig. III.4, lower line); and 2) induced final maturation, ovulation and spawning (Fig. III.4, upper line). The results show that a higher dose of LHRHa is required to stimulate maturation and spawning in fish with smaller oocytes. Several fish which matured, but did not spawn lie on or above the upper regression line. Three of these fish, represented by open squares, were sampled at eight-hour intervals to monitor the time course of oocyte maturation. The two fish represented by open diamonds were severely diseased at the time they were implanted. The stress on these fish from excessive handling or disease may have prevented them from ovulating and spawning.

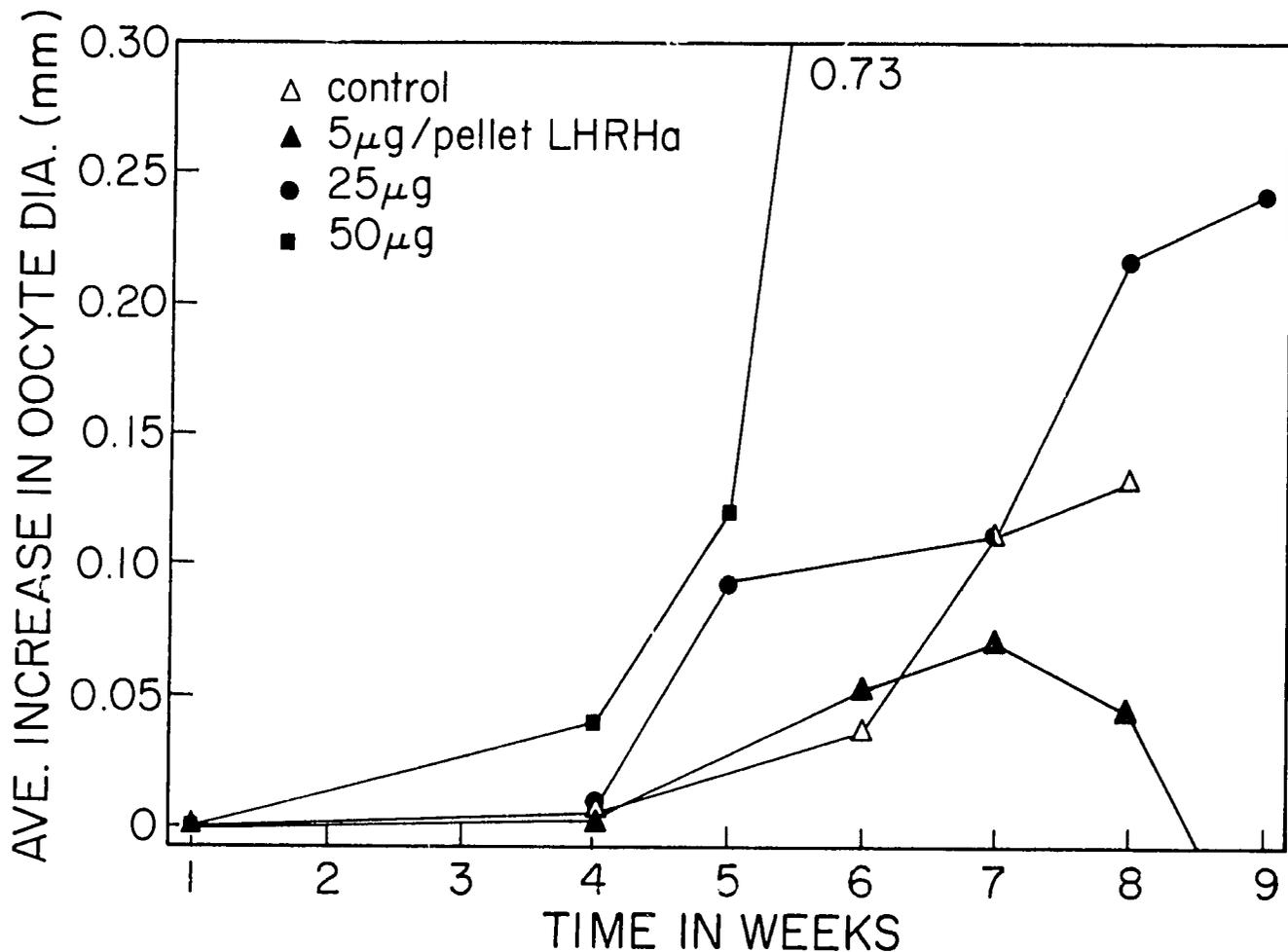


Fig. III.1. The relationship between initial oocyte diameter and dose of LHRHa on the induction of oocyte maturation in the spotted scat, *Scatophagus argus*. Female scats, all with immature oocytes of the indicated initial diameters, were implanted with cholesterol-based pellets containing LHRHa at the indicated dosage. The solid symbols (triangles and squares) indicate fish which were held in ponds (Exp. 3). The solid triangles indicate fish which underwent gonadal maturation, but failed to spawn, and whose oocytes eventually underwent atresia. The two fish represented by solid squares completed maturation in the ponds, and spawned. The open triangles indicate fish that were confined in tanks or cages (Exps. 1 and 2), none of which showed any sign of gonadal maturation.

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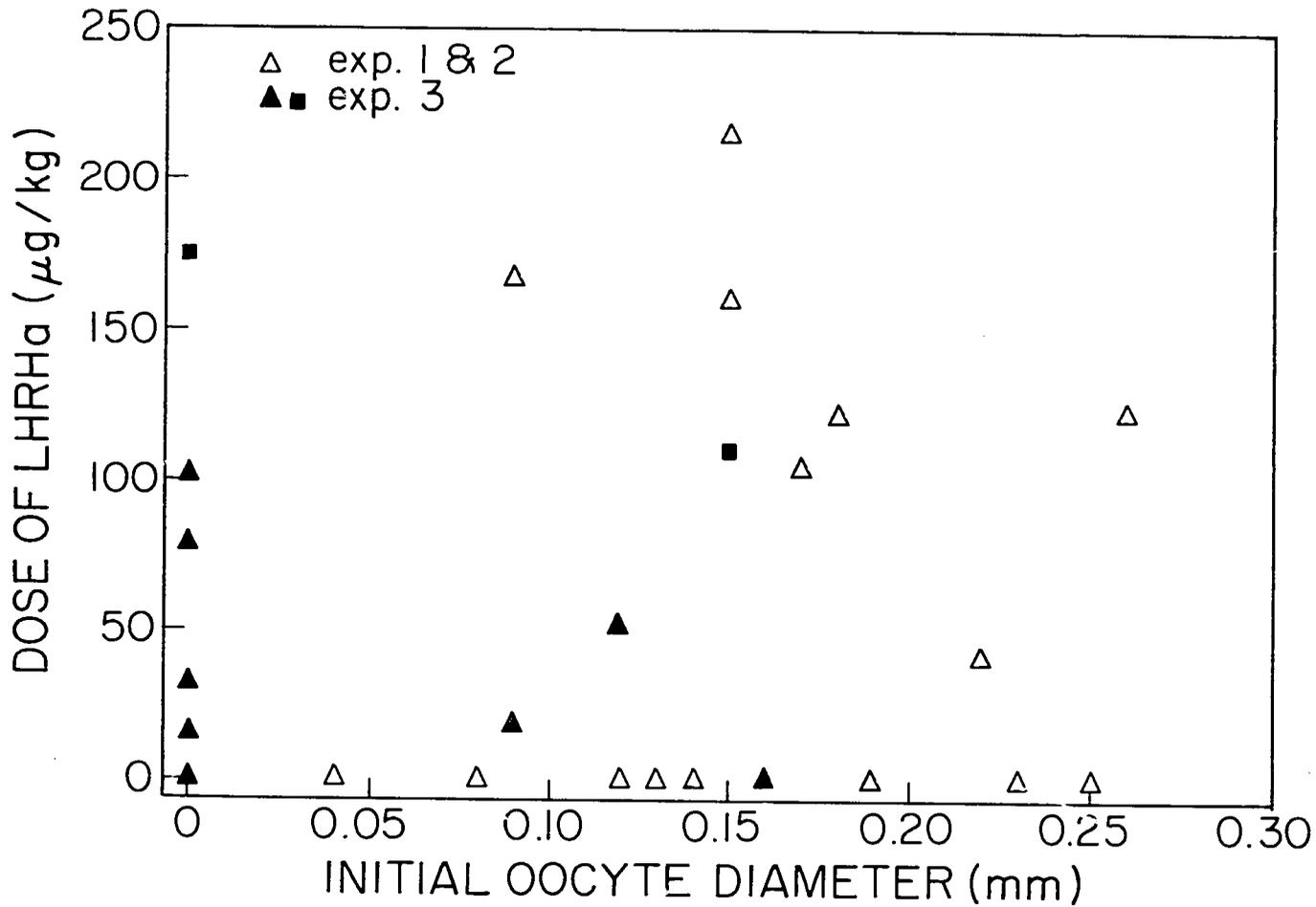


Fig. III.2, The effects of LHRHa on oocyte maturation in female *Scatophagus argus* held in a 250 m² pond (Exp. 3). Fish were implanted with cholesterol-based pellets containing 0, 5, 25, or 50 ug of LHRHa on weeks 1, 4, and 7. Each point represents the average change in oocyte diameter from the previous sampling (N = 2 or 3). Line slopes indicate the rate of oocyte development.

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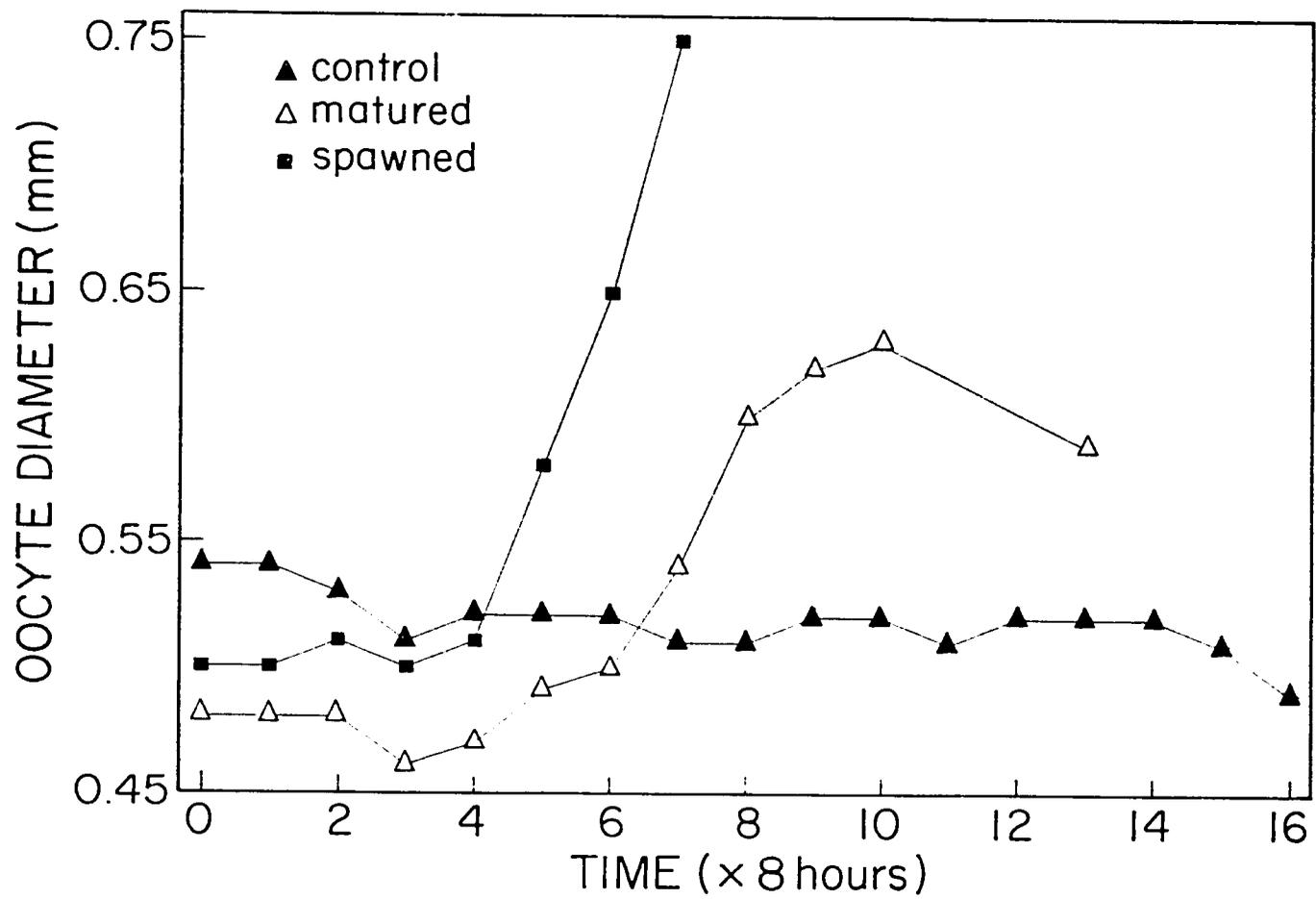


Fig. III.3. Results from a spawning experiment conducted on July 26, 1987. A sham pellet was given to the fish represented by solid triangles (control). The fish represented by open triangles received 114 ug/kg LHRHa (matured). The fish represented by solid squares received 124 ug/kg LHRHa (spawned). All fish were sampled at eight-hour intervals following pellet implantation at time 0.

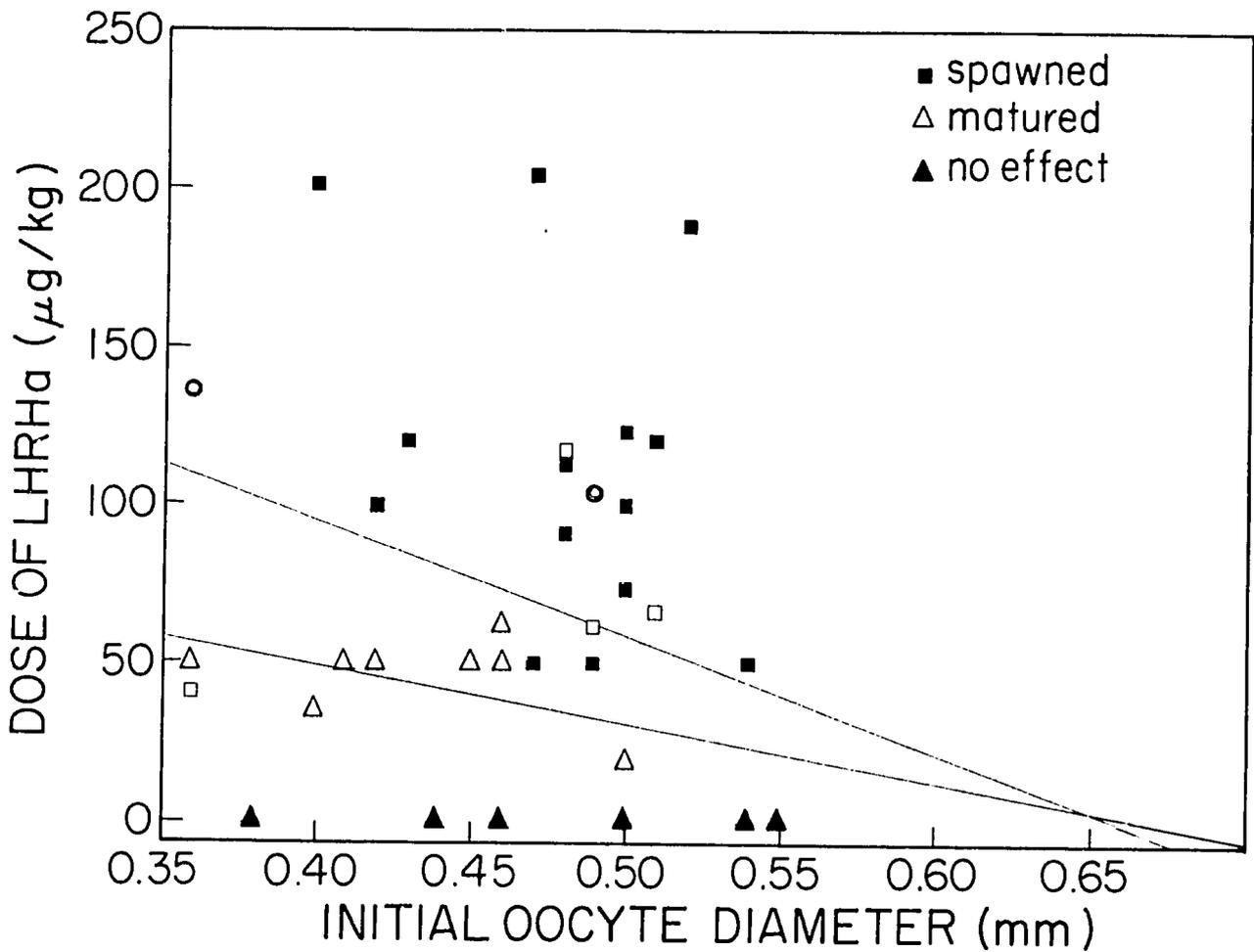


Fig. III.4. The effects of LHRHa on final oocyte maturation and spawning in female *Scatophagus argus* as a function of initial oocyte diameter. Each point represents one fish. Following LHRHa administration, each fish displayed one of the three patterns of response illustrated in Fig. III.3, i.e., 1) their oocytes failed to respond to LHRHa and eventually underwent atresia (solid triangles); 2) the oocytes matured but the fish failed to spawn (all open symbols); or 3) the oocytes underwent final maturation, and the fish ovulated and spawned (solid squares). Regression lines were fitted to the points representing the lowest doses of LHRHa, for a given initial oocyte diameter, that 1) induced final maturation and spawning (upper line), and 2) stimulated oocyte development, but failed to stimulate spawning (lower line). The 4 fish represented by open squares were sampled at 8-hour intervals. The 2 fish represented by open circles were badly diseased when implanted with LHRHa.

TABLE III.1. Body Weight, Initial Oocyte Diameter, Hormone Dose, Spawning Data, and Water Temperature for Spotted Scat Females Which Matured and Spawmed Following LHRHa Implantation

Fish	Wt. (gms)	Initial Oocyte Diameter (mm)	ug LHRHa/kg Fish	Date Spawmed	# Days from Implantation to Spawning	Temp (°C)	# of Eggs Spawmed
1	265	0.52	188	09/19/86	7	29.2	8,841
2	685	0.50	73	09/25/86	8	29.5	287,895
3	464	0.59	178	09/30/86	0		45,098
4	247	0.40	202	10/01/86	16		in pond
5 ^a	414	0.43	120	10/17/86	13	27.0	
6	244	0.47	204	10/28/86	11	27.0	94,743
7 ^a	440	0.48	113	10/30/86	3	28.3	
8	414	0.51	121	07/02/87	3		250,960 ^{b, c}
9	510	0.50	124	07/28/87	2	29.0	330,800 ^{b, c}
10	522	0.50	50	09/24/87	3	29.0	46,080
11	170	0.54	50	09/28/87	3	29.0	59,040 ^b
12 ^a	400	0.49	50	10/29/87	3	29.0	519,000
13	400	0.42	100	10/29/87	3	29.0	in pond
14	947	0.47	50	11/01/87	6	27.0	807,000 ^b

^astripped, ^btotal number of eggs present in ovary
^ctotal number of eggs from multiple spawning

of time, perhaps for as long as 12 days or more.

If a female with oocytes 0.50 mm in diameter or larger is given LHRHa, she will spawn in approximately 3 to 6 days, depending on the temperature. The minimum effective dose of LHRHa required to stimulate spawning in females having oocytes of this diameter lies within the range 40 to 75 ug/kg. Recent *in vitro* evidence suggests that cholesterol-based pellets release approximately 20% of their LHRHa in the first 24 hours (Sherwood et al., 1987). Therefore, in a scat administered 40 to 75 ug/kg LHRHa, from 8 to 15 ug/kg LHRHa is released into the blood stream on the first day. This dose of LHRHa is within the range of doses reported to be effective in stimulating oocyte maturation and spawning in a variety of cultured species.

The spotted scat now joins a growing list of species which have been shown to respond to LHRHa. This list includes the goldfish, *Carassius auratus* (Chang and Peter, 1983; Lam et al., 1976; Peter, 1980); the plaice and goby (Aida et al., 1978); sea bass, *Lates calcarifer* (Almendras et al., 1987; Garcia, L.M., 1987, person. comm.; Harvey et al., 1985; Nacario, 1987); rainbow trout, *Salmo gairdneri* (Crim et al., 1987; Weil et al., 1978); *Oryzias latipes* (Chan, 1977); and various carps and salmonids (Aida, K. and Hibiya, 1978; Billard et al., 1984; Crim et al., 1986; Crim and Glebe, 1984; Donaldson et al., 1981; Van der Kraak et al., 1983).

Maturation, and maturation with spawning in female spotted scat is influenced by many factors, including method of containment. In our experiments, oocyte maturation never occurred in control fish confined in tanks or cages, regardless of the state of development of their oocytes. Two control fish stocked in ponds, however, matured, but did not spawn.

These results suggest that the conditions in the maturation and spawning containers (tanks, hapas, or cages) may have been inappropriate, and thus failed to provide stimuli essential for normal oocyte development; or, that the stresses of capture and confinement inhibited endogenous processes that normally culminate in gonadal development or spawning (Donaldson and Hunter, 1983). The appropriate food, salinity, temperature, water depth, rainfall, current, the presence or absence of males, sex ratio, or any combination of these and other factors required to provide the proper environmental cues to initiate development may have been lacking in the tanks, but were present in the ponds. The fact that scat were able to mature in ponds, with or without hormonal intervention, is an exceptional attribute of this species which will facilitate its future mass culture.

In conclusion, spawning can be readily induced in female spotted scat which have oocytes of at least 0.35 mm in diameter. Earlier stages of oocyte maturation can take place in pond-reared females, or fish at this stage of development can be readily obtained from the wild at the right time of the year (see Barry and Fast, II, this volume). The minimum effective dose of LHRHa required to induce final maturation and spawning has been determined, and depends on the initial diameter of the female's oocytes when she is implanted with LHRHa. The approximate time when the female will spawn following LHRHa pellet implantation can be estimated at any point in time if the oocyte diameter, and the temperature of the water where the female is held, are known. The effects of LHRHa on egg "quality" should be investigated in future studies.

IV. PRELIMINARY OBSERVATIONS ON THE EFFECTS OF SEX STEROIDS AND HUMAN CHORIONIC GONADOTROPIN (HCG) ON THE FINAL MATURATION OF SPOTTED SCAT (*Scatophagus argus*) OOCYTES *IN VITRO*

Milagros T. Castaños and Terence P. Barry

INTRODUCTION

In vitro studies have given valuable information about the hormonal control of reproduction in fishes. Results from *in vitro* studies, for example, have shown that certain steroids (particularly progestogens and corticosteroids) are potent stimulators of oocyte maturation in fish. *In vitro* studies have also demonstrated that gonadotropins can act directly at the ovarian level to stimulate final oocyte maturation (Goetz, 1983). This information is of practical use since females of many cultured teleost species fail to undergo final maturation and ovulation in captivity, and hormonal intervention is required to breed these fish. Information from *in vitro* studies can suggest what may be the most appropriate hormones to administer, and at what time and dosage to deliver them. There is also reason to believe, based on the situation with domesticated mammals and birds, that a fairly complete understanding of the workings of the endocrine system may be necessary before fish culturists can gain complete control over the reproductive cycles of their stocks. *In vitro* studies have made, and should continue to make, substantial contributions in this regard (Goetz, 1983).

In vivo, spotted scat oocytes undergo obvious changes during final maturation, including lipid droplet fusion, oil droplet coalescence, germinal vesicle migration and breakdown, and ovulation (Barry and Fast, II, this volume). In preliminary *in vitro* experiments, we found that scat oocytes could be cultured for over three days, and undergo similar changes *in vitro*. We also had preliminary data suggesting that the scat has a group-synchronous ovary; that is, all the oocytes of a single clutch undergo vitellogenesis and ovulation at approximately the same time, and are stimulated by the same environmental clues. All of these qualities contribute to making the spotted scat an excellent species with which to conduct *in vitro* studies on oocyte maturation. Realizing these advantages, and knowing the potential practical benefits basic information derived from *in vitro* studies could have, we conducted several experiments to learn more about the hormonal control of oocyte maturation in the spotted scat.

MATERIALS AND METHODS

Histology. The ovaries of two female scats with oocytes at different developmental stages were removed, fixed in Bouins solution, and sectioned according to the histological procedure described by Tan (1985). To quantify the percentage of oocytes at different developmental stages in the ovary of the spotted scat, the diameters of all the oocytes with a visible nucleus (oocytes sectioned through their center) were measured to the nearest 0.01 mm. Approximately 100 oocytes from three slides representing sections through different parts of the ovary, were measured.

Culture Medium. The culture medium was a modified Kreb's bicarbonate ringers solution. To make 100 ml of this medium, approximately 70 ml of distilled deionized water was added to a 100-ml volumetric flask and the indicated volumes of the following stock salt solutions were added in the given order: 2.8 ml of 0.5 M NaCl; 0.47 ml of 0.5 M KCl; 0.7 ml of 0.3 M CaCl₂; 0.28 ml of 0.5 M MgSO₄; 0.25 ml of 0.5 M KH₂PO₄; and 5 ml of 0.5 M NaHCO₃. The flask was gently swirled after the addition of each solution. The following were then added: 50 mg dextrose; 29 mg glutamine; 0.4 g HEPES buffer; 0.2 ml gentamycin sulfate; 0.1 ml penicillin; and 2 ml of Eagles MEM (Sigma Chemical Co., St. Louis, MO,

USA). The volume was adjusted to 100 ml with deionized, distilled water. The pH of the medium was altered by gently blowing into the flask, and measured with pH paper. Finally, the medium was filtered through a 0.2 µm filter (Acrodisc) into a sterile, sealed culture bottle.

Oocyte Samples. Oocyte samples from mature female scats were taken by cannulation, fixed, cleared, and measured as described previously (Barry and Fast, II, this volume). Female scat with oocyte diameters greater than 0.50 mm and a centrally located germinal vesicle were selected for the experiments.

Oocyte samples for culture were taken by one of two methods. In method one, the ovaries were removed from a sacrificed female, and placed immediately into culture medium in a sterilized 250-ml beaker. Oocytes were separated using fine forceps. In method two, the female was cannulated and the oocyte sample obtained was blown gently into culture medium in a sterile plastic petrie dish. Oocytes obtained in this manner usually did not need to be separated with forceps.

Culture. Concentrated stock solution of the test steroids, 17 α ,20B-dihydroxyprogesterone (17,20P) and 11-deoxycorticosterone (DOC), were prepared in ethanol, and hormone dilutions were made from these stock solutions in individual, sterile culture bottles. Ethanol concentrations were adjusted to the same level in all treatments, including the control, and never exceeded 1%. The experiments were conducted in 24-well, flat-bottomed culture plates (Falcon). Typically, there were 6 replications per treatment per trial. Each well was filled with 500 µl of medium containing the appropriate hormone dilution, or control medium. Twenty oocytes were introduced into each well with a glass pipette. Broken or deformed oocytes were removed and replaced. After the wells were filled, the plate was covered, and placed into an airtight container which was lined with damp paper, and fitted with a valve. Air was blown through the valve which was then closed to maintain a constant humid environment within the container. The container was placed into a 27 C incubator and the oocytes were cultured for various lengths of time, typically 24 to 48 hrs. After the culture period, the plates were removed from the incubator and the percentage of oocytes in each well that underwent a particular developmental change was recorded, i.e., complete oil droplet fusion, ovulation as evidenced by the presence of post-ovulatory follicles, or germinal vesicle breakdown (GVBD).

In preliminary experiments, we found that the best osmotic pressure to culture spotted scat oocytes was close to the osmotic pressure of the scat's blood plasma, 315 ± 1.7 mOsm/kg (see Barry and Macahilig, VI, this volume). The osmotic pressure of the culture medium was adjusted to this level in all subsequent experiments by adding NaCl to the medium. A refractometer was used to measure the salinity of the culture medium, and an empirically-derived formula was used to convert the salinity reading to osmotic pressure.

Experimental Design and Statistical Analysis. Experimental designs are shown in the results section. The results were statistically analyzed by analysis of variance followed by the Duncan's multiple range test on the arcsine-transformed data (Sokal and Rohlf, 1981).

RESULTS

Histology. Only two clutches of oocytes, immature and developing, were present in the ovary. The immature oocytes accounted for 62% of all the oocytes measured. They were all less than 0.1 mm in diameter, or primary oocytes (Barry and Fast, II, this volume). Of the remaining oocytes, 63% were within the size range 0.31 to 0.40 mm; 22% were in the range 0.11 to 0.2 mm; 6% in the range 0.21 to 0.30; and 9% greater than 0.40 mm in diameter. These results suggest that only one clutch of spotted scat oocytes develop at a time, in synchrony (Fig. IV.1).

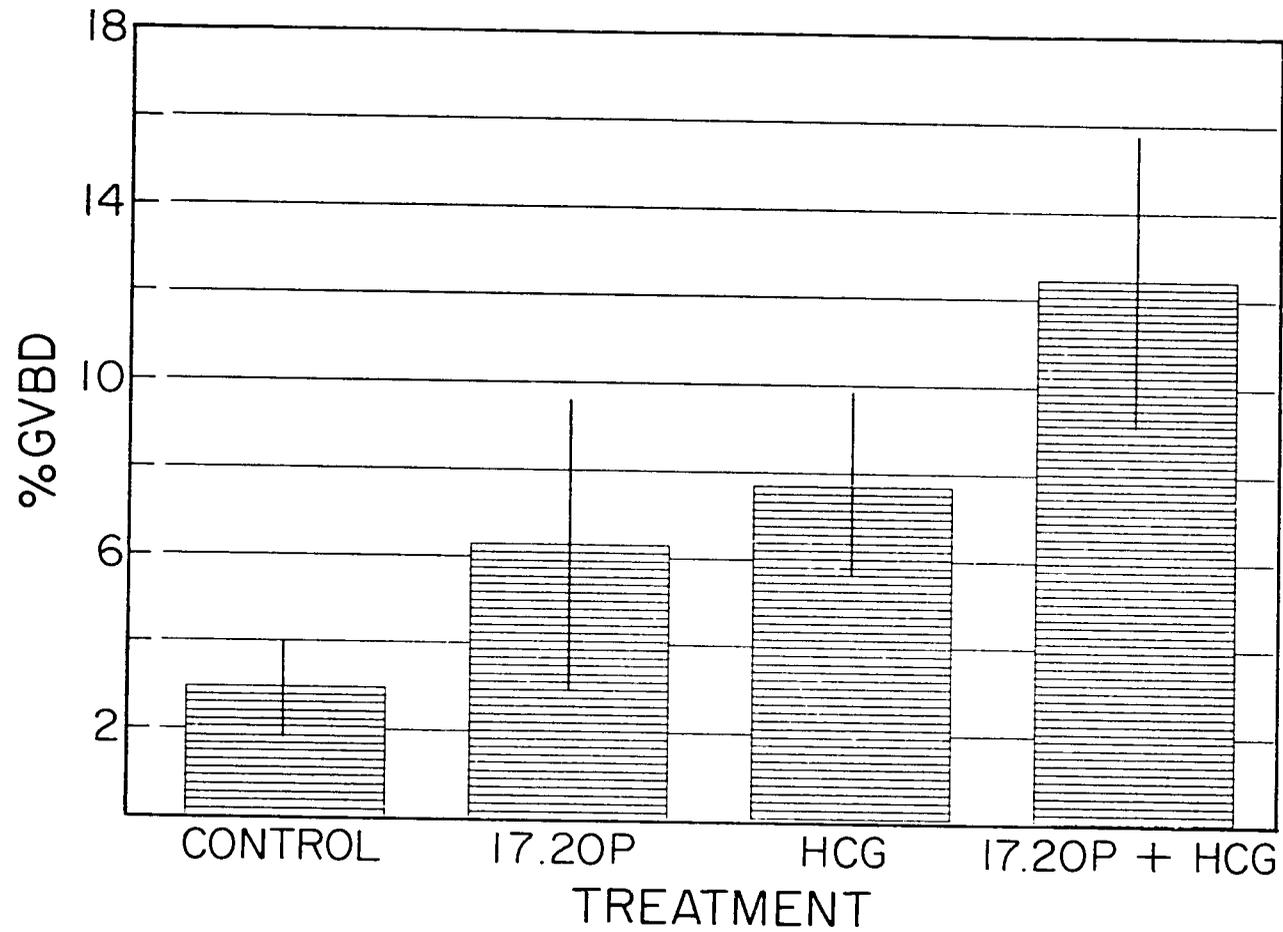


Fig. IV.1. Size classes of the oocytes in the ovary of a maturing female spotted scat. The ovary was removed, fixed and histologically sectioned. The diameters of approximately 300 oocytes with visible nuclei, from sections representing three different parts of the ovary, were measured (± 0.10 mm).

Experiment 1. The effects of 0, 0.1, 1 and 10 μM of 17,20P and DOC on the stimulation of complete oil droplet fusion were investigated after 24, 48 and 72 hrs of culture. There was very little response in any of the cultures after 24 hrs.

After 48 hrs, 12.5% of the oocytes underwent complete oil droplet fusion in the control incubations. The best responses were at 1 μM 17,20P, and 0.1 μM DOC which stimulated $31.2 \pm 15.4\%$ and $28.0 \pm 8.3\%$ of the oocytes to undergo complete oil droplet fusion, respectively (Table IV.1). The differences between these values and the control were not significant ($p = 0.107$). There was very little change in the response between 48 and 72 hrs. Based on the results of this experiment, steroid hormone concentrations of 0.1 and 1 μM , and 48-hr culture periods were used in subsequent experiments.

Experiment 2. Post-ovulatory follicles were observed in the 17,20P incubations of Exp. 1 after 2 days of culture. Exp. 2 was designed to investigate the effects of 17,20P on ovulation *in vitro*. There were two treatments, control and 1 μM 17,20P. After 48 hrs of culture, $16.0 \pm 21\%$ (range 0 to 57%) and $30.1 \pm 29\%$ (range 0 to 71%) of the oocytes in the control and experimental treatments ovulated, respectively. The difference was not significant. Ovulation usually occurred when most of the eggs had yolk uniformly distributed throughout the cytoplasm, and yolk vesicle and oil droplet coalescence had just begun.

Experiment 3. The following treatments were set up: Control, 0.1 μM 17,20P, 100 IU/ml HCG, and 0.1 μM 17,20P plus 100 IU/ml HCG. After 48 hrs in culture, the oocytes were cleared and the position of the germinal vesicle was recorded.

In the controls, $3.0 \pm 1.1\%$ ($N = 18$) of the oocytes underwent GVBD. In the 17,20P and HCG cultures, $6.3 \pm 3.6\%$ ($N = 9$) and $7.7 \pm 2.1\%$ ($N = 18$) of the oocytes underwent GVBD, respectively. In neither of these treatments was the percent GVBD significantly greater than the control. In the combined treatment, however, a significant ($p < 0.05$) percentage of the oocytes, $12.5 \pm 3.5\%$, underwent GVBD compared to the control (Fig. IV.2).

DISCUSSION

The oocytes of the spotted scat proved to be an excellent system for studying the hormonal control of oocyte maturation *in vitro*. Three distinct maturational events - oil droplet fusion, germinal vesicle breakdown, and ovulation were quantified in the present study, and all three processes responded in some degree to hormonal treatment.

Experiment 1. The most effective steroid yet tested for stimulating final maturation in teleosts, 17,20P, is effective in at least eight species representing several orders of teleosts. In other species, however, 11-deoxygenated, or 11-oxygenated corticosteroids produced by the interrenals are more potent stimulators of final oocyte maturation (Goetz, 1983). The results of Exps. 1 and 3 suggest that the final maturation of spotted scat oocytes may not be stimulated by either 17,20P or DOC. Additional steroids must be tested.

Experiment 2. Ovulation may not be controlled by the steroid hormone, 17,20P, in the spotted scat. Unfortunately, we were not able to carry our investigations further and the present results should be considered preliminary. The variation we found between replicates within treatments can not be easily explained, especially considering that the oocytes used in any one experiment all came from the same female and were at the same developmental stage. Perhaps further refinements in the culture conditions are needed. In our initial experiments, we found that spotted scat oocytes were highly affected by changes in medium osmotic pressure. If the osmotic pressure was too high or too low, the oocytes failed to develop *in vitro* or died. Other adjustments in the culture medium may still be needed. On the other hand, it may be that once scat oocytes reach a particular developmental stage *in vivo*, they are "committed" to ovulate even if removed from the female and cultured *in vitro*. This has been reported for another euryhaline teleost,

TABLE IV.1 THE EFFECTS OF 17a,20B-DIHYDROXYPROGESTERONE (17,20P) AND 11-DEOXYCORTICOSTERONE (DOC) ON OIL DROPLET COALESCENCE OF SPOTTED SCAT OOCYTES IN VITRO

CULTURE PERIOD (HRS)	CONTROL	[DOC] (uM)			[17,20P] (uM)		
		0.1	1	10	0.1	1	10
24	15.0 ± 36.7 ¹	0	0	0	1.5 ± 3.7	8.2 ± 16.0	13.5 ± 17.8
48	12.5 ± 15.9	28.0 ± 8.3	20.0 ± 16.7	14.2 ± 13.0	20.5 ± 10.6	31.2 ± 15.4	11.0 ± 14.0
72	24.0 ± 29.7	29.8 ± 10.9	35.2 ± 21.8	23.5 ± 21.0	25.3 ± 14.4	38.0 ± 21.4	10.7 ± 15.6

¹ Mean ± SEM of the percentage of scat oocytes which underwent complete oil droplet coalescence after the indicated times in culture.

48

4h

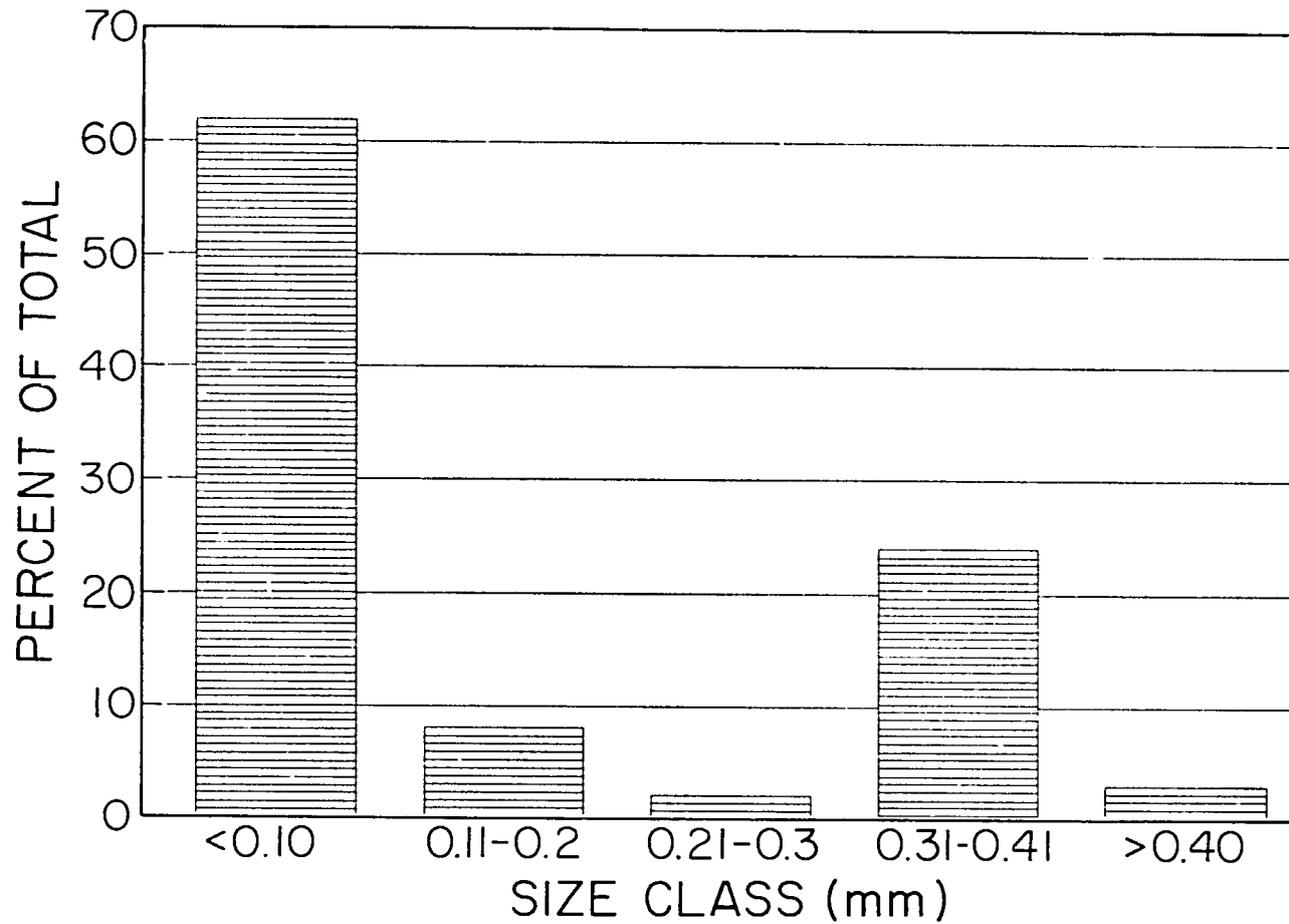


Fig. IV.2. The effects of 0.1 μ M 17 α ,20B-dihydroxyprogesterone (17,20P), 100 IU/ml human chorionic gonadotropin (HCG), and the combined effects of both 17,20P and HCG on germinal vesicle breakdown (GVBD) in mature spotted scat oocytes cultured *in vitro*. Shown are the combined results of 2 or 3 separate experiments (N = 9 or 18). There was a significant difference in percent GVBD between the control and combined treatment ($p < 0.05$).

Fundulus heteroclitus (Wallace and Selman, 1978).

Experiment 3. HCG did not have a significant effect in stimulating oocyte maturation *in vitro* in the spotted scat. In the Indian catfish, gonadotropins stimulate the interrenal to produce corticosteroids which in turn stimulate final maturation. Gonadotropins do not act directly on the ovary. A similar mechanism could be operating in the scat, although this hypothesis is not supported by our finding that HCG and 17,20P together stimulated GVBD. Indeed, this result supports the conclusion that gonadotropin receptors are present on the scat oocytes. HCG is a mammalian hormone and may not bind appropriately to the gonadotropin receptors on the scat's oocytes, resulting in its failure to give a significant maturation response. Additional studies using other doses of HCG and gonadotropins of piscine origin are required.

The significant effect of the combination of 17,20P and HCG suggests that there is a cooperative interaction between these two hormones in stimulating final oocyte maturation in the spotted scat. In several teleost species, gonadotropins act by stimulating follicular steroidogenesis. The most potent maturational steroid in other teleosts, 17,20P, failed to stimulate maturation in the scat when administered alone, suggesting that it may not be the maturational steroid produced in the scat in response to gonadotropin. However, 17,20P may act as a precursor to the real maturational steroid in scat. Such a mechanism explains the effectiveness of the combined treatment of both HCG and 17,20P, when both hormones alone were ineffective. The hypothesis that 17,20P serves as a precursor to the real maturational steroid in the scat can be tested by adding other steroid precursors and testing the effects of other gonadotropins, preferably of piscine origin. An alternative hypothesis is that either hormone is able to "sensitize" the oocyte to the action(s) of the other (see Catt et al., 1979).

In conclusion, the results of the present investigation suggest that neither 17,20P or DOC are the maturational steroids in the spotted scat. Moreover, HCG was not an effective gonadotropin for stimulating maturation *in vitro* when administered alone, and it may not be the most appropriate hormone for inducing final maturation and spawning in this species. Indeed, in a preliminary experiment, we injected HCG (100 IU/g) into a female with oocytes at a very advanced stage of development (oocyte diameters greater than 0.55 mm) and demonstrated that HCG had no effect on final maturation and spawning. The combination of HCG and 17,20P, however, was effective in stimulating final maturation *in vitro*, and a combined treatment with both a steroid and HCG may be effective in stimulating final maturation and spawning in the spotted scat. This remains to be tested *in vivo*. The effectiveness of LHRHa in stimulating maturation and spawning in the female scat may be due to its ability to stimulate both steroidogenesis and gonadotropin release. This hypothesis also remains to be investigated.

V. GONADAL MATURATION AND SPERMATION IN MALE SPOTTED SCAT (*Scatophagus argus*)

Terence P. Barry and Milagros T. Castaños

INTRODUCTION

To successfully reproduce the spotted scat, methods are needed to induce, on demand, both final oocyte maturation in captive females, and spermatogenesis and spermiation in captive males. Success with both sexes is needed, since one without the other will obviously not produce the desired results. We were able to readily induce maturation and spawning in the female scat using pelleted implants of LHRHa (Barry et al., III, this volume). Unfortunately, we did not obtain fertilized eggs because we lacked milt-producing males. To achieve our goal of developing a standardized technique for the propagation of the spotted scat, therefore, we needed to develop a reliable method for stimulating milt production in males. The results of our efforts in this regard are reported here.

In male teleosts, testicular development is regulated by the hypothalamic-pituitary-testicular axis, and hormonal intervention at each level of this axis has been effective in inducing spermatogenesis and spermiation in various species. Unlike the situation with females, however, no single method appears to have a distinct advantage over the others with males. Perhaps this is because it has been generally much easier to induce spermiation in males than maturation and ovulation in females. A single injection of HCG, for example, is often all that is needed to obtain a sufficient quantity of milt for induced breeding purposes (Donaldson and Hunter, 1983). With male scat, therefore, rather than focus on the use of LHRHa as was done with the females, our induced spermiation experiments were designed to test the effects of several different hormones in order to determine the efficacy and economy of each. In addition, since the method of hormone administration is known to significantly influence the effectiveness of a given hormonal treatment, we used several different approaches of administering these agents.

In the present study, male scat were treated with synthetic luteinizing hormone-releasing hormone analogue, des-Gly¹⁰, [D-Ala⁶] LHRH (LHRHa) administered as a cholesterol-based pelleted implant. Human chorionic gonadotropin (HCG) was injected intraperitoneally or intramuscularly, and 17 α -methyltestosterone (MT) was delivered in cholesterol-based pellets, silastic implants, or incorporated into the fish's diet. Combinations of these treatment methods were also employed.

MATERIALS AND METHODS

Hormone Administration. The synthetic luteinizing hormone-releasing hormone analogue, des-Gly¹⁰, [D-Ala⁶] LHRH (LHRHa), 17 α -methyltestosterone (MT), and cholesterol were purchased from the Sigma Chemical Co., St. Louis, MO, USA. Human Chorionic Gonadotropin (HCG) was purchased from the Argent Chemical Co., Redmond, WA. Cholesterol-based pellets of LHRHa were made according to the method of Lee et al. (1986) with the modifications described by Barry et al. (op. cit.). The fish were usually treated in the early afternoon, within 24 hours of capture, after being tagged, weighed, and measured.

LHRHa pellets were implanted into an incision made in the dorsal musculature using a device made from two sections of a collapsible radio antenna.

The steroid hormone, MT, was administered in one of three ways: 1) silastic tubes were prepared according to the method described by Lee et al. (1986). A small incision was made in the abdominal wall and the silastic tube containing MT dissolved in castor oil was implanted into the peritoneal cavity. One or two sutures were made to close the incision. 2) Using the method described by Higgs et al. (1977), commercial milkfish pellets were sprayed with MT dissolved in 95% ethanol to produce feed containing 10 mg MT per kg of feed. The experimental fish were fed the steroid-containing feed at a rate of 5% of their

body weight per day. 3) Powdered MT was used to make MT cholesterol-based pellets which were implanted into the dorsal musculature.

Human Chorionic Gonadotropin was administered as either an intraperitoneal or intramuscular injection using a 1-ml syringe and 22-gauge needle. In all cases, one-third of the full dose was administered at time 0 in the late afternoon, and the remaining two-thirds 12 hrs later.

Experimental Designs

Experiment 1 was conducted between Oct. 10 and Nov. 7, 1986 in 2-ton fiber glass tanks, when mature females were expected to spawn. Four treatments were administered: 1) control, 2) 10 ug LHRHa pellet, 3) 1 mg MT in a cholesterol pellet, and 4) one pellet containing both 1 mg MT and 10 ug LHRHa. There were four fish per treatment; fish were sampled daily.

Experiment 2 was conducted between July 27 and July 30, 1987. Three fish received 10,000 IU HCG per kg of body weight (10 IU/g), three received 5,000 IU (5 IU/g), and three served as controls receiving saline vehicle only. One fish from each treatment group was stocked with a potentially spawning female in a 200-l tank; i.e., 3 males and 1 female were stocked together in each of 3 tanks. The males were sampled every eight hours.

Experiment 3 was conducted from July 28 to Aug. 7, 1987. Six fish were implanted with pellets containing 25 ug LHRHa; five served as controls. Four 200-l tanks were stocked with one control and one experimental fish. One tank had two experimental fish, and one control. The fish were sampled every eight hours.

Experiment 4 was conducted from Sept. 15, 1987 to Nov. 23, 1987, in three 2 m³ hapa nets set in a 1000 m² reservoir pond. On Sept. 15, four males were placed into each of the following treatments: 1) control, 2) MT-fed, and 3) MT silastic implant (250 ug MT). On Oct. 16, two fish each were added to treatment groups 1 and 2.

On Oct. 30, 45 days after the start of the experiment, all the fish from the MT-fed and control groups were implanted with the same dose of LHRHa (pellets containing 35 ug/kg) to test the effects of LHRHa on fish previously treated, or not, with MT. Only two fish from the MT implant group survived until Oct. 30. One of these fish was administered 35 ug/kg LHRHa, the other served as a control.

Data Collection and Statistical Analysis. At each sampling time, an individual was recorded as being either with (+ or ++) or without (-) expressible milt. For all the fish within one treatment group as a whole, the total number of sampling dates when fish were found with milt was divided by the total number of sampling dates. The value obtained (% milting) gives the percentage of fish within a treatment group that were milting over the entire course of the experiment. To calculate the % milting value, + and ++ fish were not differentiated. An R x C Test of Independence Using the G-Test was used to test the null hypothesis that the % milting value was independent of the hormone treatment (Sokal and Rohlf, 1981). Rejection of the null hypothesis indicated that the treatment had significantly influenced milt production.

RESULTS

Experiment 1. Single pellet implants of 10 ug LHRHa, 1 mg MT, or a combination of the two hormones did not cause significant increases in spermiation of male scat over control fish. In the controls, 36.4% were with milt, whereas for the other treatments, the percentage of males with expressible milt ranged from 14.3 to 38.1% (Table V.1). There were no significant differences between treatments.

TABLE V.1. INDUCED SPERMATION IN MALE SPOTTED SCAT,
Scatophagus argus. EFFECTS OF LHRHa,
 MT CHOLESTEROL IMPLANT, AND LHRHa PLUS MT

TREATMENT	Dates not Milting	Dates Milting	% Milting
CONTROL	7	4	36.4
10ug LHRHa	13	8	38.1 ^{n.s.}
1 mg MT	18	3	14.3 ^{n.s.}
LHRHa & MT	12	4	25.0 ^{n.s.}

TABLE V.2. INDUCED SPERMATION IN MALE SPOTTED SCAT,
Scatophagus argus. EFFECTS OF HCG

TREATMENT	Dates not Milting	Dates Milting	% Milting
CONTROL	28	14	33.3
5,000 IU	33	7	17.5 ^{n.s.}
10,000 IU	33	9	21.4 ^{n.s.}

TABLE V.3. INDUCED SPERMATION IN MALE SPOTTED SCAT,
Scatophagus argus. EFFECT OF LHRHa

TREATMENT	Dates not Milting	Dates Milting	% Milting
CONTROL	29	29	50.0
25 ug LHRHa	22	39	63.9 ^{n.s.}

Experiment 2. Single injections of HCG into male scat at 5 and 10 IU/g body weight resulted in lower spermiation rates than in the control fish; with 33.3% for controls, and 17.5 and 21.4% in the two HCG treatments (Table V.2). These differences were not significant.

Experiment 3. LHRHa pellet implants at 25 ug resulted in 63.9% spermiation, compared with 50.0% for controls (Table V.3). This difference, however, was not significant.

Experiment 4. There was no significant spermiation in either control, MT-fed or MT-implanted fish between September 15 and October 30 (Table V.4). One of the control fish, and one of the MT-implanted fish were spermiating when captured on October 16, but neither was spermiating when next sampled on October 29. Two MT-implanted fish died before Oct. 16, perhaps because their internal organs were damaged during the implantation of the silastic tubes.

Following the implantation of LHRHa on Oct. 30, there was a dramatic increase in the number of milting males. One control and 2 MT-fed fish responded to LHRHa within 24 hours. Within 48 hours following implantation, 4 out of the 5 MT-fed males were showing a marked milting response. Three of these 4 fish were still producing measurable amounts of expressible milt 24 days later, on Nov. 23, when the experiment was terminated. In contrast, not a single control fish was found milting 48 hrs after LHRHa implantation. One male from the control group was found milting on Nov. 8 through Nov. 23. In this fish, there was an 8-day latency period following LHRHa implantation before it initially began to produce milt. Over the course of the experiment, following implantation of LHRHa, 18.2 and 61.8% of the control and MT-fed fish were spermiating, respectively. This difference was highly significant ($p < 0.001$).

In the MT-implanted group, only the male that received LHRHa began milting; the fish that received a control pellet failed to respond.

In summary: MT alone had no effect on milt production in male scat (Table V.4). When 35 ug/kg of LHRHa was administered to MT-fed or MT-implanted fish, however, there was a highly significant spermiation response. LHRHa had little or no effect on males not previously exposed to MT.

DISCUSSION

LHRHa is an effective agent for inducing spermatogenesis and spermiation in male teleosts (Donaldson and Hunter, 1983). In adult, pre-spawned Atlantic salmon (*Salmo salar*) for example, spermiation began two days following implantation of cholesterol-based pellets containing the synthetic LHRH analog, (D-Nal¹(2)⁶LHRH, (dose = 270ug/kg), whereas spermiation began after six days in control fish. Plasma gonadotropin levels were elevated for twelve days following LHRHa implantation, however, in the controls, GtH levels never rose above basal values (Weil and Crim, 1982). Apparently, LHRHa delivered in long-term-release pellets, is capable of continually stimulating spermiation via its ability to maintain elevated GtH levels for extended periods of time.

In adult milkfish, *Chanos chanos*, the combination of a cholesterol pellet containing 200 ug LHRHa, plus a silastic implant containing 250 ug of 17 α -methyltestosterone, produced a much greater spermiation response (9/14 spermiating males) than did treatment with LHRHa alone (4/14) (Lee et al., 1986). This result is very similar to the result from our study.

The exact functional role played by MT is unknown. One possibility is that MT stimulates GtH accumulation in the pituitary (Crim and Evans, 1979). Another possibility is that androgens may regulate the production and maturation of spermatozoa (Fostier et al., 1983). GtH released in response to LHRHa could then presumably induce spermiation. Either of these modes of action could explain the results from the present study.

TABLE V.4. INDUCED SPERMIATION IN MALE Scatophagus argus
EFFECTS OF LHRHa, MT, AND LHRHa PLUS MT

TREATMENT ²	FISH	SAMPLING DATE AND SPERMIATION RESPONSE ¹																	
		SEP		OCT		LHRHa		NOV											
		15	18	02	16	29	30	31	02	03	04	08	10	13	16	18	20	23	
CONTROL	1	-	-	-	-	-	Y	-	-	-	-	-	-	-	-	-	-	-	
	2	-	-	-	-	-	Y	-	-	-	-	-	-	-	-	-	-	-	
	3	-	-	-	-	-	Y	-	-	-	-	-	-	-	-	-	-	-	
	4	-	-	-	-	-	Y	-	-	-	-	-	-	-	-	-	-	-	
	5					++	-	Y	+	-	+	-	-	-	+	+	+	-	-
	6					-	-	Y	-	-	-	-	+	+	+	+	+	+	+
MT FED	7	-	-	-	-	-	Y	-	-	-	-	-	-	-	-	-	-	-	
	8	-	-	-	-	-	Y	+	+	+	+	+	-	+	+	+	+	+	
	9	-	-	-	-	-	Y	-	+	++	-	+	+	+	+	+	+	+	
	10					-	Y	-	+	++	-	+	+	+	+	+	+	+	
	11					++	-	Y	+	+	+	+	+	-	+	-	-	-	
T IMPLANT	12	-	-	-	-	-	n	-	-	-	-	-	-	-	-	-	-	-	
	13	-	-	-	-	-	Y	-	-	+	+	+	+	+	+	+	+	+	
	15					died													
	16					died													

¹ (-) no milt; (+) some milt; (++) greater amounts of milt than (+).

² Beginning Sept. 15, 1987, male Scatophagus argus were fed commercial pellets treated with (MT Fed) or without (Control) 17a-methylsterone (10 mg/kg of feed) at a rate of 5% of their body weight per y. Other males were implanted with silastic tubes containing 250 ug MT T Implant). On October 30, 1987, fish were implanted with cholesterol-sed pellets containing 35 ug/kg LHRHa (y), or given a control pellet (n).

SUMMARY

INDUCED SPERMIATION IN MALE Scatophagus argus
EFFECTS OF MT FEEDING AND LHRHa

TREATMENT	BEFORE LHRAa			AFTER LHRAa		
	Dates not Milting	Dates Milting	% Milting	Dates not Milting	Dates Milting	% Milting
CONTROL	23	1	4.2	54	12	18.2
FED	23	1	4.2	21	34	61.8***

*** p < 0.001

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Regardless of its precise method of action, MT delivered prior to treatment with LHRHa is a highly effective method to induce spermiation in *Scatophagus argus*.

VI. THE EFFECT OF SALINITY ON SPERM MOTILITY IN THE SPOTTED SCAT (*Scatophagus argus*)

Terence P. Barry, Maria Paz Socorro C. Macahilig
and Milagros T. Castaños

INTRODUCTION

We began our research on the induced gonadal maturation and spawning of the spotted scat (*Scatophagus argus*) knowing very little about its natural spawning habits. Although we soon induced female scat to spawn, we did not succeed in obtaining fertilized eggs. We concluded that either the eggs were of poor quality, perhaps because we had overdosed with LHRHa, or that we had used an inappropriate salinity in our spawning tanks (17 to 25 ppt, and 35 ppt) which resulted in the death of the spawned eggs and sperm. Winfree (1983, unpublished report) was able to successfully spawn the scat and get viable eggs, however, he did not report salinity values. Articles in various aquaria trade publications stated that scats spawned in habitats ranging from freshwater rivers to coral reefs; i.e., in salinities ranging from 0 to 35 ppt.

The scat is an estuarine species, and our broodstock collection efforts began to suggest that scat spawned in brackishwater. We needed to know, however, what salinity was optimal for sperm and egg survival. We therefore conducted experiments to determine the survival of mature scat sperm in water of different salinities. Our underlying assumption was that sperm survival would be highest at the salinity where normal spawning occurs.

MATERIALS AND METHODS

Water samples of desired salinities were prepared by dissolving sea salt in distilled water, and adjusted to the nearest 1 ppt using a handheld optical refractometer. All males were acclimated at 25 ppt for at least 2 weeks prior to the experiment. For the experiments, milting males were anesthetized with 2-phenoxyethanol (3 ml/l), and 5- μ l milt samples were taken directly from the their genital pores using a 50- μ l glass microcapillary tube.

In experiment 1, small drops of milt (0.5 μ l) were placed into the depressions of 8 glass well-slides. At 1-min intervals, the 8 milt samples were diluted with 200- μ l water samples ranging in salinity from 5 to 45 ppt, and mixed with a toothpick. The precise time when the water was added to each milt sample was recorded. After the milt samples were diluted, they were each examined in turn at exactly 5-min intervals through a compound microscope. The time when 100% of the sperm on the slides became completely immotile was recorded. The effects of individual salinities on the duration of sperm motility were tested in this way 4 to 9 times. Milt from a new fish was used for each trial. All experiments were conducted at 25 C.

In experiment 2, one salinity was tested at a time. After the milt was diluted, the percentage of the total number of visible sperm which remained motile was estimated at 2-min intervals until all movement stopped. Other salinities were then tested using milt drawn freshly from the same male. A haemocytometer was used to estimate the percent active spermatozoa. Different males were used for each of the 2 or 3 replications.

Blood samples were taken from the caudal vessels of 9 male scat acclimated at 25 ppt. The blood was allowed to clot on ice for several hours, and the plasma centrifuged at 10,000 RPM for 10 min. Plasma osmolarities of three pooled samples were measured with a Wescor vapor pressure osmometer. The osmotic pressures of the different salt solutions used in the sperm motility experiments were also measured.

An analysis of variance (ANOVA) followed by a Duncan's multiple range test (DMRT) was used to test for significant differences among the means.

RESULTS

The osmotic pressure of male scat blood plasma acclimated to 25 ppt was 315 ± 1.7 mOsm/kg. The following regression equation ($R^2 = 0.996$) was calculated, and gives the osmotic pressure of the sea salt solutions used to dilute the scat's milt:

$$\text{Osmotic pressure (mOsm/kg)} = [31.7 \times \text{Salinity (ppt)}] - 48.5$$

In experiment 1, the longest mean survival time of scat spermatozoa was 24.4 ± 3.4 min at 25 ppt; followed by 23.1 ± 3.4 min at 35 ppt; 22.8 ± 4.5 min. at 20 ppt; 22.5 ± 3.7 min at 15 ppt; 17.5 ± 1.4 min at 40 ppt; 11.2 ± 1.2 min at 45 ppt; 7.1 ± 2.4 min at 10 ppt; and 5 ± 2.6 min at 5 ppt (Fig. VI.1). There was a 316% increase in sperm survival between 10 and 15 ppt. There were no significant differences in the time spermatozoa remained motile when placed into 15, 20, 25, and 35 ppt. There were also no significant differences among 5, 10, and 45 ppt. These two groups, however, were significantly different from each other ($P < 0.05$); 40 ppt was not significantly different from either of these groups.

Significant differences among the salinities: 15, 20, 25 and 35 were found in experiment 2 when the percentage of spermatozoa which remained motile over time was recorded. The sperm became immotile significantly faster ($P < 0.05$) at salinities 15, 20, and 35 ppt. After 8 min, for example, only 20% of the sperm remained motile at these salinities. In contrast, 80% of the sperm was motile after 8 min at 25 and 30 ppt. After 14 min, 40% of the sperm remained motile at 25 and 30 ppt, whereas only 5% was motile at 15, 20, and 35 ppt. By 20 min, however, the spermatozoa were immotile at all salinities (Fig. VI.2).

DISCUSSION

Spermatozoa of many marine teleosts become motile when diluted with solutions of a higher osmolarity than the fish's seminal fluid, while in freshwater oviparous species, sperm motility is stimulated by solutions of lower osmolarity (Morisawa and Suzuki, 1980). By measuring spermatozoan motility and longevity in the laboratory under simulated spawning salinities, we can infer preferred spawning salinity ranges in the natural habitat. Evidence from 4 freshwater species and 5 marine teleosts indicate that fish spermatozoa are adapted to the salinity of the environment where they are naturally released (Morisawa and Suzuki, 1980).

Scat sperm survival was low at 5 and 10 ppt (110 and 269 mOsm/kg). These salinities are hypotonic to the scat's blood plasma (315 mOsm/kg). At salinities well below the osmotic pressure of its blood, the scat's sperm is not stimulated, and dies rapidly, probably because of hypoosmotic shock. This suggests that the scat does not spawn at water salinities of 10 ppt or less.

Although differences in sperm survival at salinities between 15 and 35 ppt could not be determined using the methodology of experiment 1, the results clearly indicate that sperm survival is highest in brackishwater. The results from experiment 2 do show, however, that more spotted scat spermatozoa remain motile longer at salinities between 25 and 30 ppt. This suggests that spotted scat may spawn preferentially at salinities within this range. Although the time to 100% immobility is not significantly different between 25 and 35 ppt, there is a more rapid decline in sperm survival at 35 ppt. This suggests that the scat may not spawn at sea.

Based on the broodstock and fry catch data, we concluded that scat spawn after the start of the rainy season. In Iloilo, the SW monsoons bring rain and river runoff which result in reduced salinities in the scat's coastal, estuarine habitat. This habitat is where we collected our scat broodstock. The hypothesis that spawning in spotted scat is

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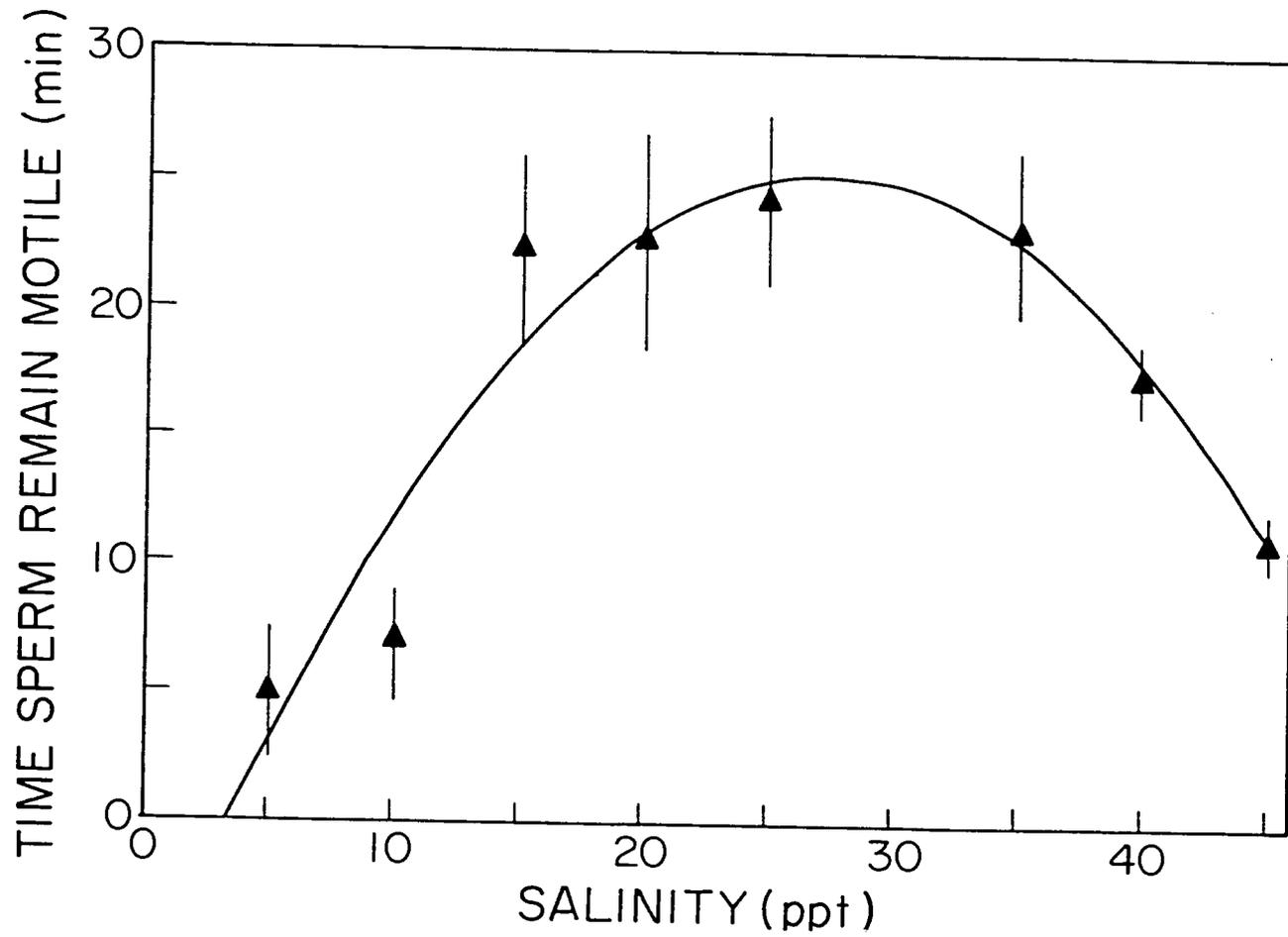


Fig. VI.1. The length of time spotted scat spermatozoa remain motile when diluted with water, as a function of salinity. Each point represents the mean \pm the standard error of 4 to 9 trials.

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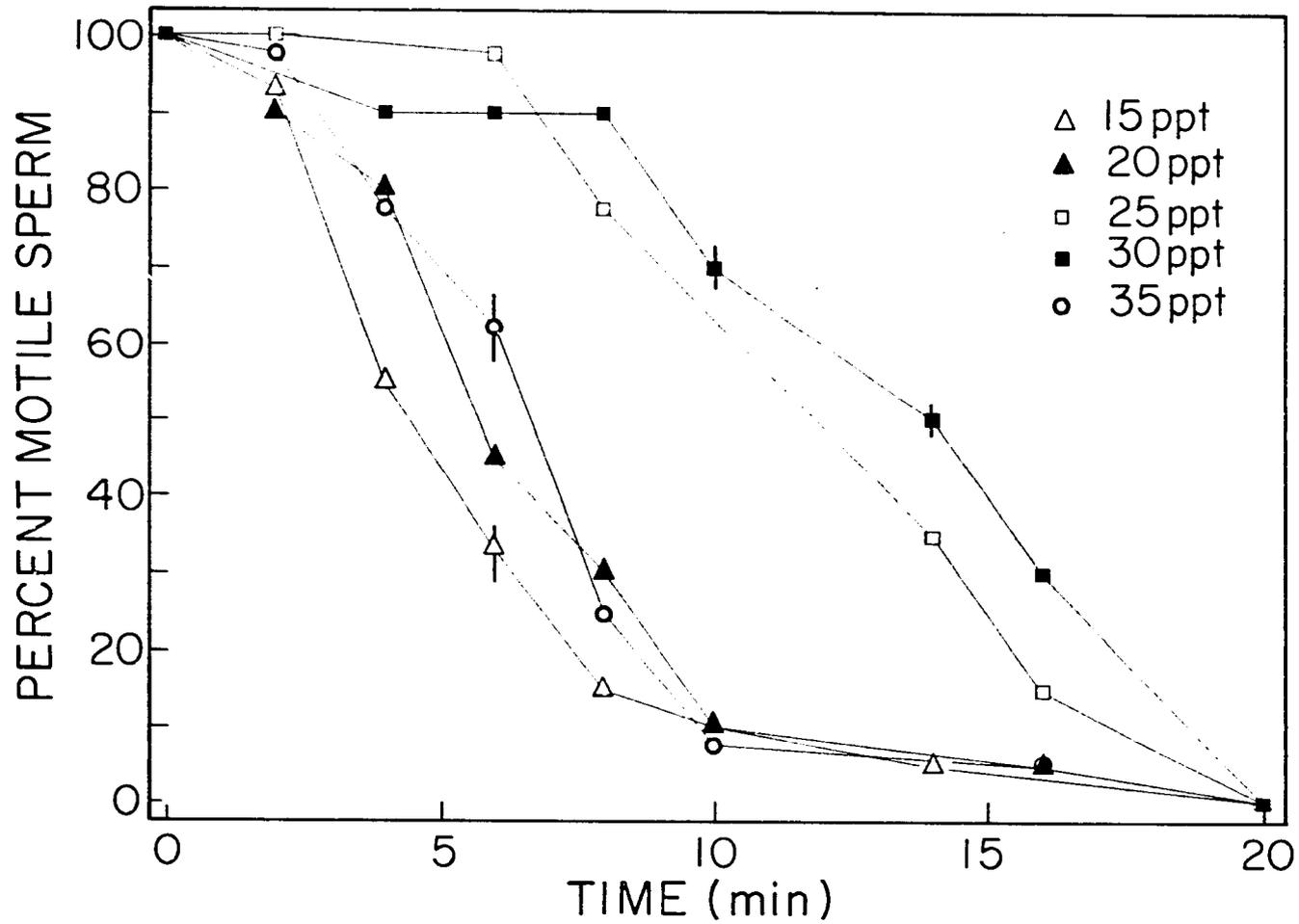


Fig. VI.2. Spotted scat spermatozoa motility as a function of time and five salinities. Diluted milt samples were observed at 2-min intervals, at which time the percentage of motile spermatozoa was estimated. Each point shows the mean \pm the standard error of 2 or 3 trials.

stimulated by rain and river runoff is further supported by the data presented here, which suggests that scat spawn in brackishwater with salinities lower than sea water, in the range 25 to 30 ppt.

VII. PRODUCTION OF MILKFISH (*Chanos chanos*) AND SPOTTED SCAT (*Scatophagus argus*) IN POLY CULTURE

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INTRODUCTION

The Filipinos have reared milkfish (*Chanos chanos*) in brackishwater ponds for centuries (Villaluz, 1953). Over time, marked improvements in culture techniques have evolved, and there has been a steady increase in the number of fish ponds throughout the Philippines. Presently, the Philippines has a potential area of 425,368 ha for brackishwater ponds, of which 176,230 ha are developed (BFAR, 1976).

Milkfish production in the traditionally-managed Philippine ponds has significantly increased in recent years through the application of science and technology. Nevertheless, the national average milkfish production in Philippine ponds is still only 640 kg/ha/yr (Lijauco, 1979), well below Taiwan's average production of over 2,000 kg/ha/yr (Rabanal and Shang, 1976). This difference can be largely attributed to differences in the feeding strategies employed in the two countries. In the Philippines, almost all milkfish production relies on feeding via "food chain" sources. In Taiwan, however, farmers make widespread use of prepared milkfish feeds which greatly enhance yields.

Milkfish has been the traditional brackishwater pond fish in the Philippines. Only tilapia have been cultured widely in large quantities in these same ponds, and only very recently. Many other fish species are cultured "incidentally" in ponds, often due to accidental intrusion rather than by design. Spotted scat (*Scatophagus argus*) is one such species. Even though it is a high priced and preferred food fish in the Philippines, it has not been pond-cultured in large numbers. In a recent market survey, the cost per kilogram of a marketable-sized scat, locally known as "kitang" or "kikiro", ranged from 60 to 80 pesos (Becerial, 1987). The Fish Marketing Review (1979) reported that from July to December 1976, 619 and 2,115 kg of scats were sold at the Iloilo Palapala Fish Market and Navotas Fishing Port and Fish Market, respectively.

Very little data exists on the incidental pond growout of spotted scat, and the limited experimental data that exists is generally unpublished and unavailable. If successful growout strategies and management techniques for the scat do exist, therefore, they are largely undocumented. We initiated a series of experiments to evaluate different growout strategies for the scat. The experiments reported here on the polyculture of milkfish and scat in traditional, unfed lab-lab ponds had two primary objectives:

1. To establish benchmark information on the survival and growth of scat in brackishwater ponds stocked at two densities, in polyculture with milkfish stocked at a single density.
2. To evaluate the effect of two scat stocking densities on the survival and growth of milkfish.

MATERIALS AND METHODS

Experimental Ponds and their Preparation. The experiment was conducted in six 250 m² brackishwater ponds of the ISCOF Brackishwater Fishpond Center at Tiwi, Barotac Nuevo, Iloilo. Each pond had an individual wooden gate provided with fine meshed screen (0.50 mm) to prevent the entrance of wild fish and the escape of experimental animals.

Prior to stocking, the ponds were drained, levelled and dried for 15 days until the soil cracked. After drying, the ponds were flushed with new tidal water. Thereafter, agricultural lime was evenly broadcasted in all ponds at the rate of 2 metric tons/ha. The next day, carabao (domestic water buffalo) manure was applied at 2 tons/ha. One day later,

carabao manure was again applied at 2 tons/ha. Ponds were then filled with tidal water to a depth of 5 cm. The next day, 2 inorganic fertilizers, 16-0-0 and 45-0-0, were applied at 50 kg each per ha. After 5 days, pond water depth was raised to 10 cm. This depth was maintained until lab-lab had grown. Once lab-lab was well established, the pond water was gradually increased to 40 cm over one week. Seven days before stocking the milkfish and scat, teaseed cake was applied at 15 ppm to eradicate undesirable fish species.

Fish Stocking. Milkfish fingerlings, with average weights and lengths of 4.0 g and 6.4 cm, respectively, were stocked in combination with spotted scat. Scat average weights and lengths were 0.2 g and 0.9 cm, respectively. The fishes were acclimated to the temperature and the salinity of the ponds before they were randomly stocked. Stocking was done in the early morning of August 10, 1987.

Experimental Design and Statistical Analysis. The experimental design consisted of two treatments with three replications each. A randomized complete block design was employed because we suspected that the ponds lay along a fertility gradient. The treatments were as follows:

Treatment	Stocking Density(#/m ²)
I	1.30 scats 0.53 milkfish
II	2.61 scats 0.53 milkfish

In all, 133 milkfish were stocked into each pond (total milkfish = 798), 326 spotted scat were stocked into each pond of treatment I, and 652 spotted scat were stocked into each pond of treatment II (total scat = 2,934).

Results obtained from this study were analyzed using a two-level nested ANOVA. Results with significant differences were further analyzed using the Duncan's multiple range test to detect the differences between treatment means.

Water Management and Fertilization. The pond water depth ranged from 40 to 45 cm throughout the culture period. Water was replenished 3 times each spring tide by partially draining the ponds during low tide and replacing the same volume of water during high tide.

Inorganic fertilizer (16-20-0) was applied to all ponds every week at the rate of 12.5 kg/ha to provide sufficient nutrients for "lab-lab" and other organic producers.

Sampling. Weights and lengths were taken on the 60th day of culture by seining and randomly selecting a sample of 5 milkfish and at least 15 scats per pond.

Determination of Physio-Chemical Parameters. Water temperature and dissolved oxygen (DO) concentration at a depth of 25 to 30 cm were measured twice daily, at 0530 to 0600 hrs and 1500 to 1700 hrs, throughout the culture period. Salinities were measured daily between 1500 and 1530 hrs with an Atago Refractometer. Water pH was measured three times a week between 0500 and 0530 hrs with a Broadley James pH meter.

Harvesting. Fish in all treatments were harvested after 90 days of culture. Harvesting was done by first seining each pond three times, and then totally draining the ponds and collecting and counting the fish by hand.

RESULTS

Average weekly morning water temperatures ranged from 26.5 to 31 C (Fig. VII.1). Afternoon temperatures were typically 4 C warmer, and ranged from 30 to 36 C. Week 4 had the coolest average temperatures, while week 6 had the highest.

Average morning dissolved oxygen ranged between 2 and 4 mg/l, with an upward trend during growout (Fig. VII.2). Afternoon dissolved oxygen was always greater, and ranged between 3 and 22 mg/l. The highest afternoon dissolved oxygen values were during the last month.

Secchi disc transparencies were the inverse of dissolved oxygen. The highest transparencies occurred during week 2 with 32 cm, while the lowest occurred during weeks 10 and 12 with 23 and 24 cm, respectively. (Fig. VII.3).

Salinities varied substantially during growout, from a high of 30 ppt during weeks 3 and 4, to a low of 16 ppt during weeks 8, 9 and 10 (Fig. VII.4). Salinities again increased to 26 ppt by the end of the experiment.

Water pH increased from an average of 6.7 at the beginning of growout, to 8.0 during the last week (Fig. VII.5). There were no differences in pH between the ponds in treatments I and 2 over the growout.

During the 3-month growout, the milkfish weight increase was approximately linear, with average final weights of 85.8 and 83.3 g for treatments I and II, respectively (Fig. VII.6; Table VII.1). There was not a significant difference between these two means. Among the three ponds within treatments, however, there were significant differences in milkfish weight ($p < 0.01$). Milkfish survival was very high in all ponds, ranging from 92.5 to 100%, with an overall average survival of 96.2%. Average milkfish yields were 446.5 and 422.2 kg/ha for treatments I and II, respectively. The difference between these two means was not significant.

The rate at which the spotted scat gained weight during the 3-month growout declined after the eighth week of culture. (Fig. VII.6). The final average weights for the scat were 2.78 and 2.23 g for Treatments I and II, respectively (Table VII.1). The difference between these means was significant ($p < 0.01$). There were no significant differences in average scat weights among the three ponds within treatments. Spotted scat survival was 74.2 and 66.9% for treatments I and II, respectively, and ranged from 52.4 to 76.7%. Average scat yield was 26.9 kg/ha for treatment I (low stocking density), and 38.5 kg/ha for treatment II (higher densities). The difference between these means was highly significant ($p < 0.01$).

DISCUSSION AND CONCLUSION

Spotted scat grow slowly in unfed ponds when compared to milkfish and other commonly cultured pond fishes. In our study, scat weight gain during three months in unfed earthen ponds was 3% that of milkfish with overall gains of 2.3 g for scat vs. 81 g for milkfish. Survival was good, but growth in these ponds was not adequate enough to justify their production as a food fish. They would perhaps need to reach 50 g each before they would be marketable as a food fish. In unfed ponds, even at low densities, it might take perhaps one year to reach this size range. This might be achievable if grown in polyculture with milkfish through three milkfish crops, but this would require live separation and hand sorting of the scat through two of the harvests so that the scat could be restocked for further growout.

Magistrado (unpublished data) also observed very slow growth of spotted scat in unfed brackishwater and freshwater ponds. She found that scat stocked at 3.0/m² and 0.5/m² in these ponds grew to about 1.75 g in 3 months, and to 6 g in 10 months (Fig. VII.7). Growth was about the same in both fresh and brackishwater. Although our fish grew faster, growth was still very slow in these unfed pond systems.

Perhaps as an alternative to growout for food, the scat could be collected as small

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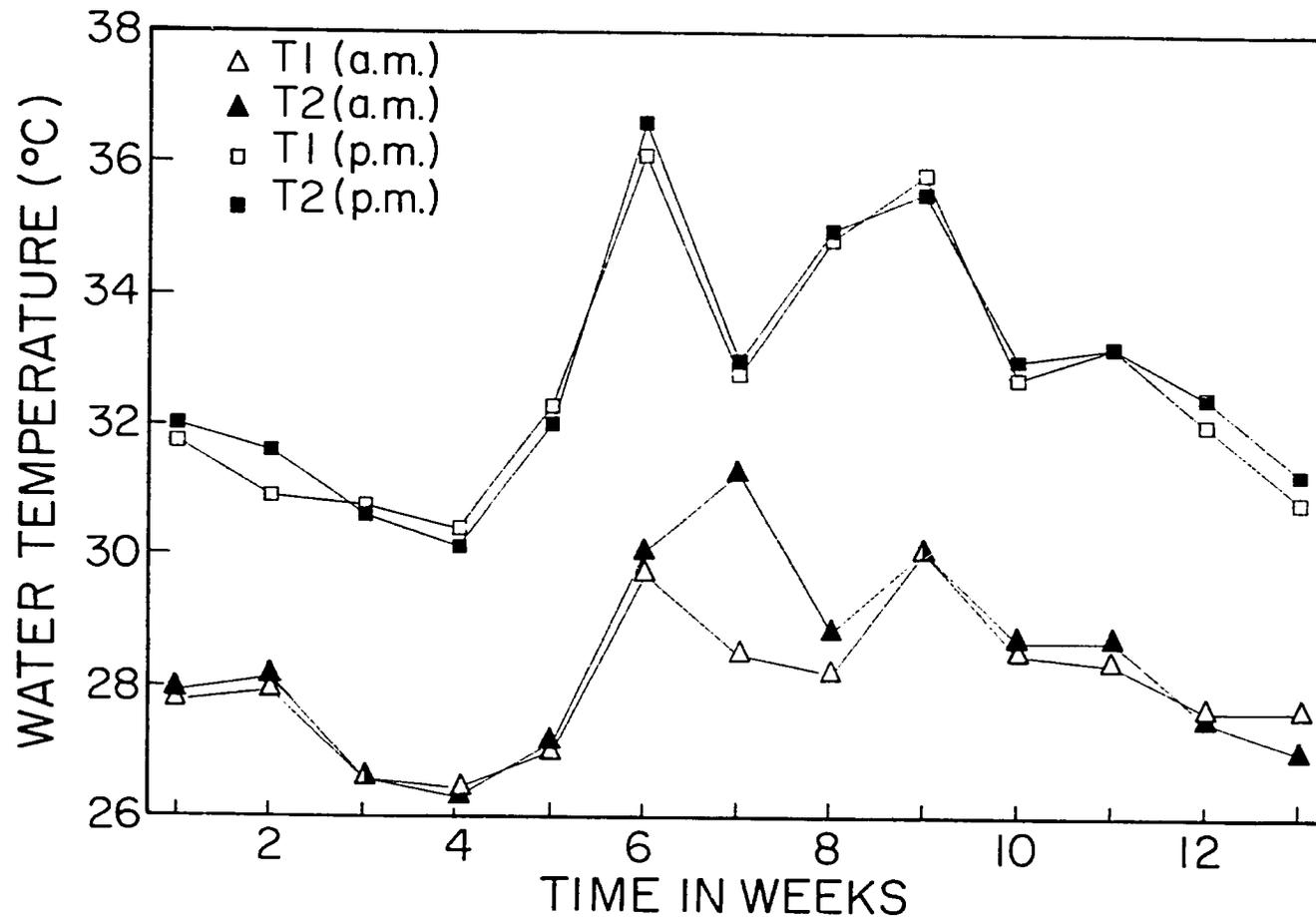


Fig. VII.1. Average weekly morning (lower lines) and afternoon (upper lines) water temperatures in each of the two treatment groups of the milkfish and spotted scat polyculture experiment. Milkfish were stocked at one density in both treatments (0.53 fish/m^2), while scat were stocked at 1.30 and 2.61 fish/m^2 in treatments 1 (T1) and 2 (T2), respectively. There were three 250 m^2 ponds per treatment. Start date was 10 September 1987.

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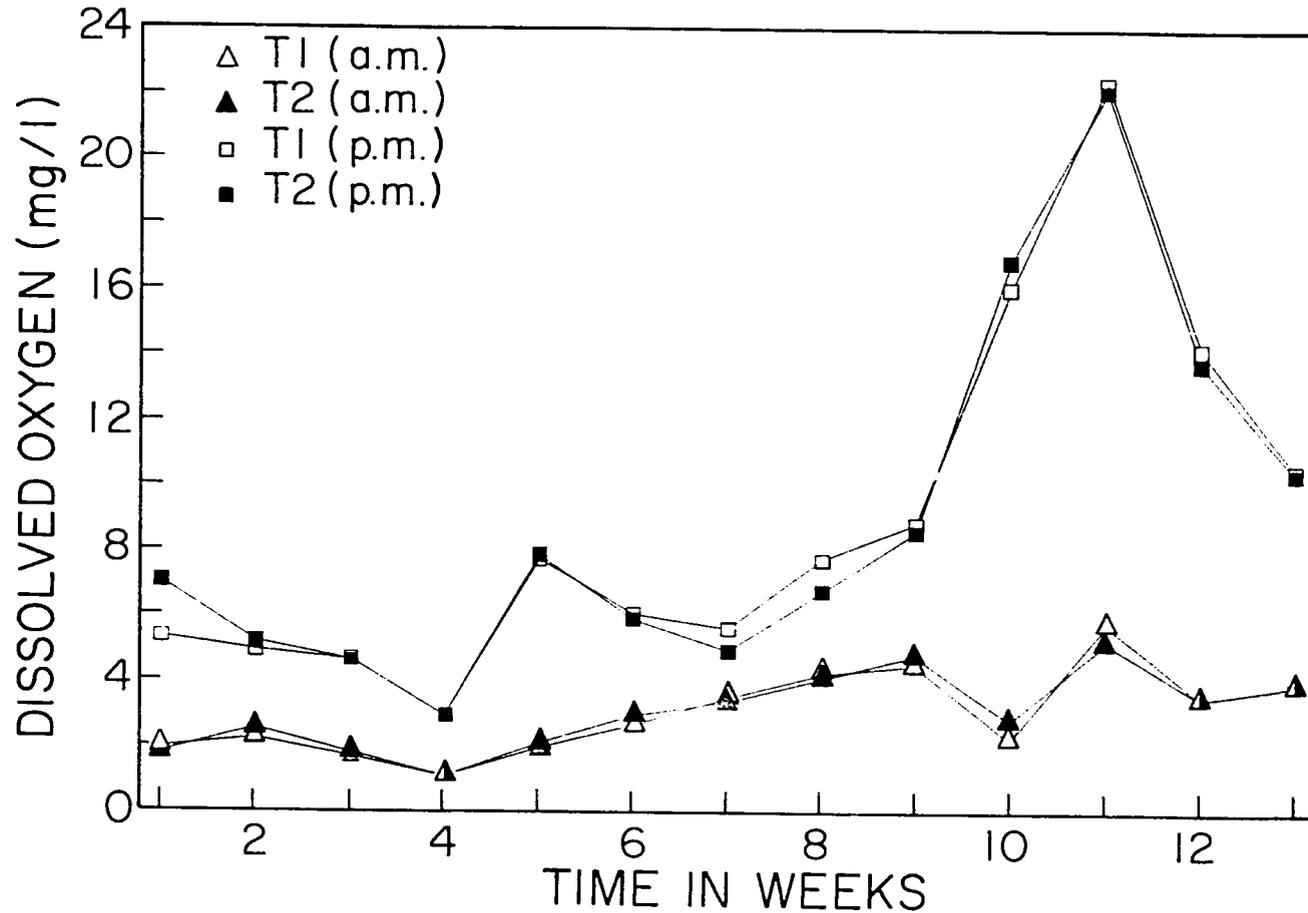


Fig. VII.2. Average weekly morning (lower lines) and afternoon (upper lines) dissolved oxygen in each of the two treatment groups of the milkfish and spotted scat polyculture experiment. Milkfish were stocked at one density in both treatments (0.53 fish/m^2), while scat were stocked at 1.30 and 2.61 fish/m^2 in treatments 1 (T1) and 2 (T2), respectively. There were three 250 m^2 ponds per treatment. Start date was 10 September 1987.

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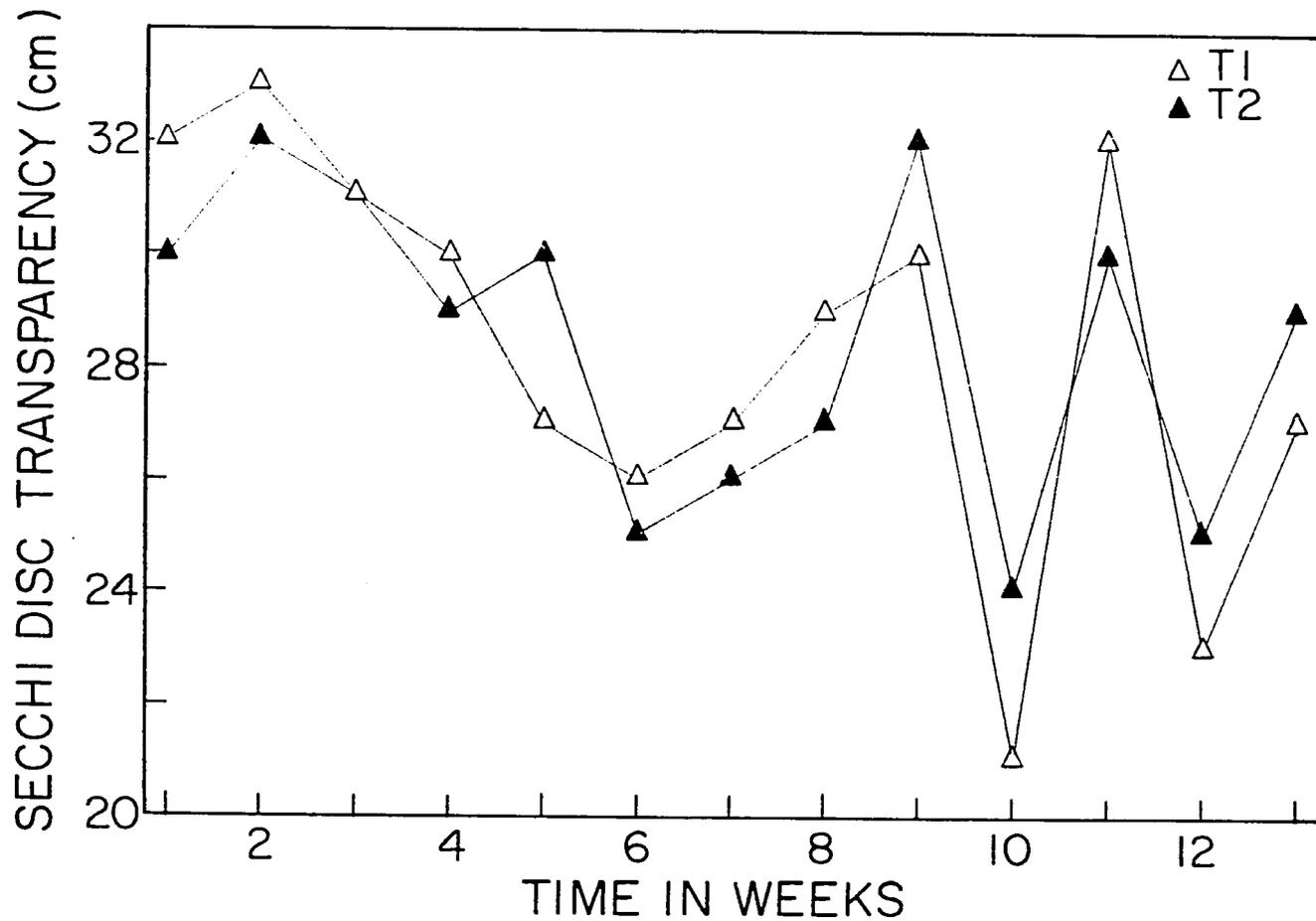


Fig. VII.3. Average weekly secchi disc transparencies in each of the two treatment groups of the milkfish and spotted scat polyculture experiment. Milkfish were stocked at one density in both treatments (0.53 fish/m^2), while scat were stocked at 1.30 and 2.61 fish/m^2 in treatments 1 (T1) and 2 (T2), respectively. There were three 250 m^2 ponds per treatment. Start date was 10 September 1987.

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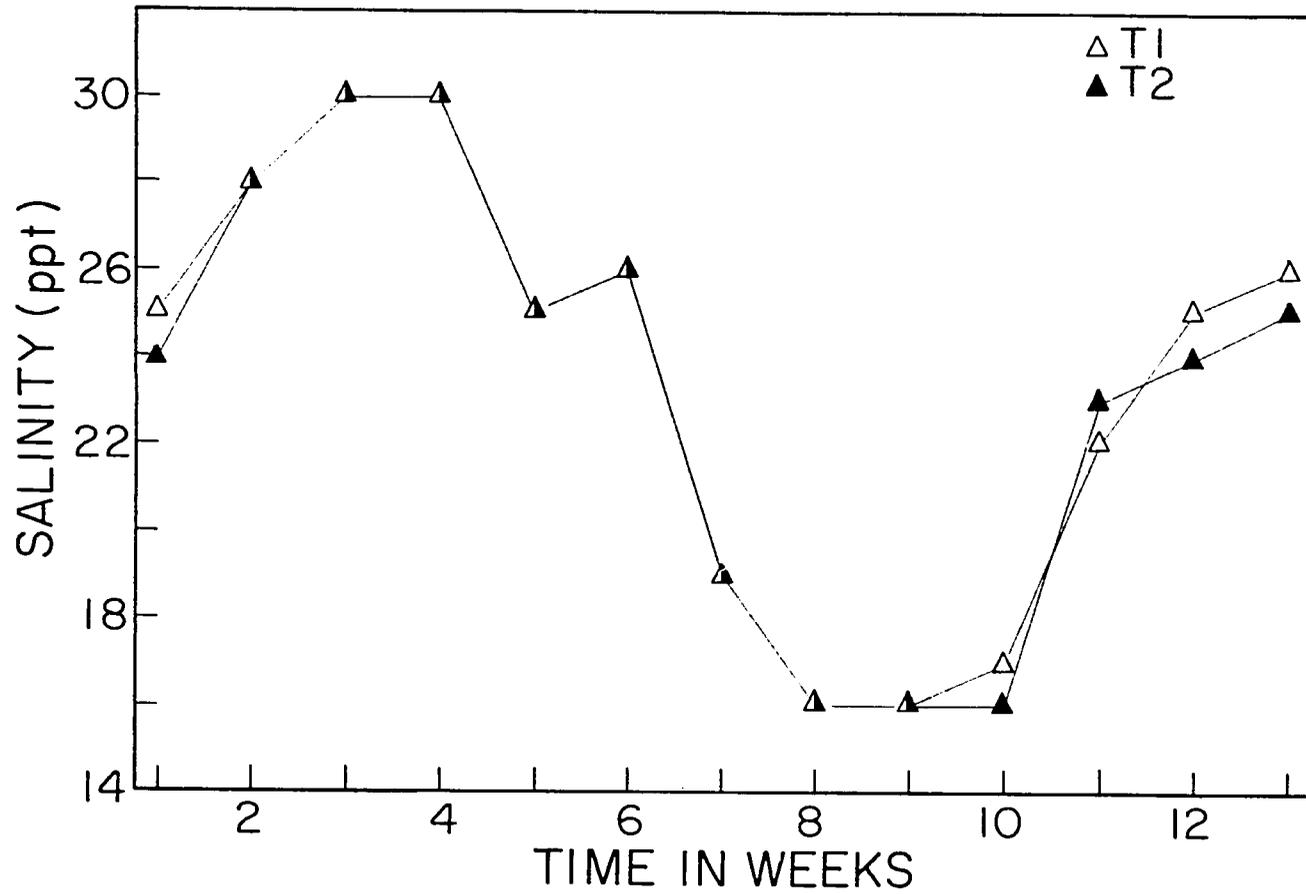


Fig. VII.4. Average weekly salinities in each of the two treatment groups of the milkfish and spotted scat polyculture experiment. Milkfish were stocked at one density in both treatments (0.53 fish/m^2), while scat were stocked at 1.30 and 2.61 fish/m^2 in treatments 1 (T1) and 2 (T2), respectively. There were three 250 m^2 ponds per treatment. Start date was 10 September 1987.

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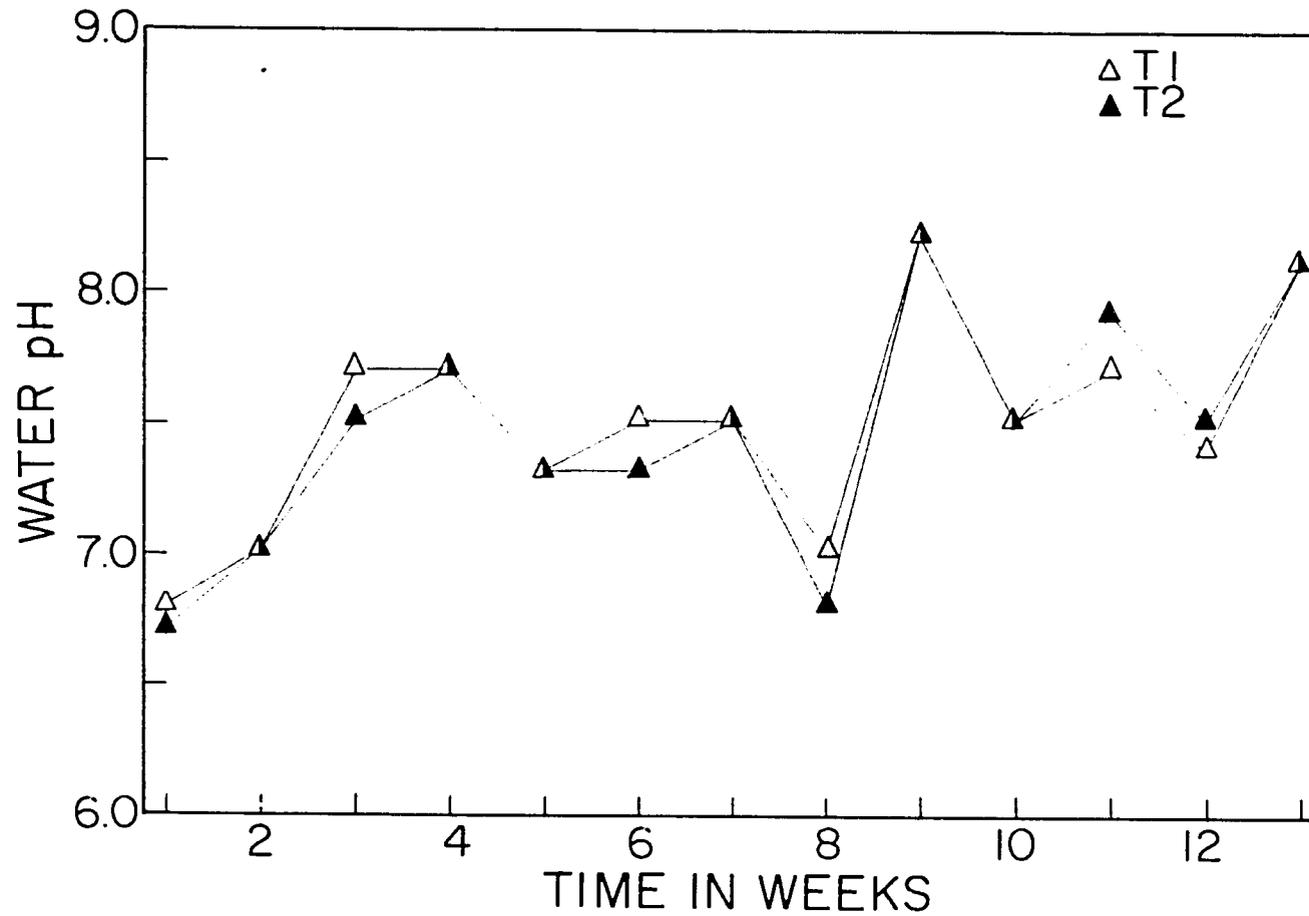


Fig. VII.5. Average weekly water pH in each of the two groups of the milkfish and spotted scat polyculture experiment. Milkfish were stocked at one density in both treatments (0.53 fish/m^2), while scat were stocked at 1.30 and 2.61 fish/m^2 in treatments 1 (T1) and 2 (T2), respectively. There were three 250 m^2 ponds per treatment. Start date was 10 September 1987.

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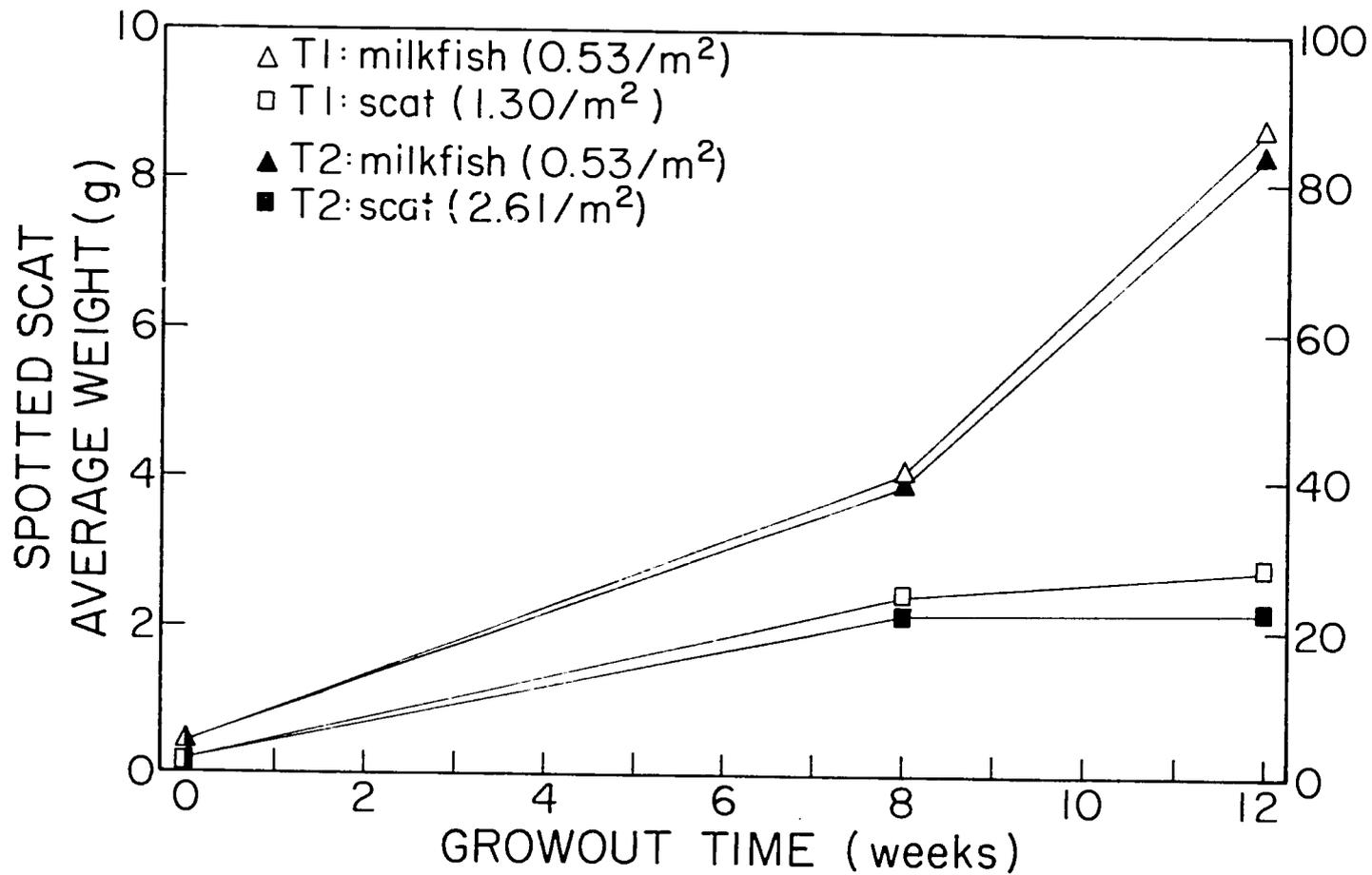


Fig. VII.6. Individual average growth of milkfish and spotted scat in non-fed, 250 m² brackishwater ponds. Milkfish were stocked at one density in both treatments (0.53 fish/m²), while scat were stocked at 1.30 and 2.61 fish/m² in treatments 1 (T1) and 2 (T2), respectively. There were three 250 m² ponds per treatment. Start date was 10 September 1987.

TABLE VII.1. FINAL SURVIVAL, GROWTH AND YIELD DATA FOR MILKFISH AND SPOTTED SCAT IN POLYCULTURE

		MILKFISH					SPOTTED SCAT				
Treatment Pond		Survival		Average Weight (g)	Average Length (cm)	Yield (kg/ha)	Survival		Average Weight (g)	Average Length (cm)	Yield (kg/ha)
		No.	%				No.	%			
I	1	128	96.2	106.3	19.7	544.2	239	73.3	2.94	4.8	28.1
	2	128	96.2	83.6	22.2	428.2	250	76.7	2.65	4.4	26.5
	3	130	97.7	70.6	24.1	367.1	237	72.7	2.75	4.6	26.0
Average		128.7	96.7	86.8	22.0	446.5	242	74.2	2.78	4.6	26.9
II	1	126	94.7	108.2	21.2	545.3	497	76.2	2.02	4.0	40.2
	2	123	92.5	81.9	22.2	402.9	342	52.4	2.48	4.3	33.9
	3	133	100.0	59.9	24.1	318.5	470	72.1	2.20	4.2	41.3
Average		127.3	95.7	83.3	22.5	422.2	436.3	66.9	2.23	4.2	38.5

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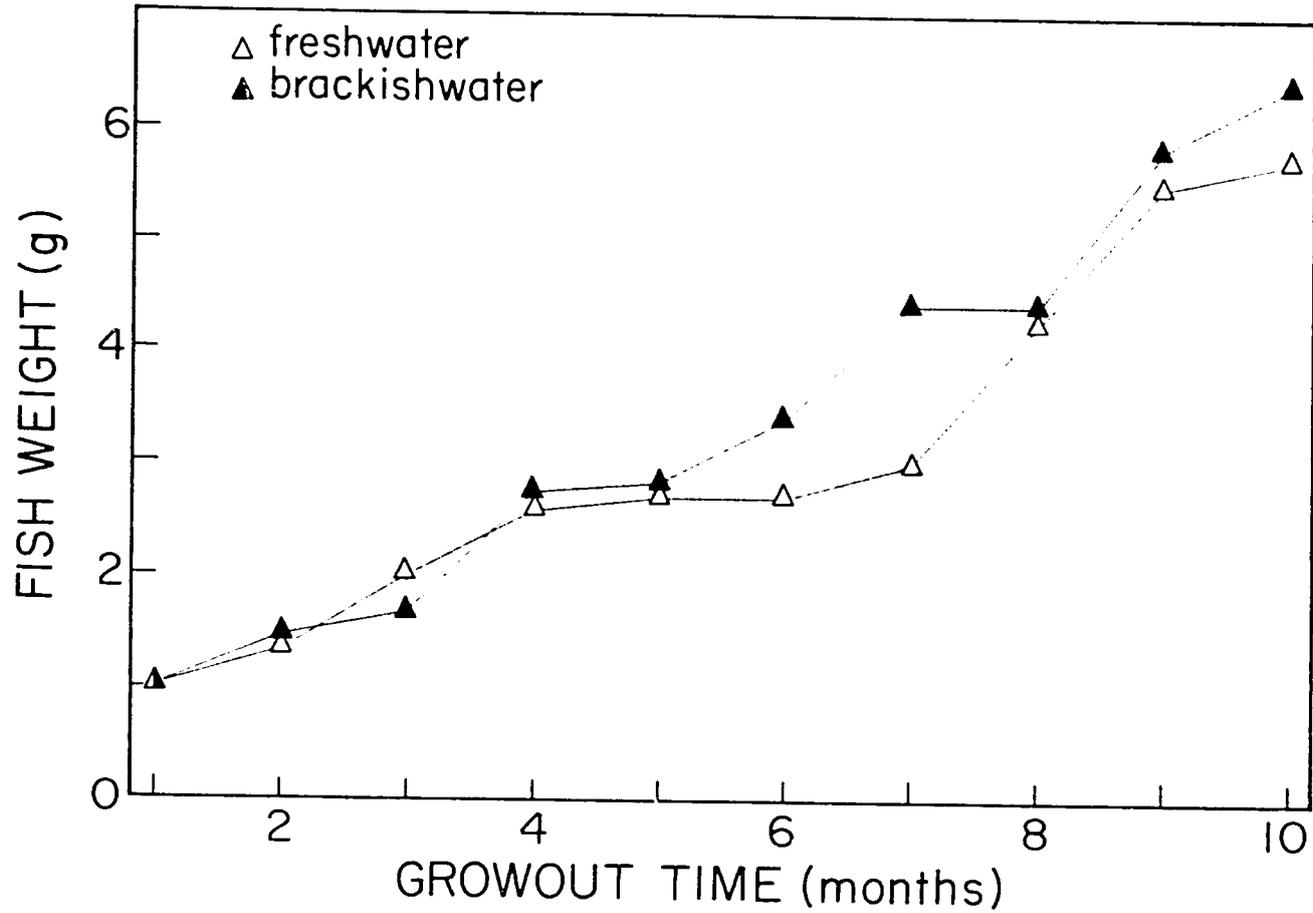


Fig. VII.7. Growth of spotted scat (*Scatophagus argus*) in brackishwater and freshwater ponds at the BFAR Station, Butong, Batangas, Philippines. Stocking densities were 0.50 fish/m² and 3.0 fish/m² in the respective trials. Data from Magistrado (unpublished; personal communication, 1987).

fry (0.2 g or less) and then grown in ponds to 2 g or larger for the aquarium trade. The larger fish command a higher price, and may be hardier for shipment, if good husbandry techniques are used in the ponds. They could also be grown in polyculture with milkfish as an add-on crop.

When grown in polyculture with milkfish, spotted scat did not affect the growth rate or the yield of the milkfish at the stocking densities that we tested. That is, scat stocked at 1.3 and 2.6 fish/m² did not cause significant differences in milkfish growth when each species reached final standing crops of 38.5 kg/ha for scat and 422 kg/ha for milkfish. Even with the highest scat average yield in our trials, scat standing crop was less than 10% of that for milkfish. Perhaps at some higher standing crop of scat, there would be a negative interaction on milkfish yields, but not in our case.

Although scat did not affect milkfish yield in polyculture with milkfish, there was a density-dependent interaction among scat stocked in our trials. At 2.6 scat/m² stocking density, growth was significantly less than when stocked at 1.3 scat/m² (Table VII.1). Although mean growth was significantly slower at the higher stocking densities, it was only 20% less at the higher stocking densities in absolute terms, but yield was 45% greater. A wider range of scat stocking densities and final yields are needed to better define the stocking density vs. yield effects.

VIII. THE EFFECT OF TWO STOCKING DENSITIES AND METHYL-TESTOSTERONE FEEDING ON GROWTH OF SPOTTED SCAT (*Scatophagus argus*) IN EARTHEN PONDS

Henry D. Biona, Sr., Rizaline Tabanda, Rodney Bayogos,
Arlo W. Fast, and Terence P. Barry

INTRODUCTION

Some of the main concerns of aquaculturists are the length of time it takes to bring a cultured crop to market, crop yield, and the size of the individual animals at harvest. These variables are not independent, but instead depend on a number of other factors such as: stocking density; feeding rate and feed quality; water quality management; and most importantly, the intrinsic growth rate of the cultured species.

Lowest yields are typically produced in extensive pond growout, where nutrition for the cultured crop comes from food chain production, with no formulated feed additions. Crop stocking densities (number of animals/m²) in extensive systems are low to assure that when carrying capacity (kg/m²) is reached and the crop harvested, the individual animals have reached marketable size.

The growth rate of spotted scat (*Scatophagus argus*) polycultured with milkfish (*Chanos chanos*) in extensive pond growout is quite low. During a 3-month growout, the scat grew from 0.20 to 2.8 g at a stocking density of 1.3/m², while at the same time, the milkfish grew from 4.0 to 86.8 g (Biona et al., VII, this volume).

When scat are cultured in ponds receiving prepared, pelleted feeds, their growth, yield, and individual size at harvest can be markedly increased. We cultured scat in a semi-intensive polyculture system with tiger prawns (*Penaeus monodon*) and found that during a 3-month growout, the fed scat stocked at 1.15/m² grew from 0.41 g to 10.32 g. At the same time, the prawns grew from 1.7 mg to 28.3 g (Biona et al., IX, this volume).

Anabolic hormones, incorporated into prepared feeds, have been used to accelerate the growth of some fishes, even beyond their intrinsic growth rate. For example, the growth of tilapia and many salmonids is greatly accelerated when they consume prepared feeds containing the steroid hormone methyltestosterone (MT) (for extensive review, see Cruz and Barry, X, this volume). With this in mind, we decided to assess the growth enhancement potential of MT as a prepared food additive on scat cultured in earthen ponds. The principal objective was to determine if the anabolic hormone, MT, could increase scat growth such that a crop of marketable-sized fish could be produced within a reasonable growout period.

MATERIALS AND METHODS

Experimental Ponds and Their Preparation. The 250 m² experimental ponds and their preparation are similar to those described by Biona et al. (IX, this volume), with two exceptions. For this experiment, no peripheral canal was dug in the ponds; instead, the pond bottom was level. During growout, the water depth was maintained at 50 to 55 cm. In all, 12 such ponds were used in the scat monoculture/hormone experiment.

Experimental Animals and Stocking. Wild scat fry with average weights ranging from 0.09 to 0.13 g were stocked on 3 dates. On 13 August, 4 ponds (block 1, one of 4 treatments per pond) were stocked with 0.09 g scat fry which had been acclimated for 2 days in tanks; on 14 August, the 4 ponds of block 2 were stocked with 0.126 g acclimated fry; and on 26 August, the 4 ponds of block 3 were stocked with 0.053 g fry which were not acclimated in tanks, but instead stocked directly into the ponds following capture and transport to ISCOF.

Experimental Design and Statistical Analysis. The experimental design consisted of four treatments with three replications each in a randomized block design as follows:

Treatment	Scat Stocking Density (#/m ²)	Hormone (MT, 10 mg/kg)
I	1.15	(-) without
II	5.76	(-) without
III	1.15	(+) with
IV	5.76	(+) with

In all, 288 scat fry were stocked into each low-density pond, and 1,440 into each high-density pond (grand total = 10,368). Data were analyzed using a two-way ANOVA. Results with significant differences were further analyzed using the Duncan's Multiple Range Test to detect differences between treatment means.

Water Management. The pond water in all treatments ranged from 50 to 55 cm throughout the culture period. Pond water was replenished every other day during spring tide by draining 10 to 15% of pond water during low tide and replacing the same volume during high tide. Water depth was maintained by pumping water from a reservoir, as needed, throughout the culture period.

Feeding and Sampling. Scat were fed with commercial floating pellets twice daily. The feeding rates used were 15% (days 1-30), 7% (days 31 to 60), 5% (days 61 to 76), 3% (days 77 to 92), and 2% (days 93 to 120). The feed containing MT was prepared as described by Cruz and Barry (X, this volume) and contained 10 mg MT per kg of feed. Beginning 60 days after stocking, the fish were sampled every 15 days, and their feed allowances adjusted.

Physio-Chemical Parameters. Water temperature, dissolved oxygen, salinity, pH and secchi disc transparency were monitored on the same schedule as described by Biona et al. (VII, this volume).

Harvesting. The fish were harvested after 120 days of culture (15 December 1987). The ponds were totally drained and the fish picked up by hand. Based on the previously determined size variation between fish within ponds, a statistical procedure was employed to find the correct sample size at harvest in order to estimate the true population mean within 10% (Gomez and Gomez, 1976). The weights of the estimated number of fish were measured to the nearest 0.1 g. Fish were counted to determine survival. In addition, the total weights of intruding species were measured.

RESULTS

Average weekly morning water temperatures ranged from 25 to 31 C (Fig. VIII.1) Afternoon temperatures were typically 4 C warmer. Weeks 6 and 9 had the highest temperatures, with a gradual cooling through week 18.

Average morning dissolved oxygen (DO) ranged between 1 and 6 mg/l (Fig. VIII.2), with an upward trend during growout. Afternoon DO was always greater, and ranged from 3 to 24 mg/l. The highest average afternoon DO values were during weeks 10 and 11.

Secchi disc transparencies were the reverse of dissolved oxygen, with a general downward trend throughout growout (Fig. VIII.3). The highest values were during weeks 2 through 5, with values between 37 and 45 cm. By the last week of growout, the average was below 30 cm.

Salinities varied greatly during growout, from a low of 15 ppt during week 9, to

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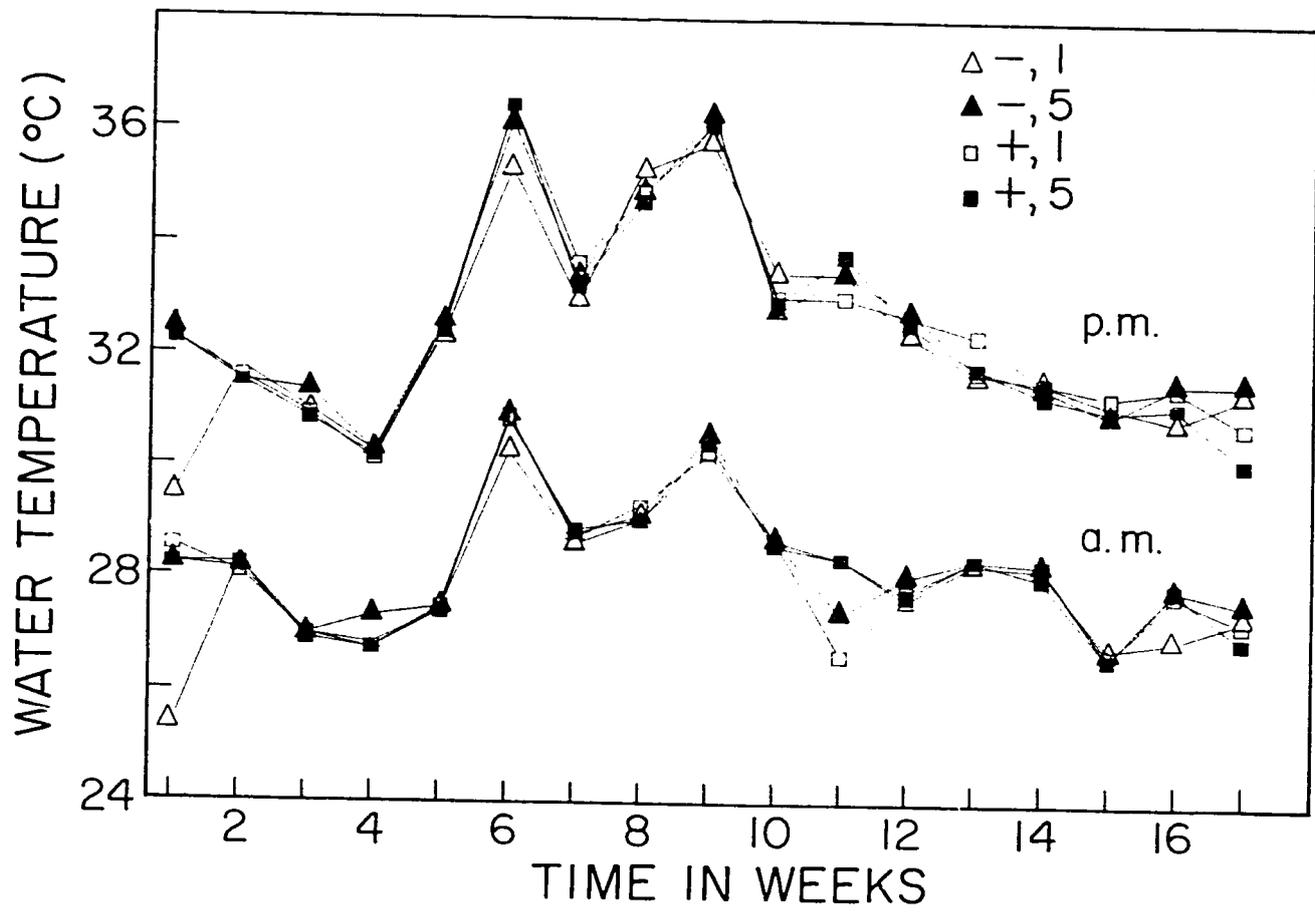


Fig. VIII.1. Average weekly morning (lower lines) and afternoon (upper lines) pond water temperatures for each of the 4 treatments of the monoculture growout experiment with spotted scat. There were three 250 m² ponds per treatment; with and without 17a-methyltestosterone (10 mg/kg feed) (+, -); and 2 stocking densities (1.15 and 5.76/m²).

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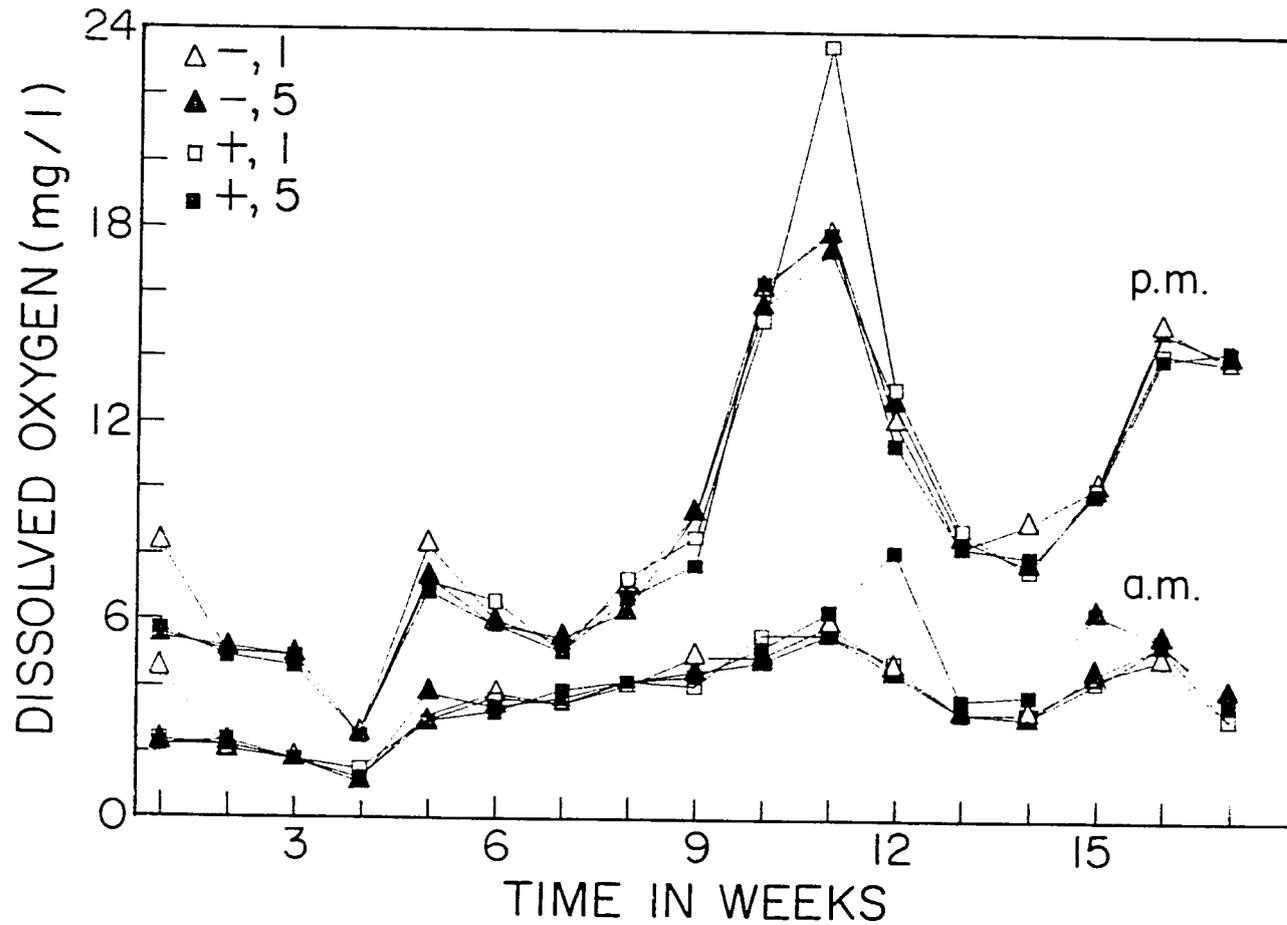


Fig. VIII.2. Average weekly morning (lower lines) and afternoon (upper lines) pond water dissolved oxygen for each of the 4 treatments of the monoculture growout experiment with spotted scat. There were three 250 m² ponds per treatment; with and without 17 α -methyltestosterone feeding (+, -); and 2 stocking densities (1.15 and 5.76/m²).

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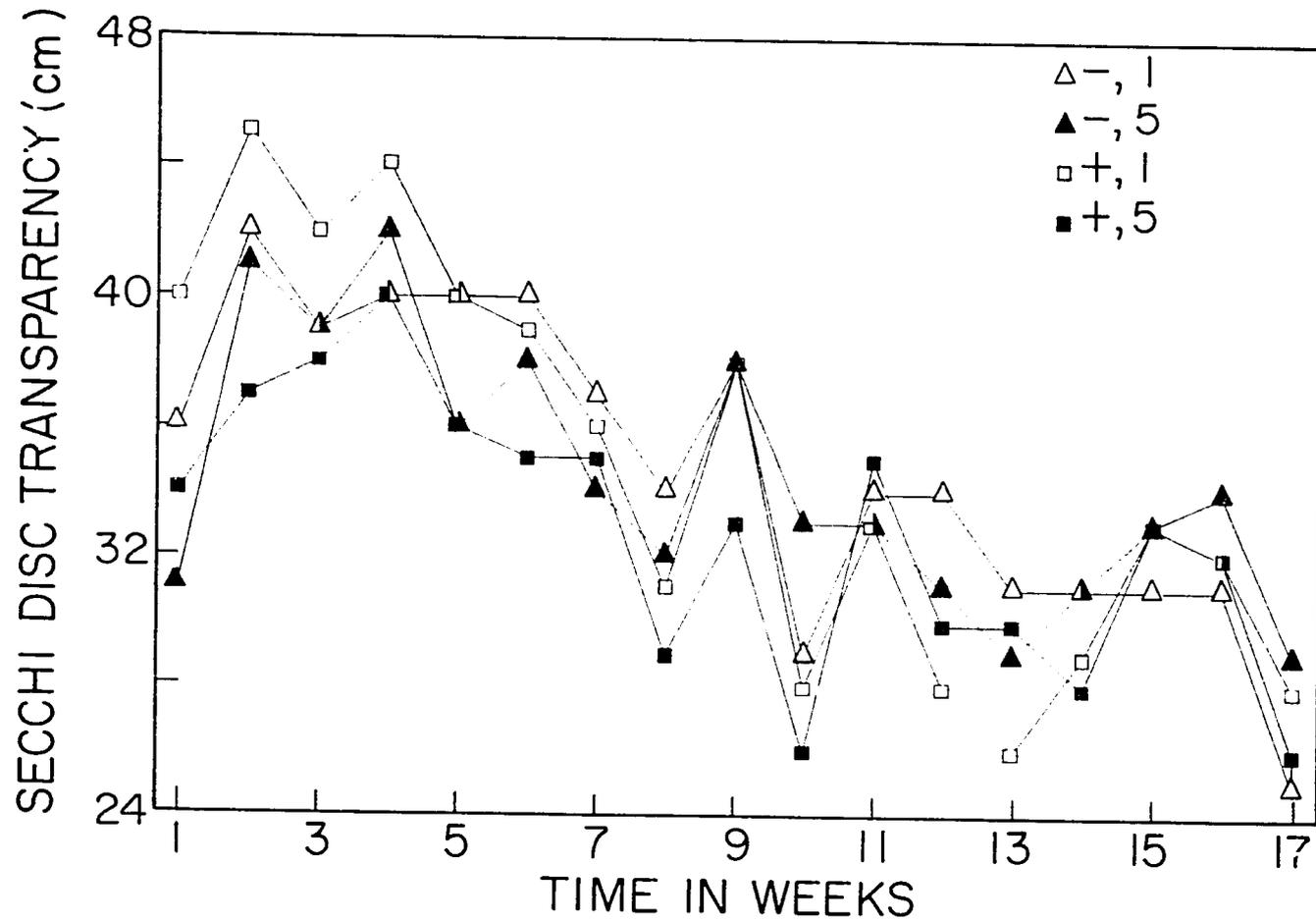


Fig. VIII.3. Average weekly pond secchi disc transparencies in each of 4 treatments of the monoculture growout experiment with spotted scat. There were three 250 m² ponds per treatment; with and without 17a-methyltestosterone feeding (+, -); and two stocking densities (1.15 and 5.76/m²).

highs of 30 and 27 ppt during weeks 3 to 4 and 13, respectively (Fig. VIII.4). Salinity was influenced by rainfall and runoff into the water source.

Water pH increased from an average of 7.0 at the beginning of the growout, to between 7.5 and 8.0 during the last weeks (Fig. VIII.5). This trend reflects the increased photosynthesis during growout, and correlates with both water transparency (algal density), and dissolved oxygen values (photosynthesis).

Average final weights of scat ranged from 3.33 g for those stocked at 5.76/m² and fed MT, to 8.39 g for those stocked at 1.15/m² and not fed MT (Fig. VIII.6, Table VIII.1). At both stocking densities, fish fed the hormone MT grew slower than fish not fed MT. MT had a highly significant effect on average scat weight ($p < 0.01$, $F = 38.652$, $df = 1,896$). Likewise, stocking density had a highly significant effect on average scat size at harvest ($p < 0.001$, $F = 405.897$, $df = 1,896$). The average weight of scat stocked at 1.15/m² was 7.6 g while that for scat stocked at 5.76/m² was 3.78 g.

Scat survival ranged from 39.4 to 86.8%, with an overall average of 58.1% (Table VIII.1). Treatment I (1.15/m², without hormone) had the highest survival rate. Stocking density and MT feeding, however, had no significant treatment effect on survival.

Average scat yield ranged from 53.6 kg/ha in treatment II (1.15/m², hormone added) to 143.5 kg/ha in treatment III (5.76/m², without hormone). Stocking density had a significant effect on average yield ($p = 0.019$; $F = 8.574$, $df = 1,8$). Hormone feeding had no significant effect on average yield. Feed conversion ratios among the 12 growout ponds ranged from 0.03 to 0.19 (Table VIII.1). The lowest, or best, feed conversions were at the lower stocking densities.

The total weight of intruders in the 12 scat monoculture ponds ranged from 0.24 kg to 1.72 kg (Table VIII.2). The most common and abundant intruders were tilapia and gobies.

DISCUSSION AND CONCLUSIONS

Average growth rate, or size at harvest, was strongly affected by stocking density. This is a common effect in fish culture, and can be due to one or more factors. In our case, the two most likely factors are: 1) the standing crop approached the carrying capacity of the pond, or 2) aggressive interactions between individuals increased with stocking density and had a negative impact on growth. It is not likely that the first factor is responsible for the decreased growth of scat at the higher density. The maximum standing crop of scat was 43.5 kg/ha (14.4 g/m²), which is well below the density where growth is depressed in other less aggressive species. In our prawn polyculture experiment, for example, there was no density-dependent effect on prawn growth in fed ponds, even at densities of 110 g/m² (Biona et al., IX, this volume). Similarly, stocking density had no effect on milkfish growth in unfed ponds, even at densities of 45 g/m² (Biona et al., VII, this volume).

Most likely, the scat density-growth effect was due to aggressive interactions between scat. We have commented on this characteristic of scat throughout our report. This behavior is most obvious in fish held in aquaria or tanks, and can even result in death. Although this aggression apparently caused reduced individual growth, it did not increase mortality since stocking density had no effect on survival. This suggests that in ponds, unlike aquaria or tanks, the "submissive" fish can escape serious injury, but at the same time individual growth is affected.

An unexpected finding was reduced growth in those fish fed with the hormone MT. We had expected a growth increase, but found the opposite. We have no good explanation for this, although Cruz and Barry (X, this volume) also found that MT did not increase the growth rate of scat held in aquaria or cages in ponds.

Overall growth of the scat in this experimental set was low. Even the best case growth is probably too slow to justify monoculture of scat for food fish. Monoculture of scat may still be justified for the aquaria trade, however, especially if larval scat can be purchased at a very low price, reared to 2 g or larger, and sold for a much higher

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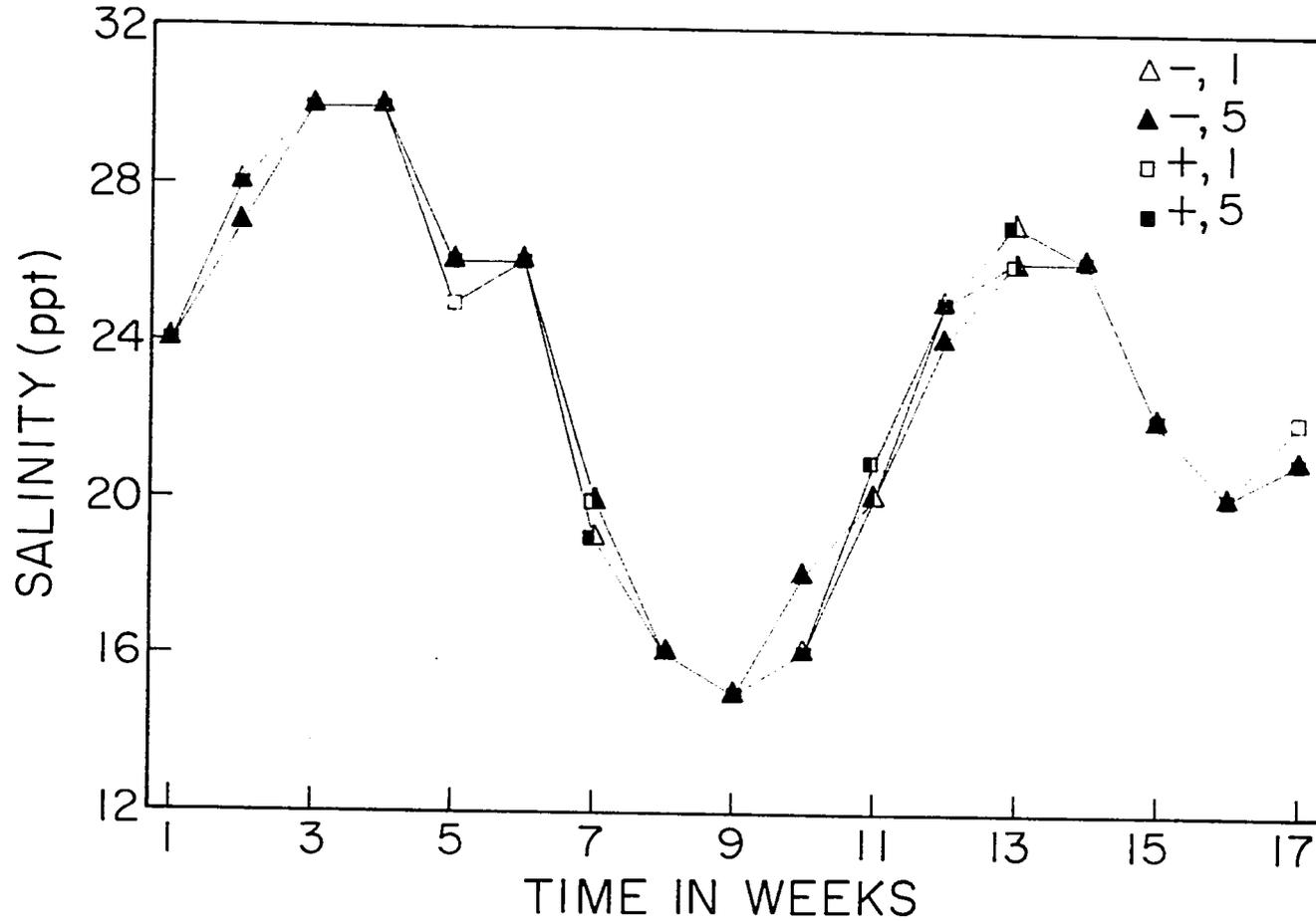


Fig. VIII.4. Average weekly pond salinities in each of the 4 treatments of the monoculture growout experiment with spotted scat. There were three 250 m² ponds per treatment; with and without 17a-methyltestosterone feeding (+, -); and two stocking densities (1.15 and 5.76/m²).

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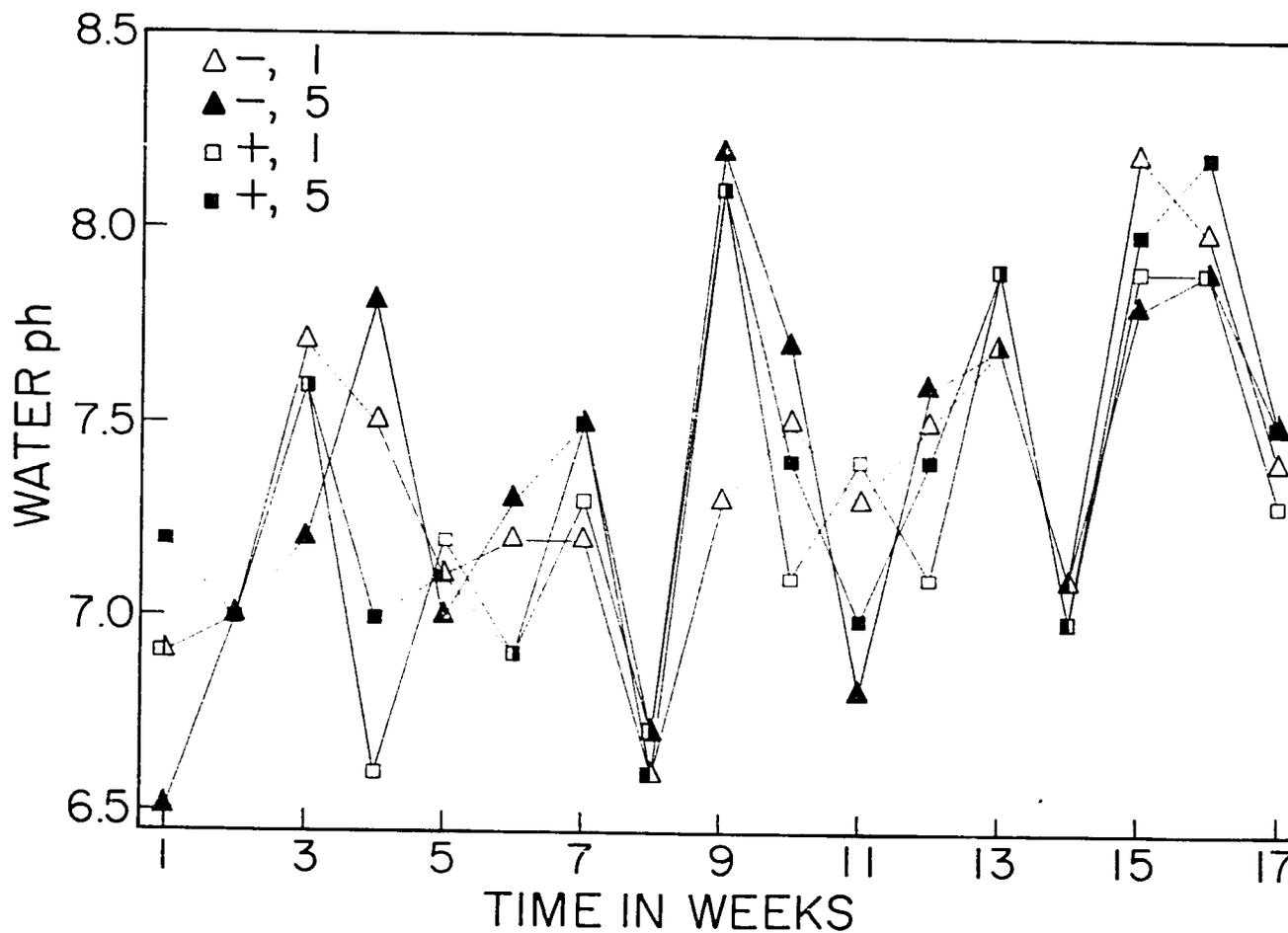


Fig. VIII.5. Average weekly morning pond pH values in each of 4 treatments of the monoculture growout experiment with spotted scat. There were three 250 m² ponds per treatment; with and without 17 α -methyltestosterone feeding (+, -); and two stocking densities (1.15 and 5.76/m²).

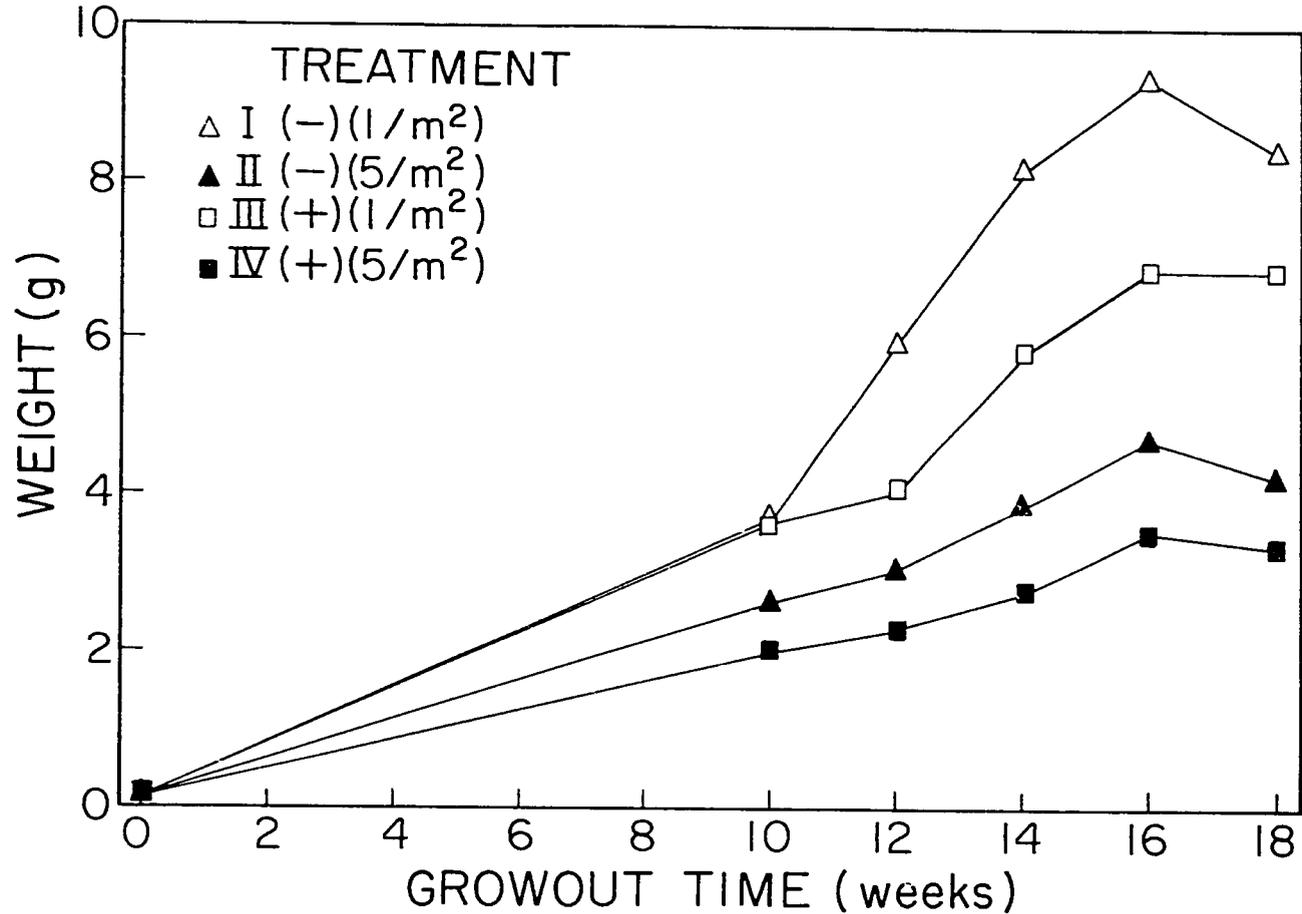


Fig. VIII.6. Average weights of spotted scat during growout in monoculture for 100 days in 250 m² earthen ponds. There were four treatments, testing all combinations of 2 stocking densities (1.15 and 5.76/m²) with or without feeding 17 α -methyl-testosterone. Treatment I, low density and no hormone feeding; Treatment II, high density, no hormone feeding; Treatment III, low density, hormone feeding; Treatment IV, high density, hormone feeding.

TABLE VIII.1. FINAL SURVIVAL, GROWTH, YIELD DATA AND FOOD CONVERSION (FCI) DATA FOR SPOTTED SCAT (*Scatophagus argus*) STOCKED AT TWO DENSITIES WITH OR WITHOUT METHYLTESTOSTERONE FEEDING IN MONOCULTURE

		Stocking Density											
		1/m ²						5/m ²					
Treatment Pond		Survival		Average Weight (g)	Total Weight (kg)	Yield (kg/ha)	FCI	Survival		Average Weight (g)	Total Weight (kg)	Yield (kg/ha)	FCI
		No.	%					No.	%				
No MT	1	250	86.8	9.91	2.27	99.1	0.030	1015	70.5	4.02	0.58	163.2	0.045
	2	178	61.8	4.97	0.88	35.4	0.085	840	58.3	4.99	1.11	167.6	0.055
	3	165	57.3	10.28	1.69	67.6	0.044	671	46.6	3.71	1.55	99.6	0.040
	Average	197.6	68.6	8.39	1.61	67.4	0.053	842	58.5	4.23	1.08	143.47	0.047
MT FED	1	195	67.7	3.01	4.08	54.2	0.051	567	39.4	1.49	0.84	33.75	0.19
	2	147	51.0	7.56	4.18	44.4	0.050	852	59.2	3.43	2.91	116.79	0.055
	3	153	53.1	10.16	2.49	62.2	0.084	648	45.0	5.08	3.29	131.67	0.049
	Average	165.0	57.3	6.9	3.58	53.6	0.062	689	47.9	3.33	2.35	94.00	0.098

TABLE VIII.2. TOTAL WEIGHT (kg) OF "INTRUDERS" TAKEN FROM EACH OF TWELVE PONDS AT HARVEST

SPECIES	TREATMENT											
	I			II			III			IV		
	1	2	3	1	2	3	1	2	3	1	2	3
<u>Tilapia Oreochromis spp</u>	0.3985	0.6364	0.2927	0.1736	0.7282	0.1438	0.0991	1.635	0.1137	0.366	0.3506	0.0222
<u>Goby Glossogobius spp</u>	0.6724		1.5199	0.4423		0.1194	0.0568	0.0207		0.0159		
<u>Milkfish Chanos chanos</u>		0.0886					0.3065					
<u>Seabass Lates calcarifer</u>	0.0614						0.2455	0.0419				0.6767
<u>Megalops spp</u>	0.254	0.1875	0.614	0.1301	0.8107	0.7729	0.1196	0.0906	0.104	0.1227	0.1843	0.0222
<u>Greasy Back Shrimp Metapenaeus ensis</u>	0.254	0.1875	0.614	0.1301	0.8107	0.7729	0.1196	0.0906	0.104	0.1227	0.1843	0.1258
TOTAL	1.386	0.9125	2.427	0.746	1.565	1.036	1.7191	1.7882	0.2428	0.5046	0.5349	0.8247

price. Pond-reared scat may have greater stamina and hardiness than fish captured from the wild and directly exported. Our experience was that fish 2 g and larger were difficult to capture in quantity from the wild, whereas the larval scat were seasonally abundant.

IX. PRODUCTION OF TIGER PRAWN (*Penaeus monodon*) AND SPOTTED SCAT (*Scatophagus argus*) IN POLYCULTURE

Henry D. Biona, Sr., Rizaline Tabanda, Rodney Bayogos
Arlo W. Fast and Terence P. Barry

INTRODUCTION

The polyculture of fishes and crustaceans is an effective method of maximizing yield and income from earthen ponds. The effectiveness of this approach, however, depends on the species used in polyculture, the culture conditions, and the economic value of the crops.

For our present growout experiment, we chose to evaluate a growout scheme using spotted scat, or "kitang" (*Scatophagus argus*) and tiger prawn or "sugpo" (*Penaeus monodon*). At large sizes (50 g or larger), the spotted scat is a popular food fish for domestic consumption in the Philippines. At small sizes (2 to 10 g), the scat is a popular aquaria fish, especially for export. The tiger prawn is a highly preferred food item in the Philippines, Japan, Europe, and the USA.

The Philippine tiger prawn industry is responding rapidly to meet the export potential for tiger prawns (Apud, 1985). In 1983, the Philippines exported 4,321 metric tons of tiger prawn, which accounted for only 2.9% of Japan's total shrimp imports. Revenues from prawn sales are expected to become the Philippines' principal source of foreign currency from exports.

Thousands of hectares of brackishwater fishponds, formerly used to grow milkfish (*Chanos chanos*), have been converted over to prawn culture. In addition, many new prawn ponds are being built. The rapid growth of prawns to a large size, and their high price, make the tiger prawn an attractive species for culture in the Philippines.

From our other pond growout experiments with scat, in polyculture with milkfish (unfed), and in monoculture (fed), we knew that scat grow very slowly, especially in unfed ponds. (Biona et al., VII, this volume) The growth rate of scat can be increased substantially by feeding, even at higher stocking densities (Biona et al., VIII, this volume). Nevertheless, growth is slow in both cases, and if scat are grown as a food fish, most likely the growout techniques will require polyculture with a faster growing species, such as milkfish or prawn. Moreover, multiple crop cycles will most likely need to be employed, where, from the same pond, perhaps 2 or 3 crops of the faster growing species will be harvested for every one crop of scat. Even with multiple crop cycles, growout of scat in a reasonable time frame may require the application of prepared supplemental feeds throughout the growout.

The objectives of our present growout experiment were to:

1. Establish benchmark information on the survival and growth of scat in polyculture with prawns.
2. Evaluate the effects of scat on the growth and survival of prawns in fed ponds.

MATERIALS AND METHODS

Experimental Ponds and Their Preparation. The 250 m² experimental ponds, and their preparation are similar to those described by Biona et al. (VII, this volume), with the following exceptions.

Prior to pond bottom drying, all ponds were drained and undesirable fish species and snails were removed manually. A peripheral canal, 2 m wide and 0.3 m deep was constructed in each pond. This canal served as a refuge area during sunny days and facilitated harvesting. The soil excavated from the peripheral canal was used to reinforce the dike of each pond. Thereafter, the ponds were completely drained and dried until the soil cracked. During drying periods, the gates were soil-sealed and provided with fine meshed screen to

prevent the entrance of extraneous species.

After drying, the ponds were flushed with tidal water. After flushing, agricultural lime and carabao manure were broadcasted at 2 ton/ha each. The following day, water was admitted to a depth of 5 cm. After three days, inorganic fertilizers, 16-0-0 and 45-0-0, were applied to the ponds at 50 kg/ha each. Thereafter, pond water was increased to 10 cm. This depth was maintained until benthic algae (lab-lab) had grown. Teaseed cake (15 ppm) was applied in all ponds two weeks before the scheduled stocking. After the application of teaseed cake, water depth was gradually increased to 80 cm by adding 10 cm every two days to prevent detachment of growing lab-lab.

Experimental Animals and Stocking. Hatchery-cultivated prawn post-larvae and wild-caught scat fry with average weights of 1.7 mg and 0.41 g, respectively, were used. Prior to stocking, the prawn and scat seed were acclimated in the holding tanks to the salinity and temperature of pond water. Randomized stocking was done in the evening by taking seed from the holding tanks and releasing them into designated ponds.

Experimental Design and Statistical Analysis. The experiment consisted of two treatments, with three 250 m² pond replicates each in a randomized block design as follows:

<u>Treatment</u>	<u>Stocking Density</u>
I	4.68 prawn/m ²
II	4.68 prawn/m ² 1.0 scat/m ²

In all, 1,170 prawn post-larvae were stocked into each pond of both treatments (total prawn = 7,020); and 250 scat fry were stocked into each pond of treatment II (total scat = 750). The ponds were stocked the week of September 13, 1987.

The results were analyzed by an analysis of variance (ANOVA) followed by the Duncan's multiple range test (DMRT).

Water Management. Pond water depth in all treatments ranged from 85 to 90 cm throughout the culture period. Pond water was changed every other day during spring tide by draining 10 to 15% of the pond water volume during low tide and replacing the same volume during high tide. Water depth was maintained constant during neap tides by pumping water from a reservoir as needed throughout the culture period.

Feeding and Sampling. After stocking, the ponds were fed daily with commercial shrimp diet. The feeding rates were 15% (days 1 to 15); 5-6% (days 16 to 53); 3-4% (days 54 to 75); and 2-3% (days 76 to 100). Feed rations were calculated following cast-net sampling for weight, and an assumed prawn survival rate of between 90 and 100%.

Physio-Chemical Parameters. Pond water temperatures and dissolved oxygen were measured twice daily throughout the culture period between 0530 and 0600 hrs, and 1500 to 1530 hrs at a depth of 25 to 30 cm. Salinities were determined between 1500 and 1530 hrs with an Atago Refractometer. Water pH was measured three times per week between 0530 and 0600 hrs with the Broadly James pH meter.

Harvesting. Fish and prawns in all treatments were harvested after 100 days of culture, on December 16, 1987. At first, a cast net was thrown ten times in each pond to collect the animals. The ponds were then totally drained and the animals collected by hand. A statistically-determined sample (Gomez and Gomez, 1976) of fish and prawn from each pond were counted and individually weighed to determine survival, growth and yield.

RESULTS

Average weekly morning water temperatures ranged from 26.5 to 30 C (Fig. IX.1). Afternoon temperatures were typically 4 C warmer. Weeks 3 and 6 had the highest temperatures, with a gradual decrease after week 6.

Average morning dissolved oxygen (DO) ranged between 1 and 6 mg/l (Fig. IX.2), with an upward trend during the first 8 weeks of growout. Afternoon DO ranged from 2.5 to 15 mg/l. The highest average afternoon DO values were during week 8.

Secchi disc transparencies were high during weeks 1, 6 and 8, with average values of 40 cm for both treatments (Fig. IX.3). There was a sharp decrease in transparency between weeks 8 and 11, with a partial recovery thereafter.

Average weekly salinities ranged from 30 ppt during week 1 to 18 ppt during week 4 (Fig. IX.4). Salinities then increased to over 26 ppt by week 10, before dropping again to less than 22 ppt.

Average weekly water pH values ranged between 6.7 and 8.0 (Fig. IX.5). There is no trend in these data, which fluctuated from week to week within the range.

During the growout, prawn weight increased from 1.7 mg to more than 28 g (Fig. IX.6, Table IX.1). Final average prawn weights were 29.4 g for prawn-only ponds, and 28.3 g for ponds with prawns and scat. There was no significant difference between these mean weights at $p = 0.05$. Prawn survival ranged from 78.2 to 87.9% in all ponds, with means of 79.4 and 84.4%, respectively, for prawns only, and prawns with scat. These mean survivals were not significantly different at $p = 0.05$. Prawn yields for the two treatments averaged 1,091 kg/ha and 1,120 kg/ha, respectively, for prawns only, and prawns with scat. Again, there was no significant difference in average yields of prawns between the two treatments at $p = 0.05$.

During growout, spotted scat grew from 0.41 g to 10.32 g final weight (Fig. IX.6, Table IX.1). Average weights in the three ponds ranged from 7.72 to 14.96 g. Percent survival averaged 54.8%, and ranged from 47.6 to 59.2%. Survival did not correlate with the growth data. Scat yield averaged 57.5 kg/ha, and ranged from 36.7 to 86.2 kg/ha.

Feed conversion indices (FCI) for the prawn-only ponds ranged from 1.16 to 1.29, with a mean of 1.24 (Table IX.1). Feed conversion indices for the prawns with scat ponds ranged from 0.97 to 1.30, with a mean of 1.13. The latter FCI includes yields of both prawns and scats. These mean FCIs were not significantly different from each other at $p = 0.05$.

The total weight of intruders in each pond ranged from 0.0 kg to 4.4 kg (Table IX.2). Intruders were undesired fish and crustaceans which found their way into the growout ponds despite efforts to keep them out.

DISCUSSION AND CONCLUSIONS

Clearly, at the stocking densities we used, culturing spotted scat together with tiger prawn did not affect the growth, survival, yield, or feed conversion of the prawns. This result is perhaps due to the fact that the yield of scat relative to that of prawns was very low. Scat yields ranged from only 3.6 to 7.4% of the total yield (prawn plus scat) (Table IX.1). Another explanation is that each species occupied a different ecological niche, and there was little or no competition for space and resources between the prawn and scat. Although there was probably at least some competition for the prepared feeds, this was not significant enough to affect the production parameters of either species. On the contrary, the growth of scat cultured with prawns was greater than for any other growout that we conducted.

Growth of scat stocked at $1/m^2$ with prawns in fed ponds was much greater than scat stocked at about $1/m^2$ in polyculture with milkfish in unfed ponds, 10.3 g and 2.8 g, respectively (Biona et al., VII, this volume). Scat also grew faster in polyculture with

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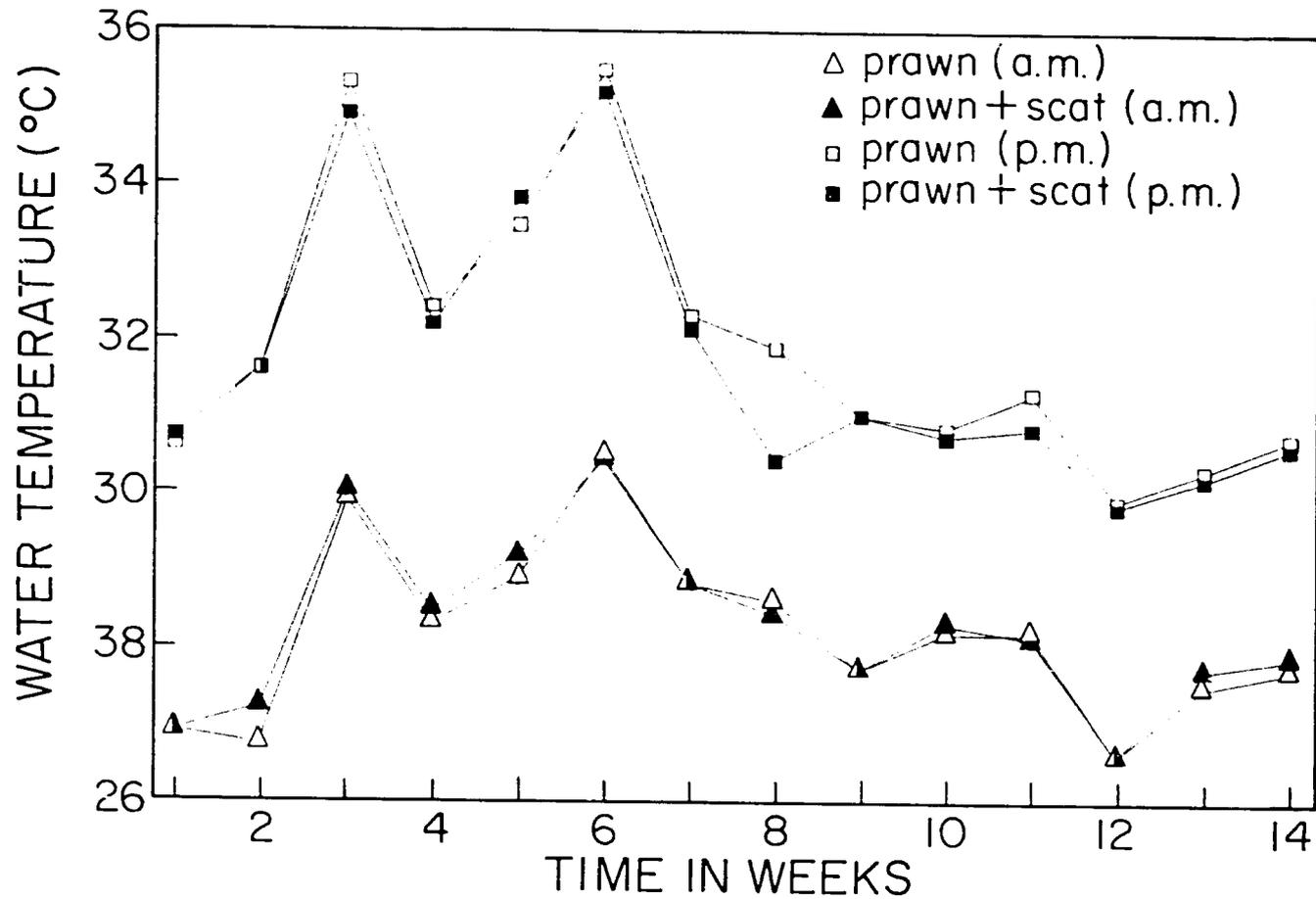


Fig. IX.1. Average weekly morning and afternoon water temperatures for each of the 2 treatments of the prawn and scat polyculture experiment. Prawn alone were stocked at $4.68/m^2$ in treatment 1, while scat and prawn were stocked together at 1.0 and $4.68/m^2$, respectively, in treatment 2. There were three $250 m^2$ ponds per treatment.

06

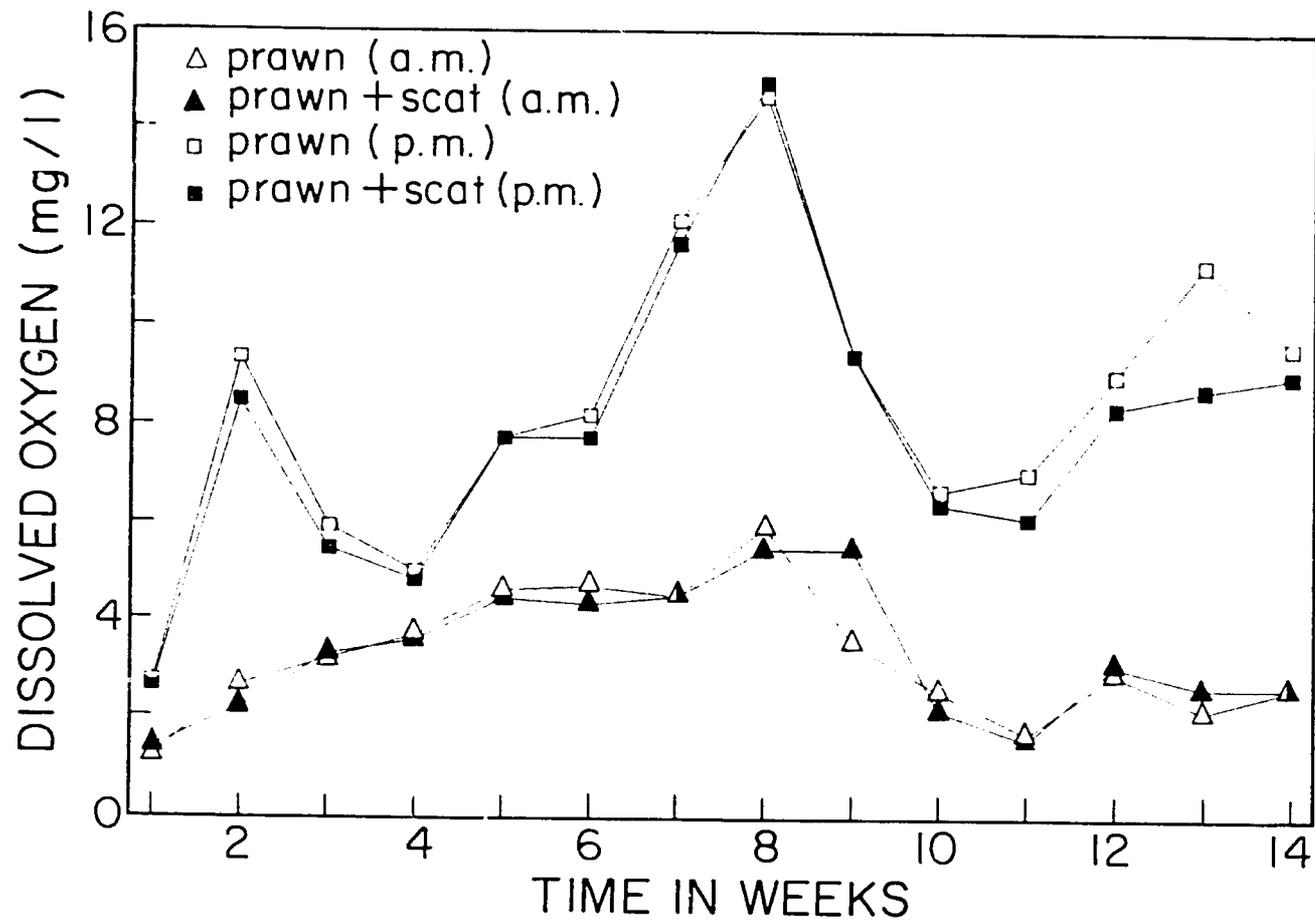


Fig. IX.2. Average weekly morning and afternoon dissolved oxygen concentration for each of the 2 treatments of the prawn and scat polyculture experiment. Prawn alone were stocked at $4.68/m^2$ in treatment 1, while scat and prawn were stocked together at 1.0 and $4.68/m^2$, respectively, in treatment 2. There were three $250\ m^2$ ponds per treatment.

16

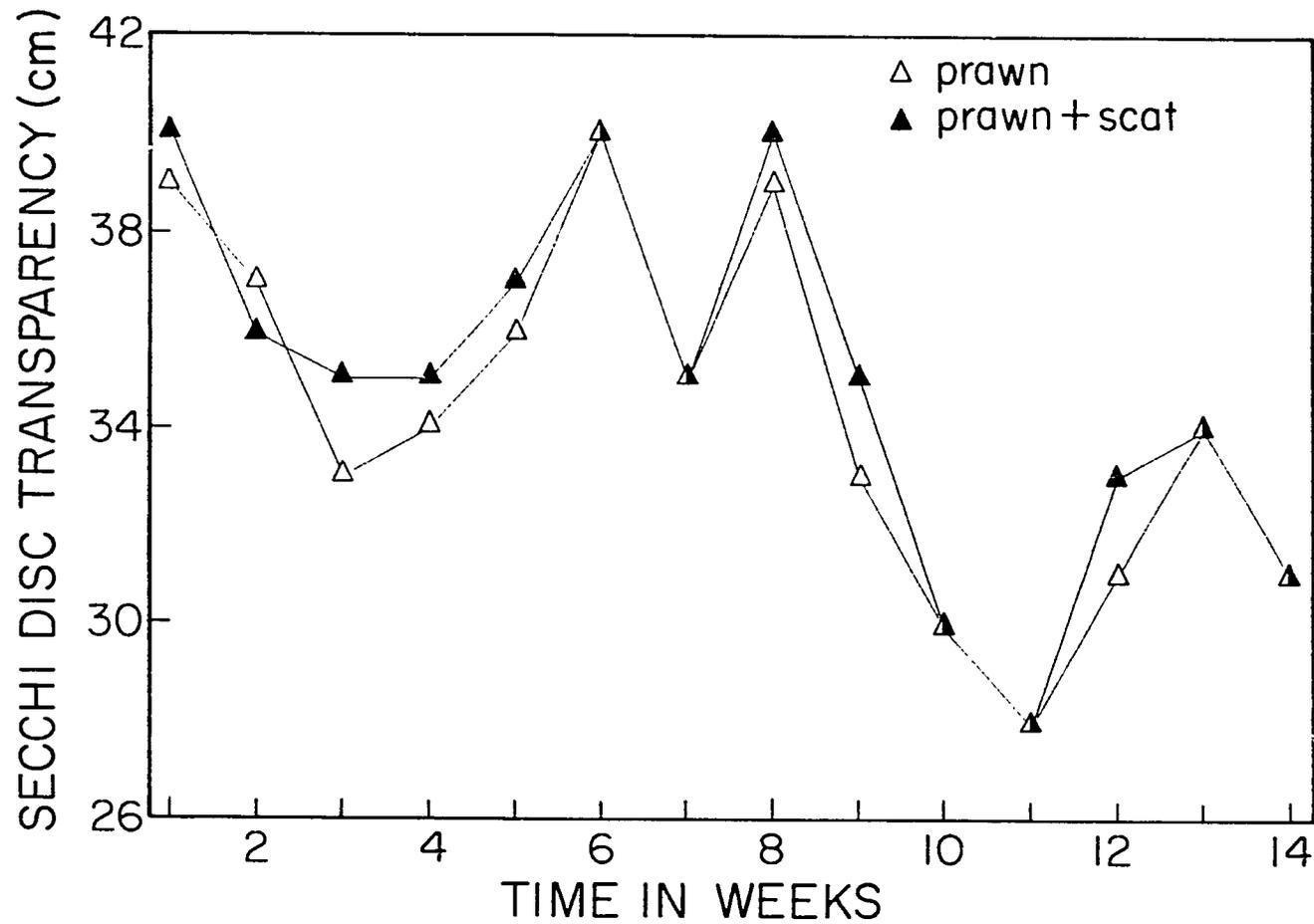


Fig. IX.3. Average weekly secchi disc transparencies for each of the 2 treatments of the prawn and scat polyculture experiment. Prawn alone were stocked at $4.68/m^2$ in treatment 1, while scat and prawn were stocked together at 1.0 and $4.68/m^2$, respectively, in treatment 2. There were three $250 m^2$ ponds per treatment.

lb

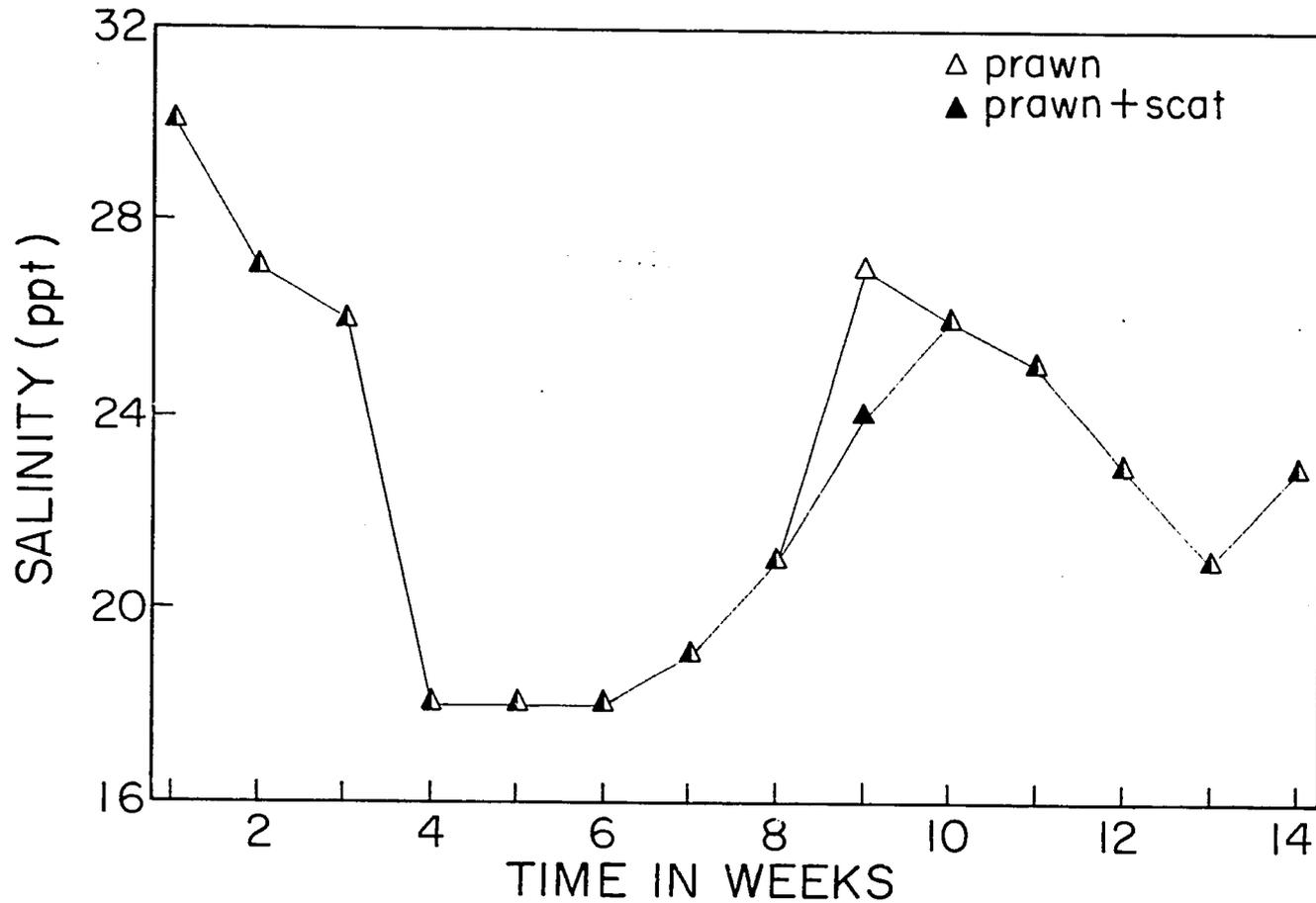


Fig. IX.4. Average weekly salinities for each of the 2 treatments of the prawn and scat polyculture experiment. Prawn alone were stocked at $4.68/m^2$ in treatment 1, while scat and prawn were stocked together at 1.0 and $4.68/m^2$, respectively, in treatment 2. There were three $250 m^2$ ponds per treatment.

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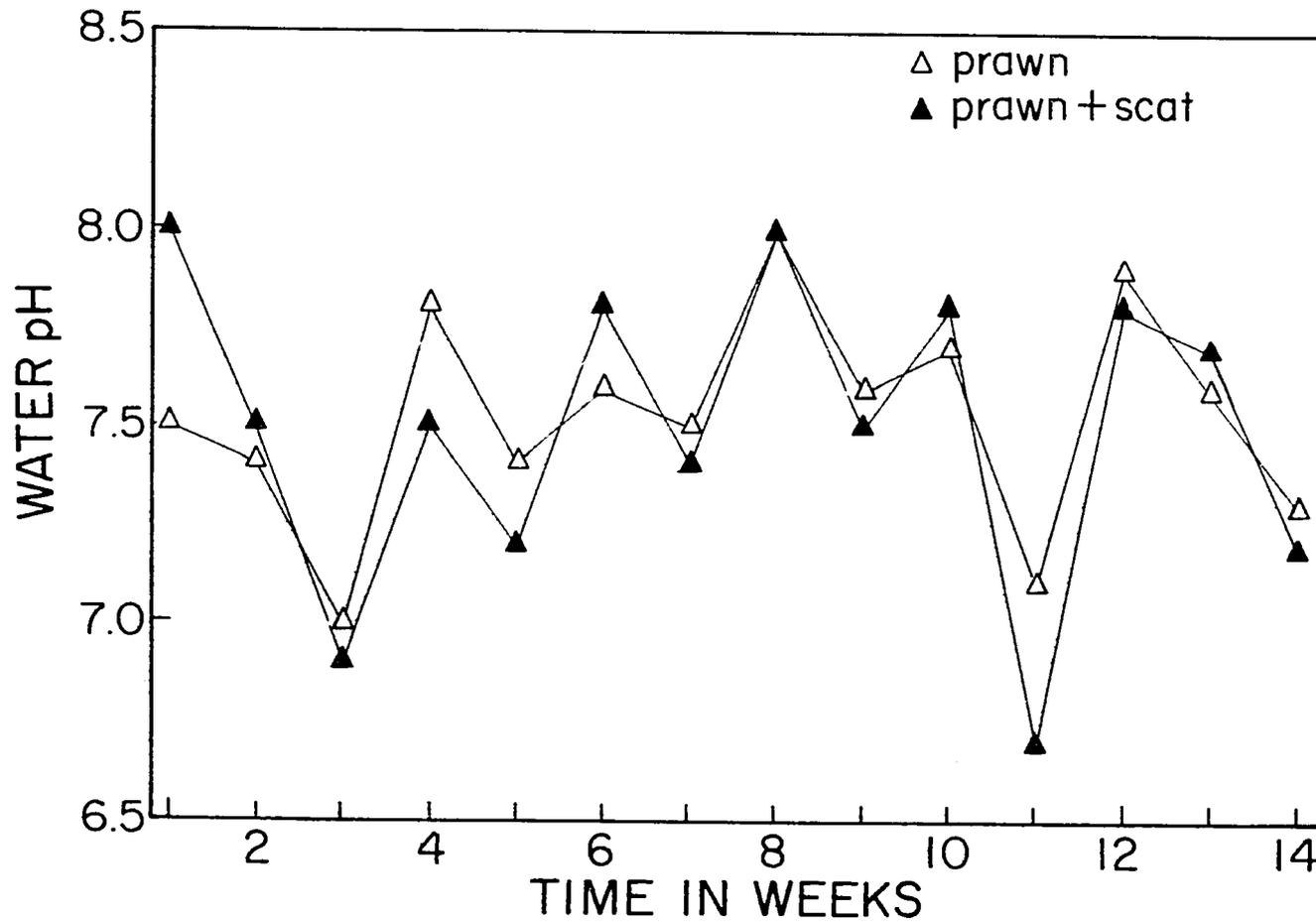


Fig. IX.5. Average weekly water pH values for each of the 2 treatments of the prawn and scat polyculture experiment. Prawn alone were stocked at $4.68/m^2$ in treatment 1, while scat and prawn were stocked together at 1.0 and $4.68/m^2$, respectively, in treatment 2. There were three $250 m^2$ ponds per treatment.

94

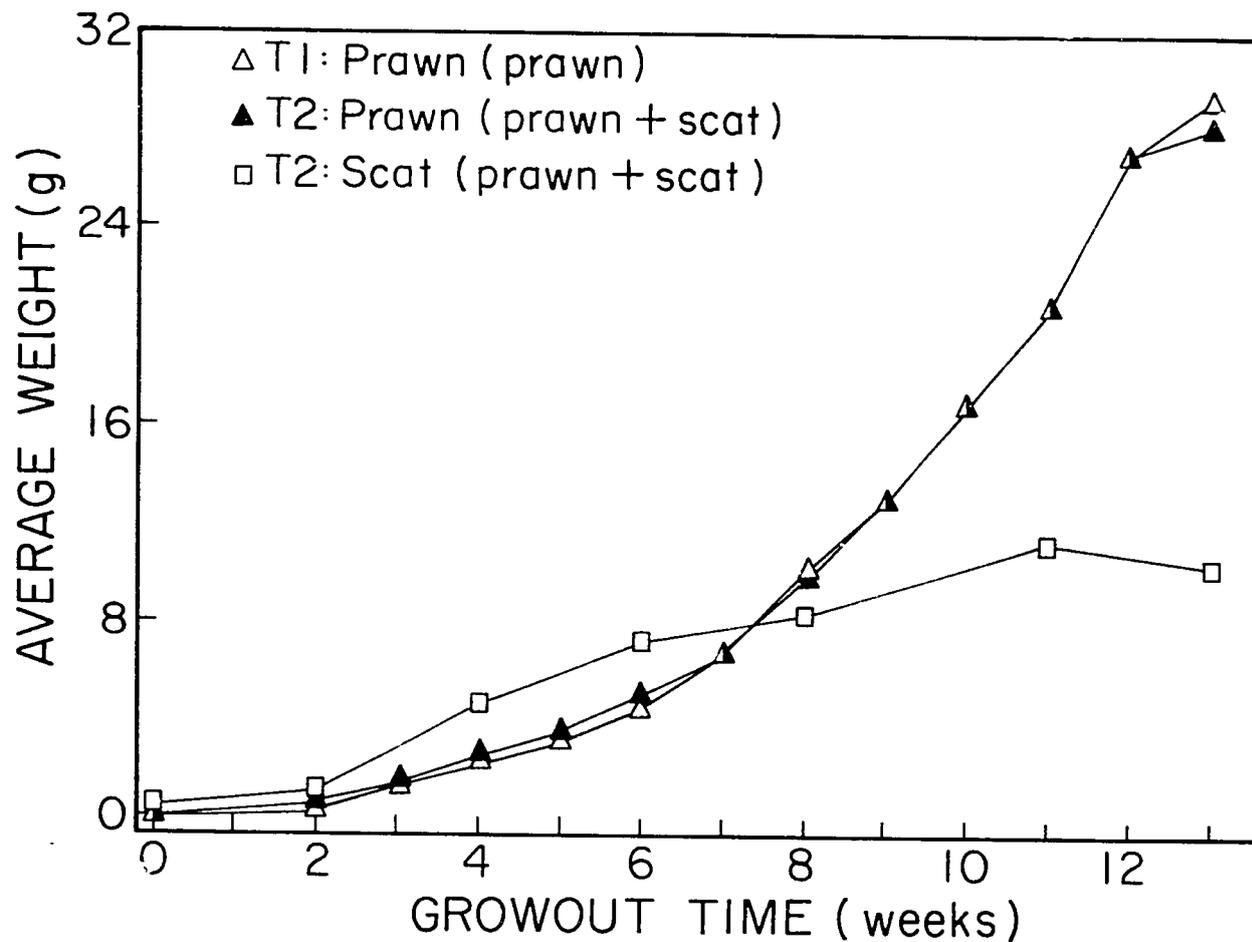


Fig. IX.6. Average weights of prawn (*Penaeus monodon*) and spotted scat (*Scatophagus argus*) during growout in earthen ponds. Three ponds of prawn only and three ponds of prawns and scat were cultured. Stocks were grown in 250 m² ponds and fed a formulated (pelleted) diet.

TABLE IX.1. FINAL SURVIVAL, GROWTH AND YIELD DATA, AND FEED CONVERSION INDEX (FCI) FOR TIGER PRAWN (*Penaeus monodon*) AND SPOTTED SCAT (*Scatophagus argus*) IN POLY CULTURE

Treatment	Pond	TIGER PRAWN						SPOTTED SCAT				
		Survival		Average Weight (g)	Total Weight (kg)	Yield (kg/ha)	FCI ^a	Survival		Average Weight (g)	Total Weight (kg)	Yield (kg/ha)
		No.	%					No.	%			
I Prawn Only	1	915	78.2	29.04	26.56	1062.8	1.26					
	2	919	78.5	31.64	29.08	1163.0	1.16					
	3	953	81.5	27.46	28.17	1046.8	1.29					
	Average	929	79.4	29.38	27.94	1090.9	1.24					
II Prawn & Scat	1	966	82.6	25.26	24.41	976.4	1.30	119	47.6	7.72	0.92	36.7
	2	968	82.7	27.81	26.92	1077.0	1.13	144	57.6	14.96	2.16	86.2
	3	1028	87.9	31.76	32.65	1306.2	0.97	148	59.2	8.27	1.22	49.0
	Average	987	84.4	28.28	27.99	1119.9	1.13	137	54.8	10.32	1.43	57.5

^a FCI was calculated from combined yields of both prawn and scat.

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TABLE IX.2. TOTAL WEIGHT OF INTRUDERS^a TAKEN AT HARVEST FROM THE SIX GROWOUT PONDS OF THE PRAWN PLUS SCAT POLY CULTURE EXPERIMENT

SPECIES	Prawns Only			Prawns and Scat		
	1	2	3	1	2	3
Tilapia <u>Oreochromis spp</u>	3.701 ^b	-	2.773	0.391	0.319	-
Goby <u>Glossogobius spp</u>	-	-	-	0.008	0.309	0.50
Milkfish <u>Chanos chanos</u>	0.046	-	-	-	-	-
Sea bass <u>Lates calcarifer</u>	0.106	-	-	-	-	-
Greasy Back Shrimp <u>Metapenaeus ensis</u>	0.536	-	-	1.353	0.026	0.479
TOTAL	4.389		2.773	1.7515	0.6748	0.979

^a undesired animals which found their way into the ponds
^b total weight in kg

prawns (average final weight, 10.3 g) than they did at approximately the same stocking density in monoculture under fed conditions (8.4 g and 6.9 g, without and with hormone added to the feed, respectively; Biona et al., VIII; this volume).

If scat are ever raised as a food fish, our findings suggest that they must be fed a prepared diet in order to achieve a reasonable growth rate. Furthermore, it may be suitable to raise the scat in polyculture with prawn, since the scat at a stocking rate of 1/m² did not negatively affect the prawn in any detectable way. Even in fed ponds, however, the scat's growth rate is very slow. Almost certainly, 2 or 3 crops of prawn could be serially produced before even a single crop of scat at the minimum market size of 50 g could be produced. If scat are grown for the aquarium trade, however, just one crop in polyculture with prawn would be adequate for the scat to reach market size, perhaps at stocking densities even greater than 1 scat/m².

X. DIETARY USE OF 17 α -METHYLTESTOSTERONE, ESTRADIOL-17 β , AND 3,5,3'-TRIIODO-L-THYRONINE AS POTENTIAL GROWTH PROMOTERS FOR THE SPOTTED SCAT (*Scatophagus argus*)

Paul Felipe S. Cruz¹ and Terence P. Barry

X.A. INTRODUCTION

X.A.1. Relevance of the Study to Fish Culture Development.

The spotted scat (*Scatophagus argus*) is one of the most prized estuarine food fishes in the Philippines, yet it is one of the least exploited species for fish culture. The scat is endemic to coastal Indo-Pacific fresh, brackish, and marine waters (Nelson, 1976) and is commonly found incidentally in most of the country's brackishwater fishponds.

The scat has several excellent characteristics for culture, including: a calm nature, hardiness, remarkable euryhaline capacity, favorable taste and appearance, good market price and omnivorous eating habits. Nevertheless, in spite of these traits, progress in the development of scat culture techniques has been minimal. This lack of progress is probably due to the fish's inherently slow growth rate (Biona et al., VII, this volume). Although scats command a high price in the market, comparable to grouper and sea bass, the relatively lengthy culture period for this species to reach marketable size discourages its propagation. An improvement in the growth rate of the scat would clearly influence its profitability for culture. Progress in scat culture may therefore depend on developing strategies which will improve its growth rate.

The purpose of our study was to test the somatic-growth-promoting effects of 17 α -methyltestosterone (MT), estradiol-17 β (E₂) and the thyroid hormone, 3,5,3'-triiodo-L-thyronine (T₃), incorporated into the diet of the spotted scat. Based on the results from studies on several other teleost species, we hypothesized that the administration of these substances would substantially increase the growth rate of the spotted scat, and thereby reduce its culture period, and feed costs.

X.B. LITERATURE REVIEW

X.B.1. Anabolic Agents in Fish Culture.

Growth in fishes, as in all vertebrates, is controlled through the orderly release of anabolic hormones from the endocrine system. The application of these anabolic hormones, or their synthetic chemical analogs, can markedly improve the growth rate of an animal beyond its normal intrinsic capacity. Of particular interest in food production are the naturally occurring anabolic hormones, or their derivatives, which are rapidly cleared from the body. Steroid and thyroid hormones are implicated in this respect (Donaldson et al. 1979) and are recognized for their anabolic potency and commercial feasibility as growth promoters in fish culture (Higgs *et al.*, 1982).

¹ Conducted in partial fulfillment of the Masters of Science in Fisheries degree, College of Fisheries, University of the Philippines in the Visayas, Iloilo City.

X.B.1.a. Sex Steroids.

No natural or artificial chemical has so far been found to promote weight increments in *Salmo* species comparable to those obtained with sex steroids. To date, there are at least 18 species known to exhibit an anabolic response to steroid treatment (Donaldson *et al.*, 1979).

Of the androgens, MT appears to have the greatest anabolic potency in fishes. In the common carp, *Cyprinus carpio*, for example, application of 1 mg MT/kg of feed for 90 days resulted in a 32% weight gain over the controls (Lone and Matty, 1980). The same chemical applied to a tilapia at high doses for short intervals (30 to 60 mg/kg of feed for 21 days), also resulted in remarkable weight gains (Guerrero, 1975, 1976). One encouraging study on the economic viability of using MT as a growth promoter throughout the entire culture period was reported by Chua and Teng (1980). They were able to produce marketable-sized (≥ 500 g) estuarine grouper, *Epinephelus salmoides*, in only 93 days instead of the usual 139 when the fish's diet was supplemented with 9 mg MT/kg of feed. The culture period was reduced by 33%, cutting the cost of production by over 25%. Howerton (personal communication, 1987) fed the tilapia (*Oreochromis mossambicus*) a diet enriched with 10 mg MT/kg of feed, and found that the production cost for this species could be cut close to half with the use of MT as a feed additive. He found that the growth of the MT-fed tilapia was improved by nearly 100% over the controls during an 8-month growout.

In contrast to the androgens, estrogens appear to have little or no growth-promoting ability among *Oncorhynchus spp.* (McBride *et al.*, 1982). In some fishes, however, including, perhaps the spotted scat (Winfrey, 1983), females grow significantly larger than males, and it is reasonable to hypothesize, that in these species, estrogens may play a role in promoting somatic growth. In addition, altering the sex ratio of these species to favor the larger females using estrogen treatment might be an effective way to enhance production. Of the naturally occurring female sex hormones, estradiol-17 β (E_2) has the greatest physiological activity (Hawley, 1971), and has been identified as a major plasma estrogen in several teleost species (Hansson and Rafter, 1983).

Administration of E_2 to genetically male fish, beginning in the sexually undifferentiated stage, and continuing through the subsequent stages of sex differentiation, can result in their complete sex reversal to functional females (Yamamoto, 1969). Normal feminization of close to 100% was observed when masu salmon (*Oncorhynchus masou*) and chum salmon (*O. keta*) fry were treated with moderate doses of E_2 (Nakamura, 1984). Estradiol-17 β treatment in different fish species following testicular differentiation can also effectively induce feminization of gonads in genetic males (Miyamori, 1964; Takahashi, 1975; Nakamura, 1984). Assuming a 1:1 sex ratio, successful feminization of genetically male scats using E_2 treatment could increase production by as much as 25%. Such a theoretical increase in yield does not even consider possible anabolic actions of E_2 which might further augment yield beyond what could be expected on the basis of its actions on sex reversal alone.

X.B.1.b. Thyroid hormones.

The actions of thyroid hormones in fishes other than salmonids are not well characterized. Thyroid hormones influence growth, skin pigmentation (guanine deposition in scales), seawater adaptation, schooling and a variety of other morphological and behavioral changes related to smoltification (Folmar and Dickoff, 1982; U.H.M. Fagerlund *et al.*, unpublished data, cited in Higgs *et al.*, 1982; Gorbman *et al.*, 1983). These thyroid hormone actions conform with the natural thyroid hyperactivity observed in salmon upon transformation into juvenile smolt stage and during spawning migrations (Lagler *et al.*, 1977; Gorbman *et al.*, 1983).

Thyroid hormones promote growth in a variety of ways. There is evidence for thyroidal involvement in the regulation of appetite, digestion, nutrient absorption, protein anabolism, and non-protein energy deposition in salmonids (Higgs *et al. op. cit.*). A

positive growth response to orally administered thyroid hormone has been recorded for Atlantic salmon (*Salmo salar*), coho salmon (*Oncorhynchus kisutch*), chinook salmon (*Oncorhynchus tshawytscha*), and steelhead trout (*Salmo gairdneri*).

It is well established that thyroid hormones are required for the growth and development (metamorphosis) of larval amphibians. The role of the thyroid in the larval growth and development of teleosts is only now beginning to emerge. Recently, Lam and co-workers (Lam and Reddy, 1986) found that the thyroid hormones, T₃ and T₄, accelerate growth, development and survival of larval tilapia (*Oreochromis mossambicus*), milkfish (*Chanos chanos*) and dwarf gourami (*Colisa lalia*). Inui and Miwa (1985) showed that T₄ induces metamorphosis in larval flounder (*Paralichthys olivaceus*), an action comparable to T₄'s actions in amphibians. Comparatively little is currently known about the role of thyroid hormones in regulating post-larval growth and development in teleosts.

X.B.2. Mechanisms of Action

The growth-promoting effects of anabolic hormones may be explained, at least in part, by their actions at several levels of the hierarchy of control mechanisms which regulate protein synthesis. Anabolic hormones may increase amino acid uptake, ribosome availability, and the numbers and kinds of mRNA (Manchester, 1976). Somatic growth in fishes depends on the rates at which food is ingested, digested, and assimilated, and all of these processes can be enhanced through thyroid hormone application (Donaldson *et al.*, 1979 and Higgs *et al.*, 1982).

X.B.2.a. Sex Steroids.

The growth-promoting potency of anabolic sex steroids in fish may be largely due to three observed responses: *a*) increased protein assimilation (U.H.M. Fagerlund *et al.*, *op. cit.*); *b*) enhanced appetite; and *c*) suppressed gonadal development.

X.B.2.a.i. Increase in protein assimilation. Application of MT can increase protein assimilation by increasing gut proteolytic enzyme activity. Yamazaki (1976) found histological evidence for increased proteolytic activity of the pancreas and intestine of masu salmon treated with MT for 2 weeks. Lone and Matty (1981a) had similar findings with common carp treated with testosterone, 11-ketotestosterone, or adrenosterone at doses of 1 to 10 mg/kg of feed for 60 days. In chinook salmon, protein assimilation and feed conversion efficiency (FCE) were increased by 18 and 23% over controls, respectively, when diets contained 10 and 15 mg testosterone per kg of feed (Schreck and Fowler, 1982). Coho salmon fed a diet containing 1 mg MT/kg for 8 mos had a FCE of 20.5% over controls (Fagerlund *et al.*, 1979b).

The mechanism of action of these anabolic hormones on FCE at the cellular level is suggested by the studies of Lone and Matty (1980, 1981b) on the common carp. They observed a trend of decreasing DNA content and increasing RNA, RNA/DNA, protein/RNA and protein/DNA in the muscle, kidney, and liver as the dosage of MT in the diet was increased. This data suggests that the hormones stimulate increased protein synthesis, which in turn increases the fish's somatic growth.

X.B.2.a.ii. Enhancement of appetite. Coho salmon consumed 17-23% and 6% more feed when given formulated diets containing 1 mg MT/kg of feed, and 5 mg testosterone/kg of feed, respectively (Fagerlund *et al.*, 1979a; 1980). Enhanced appetite and ingestion may be brought about indirectly by an increased nutrient requirement resulting from the stimulation of anabolic processes by MT. Alternatively, MT could have a direct effect on the neural control of feeding behavior, possibly stimulating the brain's feeding center. In the male goldfish (*Carassius auratus*), elevated levels of sex hormones during the prespawning state increase olfactory sensitivity to food odors (Partridge *et al.*, 1976). This finding suggests that there may be a relationship between blood plasma levels of sex steroids, and a fish's feeding behavior.

X.B.2.a.iii. Suppression of gonadal development. Testosterone and estradiol have been

reported to retard gonadal development in brown trout (*Salmo trutta*) (Yamamoto, 1969). Howerton (*op. cit.*) observed that the dose of MT which caused the greatest growth response also caused a decrease in the gonadosomatic index (gonad weight expressed as percent body weight; GSI). Evidently, normal gonadal development can be suppressed by excessive titres of sex steroids, possibly through a negative feedback on the brain-hypothalamo-pituitary axis. Suppression of normal gonadal development could allow more energy to be diverted to somatic growth.

X.B.2.b. Thyroid hormone.

X.B.2.b.i. Increase in protein synthesis. Most of the growth-promoting actions of thyroid hormone in fishes may stem from their interaction with nuclear receptors which stimulate RNA synthesis, and subsequently protein (enzyme) production (Higgs *et al.*, *op. cit.*). *In vivo* studies in rainbow trout (Van der Kraak and Eales, 1980; Omeljanuk and Eales, 1985) and *in vitro* studies in coho salmon (Darling *et al.*, 1982) and the sea lamprey (*Petromyzon marinus*) (Lintop and Youson, 1983) have shown that saturable T₃-binding sites exist in hepatic nuclei, although it has not been established experimentally that these saturable nuclear receptors are indeed T₃ receptors. The possibility of T₄ binding to putative nuclear receptors has not been studied in fish. In mammals, Sterling (1979) speculates that the growth-promoting and developmental effects of thyroid hormones are due to actions following their binding to nuclear receptors, while their metabolic effects can be attributed to their actions following binding to extranuclear receptors, such as those in the mitochondria.

X.B.2.b.ii. Enhancement of appetite. The enhancement of appetite by thyroid hormone is probably a result of its anabolic potency. Moreover, thyroid hormone may enhance the fish's appetite by increasing olfactory and other sensory sensitivity. In estivating African lungfish (*Protopterus annectens*), general metabolism and olfaction are shut down by low thyroid levels (Atema, 1980). With the onset of the wet season, plasma thyroid hormone levels rise and evoke normal feeding behavior by stimulating olfactory acuity (Dupe, 1973). The olfactory response to food in temperate fish species which are exposed to a seasonal abundance of food and/or variation in climate may be likewise regulated (Atema, 1980). An equivalent action in the feeding behavior of tropical fishes is not known.

X.B.3. Factors Affecting Exogenous Hormone Efficiency

X.B.3.a. Treatment dosage and duration.

The most effective hormone dosage for anabolic and secondary effects depends on the duration of treatment. Below a specific dose, which varies with ambient conditions, short-term growth responses are positively correlated with dose. Growth response within this dose range may gradually increase over extended periods. When the dosage is raised above this level, short-term response may still increase with dose, but long-term response may be greater in fishes receiving lower doses (*cf.* Higgs *et al.*, 1982). The decline in response with increasing dose may be due to a reduction in target cell receptor affinity (negative cooperativity) and/or number (down regulation). Another explanation is that high levels of the hormone may interfere with the normal functioning of organs like the liver and kidney.

X.B.3.b. Relative growth rates among species.

Interspecific growth responses to exogenous growth hormone in fish can be highly variable. The degree of response seems to be influenced considerably by the relative growth rate of the species. Fish with comparatively rapid growth rates, such as chinook salmon, Atlantic salmon, and steelhead trout, appear to respond weakly to anabolic

hormones (Higgs *et al.*, 1982). Perhaps the endogenous hormonal milieu of these fishes are already close to the optimal levels required for somatic growth (McBride *et al.*, 1982). If so, application of exogenous hormones to these and other fishes which normally have rapid growth rates might not be expected to enhance growth any further. The use of exogenous hormones on fast-growing species might even be expected to have a negative effect. On the other hand, it is reasonable to hypothesize that the use of growth-promoting hormones on fishes with inherently slow growth rates, such as the spotted scat, might have a marked stimulatory effect on growth and development.

X.B.3.c. Form of hormone administered.

X.B.3.c.i. Sex steroids. There are several natural sex steroids (*e.g.*, testosterone and 11-ketotestosterone) and synthetic chemical analogs (*e.g.*, MT and oxymetholone) that can promote somatic growth in fishes (McBride *et al.*, 1982). These steroids and their analogs are chemically and structurally similar, although, they vary considerably in activity. The synthetic androgen MT is the most potent sex steroid tested in fish to date. Relative anabolic responses in juvenile coho salmon are: MT > 11-ketotestosterone > testosterone (McBride and Fagerlund, 1973).

X.B.3.c.ii. Thyroid hormone. The teleost thyroid contains T₃ and T₄ as the main plasma iodothyronines with the latter generally considered as the principal derivative. In fishes, as in mammals, T₄ seems to be a relatively inactive precursor or prohormone of the more biologically potent T₃. Up to 70% of plasma T₄ may be rapidly deiodinated extrathyroidally in trout, with T₃ as the major, if not sole, end product (Higgs and Eales, 1977; Eales, 1977 a, b). Apparently, T₄ is not a distinct growth-promoting entity and much of its effect depends on its peripheral conversion to T₃.

X.B.3.d. Method of hormone administration.

Oral application of anabolic hormones (other methods of administration include: injection, implantation, and immersion) is the most practical means for dosing fishes that readily accept formulated feeds (Higgs *et al.*, 1982). For thyroid hormone, this route appears to be most appropriate for T₃, but not T₄. Present evidence indicates that T₄ taken orally can, through several means, result in a considerable loss of hormone activity (Ibid.)

X.B.3.e. Other factors.

The intrinsic hormonal control of growth is dependent on the interactions among biotic and abiotic factors, which may also influence a fish's responsiveness to exogenous growth-enhancing hormones. Interspecific and intraspecific responses to exogenous growth promoters is also influenced by age, size, developmental stage, photoperiod, water temperature, salinity, food quantity and quality, stress, and possibly interspecific genetic variations (Higgs *et al.*, 1982). The physiological response to all these factors will depend, to a large extent, on the endogenous hormonal milieu of the animal. Nevertheless, in spite of the large range of variables which have a potential impact on growth, both anabolic steroids and thyroid hormones have been shown to produce consistent and frequently dramatic improvements in the intrinsic capacity of fish to grow.

X.B.4. Abnormal Morphological and Physiological Effects

X.B.4.a. Steroid hormone.

Steroid hormone doses, particularly at high levels, can result in morphological and physiological abnormalities. In carps which received MT for 90 days at doses ranging from 2.5 to 10 mg/kg of feed, there was a progressive increase in the craniosomatic index

(cranium weight expressed as percent body weight), a decrease in hepatosomatic index (liver weight expressed as percent body weight), and a small increase in renosomatic index (kidney weight expressed as percent body weight) (Lone and Matty, 1980). These researchers suggest that the decrease in hepatosomatic index was caused by a mobilization of liver fat to the muscle, a conclusion supported by their observed increase in the muscle lipid. Muscle protein was also significantly elevated, with a concomitant drop in water content. At 1 mg MT/kg of feed, there were no detectable changes in muscle protein, water content, or lipid levels. In salmonids, structural alterations induced by exogenous steroid hormones have been described in the integument, bone and a number of endocrine glands, including the thyroid (McBride et al., 1982). Alterations in body composition through dietary MT treatment have been noted on coho salmon (Fagerlund and McBride, 1975), rainbow trout (Simpson, 1976), steelhead trout and pink salmon (*Oncorhynchus gorbuscha*) (Fagerlund and McBride, 1977). There are no consistent trends among the various species, however, suggesting that the responses to exogenous hormone application are species-specific.

As might be expected, sex steroids influence the state of the gonad and related tissues. No anabolic steroid, either natural or synthetic, has been found to be entirely devoid of sexual effects (Higgs et al., 1982). As was mentioned earlier, MT caused a marked decrease in gonadosomatic index in tilapia (Howerton, *op. cit.*). In salmonids, androgen treatment can induce premature spermatogenesis; degeneration of the testis, but rarely of the ovary; and development of intersexed gonads, containing male and female components (McBride *et al.*, 1982). These undesirable secondary sexual effects generally develop when the steroid doses are at, or near, the maximum concentration of hormone needed to stimulate a significant increase in somatic growth, or when the duration of application is prolonged.

X.B.4.b. Thyroid hormone.

Thyroid hormones at concentrations beyond normal circulating levels can cause a number of morphological aberrations. In salmonids, oral doses of T_3 greater than 20 mg/kg of feed, or lower for a prolonged period, can induce: fin elongation, increase in width and/or height of the skull, elongation or curling of the operculum, and enophthalmos (Higgs *et al.*, 1982). In addition, condition factor of the fish progressively decreases due to greater growth in length relative to weight.

X.B.5. Interaction Between Sex Steroid and Thyroid Function

The activity of a hormone can be greatly influenced by its interaction with other hormones involved in the regulation of the same, or related bodily functions (Dickson, 1984). Gonadal and thyroid function are thought to be related in teleosts (Fostier *et al.*, 1983) although the supposed relationship is not well understood. Administration of sex steroids, such as MT, testosterone, or E_2 in several fishes, have been reported to increase (Higgs *et al.*, 1982; Leatherland, 1985), decrease (Leatherland, 1985), or have no effect (Milne and Leatherland, 1980) on the thyroid activity. A possible synergistic interaction between steroid and thyroid hormone was considered in our present study. Higgs *et al.* (1977) reported that the combined use of MT (oral) and T_4 (intramuscular) in coho salmon resulted in a growth response greater than the single effects of either hormone alone. Howerton (*op. cit.*) also noted such an interaction between the 2 hormones in tilapia. Available evidence suggests that growth stimulation by steroid hormones may be at least partially mediated through the thyroid (Higgs *et al.*, 1982). Thyroid hormones are considered to play a permissive or synergistic role in growth processes of fish, potentiating the effects of other anabolic hormones.

X.C. OBJECTIVES

The overall objective of the present study was to determine the effects of MT, E₂, and/or T₃ in promoting somatic growth in the spotted scat.

Our specific objectives were to:

1. test a range of doses of MT, E₂, and T₃ for their ability to promote growth in the spotted scat raised in aquaria;
2. evaluate the interactions, if any, between T₃ and the steroids in stimulating growth, and
3. evaluate the feasibility, from both a practical and economic point of view, of using hormone feeding in pond-reared spotted scat.

X.D. MATERIALS AND METHODS

The first experiment was conducted in aquaria and was designed to determine the dose-response effects of T₃ on scat fry. The second experiment was conducted in floating cages in a pond and was designed to test the effects of E₂ and MT, with or without T₃, on the growth of sexually mature male and female scat.

X.D.1. Determination of the optimal dose level of T₃.

The following doses of T₃ were evaluated: 0, 0.1, 1, 5, and 10 mg/kg of feed.

The setup consisted of fifteen 40-l glass aquaria, each equipped with an undergravel filter and airlift pump. A portion of the tank bottom was left devoid of the filter bed to serve as the feeding area. This feeding space facilitated observation and prevented the feed from being drawn into the filter.

A commercial prawn feed (40% protein) was used. The T₃ was incorporated into the feed by first preparing a 0.5 mg/ml stock solution in 95% ethanol, and then spraying this on the feed. The sprayed feeds were left exposed to the air overnight for the ethanol to evaporate, and then stored in a freezer.

After conditioning the undergravel filters for 2 weeks, scat fry (0.2 g) were stocked at 12 per aquarium. The fish were conditioned to the aquaria for one week, and fed a control diet to acclimate them to prepared feeds. The experiment started on 26 September 1986. Feeding levels were initially set at 10% of the body weight of the fish per day, divided into 3 rations. Later, the feeding rate was gradually lowered to 3% per day during the latter part of the experiment. The fish were not fed on sampling days. The total wet weight of all the fish in each aquarium was sampled every two weeks.

From 80 to 100% of the aquarium water in each tank was changed every sampling period over the first 2 months. Water changes were increased to 2-3 times every 2 weeks when the fish biomass increased. Salinity was maintained at 20 ppt during the earlier part of the experiment when freshwater was available, but later left to range from 20 to 40 ppt.

After 6 months (26 March 1987), the total biomass and survival in each tank was measured. All specimen were sacrificed; the weight, total length, and standard length of individual fish were measured.

X.D.2. Effects of MT, E₂, and/or T₃ on the growth of scats in ponds.

The design of this experiment was as follows:

Treatment No.	Treatment (mg hormone/kg of feed)	Number of fish/cage
1	10 mg E ₂ + 5 mg T ₃	7
2	10 mg MT + 5 mg T ₃	7
3	20 mg E ₂	7
4	control	10
5	20 mg MT	8
6	10 mg MT	8
7	10 mg E ₂	7

Each fish was uniquely tagged with color-coded beads (Barry and Fast, II, this volume).

The setup consisted of 7 nylon net cages (1.2 m x 1.8 m x 1.2 m) installed adjacent to each other in an 800 m² pond. Seven to ten scats, ranging from 50 to 200 g, were stocked in each cage. The size and sex ratio in each cage was made as uniform as possible. Sexing was based on the shape of the head; cannulations were not attempted to verify sex (see Barry and Fast, II, this volume).

A commercial prawn feed (35% protein) was used. The hormones were incorporated as previously described.

Prior to starting the experiment, the fish were conditioned for 2 weeks on the control feed. The experiment started on 18 April 1987. Feed was initially provided at 3% of the fish's body weight/day in 2 rations. The fish were later fed to satiation. No feed was given on sampling days. Samplings and water changes were scheduled biweekly, during spring tides.

After 10 weeks (1 July 1987), all fish were sacrificed, and the body weight, liver weight, gonad weight, total length, and standard length of each specimen was measured.

X.E. RESULTS

X.E.1. Determination of the optimal dose level of T₃ on the growth of spotted scat fry in aquaria.

After 6 months, no significant differences in percent weight gain and feed efficiency ratio were detected between the five treatments (Table X.i). Survival was significantly lower ($p < 0.001$) in the control group, although this was due to an accidental mass mortality which occurred in one of the replicates, and was not a treatment effect.

The condition factor (CF) of each fish was calculated based on its standard length and weight. A significant ($p < 0.01$) linear decrease in condition factor occurred with increasing doses of T₃ (Fig. X.1). The condition factor was calculated as follows:

$$CF = (\text{weight}/\text{standard length}^{2.659}) \times 100$$

A significant ($p < 0.01$) linear decrease in body/tail ratio (BTR = standard length/(total length - standard length)) was also observed with increasing doses of T₃

TABLE X.1. THE DOSE-RESPONSE EFFECTS OF 3,5,3'-TRIIODO-L-THYRONINE ON PERCENT WEIGHT GAIN, SURVIVAL, FEED CONVERSION EFFICIENCY, CONDITION FACTOR, AND THE BODY/TAIL RATIO IN SPOTTED SCAT (Scatophagus argus) FRY RAISED IN AQUARIA

PARAMETER	DOSE (mg T ₃ /kg feed)				
	0	0.1	1	5	10
Percent Wt. Gain	1705.6	1805.6	1706.1	1637.9	1708.3
Percent Survival	55.5	83.3	86.1	75.0	88.9
Feed Conversion	38.6	34.1	32.8	33.4	33.4
Condition Factor	9.7	8.8	8.9	8.8	8.0
Body/Tail Ratio	4.5	4.6	4.4	4.2	3.9

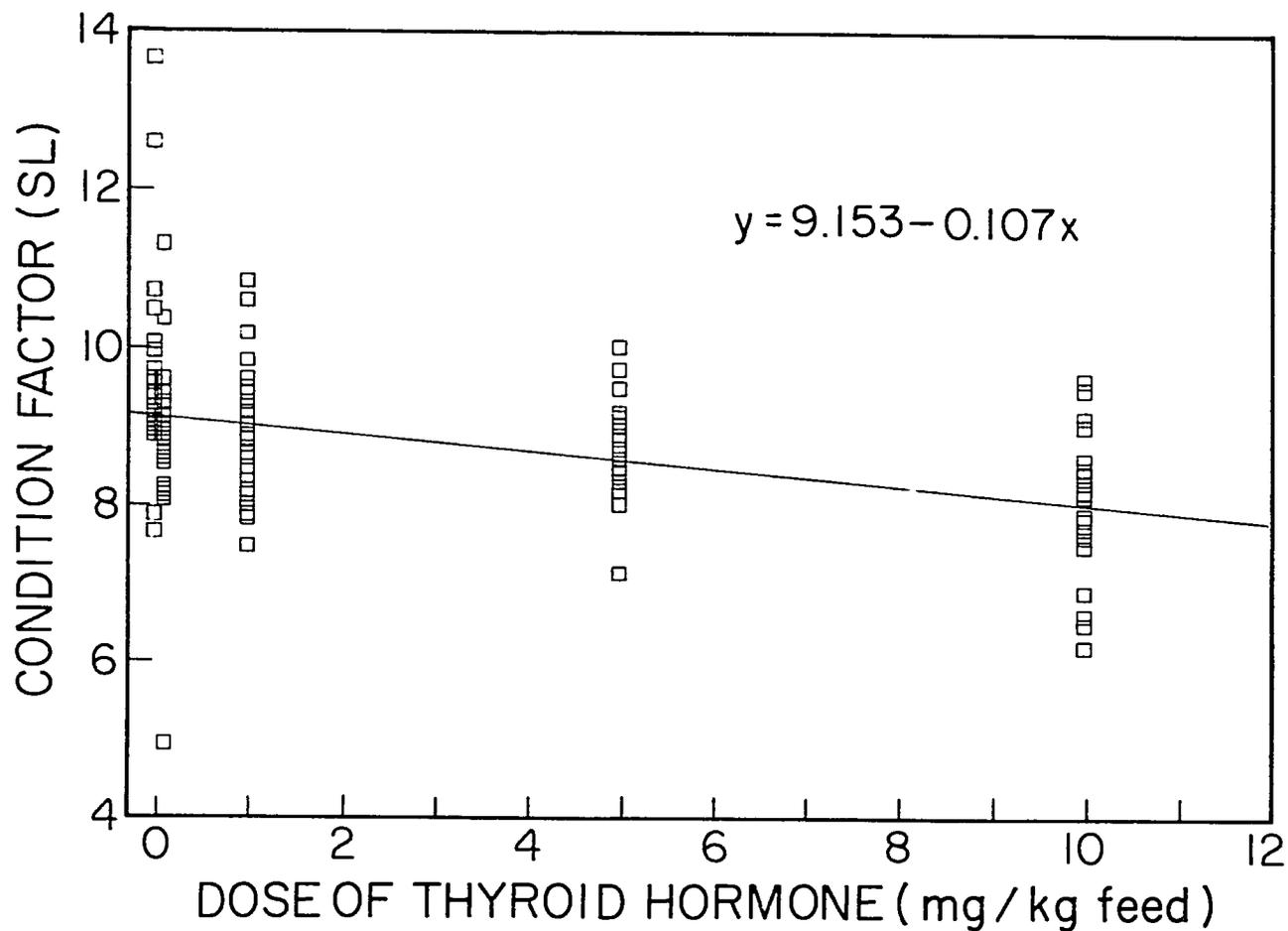


Fig. X.1. The effects of various doses of 3,5,3'-triiodo-L-thyronine (T_3) on the condition factor [(weight/standard length^{2.659}) x 100] of spotted scat fry raised in 40-l aquaria for 6 months. The hormone was incorporated into the diet fed to the fry. Each point represents one fish. A linear regression was fitted to the points, and statistical analysis indicated a significant decrease in condition factor with an increasing dose of T_3 ($p < 0.01$).

(Fig. X.2). The following model describes the relationship between hormone dose and BTR:

$$\text{BTR} = 4.560 - 0.072 \times (\text{dose of } T_3)$$

Aside from changes in condition factor and body/tail ratio, certain hormone-induced external morphological abnormalities were noted, which were most prominent at the highest hormone doses. Features affected included fin length, fin pigmentation, and body shape (Table X.2 and Fig. X.3). Changes in external morphology induced by T_3 were noted as early as the 2nd month, and became distinct by the 4th month.

X.E.2. Effect of MT , E_2 , and T_3 on the growth of scats in ponds.

Beginning on the 4th week, we observed that all of the hormone-treated fish had small appetites. This was particularly evident on week 6 when we began to feed the fish to satiation; the controls ate twice as much as the hormone-treated fish. The experiment was terminated on week 10 when this pattern did not change. The hormone-treated fish grew significantly slower than the controls, probably because the hormones depressed appetite.

The control fish grew most quickly between the time they were stocked and the 2nd sampling (4th week). By the 6th week, the controls had gained significantly more weight than the fish in all of the hormone treatment groups ($p < 0.01$). The control fish continued to grow faster than the hormone-treated fish until the end of the experiment. There were no differences at any sampling time in the percent weight gain among the 6 groups of hormone-fed fish. Linear regressions were fitted to the average cumulative weight gains for each treatment group. Comparisons of these regressions showed that the control fish grew at a significantly faster rate ($p < 0.01$) than all of the hormone-treated fish, which did not significantly differ from each other (Fig. X.4).

Survival in all treatments, except treatment 7 where there was a single mortality, was 100%.

Feed conversion efficiencies (FCE) were computed every sampling period and were consistently highest in the control fish. The overall FCEs, calculated as the mean of the FCEs found each sampling period, are shown in Table X.3. Statistical analysis was not performed on the FCE data.

When the specimens were dissected to get gonad and liver weights, it was discovered that our sexing method was only 64% reliable for the relatively small fish used in our experiment. Some putative males turned out to be females, and several putative females turned out to be males. Treatment 2, for example, had only one female instead of the expected 4.

X.E.3. Problems encountered with the spotted scat.

We encountered several problems while working with the spotted scat. These are summarized below.

1) Fry were not abundant, and only seasonally available. Fry did not readily accept formulated diets, and when conditioning them to eat these feeds, there was high mortality (50 to 90%). At this time, the fry became cannibalistic when food was inadequate. These problems were solved by feeding the fry filamentous algae, artemia, or other zooplankton in steadily decreasing amounts during the conditioning period.

2) Juveniles. Starting at approximately 3 cm, juvenile scat become extremely aggressive and territorial. The aggressive behavior is very pronounced when the fish reach 5 to 8 cm. At this size, casualties as the result of aggressive conflicts are common, especially in closed containers. Providing shelter did not solve the problem. In ponds, even at high stocking densities in closed cages, there were fewer mortalities related to aggressive behavior, perhaps because of the decreased visibility in the ponds.

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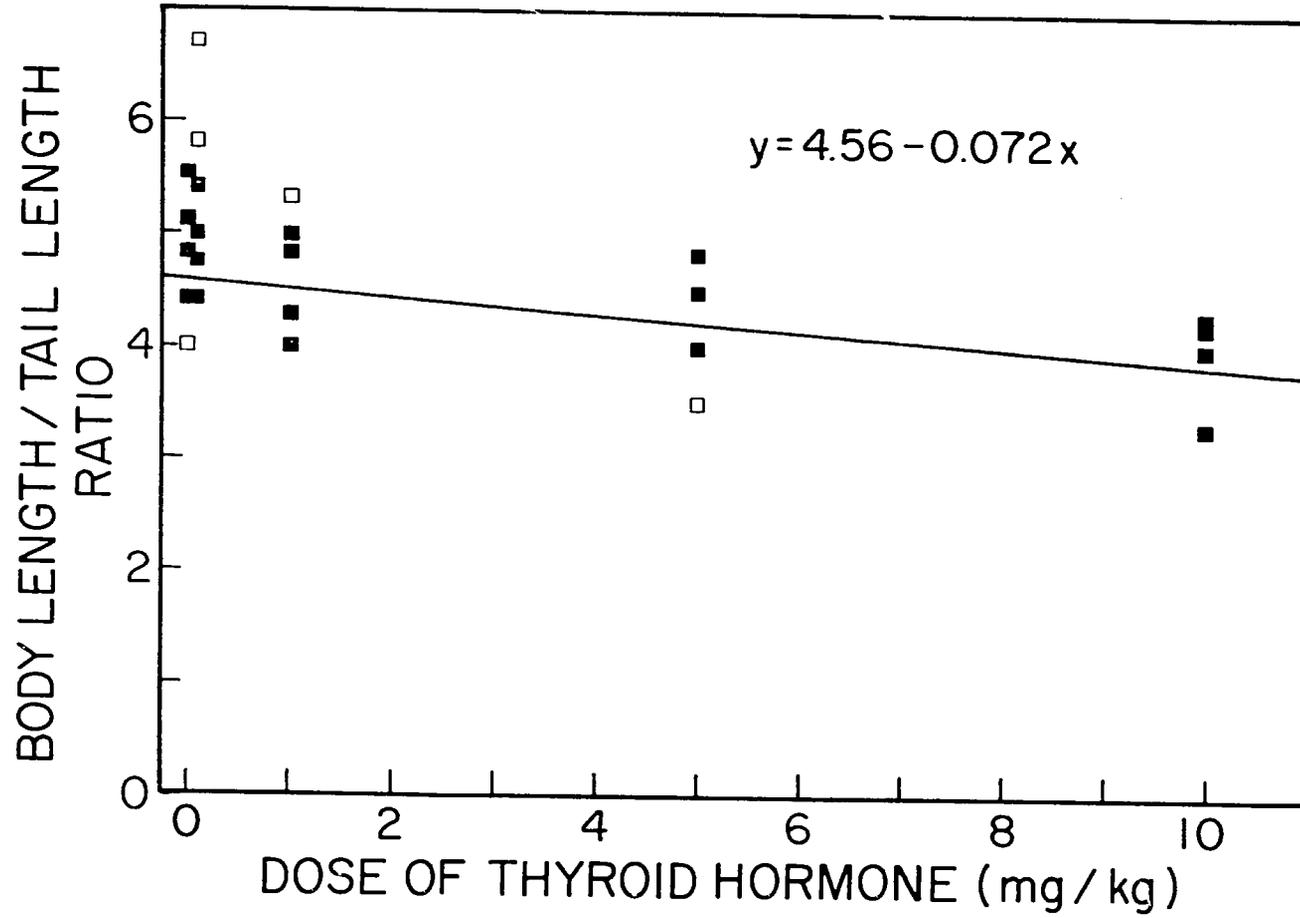


Fig. X.2. The effects of various doses of 3,5,3'-triiodo-L-thyronine (T_3) on the ratio of standard body length to tail length (Body/Tail Ratio, BTR) of spotted scat fry raised for 6 months in 40-l aquaria. The hormone was incorporated into the diet fed to the scat fry. Open symbols represent one fry, and closed symbols more than one fish. Statistical analysis indicated that there was a significant linear decrease in the BTR with increasing dose of T_3 ($p < 0.01$).

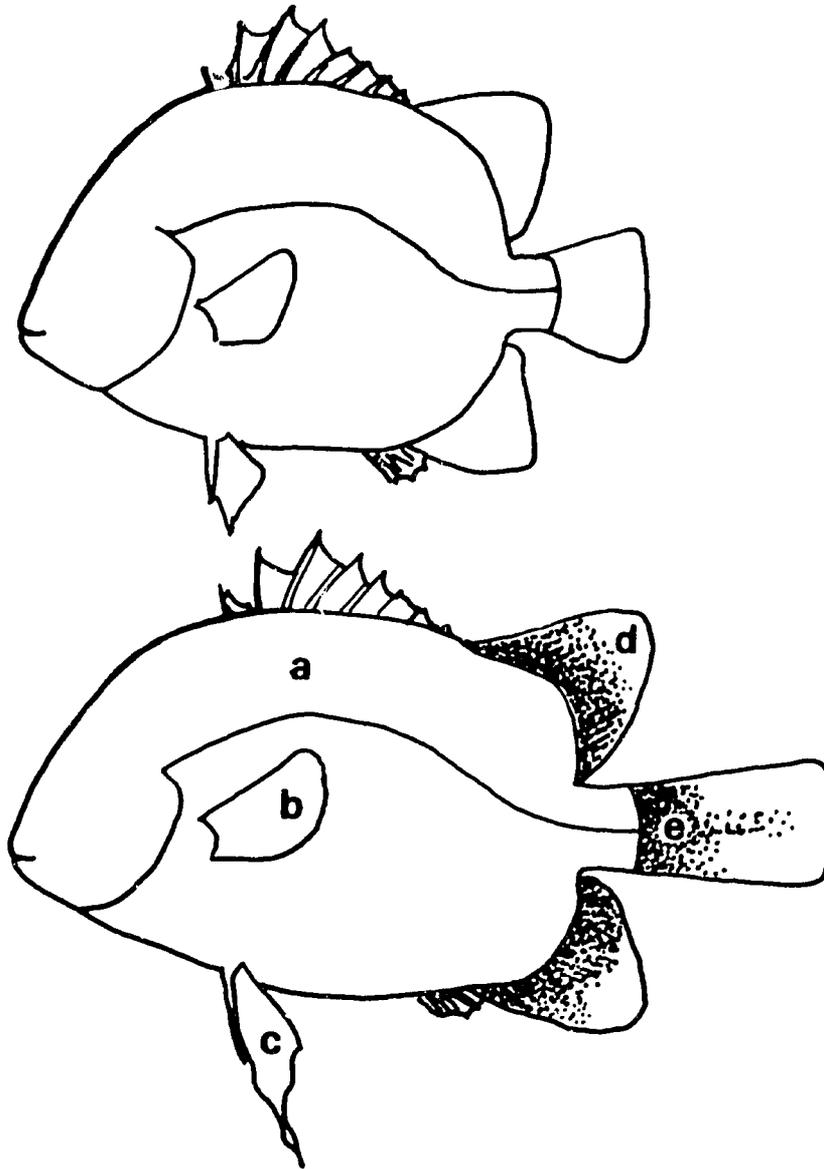


Fig. X.3. Drawings of spotted scat fry illustrating abnormalities which develop when fry are fed high doses (5 or 10 mg/kg feed) of 3,5,3'-triiodo-L-thyronine (T_3). The upper and lower drawings represent fry of the same weight which received 0 or 10 mg T_3 /kg of feed for 6 months, respectively. The following abnormal morphological changes were observed in scat fry administered high doses of T_3 : a) elongated body, b) elongated pectoral fins, c) elongated pelvic fins, especially at the tips, d) orange tinge on the tips of the soft dorsal and anal fins, e) elongated soft-dorsal, anal, and caudal fin rays, and a darkening of these fins at their bases.

TABLE X.2. ABNORMAL MORPHOLOGICAL CHANGES CAUSED BY 3,5,3'-
L-THYRONINE ADMINISTERED IN THE DIET TO SPOTTED SCAT
(*Scatophagus argus*) FRY RAISED IN AQUARIA

Body Structure	Morphological Change	Effective Dose(s) (mg T ₃ /kg feed)
Pectoral Fin	slightly elongated	10
Pelvic Fin	elongated, especially at tips; tips curled or undulate	10, slight at 5, very slight at 1
Second Dorsal Fin	elongated, darkened, distinct orange pigmentation on upper half	10, slight at 5
Anal Fin	elongated, darkened	10
Caudal Fin	elongated	10
Body Shape	elongated	10, slight at 5, very slight at 1

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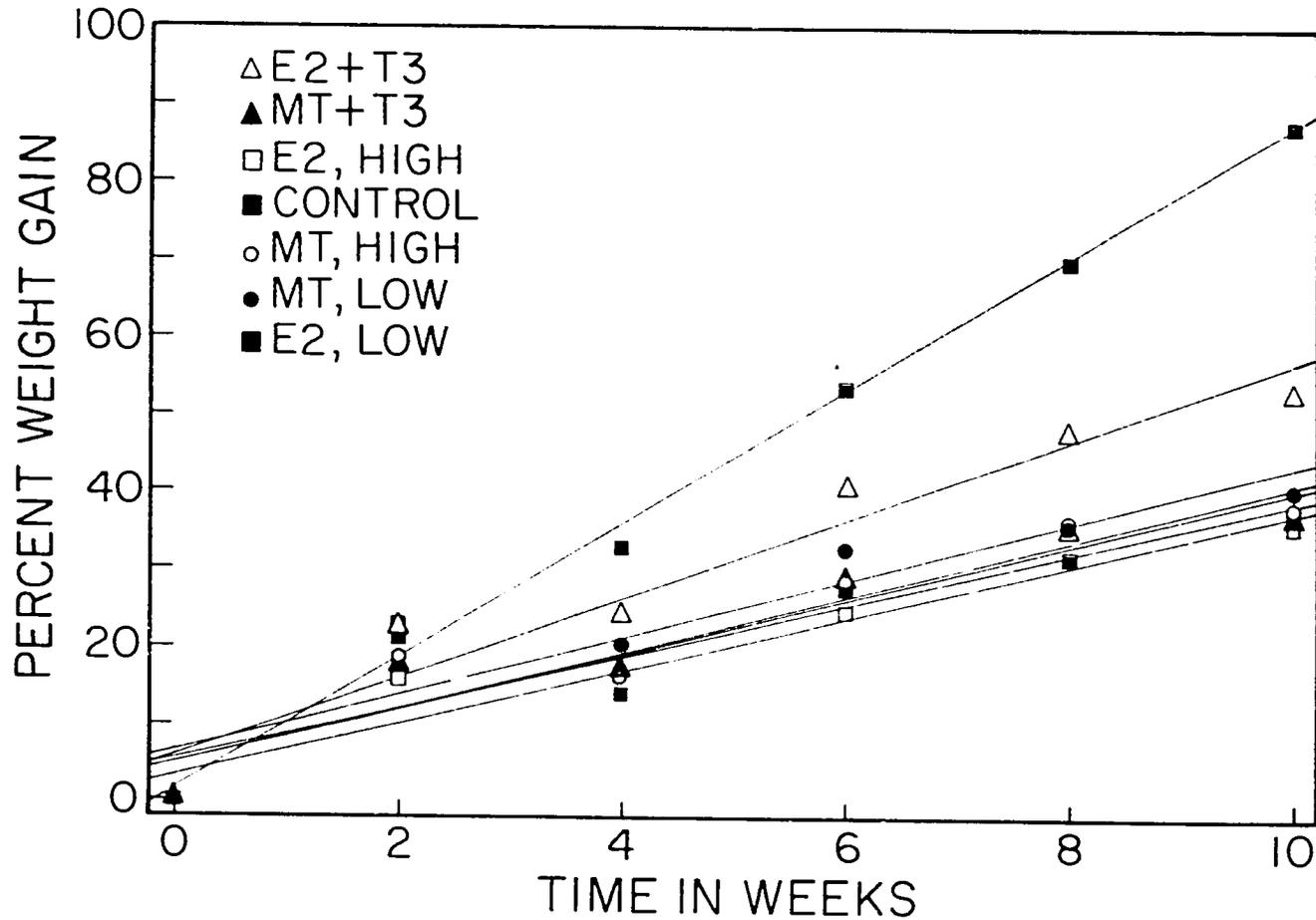


Fig. X.4. The effects of various hormone treatments on the growth of adult spotted scat (*Scatophagus argus*). The fish were held in floating cages in a 500 m² brackishwater pond, and the hormones were incorporated into their diet. Dose levels were 20 mg/kg feed (high), 10 mg/kg feed (low), or in the combined treatments, 10 mg/kg (E₂ or MT) plus 5 mg/kg (T₃). Regression lines of average percent weight gain on time were fitted to the points of all treatment groups. The control fish (closed squares, upper-most line) grew significantly faster than all the treated fish ($p < 0.01$). The growth rates of the hormone-fed fish were not significantly different from each other.

TABLE X.3. FEED CONVERSION EFFICIENCIES FOR POND-REARED ADULT SPOTTED SCAT (Scatophagus argus) FED VARIOUS HORMONE-ENRICHED FEEDS

Hormone Dose/kg feed	FCE ^a
control	46.2 ± 4.8
10 mg E ₂	36.5 ± 9.1
10 mg E ₂ + 5 mg T ₃	34.9 ± 8.7
10 mg MT	33.8 ± 7.5
10 mg MT + 5 mg T ₃	29.3 ± 7.4
20 mg MT	29.2 ± 7.1
20 mg E ₂	29.1 ± 5.0

^a Feed conversion efficiencies (FCE) were determined 4 or 5 times for each treatment group during the 10-week experiment. The means ± the SEM of these 4 or 5 determinations are presented.

Table X.4. GENERAL OBSERVATIONS ON PROBLEMS CONCERNING THE BIOLOGY OF THE SPOTTED SCAT (Scatophagus argus)

Subject	REMARKS
1) fry	: not so abundant, seasonal availability; high mortality (50-90%) especially when conditioning to artificial diets; cannibalistic when food is inadequate
2) juvenile	: Starting at approx. 3 cm, the fish becomes territorial. This aggressive behavior is very pronounced when they reach 5-8 cm where casualties are common due to bite wounds. : Provision of shelters in the aquaria did not solve this problem. This was not observed in cages in ponds with high stocking densities, possibly due to lower visibility.
3) dermal bacterial infections (hemorrhagic lesions, ulcers, tail and fin rot)	: Scats are very prone to bacterial attack especially when stressed and bruised from handling. Advanced cases of the disease are often fatal. Chloramphenicol has been found to be very effective either orally (750 mg/kg feed at 3% bw/day for 1 week for juveniles and adults; 500 mg/kg feed at 10% bw/day for 5 days for fry), or as bath (50 ppm for 10 hours for all sizes). Injectable oxytetracycline was also effective intramuscularly at 3 mg/100-150 g fish.
4) ectoparasites	: Epizootics of the ectoparasites <u>Caligus sp.</u> and <u>Argulus sp.</u> are common in aquaria and ponds. FW bath treatment has been successful for a minimum of 30 min. Formaldehyde was observed to be very effective at 100 ppm for 20 min or 300 ppm for 5 min. Scats can tolerate 300 ppm formaldehyde even for 20 min.

Both fry and juvenile spotted scat are susceptible to diseases and parasitic infestation (Natividad and Gerundo, XIII; Lio-Po and Barry, XIV; and Cruz, XV, this volume).

X.F. DISCUSSION

Experiment 1. The T_3 dose-response experiment indicated that the hormone had no effect on growth, FCE, or survival after 6 months of treatment, under the given experimental conditions. The effects of the hormone were instead characterized by external morphological abnormalities, particularly in treatments with high levels of T_3 . The ineffectiveness of T_3 as a growth promoter does not support our hypothesis that a slow-growing fish species would respond with greater growth increases to anabolic hormone treatment than would a faster-growing fish. Distinct morphological aberrations were induced by 5 and 10 mg T_3 /kg of feed, perhaps because these treatments increase plasma hormone levels to abnormally high, perhaps pharmacological, levels. The thyroid hormone levels which elicited morphological abnormalities in the scat were considerably lower than those reported to produce similar changes in salmonids.

The most distinct and consistent dose-related abnormalities caused by T_3 in the scat were the elongation of the body and fins. The condition factor, which provides a means of evaluating the fish's weight relative to its standard length, was used as an index to quantify the effect of T_3 on body lengthening. The hormone caused a significant dose-dependent decrease in condition factor; higher doses of T_3 resulted in longer fish.

The T_3 -induced elongation of the fish's body length was independent of T_3 's effects on tail fin elongation. This was evidenced by the significant effect of T_3 on condition factor, and the decreased BTR with increasing doses of T_3 .

The sensitivity of the fins to thyroid hormone treatment, particularly the caudal fin, suggests that thyroid hormone receptors are concentrated in these areas, and that thyroid hormones may play an important role in the growth and development of these appendages. Indeed, the spotted scat caudal fin might prove to be an excellent model system for studying the mechanisms by which T_3 promotes growth in general.

Experiment 2. The pond study was originally designed as a 20-week feeding experiment, but was terminated on week 10 when it became evident that the hormones were depressing appetite and growth.

The doses used in the present study--chosen by their ability to promote rapid weight gains in *Epinephelus salmoides* (9 mg MT/kg feed) (Chua and Teng, 1980), and *Oreochromis mossambicus* (10 mg MT/kg feed) (Howerton, op. cit.)--may have been too high. The fish used in the experiment were undergoing sexual maturation and the concentrations of endogenous sex steroids may have already been high. The addition of exogenous hormone may have increased the blood hormone concentrations beyond pharmacological levels. Another factor that could have prevented an anabolic response in the fish would be a reduction in the affinity and/or number of hormone receptors on the target tissues responsible for growth. This situation would be expected during sexual maturation, since energy is diverted at this time from somatic to gonadal growth. The potentials of MT, E_2 , and these steroids combined with T_3 need to be evaluated in immature spotted scat.

XI. TEMPERATURE, SALINITY, AND PH TOLERANCE OF SPOTTED SCAT FRY (*Scatophagus argus*)

Maria Paz Socorro C. Macahilig, Milagros T. Castaños,
and Terence P. Barry

INTRODUCTION

To fully evaluate the culture potential of the spotted scat, it is necessary to know its tolerance limits to environmental parameters which can fluctuate widely in brackishwater ponds. These parameters include temperature, salinity, pH, and dissolved oxygen. Scat fry are captured in waters with salinities ranging from 5 to 40 ppt (mean = 27 ppt), and temperatures ranging from 27 to 37 C (mean = 28) (personal observation). Salinities and temperatures in the ponds where scat may be cultured, however, might vary over even greater ranges. To determine whether or not the scat's physiological adaptations which allow it to survive in its ever-changing natural habitat will also allow it to thrive in culture, we conducted experiments designed to evaluate the temperature, salinity, and pH tolerances of spotted scat fry.

MATERIALS AND METHODS

Water samples of different salinities were prepared by diluting sterile pond water (25 ppt) with distilled water, or by adding salt from evaporated seawater. Scat fry ranging in size from 2 to 4 g were used in the experiment. The fry had been reared in earthen ponds for 3 months, during which time pond salinities ranged from 16 to 30 ppt, and pond temperatures ranged from 26 to 36 C. Over the three weeks prior to the start of the experiment, salinities ranged from 22 to 25 ppt, and temperatures ranged from 28 to 32.5 C. The fish were at 25 ppt and 27.5 C when the experiments began.

Temperature Tolerance. The physiological tolerance of fish to elevated temperature was quantified by determining the critical thermal maximum (CTM). Ten scat fry were placed in water of the following salinities and acclimated for at least 24 hrs: 0, 10, 20, 25, 30, 35, 40, and 50 ppt. Five fish from each salinity group were placed together into a 1-l beaker filled with water of the acclimation salinity. At time 0, the temperature in the beaker was heated at a constant rate of 0.2 C per min by placing the beaker into a large basin of water which was in turn placed onto a laboratory heating plate. The temperature at which each fish turned upside down, began to swim weakly, or was in obvious distress was recorded as the critical thermal maximum (CTM) for that individual. It was not difficult to determine when the CTM was reached. After the CTM was recorded for all the fish in the beaker, they were transferred to a bucket containing water of the acclimation salinity. The water in the experimental basin was replaced with cool water and the experiment was continued with fish from a different acclimation group.

Salinity Tolerance. Ten scat fry per treatment were stocked directly from the pond into 20-l basins with water of salinities ranging from 0 to 60 ppt. Two temperature ranges were tested: ambient (range, 25 to 27.5 C), and high (range, 33 to 34.4 C). High temperatures were maintained using thermostatically-regulated, 150-watt aquarium heaters. The fish were fed "lab-lab" and filamentous algae twice daily over the 4-day experimental period. Dead fish were removed at regular intervals. Survival rate (% survival) was determined after 24 hrs for both test temperatures, and after 96 hrs for the groups tested at ambient temperature. The experimental procedure was repeated 2 to 4 times.

pH Tolerance. Scat fry were acclimated for over 48 hrs in a holding tank with water at pH 6.3. Ten scat fry per treatment were stocked directly from the tank into 20-l basins containing water of pHs 4, 6, 8 and 10, respectively. Low and high pHs were adjusted with

HCl and NaOH respectively, and measured using pH paper. The experiment was conducted at ambient temperature (range, 25 to 27.5 C). Survival rate (% survival) was determined after 24 hrs. The experimental procedure was repeated 3 times.

Statistical Analysis. Means and standard errors of the means were calculated, and compared by analysis of variance (ANOVA). Differences between means were determined using Duncan's multiple range test (DMRT).

RESULTS

Temperature. The highest average CTM was recorded for fish acclimated to 25 ppt (41.9 ± 0.08 C, N = 6); followed by 30 ppt (41.4 ± 0.40 C, N = 5); 35 ppt (41.3 ± 0.35 C, N = 5); 40 ppt (41.1 ± 0.24 C, N = 5); 10 ppt (40.8 ± 0.46 , N = 5); 20 ppt (40.7 ± 0.24 , N = 8); 0 ppt (40.6 ± 0.29 , N = 5); and 50 ppt (38.4 ± 0.49 , N = 5). This data is also shown in Fig. XI.1.

The ANOVA revealed significant differences among the means ($p < 0.001$). The DMRT showed that there were no differences among salinities 10, 25, 30, 35, and 40 ppt; 0 and 20 ppt were significantly different from 25 ppt; and 50 ppt was significantly different from all other groups ($p < 0.05$).

Salinity Tolerance. At ambient temperature, a mean of 92.5% or more of the scat fry survived for 96 hrs after a direct transfer from an acclimation salinity of 25 ppt to salinities ranging from 0 to 40 ppt (Fig XI.2). The percent survival decreased significantly when the fry were transferred to 50 ppt. On the average, only 30% of the fry transferred to 50 ppt survived after 24 hrs; 50% were dead within 3 hours. At 60 ppt, 100% of the fish died within 6 hrs. Analysis of variance showed that the differences in survival among salinities were highly significant ($p < 0.001$). The DMRT showed that there were no significant differences among 0, 10, 20, 30, and 40 ppt. These salinities, however, were significantly different from 50 and 60 ppt ($p < 0.01$).

The medium lethal salinity (MLS) is defined as the salinity where 50% of the acclimated fry survive for 96 hrs following a direct transfer to water of a different salinity. The MLS for scat fry transferred to water of ambient temperature was determined graphically as 46 ppt. The MLS could not be determined for the scat transferred to water of higher temperature because the experiment was carried out for only 24 hrs. For comparison, however, the salinity where 50% of the fry survived for 24 hours was determined for the ambient and high temperature groups; the values were 47 and 35 ppt, respectively (Fig. XI.2). A two-way ANOVA showed that there was a significant interaction between salinity and temperature on scat fry survival ($p = 0.047$).

pH. In all three trials, 100% of the scat fry survived transfer to water of pHs 6, 8, and 10. In all three trials, every scat fry died following transfer to water of pH 4.

Dissolved Oxygen. Dissolved oxygen was measured at each salinity and temperature combination. Oxygen levels decreased with increasing salinity and temperature. Values ranged from 7.8 ppm at 0 ppt salinity and ambient temperature to 6.2 ppm at 50 ppt salinity and 33 C.

DISCUSSION

The results of the temperature tolerance test (CTM) indicated that scat fry have a very high upper temperature tolerance limit. Menasveta (1981) measured the CTM of 24 marine fish from the Gulf of Thailand. Of all the species tested, the spotted scat had the highest CTM, 37.5 C in seawater. This is lower than the value we found in the present study (41.3 C). The difference can be explained by the fact that the scat used in our study were held in ponds and had previously experienced temperatures of at least 32.5 C. The scat from the Thailand study were captured at sea where the water temperature was an almost constant 28 C. Menasveta (1981) found that the temperature tolerance, and CTM, was

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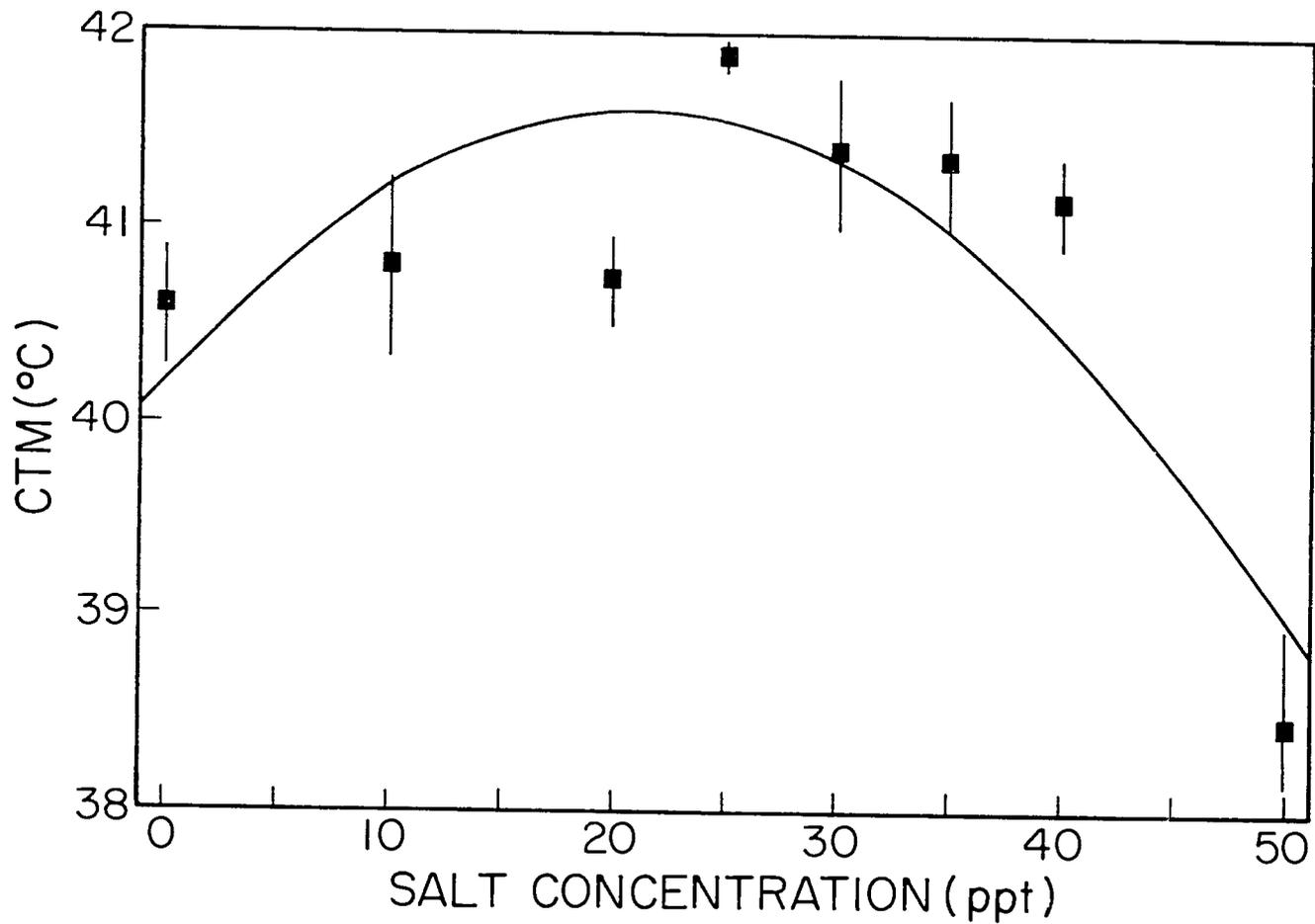


Fig. XI.2. The survival of spotted scat fry as a function of salinity and temperature. Fry acclimated to 25 ppt and ambient temperature (range, 25 to 27.5 C) were immediately transferred to water of the indicated salinity. For fry transferred at ambient temperature, the percent survival was determined after 24 (open triangles) and 96 hrs (open squares). The survival of fry transferred at a higher temperature (range, 33 to 34.4 C) was determined after 24 hrs (closed triangles).

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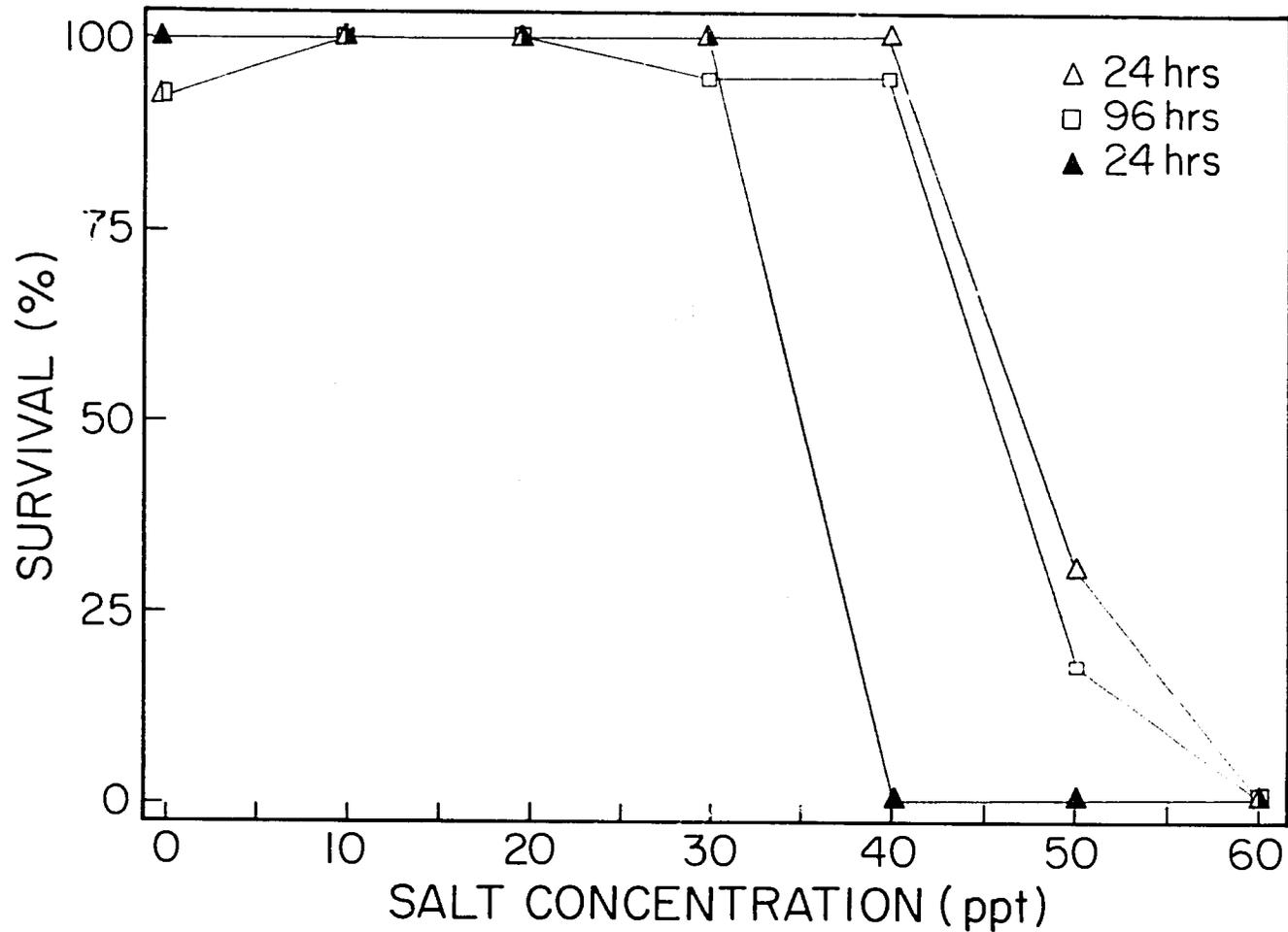


Fig. XI.1. The critical thermal maxima (CTM) of spotted scat fry acclimated to various salinities. Each point represents the mean \pm the standard error of 10 independent trials. Scat fry were acclimated at the indicated salinity for 48 hrs and placed into a beaker of water which was heated at a rate of 0.20 C per min. The temperature at which each fish first lost equilibrium was recorded as its CTM.

higher with increased acclimation temperature.

The results of the salinity tolerance test indicated that scat fry have a very wide salinity tolerance range, and are more tolerant of transfers to lower salinities. Scat acclimated at 25 ppt tolerated transfer to 0 ppt water, or a 25 ppt salinity change, but, when transferred to a higher salinity, the fish could only tolerate a change of less than 20 ppt. This pattern of survival probably reflects an ability of the scat fry to osmoregulate better at lower osmotic pressures than in a hypersaline environment. This is true for many euryhaline fishes. To maintain a constant internal environment at lower osmotic pressures, water entry and ion loss through the fish's exposed exchange surfaces, such as gut and gills, must be prevented. To osmoregulate in hypersaline environments, however, the fish must prevent water loss from its body and ion entry from the external environment across the same exposed surfaces. The latter is generally more difficult for reasons which will be discussed below.

The results of both the salinity and temperature (CTM) tolerance tests demonstrated that salinity and temperature interact in their effects on scat survival. Within a particular temperature range, the scat is more resistant to salinity changes; and within a particular salinity range, the scat is more resistant to temperature changes. Many fish species must increase their metabolic rates and oxygen consumption to actively pump ions out of their bodies when attempting to osmoregulate at higher salinities. As a result, the gills are exposed to an even greater extent to the external medium which increases the rate of passive ion and water exchange across the gill membranes. Metabolic processes are also stimulated directly by the increase in temperature, which further increases oxygen demand. In general, a rise of 10 C increases oxygen uptake in poikilothermic fish by a factor of 2. Thus, an upper limit at which the fish can no longer effectively eliminate ion entry and water loss is soon reached at elevated temperatures and salinities (Fry, 1971). Nevertheless, even at elevated temperatures, scat fry can tolerate salinities over 40 ppt, an outstanding attribute for a cultured species.

Oxygen concentrations were always maintained throughout the experiment by constant aeration. The decreases in dissolved oxygen caused by increased temperature and salinity probably had little influence on the scat's temperature and salinity tolerances. In a pond situation, however, dissolved oxygen concentrations can reach very low levels during growout. The most successful aquaculture species are able to survive when dissolved oxygen levels are low. We did not test the scat's tolerance limits to decreases in dissolved oxygen in a controlled setting. Data from our pond growout studies, however, suggest that the scat may be able to tolerate low dissolved oxygen concentrations. When scat fry were grown in polyculture with prawn, the total pond biomass was very high just prior to harvest, and the morning DO concentrations averaged less than 2 mg/l. Survivorship of scat in these ponds was 54.8% (Biona et al., IX, this volume).

The results from the pH tolerance test did not allow us to determine the mean lethal pH for the spotted scat. It lies somewhere between pHs 4 and 6 for scat acclimated to pH 6.3. The results demonstrate, however, that the scat has a large pH tolerance range (at least 6 to 10). Scat are not likely to experience pHs outside of these limits in brackishwater fish ponds.

In conclusion, as a candidate species for aquaculture, the spotted scat is highly tolerant of changes in temperature, salinity, temperature/salinity, and pH. Its upper salinity and temperature tolerance limits are high and suggest that the scat can survive in culture ponds even when temperatures and salinities reach high values during the summer months. In our own ponds, broodstock scat survived, and even underwent gonadal maturation, when the temperature reached 38 C and the salinity was 60 ppt. These values are well beyond what our laboratory-generated data suggested the scat could tolerate. Perhaps larger fish have higher tolerance limits, or gradual acclimation to higher temperatures and salinities may affect subsequent survival when extreme temperatures and salinities are encountered.

XII. 2-PHENOXYETHANOL AS A GENERAL ANESTHETIC FOR THE SPOTTED SCAT (*Scatophagus argus*)

Rizaline Tabanda and Terence P. Barry

ABSTRACT

The dose-response relationship of 2-phenoxyethanol as a general anesthetic for *Scatophagus argus* was investigated. At a temperature of 27 C, the dose at which 50% of the fish were anesthetized (ED_{50}) was between 0.04 and 0.08 ml/l. A dose of approximately 0.30 ml/l anesthetized 100% of the fish within 10 minutes and, for practical reasons, is the recommended dose for this species when undertaking procedures that require the fish to be placed under short-term anesthesia. No attempt was made to determine the lethal dose (LD_{50}). However, fish treated with a dose of 0.64 ml/l for two hours suffered no harmful effects indicating that the therapeutic ratio (LD_{50}/ED_{50}) is greater than 8, an excellent margin of safety.

INTRODUCTION

2-phenoxyethanol is a clear, oily liquid which is an effective general anesthetic for salmonids (Sehdev et al., 1963). In addition, 2-phenoxyethanol is a useful chemotherapeutic agent for treating various fish diseases. This combination of uses makes it a very attractive agent for routine procedures that require a fish to be placed under anesthesia. Such applications include surgery, blood sampling, ovarian biopsy, hormone injections, and transport.

Our paper presents the experimental results we obtained in trying to determine the minimum effective dose of 2-phenoxy-ethanol needed to anesthetize 100% of young adult spotted scat (*Scatophagus argus*) within 10 mins of treatment.

MATERIALS AND METHODS

2-phenoxyethanol was obtained from the Sigma Chemical Co., St. Louis, MO. Eighty scat, which were of mixed sex, and ranged in size from 50 to 150 grams, were used in the experiment. The experiment was conducted using ten 40-l glass aquaria. The water salinity was 25 ppt, with ambient temperatures (26.5 to 27.5 C). The water was aerated throughout the experiment. Four fish were placed into each aquaria. At time 0, each tank received a separate, pre-measured quantity of 2-phenoxyethanol, ranging from 0.02 to 0.64 ml/l. The number of anesthetized fish, and the time required for each fish to reach this condition, was recorded for each tank (dose). A fish was defined as being anesthetized based on the following criteria: 1) it turned belly up, regardless of whether or not it continued to swim, or sank to the bottom; and 2) the fish failed to respond to a light touch, and could be handled easily. The experiment was replicated twice.

RESULTS AND DISCUSSION

No fish responded to treatment with 0.02 and 0.04 ml/l 2-phenoxyethanol. After 75 min, 75% of the fish treated with 0.08 ml/l responded to the chemical. At all the remaining doses tested: 0.16, 0.20, 0.24, 0.28, 0.32, 0.64 ml/l, 100% of the fish were anesthetized within 30 min. (Table XII.1). The dose of 2-phenoxyethanol required to anesthetize 50% of the fish (ED_{50}) lies between 0.04 and 0.08 ml/l. An ED_{50} value within this range is very comparable to the ED_{50} value reported for sockeye salmon (*Oncorhynchus nerka*) at 11 C, 0.095 ml/l (Sehdev et al., 1963). Considering the differences in temperature, salinity, weight, sexual condition, and other factors, the similarity of the

Table XII.1. The Anesthetization Response of Spotted Scat (Scatophagus argus) to Various Concentrations of 2-Phenoxyethanol in 25 ppt Seawater at 27° C

2-phenoxyethanol (ml/l)	Fish Tested (no.)	Fish Anesthetized (no.)	Fish Anesthetized (%)	Time (min)
0.02	8	0	0	120 ¹
0.04	8	0	0	120
0.08	8	6	75	75
0.16	8	8	100	30
0.20	8	8	100	16.2 ± 4.7
0.24	8	8	100	17.5 ± 1.4
0.28	8	8	100	12.5 ± 2.5
0.32	16	16	100	9.3 ± 1.7
0.64	8	8	100	3

¹Mean time ± se

ED₅₀ values between these two species is surprising. Sehdev et al. (1963) reported a pronounced temperature dependence for the actions of the chemical. At 4 C, the ED₅₀ value in sockeye salmon was 0.056 ml/l, or 1.7 times lower than that at 11 C. We did not attempt to test the effects of 2-phenoxyethanol at different temperatures with scat.

The primary purpose of the study was to find the dose of 2-phenoxyethanol which would anesthetize 100% of the treated fish within 10 minutes. This dose and time period were sought for practical reasons. Our studies with scat required routine transport, tagging, weighing, measuring, blood and oocyte sampling, and hormone implantation. These procedures all required that 1) all the fish be quickly and completely anesthetized; and 2) there be no lingering side effects following treatment.

We calculated the dose needed to anesthetize 100% of the scat in 10 min to be between 0.30 and 0.31 ml/l (Fig. XII.1). Therefore, a dose of 0.30 ml/l satisfied the first requirement. To determine if the second requirement could also be met at 0.30 ml/l, we tested the effects of an even higher dose. Eight fish were treated with 0.64 ml/l 2-phenoxyethanol for two hours, a time well beyond the longest period fish were ever expected to be exposed to the chemical, and at a dose more than twice as high as that needed to anesthetize all fish within 10 minutes. All eight fish were completely anesthetized within three min; all turned belly up and remained completely immobile. After two hours, the fish were transferred to water without the chemical. Every fish fully recovered and resumed normal activity within 2 min, with no visible harmful effects over the next two days. These results indicate that a dose of 0.30 ml/l is safe for general, short-term (less than 2 hours) use with this species. The result also indicates that the therapeutic ratio (LD₅₀/ED₅₀) for 2-phenoxy-ethanol when used on scat at 27 C is greater than 8. This ratio is high and indicates that there is a large margin of safety in the use of 2-phenoxyethanol as an anesthetic for the spotted scat.

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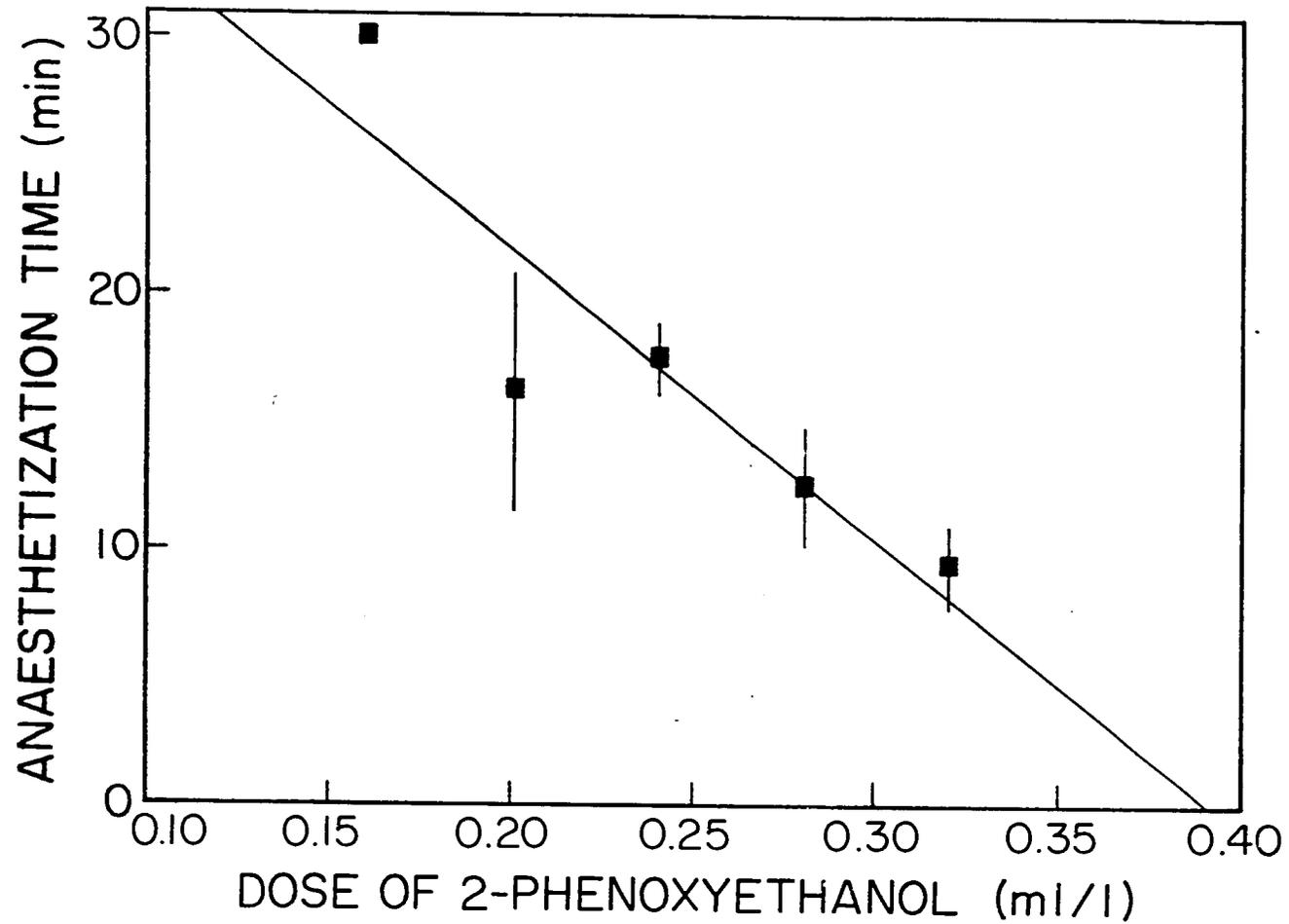


Fig. XII.1. The average time required for 5 doses of 2-phenoxyethanol to completely anesthetize young adult spotted scat (*Scatophagus argus*) at a water salinity of 25 ppt and temperature of 27 C. The means \pm the standard errors of 8 or 16 replications are shown, as is the regression through the means.

XIII. HISTOPATHOLOGICAL REPORT ON A MICROSPORIDIAN INFECTION IN THE SPOTTED SCAT (*Scatophagus argus*)

Jose M. Natividad and Nelson D. Gerundo

A postmortem examination of three specimens of spotted scat (*Scatophagus argus*) broodstock, which died during a mass die-off of fish held in tanks, revealed several cyst-like structures on their visceral organs. These cysts varied in size, and were usually confined to the intestinal region. The cysts appeared to be attached to the serosal surfaces of the intestines.

Histological sections through these structures showed that they were visceral xenomas induced by microsporidian parasites (Fig. XIII.1). The xenomas appeared to be of the Glugea type, although no effort was made to identify the species of microsporidian producing them. Species from several genera of microsporidia produce xenoparasitic cysts.

The xenomas were as large as several mm in diameter, and were composed of relatively thin layers of concentric connective tissue which were apparently produced as a result of connective tissue proliferation and collagen fiber hyperplasia (Fig. XIII. 1). The central spaces of the cysts were filled with a mass of sporophoric vesicles each of which contained several spores (Figs. XIII.2 and XIII.3). An area of uninucleate stages and larger meronts were observed at the periphery of the cysts (Figs. XIII.1 and XIII.4). Concentric foci or aggregates of mature spores, with broken down vesicles, were often observed in the central region of the xenomas (Fig. XIII.3). Capillary congestion and hemorrhages were pronounced in the areas of the xenomatous growth. There was a minimal mononuclear reaction in these areas (Fig. XIII.5). The intestine of one of the infected fish showed a diffuse necrotic alteration of the mucosal area, and marked congestion and hemorrhages in the underlying submucosal region (Figs. XIII.6).

No significant pathological changes were noted in the spleen, liver or kidney of the examined fish. The gills appeared normal except for some occasional areas of secondary lamellar hyperplasia.

The prevalence of microsporidian infections can vary widely. Cases have been reported, however, where almost 100% of the fish in a population are infected. Heavy microsporidian infection may cause inactivation of a substantial proportion of the organ or system affected, and lead to mass mortalities. The deaths are attributable to the combined effects of mechanical (pressure atrophy) and pathophysical causes.

The prevalence of the microsporidian infection in the total population of scat from which we drew our three fish could not be fully assessed because of the small number of specimens examined. The necrobiotic alterations that developed in the absorptive epithelium of the intestine were apparently a result of pressure atrophy exerted by the xenomatous growths. These morphological changes indicate that the normal functioning of the digestive system may have been significantly, or even fully, impaired. The degree of disturbance was difficult to determine however, and whether or not the observed alterations caused by the microsporidians were the cause of the high mortalities encountered could not be confirmed. Bacteriological and virological examinations are indicated, and could reveal other causative agents involved in the death of the *S. argus* broodstock.

Stress probably contributed to the mortalities in the population of *S. argus*. The fish were exposed to stress from handling and transport, and from acclimation to an artificial environment removed from their natural habitat, where they had little of their usual natural foods. These stress factors may have resulted in physiological disturbances, the consequences of which could have adversely affected the whole stock either directly, or indirectly, by rendering the fish susceptible to opportunistic organisms, such as the microsporidians.

In conclusion, it is likely that a combination of factors acted together and resulted in the deaths of the experimental fish. The specific nature of each factor, and its relative importance in the development of the problem is difficult to determine.

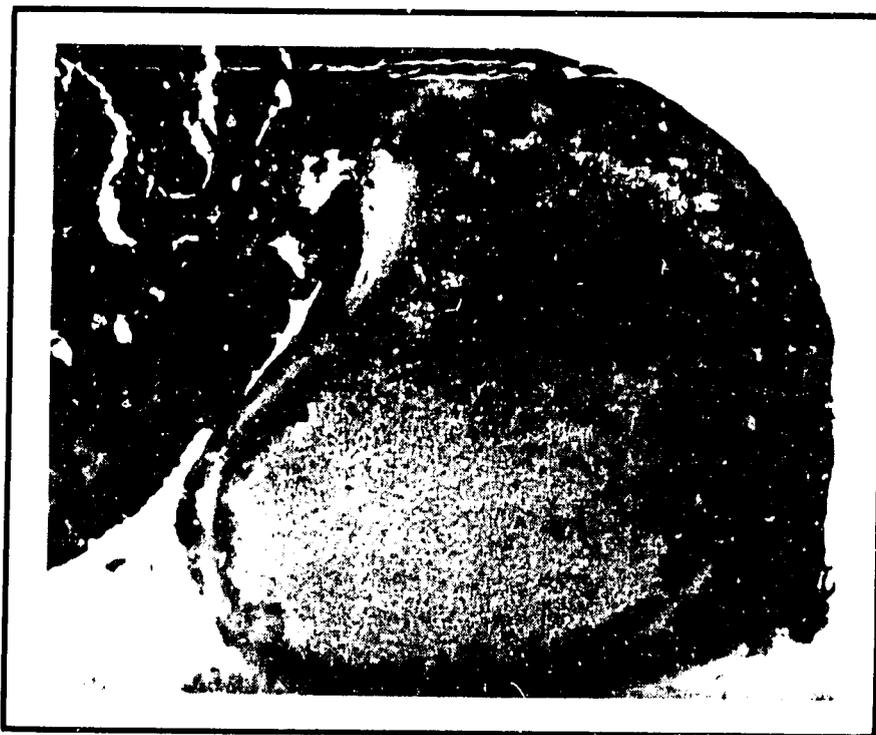


Fig. XIII.1. A xenomatous cyst induced by an unidentified species of microsporidian infecting the intestinal region of an adult spotted scat (*Scatophagus argus*). Note the center of connective tissue proliferation (arrow), the area of extensive hemorrhage (h), the peripheral zone (p) dominated by vesicles containing uninucleated spores and large meronts, and the granulomatous area (g). 75x.

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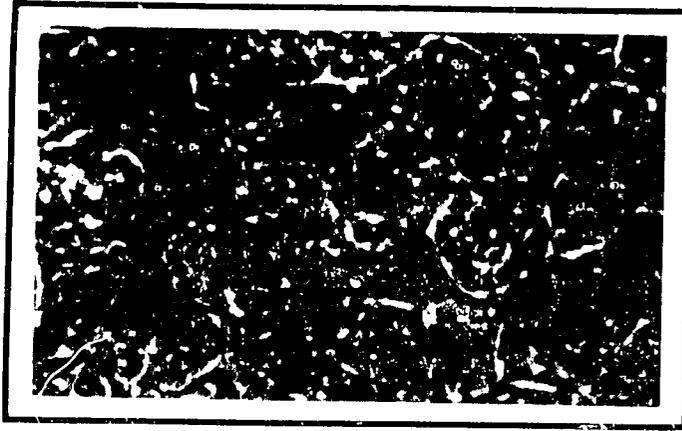


Fig. XIII.2. An aggregation of vesicles containing mature spores located in the central region of a xenomatous cyst induced by an unidentified microsporidian infecting the intestine of an adult spotted scat (*Scatophagus argus*). 150x.



Fig. XIII.3. A single mature sporophoric vesicle located within a xenomatous cyst induced by an unidentified microsporidian infecting the intestinal region of an adult spotted scat (*Scatophagus argus*). Note the concentric foci of mature spores within the vesicle (s). 150x.



Fig. XIII.4. Close-up of the peripheral area of a xenomatous cyst showing the early spore developmental stages within several sporophoric vesicles (v). Note the granulomatous area (g). The cyst was induced by an unidentified microsporidian infecting the intestinal region of an adult spotted scat (*Scatophagus argus*). 150x.

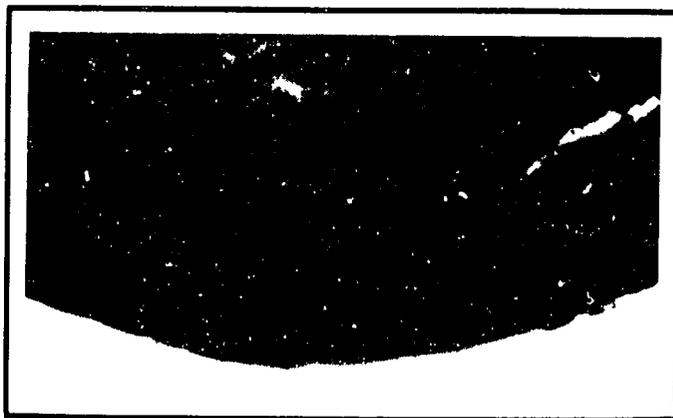


Fig. XIII.5. Cross section through the intestine of an adult spotted scat (*Scatophagus argus*) showing the extensive capillary congestion and hemorrhages in the submucosal layer (arrow). The damage was caused by an unidentified species of microsporidian. 75x.



Fig. XIII.6. The diffuse, necrobiotic alteration of the intestinal mucosal layer of an adult spotted scat (*Scatophagus argus*) caused by an unidentified species of microsporidian. 150x.

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Editor's Note: We subsequently found that over 70% of the scat fry we stocked into aquaria for a growth experiment (closed system, with undergravel water filtration) eventually died. The fry stopped feeding, became very thin, and died. Postmortem examinations of these fry revealed intestinal cysts very similar to those described in this report, suggesting that the fry had died from a microsporidian infection. Usually, all the fish in a tank died if the disease was present, although individual fish, which were apparently resistant, sometimes survived. Great care was taken to prevent the spread of disease from one tank to the other. Nets, siphons, air stones etc. were sterilized in Chlorex. The aquarium water was also sterilized before fish were stocked. Foot and hand baths (iodine-based disinfectant) were used by personnel working with the fish. These efforts were of little avail in preventing the spread of the disease from tank to tank.

All of the following treatments failed to check the disease once it was found in a tank: CuSO_4 (0.75 ppm, continuous treatment); malachite green plus formalin (0.1 ppm and 24 ppm respectively, one hour bath); Furacin (0.1 ppm continuous treatment); changing salinity from 12 ppt to 35 ppt.

XIV. REPORT ON DISEASES AND PARASITES IN THE SPOTTED SCAT (*Scatophagus argus*)

Gilda D. Lio-Po and Terence P. Barry

One hundred pieces of wild spotted scat (*Scatophagus argus*) with an average weight of 150 g were initially stocked in a 10-ton canvas tank at the Brackishwater Aquaculture Center (BAC) in Leganes, Iloilo. Rearing water with a salinity range of 35 to 50 ppt was pumped into the tank from a 500 m² pond supplied with water from the Guigui Creek and the Jalaud River. The water was changed 50% daily. The fish were fed mussel or squid meat supplemented with *Chaetomorpha* and *Cladophora*.

When the spotted scat were initially stocked, they had dermal lesions which probably resulted from the handling they received during their capture and transport to the BAC. Mortalities were first detected 16 days after stocking, and gradually increased over a week's time. The fish had become increasingly lethargic and anorectic, and many had developed hemorrhagic dermal lesions. A few hours before they died, the fish manifested imbalanced swimming activity.

The skin of the fish were heavily infested with the crustacean parasite, *Caligus sp.*, (Fig. XIV.1). Approximately 20% of the fish had single large isopods in their mouths which almost completely filled the buccal cavity. Smaller isopods were occasionally found on the gills.

The isopods were manually removed with forceps, and the fish were given a Dylox bath in freshwater to remove the *Caligus* (0.25 ppm, 0.50 hr) (Laviña, 1978). This treatment was very effective in dislodging the parasites, although it was later learned that freshwater alone worked as well as Dylox. The dermal lesions, and what appeared to be a fungal infection, were treated with 0.10 ppm methylene blue and 24 ppm formalin for one week (Brock, unpublished report). Water changes continued to be made daily.

There was a mass mortality of fishes 34 days following stocking. A total of 38 fish died within a two-hour period around noon.

At the same time, there was a mass die-off of tilapia, and *Penaeus monodon* larvae at the BAC. An investigation revealed that the water in the tilapia ponds, and the tank supplying the hatchery where the prawn and scat were held, had been changed at approximately the same time earlier that day. It was also learned that there had been an extensive oil spill from a ship cruising past the entrance to the Guigui Creek the previous night. We initially concluded, based on this evidence, that the mass mortalities were caused by this spill. However, several fish sampled and analyzed later by members of the BFAR-IDRC Fish Health Project showed that the scat were heavily infected with an unidentified species of *Microsporidian* which had induced the development of large cysts in the intestinal region of the fish. It could not be ruled out that this infection was the immediate cause of death (Natividad and Gerundo, XIII, this volume).

The surviving fish were extremely lethargic, and found to be heavily reinfested with isopods and *Caligus sp.*. The following day, five more fish died. The survivors were injected with Terramycin (50 mg/kg body weight) (Cruz, XV, this volume). Three days later, 5 fish were sacrificed for a diagnostic work-up by the SEAFDEC, AQD Fish Health Laboratory.

The sampled fish were lethargic and anorectic. They had opaque eyes, hemorrhagic lesions on their bodies, and numerous *Caligus sp.* (Fig. XIV.1) attached to their skin. Their gills were infested with *Caligus sp.*, *Trichodina sp.* (Fig. XIV.2), *Amyloodinium sp.* and an unidentified parasite which was perhaps a monogenean trematode (Fig. XIV.3). Bacterial isolations failed to demonstrate the presence of typical infectious bacteria, demonstrating the effectiveness of the earlier Terramycin treatment. Light microscopic observations of stained sections of the liver, kidney and spleen revealed that these structures were normal, except for one fish whose liver was moderately infiltrated with fat. The gills occasionally had areas of secondary lamellar hyperplasia (Fig. XIV.3). A summary of the parasite load of *S. argus* is shown in Table XIV.1.

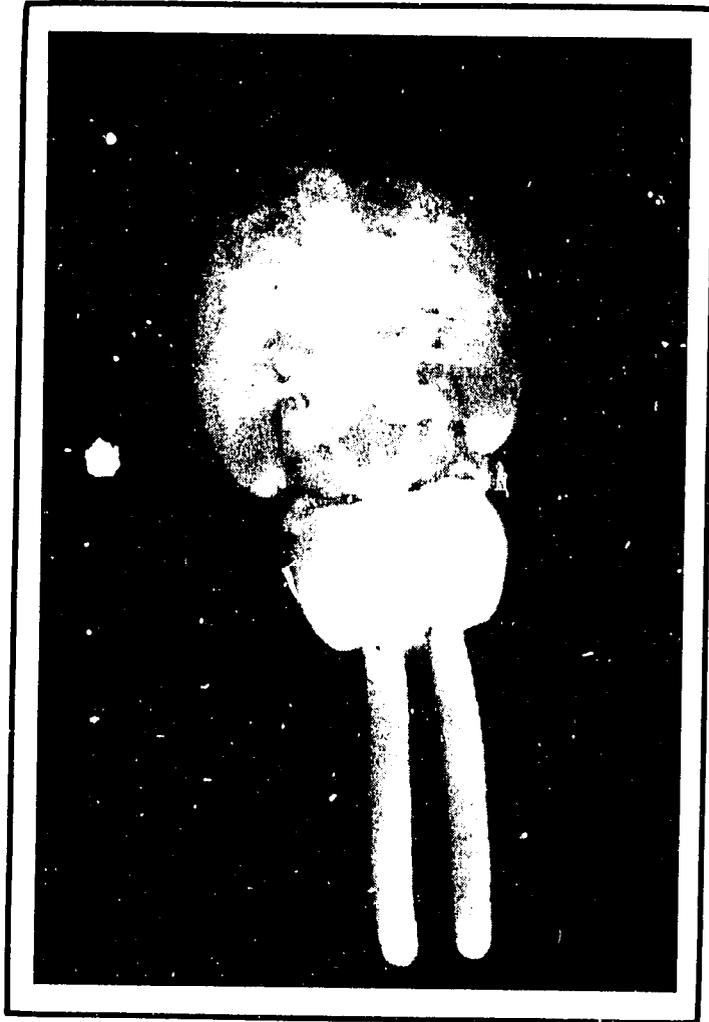


Fig. XIV.1. *Caligus sp.* Photograph of the ventral side of a specimen representative of those found infesting the skin and gills of adult spotted scat (*Scatophagus argus*). Total length, 2.85 mm.

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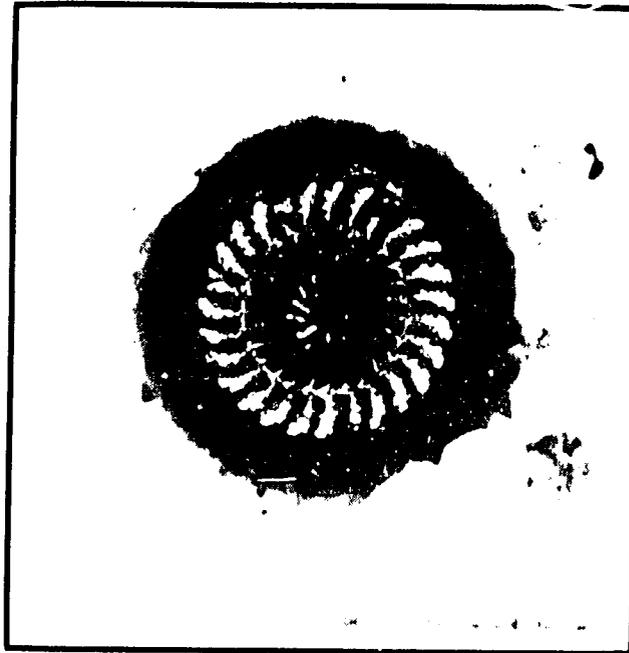


Fig. XIV.2. *Trichodina* sp. Photograph of a specimen representative of those found infesting the gills of adult spotted scat (*Scatophagus argus*). 375x.



Fig. XIV.3. Histological section through an unidentified parasite (p) attached to the gills of an adult spotted scat (*Scatophagus argus*). The specimen is perhaps a monogenean trematode. Note the secondary lamellar hyperplasia of the gill filaments in this photograph (h). 75x.

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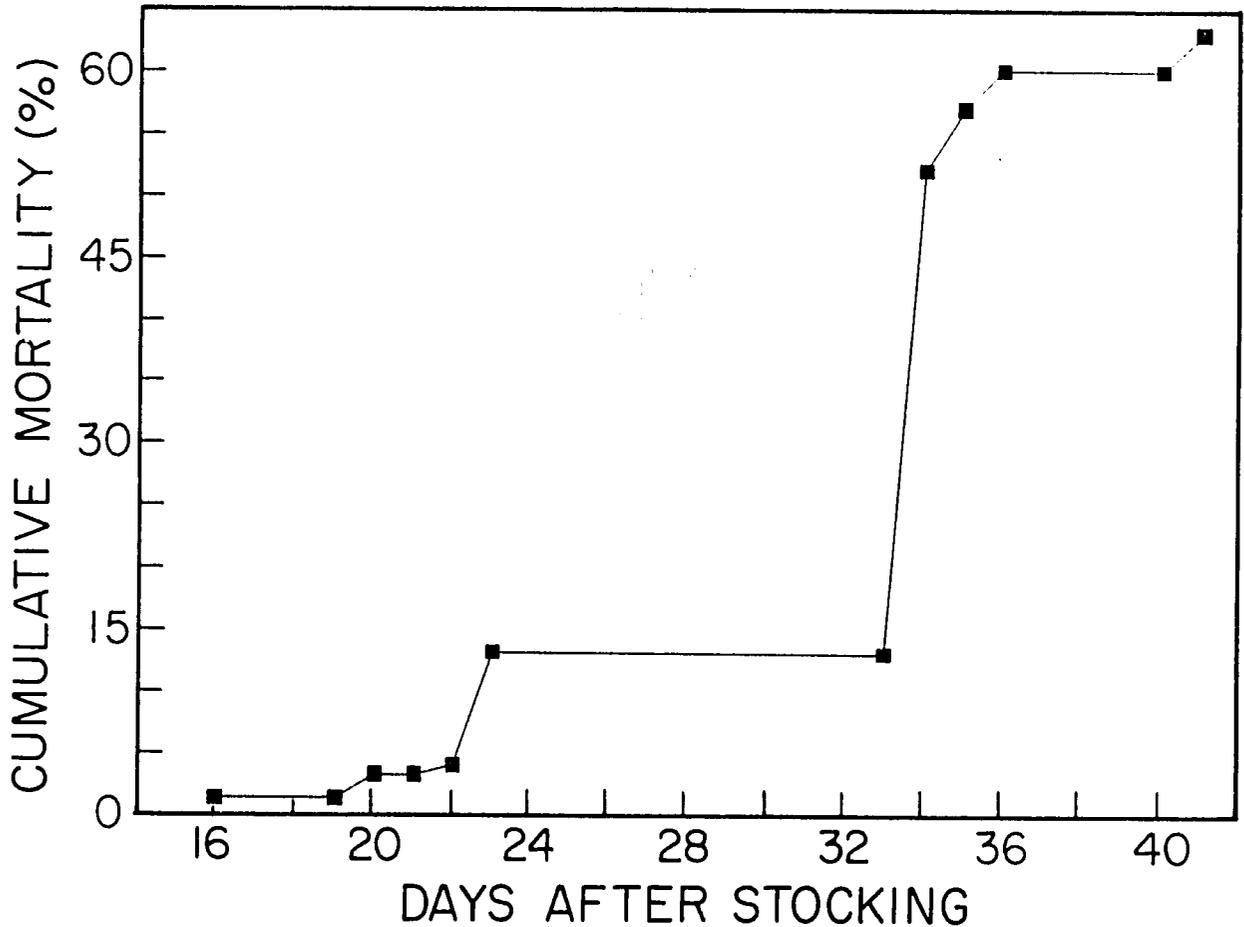


Fig. XIV.4. Cumulative percent mortalities in a population of small (average weight 150 g) adult spotted scat (*Scatophagus argus*) over a 41-day period following their capture from the wild from waters in the vicinity of Leganes, Iloilo, Philippines. The fish were confined in tanks at the University of the Philippines in the Visayas Brackishwater Aquaculture Center, Leganes, Iloilo, Philippines. The water supply used to replenish the fish's holding tanks was polluted by an oil spill on the night of the 33rd day following stocking, and was apparently the cause of the jump in mortalities observed on day 34. The earlier deaths are attributable to a multiple parasitic infestation which was detected on day 16 following capture.

TABLE XIV.1 SUMMARY OF PARASITES OBSERVED IN THE SPOTTED SCAT (Scatophagus arcus)

<u>Parasite</u>	<u>Parasite Load</u>	<u>Organs Affected</u>	<u>Effective Treatment</u>
<u>Amyloodinium sp.</u>	occasional to heavy	gills	CuSO ₄ (0.75 ppm, indefinite bath) Malachite green/formalin (0.1 ppm/24 ppm, 24 hr)
<u>Caligus sp.</u>	occasional to heavy	gills and skin	0.5 hr freshwater bath
<u>Microsporidia</u>	moderate to heavy	intestine	no effective treatment found
<u>Trichodina sp.</u>	occasional	gills	CuSO ₄ (0.75 ppm, indefinite bath) Malachite green/formalin (0.1 ppm/24 ppm, 24 hr)
Monogenean trematode	occasional	gills	0.5 hr freshwater bath
Isopod	occasional	mouth/gills	removal by hand

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Trichodina sp., *Amyloodinium sp.*, *Caligus sp.*, monogenean trematodes and isopods are all found on fish from the waters in the vicinity of Leganes, Iloilo (Natividad et al., 1986; Baticados & Quintio, 1984 and Lio-Po, personal observation). It is likely, especially in light of the fact that previously treated fish became reinfested, that the parasites came from the holding water.

The parasite load may have contributed significantly to weakening the fish, and thus rendered them more susceptible to bacterial, fungal, or protozoan (microsporidian) infection. In addition, the large isopods in the buccal cavity could have blocked the passage of food to the fish's digestive system, and the microsporidian infection may have impaired the ability of the intestine to absorb nutrients (Natividad and Gerundo, XIII, this volume). The resulting malnutrition of the fish may have put them in a very weakened state.

The hemorrhagic dermal lesions may have been caused by bacteria which were able to penetrate the fish's outer protective covering of mucus, scales, and skin when these were penetrated by the feeding and attachment organs of *Caligus sp.* and *Trichodina sp.* These lesions, in turn, could have served as portals of entry for other infectious bacteria which could have contributed to some of the earlier isolated deaths. The opaque eyes could have been caused by the bacteria *Vibrio sp.*, although this could not be verified because the fish were treated with an antibiotic. A species of *Vibrio* is responsible for the opaque eyes in milkfish sampled from the same area (Muroga et al. 1984).

Figure XIV.4 shows the cumulative mortalities in the population of spotted scat discussed in this report. There was only a 15% mortality rate from day 16, when the first heavy parasite infestation was reported, until day 33, the day before the oil spill. The parasites heavily infested many of the fish, yet the mortality rate was relatively low until the day of the spill. Apparently, it was the crude oil spill that had the most devastating effect on the fish. Oil, and related petroleum products, can severely damage fish gills, prevent oxygen uptake, and cause instantaneous death (Meyers and Hendricks, 1982). On the other hand, one or more of the infectious and parasitic organisms found in or on the scat could have suddenly reached a critical population level and been the direct cause of the mortalities. Most likely, however, the gill distress caused by the oil spill may have been aggravated by the presence of multiple parasite infestations on the gills, and the combination of assaults resulted in the mortalities.

In conclusion, the spotted scat is susceptible to a variety of parasites and diseases. However, the scat seems to have a high tolerance to these organisms, as evidenced by the low mortality rate in very heavily infested fish, prior to the oil spill. Although susceptible to disease, the scat responds very well to treatment regimes which can effectively eliminate the parasites and disease, with the exception of the *microsporidian* (Table XIV.1).

Our subsequent experience with the scat showed that it is much more susceptible to diseases and parasites if it is confined in tanks. In the ponds, even at high densities, or confined in cages, the spotted scat is far less susceptible to all of the disease and parasitic organisms reported here, including the *microsporidian*. We believe this phenomenon is best explained in terms of some missing nutritional factor(s) present in the pond and absent in tanks. Alternatively, it may be related to the fact that diurnal temperature fluctuations are much greater in the tanks than in the ponds.

XV. OBSERVATIONS AND TREATMENT OF DERMAL HEMORRHAGIC DISEASE IN THE SPOTTED SCAT (*Scatophagus argus*)

Paul Felipe S. Cruz

Occurrence, Pathology, and Treatment of the Disease

Juveniles and Adults. Beginning in July 1986 through August 1986, a disease outbreak occurred at University of the Philippines in the Visayas (UPV) Brackishwater Aquaculture Center which resulted in the mass mortality of fry and adults of spotted scat, sea bass, and tilapia. The external gross pathology of the disease was characterized in very sick scats by: opaque eyes, hemorrhagic lesions, eroded fins, and pale color. The internal gross pathology was characterized by: an empty gut with white mucus-like fluid, and spherical pus-filled, cyst-like bodies scattered along the omentum. Diseased fish did not feed, were lethargic, and often died.

The disease could be prevented by antibiotic treatment. Healthy fish were administered chloramphenicol in their diet (750 mg/kg feed; 3% body weight/day; 1 week duration). Sick fish did not feed, so they were injected intramuscularly with either chloramphenicol or oxytetracycline at 15 and 20 mg/kg fish, respectively. When samples of the diseased fish were brought to the SEAFDEC Pathology Department for diagnosis, no bacterial infection was detected after primary isolation, verifying the effectiveness of the antibiotic treatments. The SEAFDEC Pathology Laboratory found multiple parasitic infestations, primarily: *Caligus sp.* (crustacean), *Amyloodinium sp.* (flagellated protozoan), and *Trichodina sp.* (ciliated protozoan) and concluded that these organisms contributed to the outbreak of the ulcerative disease by providing a means for infectious bacteria to penetrate the fish's protective outer covering of mucus, scales and skin (Lio-Po and Barry, XIV, this volume).

Ulcerative disease outbreaks, similar to the epizootic described above, were frequently observed whenever scats were confined to tanks in the BAC's hatchery, where the quality of the water was generally poor. Even small breaks in the fish's skin would develop into infectious, hemorrhagic lesions that were usually accompanied by a fungal infection. Newly acquired fish were especially susceptible to the disease, probably because their skin was damaged during capture and transport. Most of the newly acquired fish had opaque eyes and developed skin ulcers within one day of capture. Before we began our prophylactic measures, described below, about 10% of the newly captured fish died within one day of delivery. After the prophylactic treatments were started, however, only one fish died from disease following its capture from the wild. The fish were treated as follows:

1. Anesthetize with 300 ppm 2-phenoxyethanol for at least 2 minutes.
2. Inject intramuscularly with oxytetracycline at 50 mg/kg fish.
3. Swab wounds and fins with 1% malachite green (for secondary fungal infection).
4. Bathe for 10 hours in 50 ppm of chloramphenicol.

Fry. During the disease outbreak in scat juveniles and adults in July and August, 1986, scat fry were also found infected with similar symptoms as the older fish. The infected fry had dermal hemorrhagic eruptions, were weak, and did not feed. The caudal fin was often rotten. The later stages of the disease were characterized by hemorrhagic ulcers that sometimes penetrated deep enough to expose underlying muscles. A cloud-shaped growth was sometimes present around the wound, and was probably a fungus as we observed fungal hyphae in microscopic smears from the skin of infected fish. Sometimes, the infection of the caudal fin became so severe that the entire tail of the fish was eroded away and the terminal vertebrae was exposed. Fish this severely infected ceased to feed, stayed at the bottom of the tank, or floated at the surface of the water, and soon died.

The primary cause of the disease was probably bacterial, since treatment with fungicides alone were not totally effective in preventing the disease. Treatments to eliminate fungus included: a 1% malachite green swab and/or a 10 minute bath using a

combination of 1,000 ppm formalin (10%), 0.5 ppm malachite green, and 100 ppm copper sulfate. When it became clear that these treatments were not fully effective, we treated the fish with a 10-hour bath of 50 ppm chloramphenicol. Within 3 days, the fins began to regenerate and the ulcers began to heal in 98% of the fish. A follow-up, 5-day, dietary treatment with chloramphenicol (500 mg/kg feed, 10% body weight/day) eliminated all traces of the disease. The antibiotic treatment, however, was not effective on fish in the advanced stages of the disease, suggesting that it was caused by other agents besides bacteria, or that the damage was already too severe to allow recovery.

Conclusions

The spotted scat easily becomes diseased when reared in water of poor quality. Disease outbreaks are likely following rough handling, as any break in the skin can create loci for infection. The disease agents appear to be omnipresent in the water, as evidenced by the recurrence of the diseases following improper handling of healthy fish.

Although the disease-causing organisms were not identified, the primary causative agent is probably bacterial, as evidenced by the effectiveness of antibiotic treatment in stopping the disease. Both oxytetracycline and chloramphenicol were equally effective. Both are broad spectrum antibiotics, although the latter is particularly effective against gram positive bacteria.

Antibiotic administration by immersion or injection are both suitable for newly acquired scats. Administration through the fish's diet is only possible with healthy fish which are already actively feeding on formulated feeds. Conditioning a fish to eat formulated feeds usually takes approximately one week.

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